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THE CELLS AND MEDIATORS WHICH PARTICIPATE IN THE MITOGEN-INDUCED BLASTOGENIC RESPONSES OF HUMAN CIRCULATING CELLS IN VITRO.

by

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ABSTRACT

In view of the frequent use of the mitogen-induced blastogenic response of human circulating cells in vitro in the assessment of immunocompetence, it is essential that the responder cell(s) and accessory cell(s) which respond with blastogenesis to phytomitogen (PHA, PWM, Con-A), antigen (TT, DT, CA, PPD) and allergen (AgE) stimulation, and the role(s) of soluble mediators, be identified. The T lymphocytes are the sole responder cells in the 3 day phytomitogen and 6 day antigen and allergen-induced blastogenic responses in vitro. The T lymphocytes respond optimally to phytomitogen stimulation following culture with autologous or allogeneic monocytes, or IL-1. On the other hand, the T lymphocytes respond optimally to antigen stimulation following culture with non-limiting numbers of autologous, but not allogeneic, monocytes, or with subthreshold numbers of autologous monocytes and IL-1. The T_M lymphocytes consistently respond better than the T_G lymphocytes whereas the T_C and T_N lymphocytes respond the least with respect to all the mitogenic stimuli. The T4+ and T8+ lymphocytes respond to the same extent. The B lymphocytes only respond with blastogenesis following 7 days in culture in the presence of PWM and IL-2 or BCGF. The Null lymphocytes do not respond to any of the mitogenic stimuli used. The absence of a blastogenic response by the T lymphocytes may therefore be attributed to (i) insufficient numbers of monocytes and/or (ii) defective monocytes incapable of secreting IL-1, and/or (iii) defective T lymphocyte incapable of secreting IL-2, and/or (iv) defective T lymphocyte incapable of responding to IL-2.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AB serum	Serum of blood group AB volunteers
ADCC	Antibody-dependent cell-mediated cytotoxicity
AFC	Antibody forming cell
AgE	Purified ragweed antigen E
anti-TAC	Monoclonal antiserum which detects the IL-2 receptor
B cells	Cells which stain for smIg and rosette with EAC
BCDF	B cell differentiation factor
BCGF	B cell growth factor
C′	Complement
C′3	Third component of complement
C′3R	Receptor for the third component of complement
C′3b	The cleaved b molecule of C′3
C′3bi	Inactivator cleaved bi fragment of C′3
C′3d	The cleaved d molecule of C′3
CA	Candida albicans
CF	Cytotoxic factor
CMI	Cell mediated immunity
CR1	Complement receptor for C′1, C′4, C′2, C′3b complex
CR2	Complement receptor for C′3d
CR3	Complement receptor for C′3bi fragment
CRL	Complement receptor-bearing lymphocytes
Con-A	Concanavalin-A
DARR	Direct antiglobulin rosetting reaction
DHSR	Delayed hypersensitivity reaction
DT	Diphtheria toxoid
E	Erythrocytes - red blood cells (RBC)

E rosette	Formation of rosettes by T cells-SRBC
EA	Erythrocyte complexed with antibodies
EAA indicator	erythrocytes - IgA myeloma complexed ORBC
EAC indicator	erythrocytes - C′ and IgM complexed ORBC
EAE indicator	erythrocytes - IgE myeloma complexed ORBC
EAG indicator	erythrocytes - IgG complexed ORBC
EAM indicator	erythrocytes - IgM complexed ORBC
FACS	Fluorescien activated cell sorter
FCS	Fetal Calf Serum (heat inactivated at 56^{o}C, 30 min)
FITC	Fluorescien isothiocyanate
Fc	Crystallizable fragment of IgG
FcA	Fc of IgA
FcAR	Fc receptor for IgA
FcD	Fc of IgD
FcDR	Fc receptor for IgD
FcE	Fc of IgE
FcER	Fc receptor for IgE
FcG	Fc of IgG
FcGR	Fc receptor for IgG
FcM	Fc of IgM
FcMR	Fc receptor for IgM
HBSS	Hank's Balanced Salt Solution
hrs	Hours
HI	Humoral immunity
HRBC	Human erythrocytes
IL-1	Interleukin 1

IL-2	Interleukin 2
IL-2R	T cell receptor for IL-2
IU	International units
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgD	Immunoglobulin D
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
K cells	Killer cells
LAF	Lymphocyte activating factor
LCF	Lymphocyte chemotactic factor
MAF	Macrophage activating factor
MARR	Mixed antiglobulin rosetting reaction
MCF	Macrophage chemotactic factor
MF	Mitogenic factor
MHC	Major histocompatability complex
MIF	Migration inhibitory factor
MNC	Mononuclear cells
MNC-B	MNC depleted of B cells
MNC-MO_1	MNC depleted of monocytes once
MNC-MO_2	MNC depleted of monocytes twice
MNC-Null	MNC depleted of Null cells
MNC-T	MNC depleted of T cells or non-T cells
MNC_m	Mitomycin-C treated MNC
MNCS	Supernatants of unstimulated MNC
MNCSP	Supernatants of PHA stimulated MNC

MO	Monocytes
MS	Supernatant from unstimulated monocytes
MSP	Supernatant from PHA-stimulated monocytes
MST	Supernatant from TT-stimulated monocytes
NK cells	Natural killer cells
NOCC	Natural-occuring cell-mediated cytotoxicity
NSE	Non-specific esterase staining
ORBC	Ox erythrocytes
PGE_2	Prostaglandin E_2
PHA	Phytohemmaglutinin
PPD	Purified protein derivative of tuberculin
PWM	Pokeweed mitogen
RBC	Erythrocytes (red blood cells)
smIg+	Surface membrane Immunoglobulins
SRBC	Sheep erythrocytes
SRS-A	Slow reacting substance of anaphylaxis
STA	Staphylococcal Cowan I strain Protein A
T-ARC	Antigen reacting T cells
$T\text{-}MO_1$	T cells depleted of monocytes once
$T\text{-}MO_2$	T cells depleted of monocytes twice
$T\text{-}MO_2$+MO	$T\text{-}MO_2$ cells reconstituted with monocytes
TCGF	T cell growth factor
TF	Transfer factor
TT	Tetanus toxoid
T_A	T cells with FcAR
T_C	T cells with C'3R
T_D	T cells with FcDR

T_E	T cells with FcER
T_{G+C}	T cells with FcGR and C'3R
T_G	T cells with FcGR
T_{M+C}	T cells with FcMR and C'3R
T_M	T cells with FcMR
T_N	T cells which lack detectable FcGR, FcMR and C'3R
$T\mu$	T cells with FcMR
$T\sigma$	T cells with FcGR
UV	Ultraviolet irradiation

1. INTRODUCTION

Immunity results from the interaction and cooperation of two functionally distinct systems - nonspecific (or innate) immunity and specific (or acquired) immunity. The non-specific immune system operates continually in the normal individual to provide resistance to infection with pathogenic microorganisms. It consists of (i) mechanical barriers (skin and mucous membranes), (ii) non-specific antibacterial constituents present in skin secretions, sebum, sweat and saliva, (iii) enzymes in secretions (i.e. lysozyme), (iv) the alternate pathway of complement fixation (C'3,C'5-C'9), (v) phagocytic cells, and (vi) ADCC lysis of the invading organism (Barret 1983). The FcG and C'3b receptor-bearing macrophages, monocytes and neutrophils kill microorganisms by phagocytosis and intracellular degradation most efficiently in the presence of opsonins (Stossel 1975, Klebanoff and Clark 1978). Opsonins consist of (i) conventional IgG antibodies present in subthreshold (below the level of detection by conventional immunoassays) concentration and (ii) C'3b which adheres to the antigen-antibody complex or the antigen itself following C' fixation via the classical or alternate pathways (Newman and Johnson 1979, Herman et al

1979).

The initial step in the phagocytosis of the invading microorganism (bacteria, rickettsia, fungal spores, protozoa) is the interaction of the phagocytic cell, via its receptors, with the IgG antibody and/or C'3b in the antigen-antibody-C'3b conjugate. This results in intracellular degredation of the antigen (Edelson 1980, Griffin 1982).

The FcG receptor-bearing cells can also lyse the invading microorganisms extracellularly following their interaction with minimal numbers of IgG antibodies (Segal and Hurwitz 1977, Dower et al 1981). This mechanism of target cell lysis is referred to as antibody-dependent cell-mediated cytolysis (ADCC). The effector cell is referred to as the killer (K) cell. The ADCC reaction constitutes a very important and effective immune mechanism as it can function optimally in the presence of antibodies which are non-C' fixing irrespective of their concentration or are C' fixing in too low a concentration to effect complement-mediated lysis of the target (Ziegler and Henney 1977).

An additional component of the non-specific immune system is the naturally-occurring (C' and antibody-independent) cell-mediated cytotoxic (NOCC) activity. The effector or cytotoxic cell is referred to as

the natural killer (NK) cell. The NK cells are lymphocytes or monocytes which may but need not be devoid of the conventional surface membrane receptors for SRBC, FcG, FcM and C'3b. These NK cells are considered to constitute a major defense to the emergence of malignant cells (Quan et al 1982). Although it does not provide resistance toward infectious agents, it is nevertheless considered by the discipline to constitute a component of the non-specific immune system (Kaufmann et al 1981).

The specific or acquired immune system consists of antibodies (humoral immunity or HI) and sensitized lymphocytes (cell mediated immunity or CMI) as well as phagocytic cells and the classical pathway of complement fixation (C'1-C'9) both of which are "non-specific" and not antigenically induced but function in an accessory capacity to facilitate elimination of invaders by antibodies or sensitized cells (Mayer et al 1981, Boyle and Boros 1980). Cell-mediated immunity is frequently associated with tissue destruction as occurs in tuberculosis, allograft rejection, or an organ affected by an autoimmune response (Waksman 1971). The effector cells involved in these CMI reactions in vivo are sensitized T lymphocytes which are activated by the sensitizing antigen(s). Subsequent interaction of these actively-sensitized T lymphocytes with the specific antigen results in the release of mediators referred to

as lymphokines (Cohen et al 1979). The relevant lymphokines have been identified as migration inhibitory factor (MIF), macrophage chemotactic factor (MCF), macrophage activating factor (MAF), lymphocyte chemotactic factor (LCF), mitogenic factor (MF), transfer factor (TF), cytotoxic factor (CF) and gamma interferon. These lymphokines are responsible for the tissue damage and lesions (Cohen et al 1979). The classical or prototype lesion of the CMI reaction is the delayed hypersensitivity skin reaction (DHSR) following intradermal challenge of a tuberculin-sensitized individual with tuberculin or PPD (Bloom 1967, Landsteiner and Chase 1942). The reaction evolves and progresses over a period of 24-72 hours. The induration usually attains maximum intensity by 72 hours post-challenge. Upon contact with the antigen, the sensitized lymphocytes release MIF and MCF which are specific for monocytes. Monocytes chemotactically attracted to the challenge site are prevented from emigrating and transform, upon interaction with MAF, into "angry" macrophages activated to a high level of phagocytic activity (Waksman 1971, Edelson 1980). The number of specifically sensitized lymphocytes may be increased by the action of TF which confers upon a previously uncommitted cell the properties of an actively sensitized cell (Lawrence 1969, Bloom 1967). The sensitized lymphocytes are induced to proliferate by MF

(Cohen 1979). Both TF and MF may be considered to be amplification factors in the generation of sensitized lymphocytes. The death of the invading organism and injured cells in the reaction site is attributed to the action of CF (van Loveren and Askenase 1984, Kops et al 1984).

In contrast, humoral immunity is seldom associated with tissue damage. The interaction of the precommitted antigen receptor-bearing T cell (T-ARC) with the ARC-dependent antigen results in the stimulation of previously uncommitted virgin B lymphocytes. These precursors of the antibody forming cells (AFC) transform into medium and large lymphoblasts and divide and dedifferentiate into antibody-forming and secreting lymphoblasts and plasmablasts (Richter 1982, Ashman 1982). The antibodies interact only with the original immunizing antigen as compared to the lymphokines which act indiscriminately on cells in the micro-environment. The majority of the antibody secreting B lymphoblasts and plasmablasts transform into endstage non-antibody synthesizing or secreting plasma cells with a half life of 3 - 5 days; these cells lose the capacity to secrete the antibodies before they lose the capacity to synthesize antibodies and will therefore invariably contain antibodies in the cytoplasm (Ashman 1982). A minority of the B lymphoblasts differentiate into small,

mature, long-lived (up to 30-40 years) memory lymphocytes. These lymphocytes are capable of being immediately reactivated to antibody synthesis upon reexposure to the immunizing antigen (Klinman 1972, Teale et al 1981). The requirement of memory B lymphocytes (in the presence of T helper cells and monocytes) in the secondary or anamnestic response, as opposed to clonally-selected T-ARC and uncommitted virgin B lymphocytes in the primary response, tends to explain the more explosive nature of the secondary as compared to the primary antibody response (Davies et al, Mitchell and Miller).

Antibodies, which may be considered to constitute the mediators of HI, interact with and kill the invaders following interaction with and activation of the complement system via the classical pathway (C'1 to C'9). Osmotic lysis of the invader occurs only following activation of C'9 (Borbos and Rapp 1965). The antibodies also neutralize toxins, prevent viral reinfection, and form immune complexes with the extrinsic antigens which are eliminated by phagocytosis and intracellular degradation (Metzger 1974).

The majority of the naturally-occurring antigens require interaction with T-ARC cells prior to activation of the B lymphocytes and their transformation,

proliferation and dedifferentiation into antibody-secreting lymphoblasts and plasmablasts (McDougal 1982, Tada et al 1978, Imperiale et al 1982). These antigens are referred to as T or ARC-dependent antigens. These antigens characteristically consist of a non-immunogenic component referred to as the carrier to which are attached multiple numbers of different antigenic determinants referred to as epitopes (Katz et al 1970, Paul et al 1970). A small number of antigens initiate antibody synthesis in the absence of T-ARC cells and they are referred to as T-independent antigens. These antigens have been characterized as large polymeric molecules with repeating identical antigenic determinants. Examples of such antigens are dextran, lipopolysaccharide and polymerized flagellin. The immune response to the T-dependent antigen is initially IgM antibody synthesis followed by IgG antibody synthesis whereas the response to the T-independent is primarily if not exclusively an IgM antibody response (Buck et al 1979, Layton et al 1981, Pike and Nossel 1984).

It is generally agreed that two signals are required to initiate B lymphocyte activation by the antigen. In the case of the T-ARC dependent antigens, these signals are provided by the T-ARC cell and the antigen. In the case of the T-independent antigen, one signal is provided by the antigenic determinant on the antigen while the

other is provided by the carrier constituent of the antigen (Bradley-Mullen 1982, Pike and Nossel 1984).

Immunoregulatory mechanisms operate to limit the extent of the immune response. One mechanism consists of the T helper and T suppressor lymphocytes. The T helper lymphocytes augment or enhance antibody synthesis. As they possess surface receptors for FcM, they are referred to as T_M or Tμ lymphocytes. Suppression or inhibition of antibody synthesis is effected by the suppressor T lymphocytes. Suppressor cells have cell surface receptors for FcG and are referred to as T_G or Tσ cells (Moretta et al 1975, Moretta et al 1976, Moretta et al 1977). With the emergence of monoclonal antibodies specific for subpopulations of T lymphocytes, the circulating suppressor T and helper T lymphocytes have been identified by their interaction with OKT8 and OKT4 monoclonal antibodies and are referred to as T8+ and T4+ lymphocytes, respectively (Reinherz et al 1980, Thomas et al 1980, Kung and Goldstein 1980). A second mechanism operating to limit the HI response is feedback suppression by antibody molecules themselves. The IgM antibodies stimulate the synthesis of IgG antibodies and the latter, as they increase in concentration, suppress further synthesis of IgM antibodies and thereby IgG antibodies (Herzenberg et al 1981). The stimulating IgM antibodies and the suppressive IgG antibodies may act by

stimulating the T_M and T_G lymphocytes, respectively.

The discovery and subsequent characterization of the interleukins, which are soluble factors produced by monocytes/macrophages and T lymphocytes, has led to a better understanding of the mechanisms which sustain the survival and proliferation of the immunocompetent cells. The T cell activating factor or Interleukin 1 (IL-1), secreted by the monocyte, stimulates the production by T lymphocytes of a T cell growth factor (TCGF) referred to as Interleukin 2 (IL-2) (Maizel et al 1981, Mizel 1982, Gery 1982, Gillis and Mizel 1981, Palacios 1982). T lymphoproliferation is dependent upon the interaction of IL-2 with IL-2 receptors expressed on the activated T cell (Reske-Kuntz et al 1984, Lipkowitz et al 1984). The induction of B lymphocyte proliferation and differentiation involves at least two interleukins produced and secreted by the T lymphocyte. These are referred to as B cell growth factor (BCGF) and B cell differentiation factor (BCDF) (Ford et al 1981, Howard et al 1982). The interaction of BCGF with activated B lymphocytes induces B lymphoproliferation and the synthesis or unmasking of specific receptors for BCDF. The interaction of these receptors with BCDF promotes differentiation of the blasts into antibody-secreting cells (Korsmeyer et al 1983, Muraguchi 1984).

A parameter of the immune response which can be measured in vitro is the lymphoproliferative response. It has been known for several decades that antigens are capable of inducing the proliferation of memory cells in culture (Chess et al 1975, Geha et al 1981, Morimoto et al 1981). In 1960, Nowell discovered that small, quiescent lymphocytes could be stimulated non-specifically in vitro by an extract of the red kidney bean, Phaseolus Vulgaris, to transform into large pyroninophilic lymphoblasts. The active agent in this extract is referred to as a lectin. Lectins are multivalent carbohydrate-binding glycoproteins capable of interacting with hydrophilic carbohydrate groups which are constituents of many cell surface proteins (Goldstein and Hayes 1978, Goldstein et al 1980, Lis and Sharon 1981). Several of the plant-derived lectins are mitogenic and they are referred to as phytomitogens. They include Phytohemagglutinin (PHA), Pokeweed mitogen (PWM) and Concanavalin-A (Con-A). Binding with the appropriate surface receptors results in cross linking of surface carbohydrate moieties and conformational changes in the cell membrane components triggering lymphoproliferation (Nowell, 1960, Goldstein and Hayes 1978, Lis and Sharon 1981).

Phytomitogens are nonspecific or polyclonal stimulants, activating many different clones of lymphocytes (reviewed by Lis and Sharon 1981). Specific antigens, on the other hand, are stimulants capable of binding to antigen receptors which have genetically predetermined antigenic specificity thereby, inducing proliferation. These antigens also induce the proliferation of memory (HI response) or sensitized (CMI response) lymphocytes which have been specifically generated in responses to specific antigens (Euquem and Bona 1977).

Both polyclonal phytomitogens and monoclonal antigens initiate a series of metabolic events following interaction with the responding lymphocytes which culminates in blastogenesis (Varescerio and Holden 1980, Wang et al 1978). However, lymphocyte activation by nonspecific phytomitogens obviates the requirement for presensitization and specific recognition that characterizes immunologic activation by specific antigens (reviewed by Lis and Sharon 1981). The phytomitogens are therefore useful in studying the mechanism of lymphocyte activation.

1.1. RATIONALE

Phytomitogens and antigens stimulate circulating lymphocytes in unimmunized and immunized subjects (human and animal), respectively, to undergo blastogenesis and mitosis in vitro. The phytomitogens (PHA, PWM and Con-A) and a number of soluble antigens (tetanus toxoid, diphtheria toxoid, Candida albicans and purified protein derivative or PPD) are stimulants used in the blastogenic response (Geha et al 1981, Lis and Sharon 1981, Alpert et al 1981). This response of circulating lymphocytes to phytomitogen and antigen stimulation is universally accepted as being primarily a function of T lymphocytes and is considered to be a reflection of potential or actual T cell immunocompetence (Kristensen et al 1982). The precise T lymphocyte subclass which responds to phytomitogen and antigen stimulation has not as yet been identified. The 3 day phytomitogen-induced blastogenic response is used in the clinical setting to assess and monitor T lymphocyte function in patients presenting with symptoms of immunodeficiency disease and other diseases where the immune system is suspected to have a role (Dean et al 1977, Eibl et al 1982, Hutchins and Steel 1983). It must be noted, however, that PWM also induces B lymphoproliferation and B lymphocyte differentiation into

Ig secreting cells following 7 days in culture (Rosenberg and Lipsky 1981, Fauci et al 1980, Fauci et al 1982).

Helper roles essential for optimal T lymphoproliferation have been ascribed to B lymphocytes (Delespesse et al 1976) and Null lymphocytes (Caraux et al 1982). Both B lymphocytes (Caraux et al 1978, Kasahara et al 1979b, Swain and Dutton 1980) and Null lymphocytes (Kasahara et al 1972a, Caraux et al 1982) have been shown to secrete soluble mediators necessary for T lymphocyte activation. The roles of the B and Null lymphocytes in the blastogenic response must be unequivocally defined since a minimal or absent T lymphocyte blastogenic response may reflect a defect(s) in specific subpopulations of helper B and/or Null lymphocytes rather than in the T lymphocytes.

Attention has been focused on the role of the monocyte in the induction and regulation of the blastogenic response (Williams et al 1984). The monocyte is no longer considered to be merely a passively-supporting, innocent bystander or accessory cell, but rather an active participant in the regulation of the blastogenic response. Stobo et al (1972) and Mackler (1972) have shown that phytomitogens are capable of initiating blast transformation in "monocyte depleted"

T lymphocytes; however other investigators claim that monocytes have a potentiating roles (Hedfors et al 1975, Schmidtke and Hatfield, 1976) or are absolutely required (Rosenstreich et al 1976, Raff et al 1976) for the response. The role of the monocyte in the blastogenic response must be unequivocally defined to ensure that lack of a response not be mistakenly attributed to defective T lymphocyte function when the actual defect may be in the monocytes.

A defective or absent proliferative response in vitro may therefore be the result of (i) an alteration in the function of responding T lymphocytes (ii) an alteration in the function of monocyte accessory cells, i.e. the inability of the monocyte to synthesize and secrete IL-1, (iii) an alteration in the function of the B and Null lymphocytes, (iv) inability of the T lymphocytes to synthesize and secrete IL-2, and/or (v) increased production of suppressor cells or factors. In light of the new advances in our knowledge concerning the multiplicity of soluble factors and cells involved in the blastogenic response, it is necessary to reassess the blastogenic response and to redefine the participants.

The objectives of this investigation are therefore as follows: (i) to identify the subpopulations of the

circulating human T lymphocytes which undergo blastogenesis upon stimulation with the phytomitogens, antigens and allergen (ii) to delineate the role of Null lymphocytes, B lymphocytes and monocytes in this T lymphocyte response and (iii) to define the role of the interleukins (IL-1 and IL-2), in the 3, 6 and 7 day blastogenic responses.

2. HISTORICAL REVIEW

INTRODUCTION

The historical review will acquaint the reader with the phytomitogen and antigen-induced blastogenic responses of the circulating human lymphocytes. The initial section deals with the identification of the circulating lymphocytes and the markers used to identify the lymphocyte subpopulations which participate in the blastogenic response as either responder or accessory cells. The subsequent section deals with the Interleukins and their role in facilitating and sustaining the blastogenic response. The final section deals with the mechanism of lymphocyte transformation by the non-specific (phytomitogens) and specific (antigens) mitogens.

2.1. THE CIRCULATING MONONUCLEAR CELLS IDENTIFIED AND CATEGORIZED ON THE BASIS OF CELL SURFACE MARKERS

2.1.1. INTRODUCTION

Until the mid 1960's the circulating human lymphocytes were considered to be homogeneous and were defined on the basis of morphological characteristics following staining and examination by light microscopy (Yoffey and Coutice 1956). The terms B and T lymphocytes were first proposed by Claman and his colleagues (1966) who demonstrated that thymus (T) and bone marrow (B) lymphocytes must collaborate in the rodent in order to generate an antibody response to the immunizing (SRBC) antigen. Cells of different lymphoid organs of unimmunized mice were transferred to syngeneic mice rendered immunoincompetent by sublethal total body irradiation and simultaneosly immunized with the antigen. Only those immunoincompetent mice which had been injected with a suspension of spleen cells or a combination of thymus (T) and bone marrow (B) cells synthesized and secreted antibodies 5-6 days post immunization. In contrast, the immunoincompetent mice injected with lymphocytes of the individual lymphoid organs other than the spleen did not respond with antibody production. It

was later demonstrated that the spleen contains both T and B lymphocytes. This finding explains why bone marrow and thymus cells by themselves could not restore immunocompetence whereas the spleen cells could restore immune responsiveness to the irradiated immunoincompetent mice. The T cells possess receptors for the antigenic determinants on the T-dependent antigens and are referred to as antigen receptor bearing T cells or T-ARC whereas the B cells are the precursors of the antibody forming cells or B-AFC (Richter 1982). The terms T and B lymphocytes refer to only those thymus-derived and bursa of Fabricius - equivalent lymphocytes which are immunocompetent and participate in immune responses. The distinct immunological functions of the T and B lymphocytes in antibody formation constituted the first markers for these cells (Roitt et al 1969, Cooper et al 1969).

Xenogeneic antibodies directed toward cell-surface configurations (antigens), were introduced in the late 1960's for the detection and classification of lymphocytes. Raff et al (1970) demonstrated that all of the thymus and a proportion of the circulating lymphocytes in the mouse reacted with anti-T lymphocyte antibodies. These circulating lymphocytes were referred to as T lymphocytes. The non-T lymphocytes were

subsequently referred to as B lymphocytes following the discovery of their bursa of Fabricius - equivalence (Roitt et al 1969, Cooper et al 1969). Concurrent with this discovery, human T lymphocytes were identified by their ability to interact with SRBC to form rosettes (SRBC erythrocytes or E rosettes) (Coombs et al 1970, Jondal et al 1972). The formation of E rosettes is independent of antibody or C' and is attributed to the interaction between surface glycoprotein or glycolipid configurations on the SRBC and their complementary receptors on the surface of T lymphocytes (Galili and Schlessinger 1975). Controversy concerning the nature of this receptor and its functional role, if any, has been ongoing since its original identification in the late 1960's (Biozzi et al 1968, Zaalberg et al 1968). One argument is that the substrate for the receptor is an as yet unidentified microbial antigen which cross-reacts with the SRBC. At this time, it is considered that the receptor for SRBC serves as a marker for a particular lymphocyte population and provides a means for the isolation of these cells. This property of E rosette formation has been accepted as the standard method for identifying and isolating human T lymphocytes (Wybran 1979).

Concomitant with the definition of the T lymphocytes as SRBC rosetting cells, the circulating lymphocytes previously referred to as B lymphocytes were demonstrated to bear surface membrane immunoglobulins (smIg) (Raff et al 1970, Unanue and Abbas 1975). These cells are capable of synthesizing and secreting Ig. The major Ig classes present on circulating B lymphocytes are IgM and IgG. The presence of smIg constitutes the classical (chronologically the oldest) operational definition of the B lymphocyte. It was subsequently demonstrated, in the early 1970's that B lymphocytes also possess a receptor for the cleaved products of C'3 - C'3b/d (Ross et al 1973). The B lymphocytes are thereby capable of rosetting with EAC indicator erythrocytes (Bianco et al 1970, Ross et al 1975).

A minority of circulating lymphocytes was originally referred to as L lymphocytes and were initially included within the family of B lymphocytes (Chess et al 1975, MacDermott et al 1975). These L lymphocytes, like B lymphocytes, possess surface Ig in the freshly isolated state. However, unlike B lymphocytes, L lymphocytes shed their smIg following incubation at 37°C for 30 minutes. These cells therefore have labile Ig on their surface, hence the term L lymphocytes. These L lymphocytes were initially referred to as non-T non-B, or Null

lymphocytes, as they did not bear the characteristic markers of the T (receptors for SRBC) and B (smIg) lymphocytes. The Null lymphocytes were subsequently shown to possess surface receptors for the Fc of IgG (FcG), of being capable of rosetting with the EAG indicator erythrocytes, and to have the capacity to carry out the ADCC cytolytic reaction (Horwitz and Garrett 1977, Lobo 1981).

The circulating lymphocytes, especially the T lymphocytes, have also been catagorized by the use of xenogeneic mouse monoclonal antibodies (Reinherz and Schlossman 1980, Kung et al 1980). Monoclonal antibodies are synthesized by hybridomas produced by fusing antibody-forming splenic B lymphocytes of mice immunized with human T cells with continuously replicating malignant (lymphoma or myeloma) cell lines. The hybridoma cells retain the capacity to synthesize antibodies to the original immunizing antigen, the human T lymphocyte. The different clones of hybridoma cells may synthesize antibodies to unique T lymphocyte configurations or antigens expressed by the T lymphocytes at different stages of maturation and differentiation (Kung et al 1980). Monoclonal antibodies have ushered in a new approach toward the classification of the circulating T lymphocytes.

In the following sections, the human circulating lymphocytes are discussed in terms of their surface receptors, smIg, and antigenic determinants, and the methods used to identify and isolate these cells.

2.1.2. THE T LYMPHOCYTES

2.1.2.1. CIRCULATING T LYMPHOCYTES IDENTIFIED ON THE BASIS OF CELL SURFACE RECEPTORS FOR SRBC

The term rosette and the identification of lymphocytes by rosette formation date back to the late 1960's. Biozzi et al (1968) and Zaalberg et al (1968) demonstrated that splenic lymphocytes of SRBC immunized mice were capable of binding to SRBC and thereby forming clusters called rosettes. It was initially assumed that the rosetting cells constitute the antibody-producing B cells in the immunized mouse. It was subsequently demonstrated that the T lymphocytes initially formed rosettes and did not form hemolytic plaques upon incubation with SRBC and C' (Zaalberg et al 1968).

Investigations with normal human lymphocytes demonstrated that rosette formation with SRBC is a unique property of the T lymphocytes (Coombs et al 1970, Jondal et al 1972, Wybran 1979, Palacios and Martinez-Maza 1982). This property of E rosette formation was attributed to the presence of a receptor for SRBC on the T lymphocyte. Pig, goat and horse erythrocytes were also

found to be capable of forming rosettes with human T lymphocytes but they are not as easily obtainable as are the SRBC. Wybran et al (1972) and Dickler (1976) found that the circulating lymphocytes of patients with hypogammaglobulinemia (B cell deficiency) had normal to increased proportions of E rosette-forming cells whereas the circulating lymphocytes of patients with the Di George's syndrome (T cell deficiency) displayed decreased proportions of E rosetting cells. The exclusive capacity of T lymphocytes to form rosettes with SRBC is today the accepted conventional method for the isolation and the identification of T lymphocytes. Approximately 55-75% of human circulating lymphocytes are E rosette-forming T lymphocytes.

2.1.2.2. CIRCULATING T LYMPHOCYTES CLASSIFIED ON THE BASIS OF CELL-SURFACE RECEPTORS FOR FcG, FcM and C'3

Lo Buglio et al (1967) and Uhr and Moller (1968) demonstrated that a proportion of non-T human lymphocytes possess cell-surface receptors which bind antibody-antigen complexes (immune complexes) and aggregated IgG. These receptors were demonstrated to have a high avidity for a configuration at the Fc region of IgG and were referred to as FcG receptors (FcGR). The FcGR were detected on T lymphocytes in the mouse and guinea pig by Grey (1972) and Yoshida and Anderson (1972). Yoshida and Anderson (1972) reported that T lymphocytes of allogeneic mice transferred into irradiated mice result in the activation of T lymphocytes and an increase in the number of FcGR. Further studies on lymphocyte populations have shown that T lymphocytes bear FcGR (Lee and Paraskevas 1972, Yoshida and Anderson 1972, Ferranini et al 1976, Colombatti et al 1981). Utilizing soluble fluoresceinated-IgG immune complexes and a fluorescent activated cell sorter (FACS), Dickler et al (1976) separated human circulating T lymphocytes into their FcGR positive (T_G) and FcGR negative (non-T_G) cell populations. Further investigations revealed cell surface receptors for the Fc of other Ig

isotypes on the T lymphocyte (Moretta et al 1975, Preud'homme et al 1977, Spiegelberg 1981, Richter et al 1983). Receptors for the Fc of IgM (FcM receptors or FcMR) were first demonstrated on normal T lymphocytes by Moretta (1975). Approximately 50-70% of human circulating T lymphocytes, following culture of the T lymphocytes for 24 hours at 37°C in medium supplemented with fetal calf serum (FCS) possess FcMR detected by using EAM indicator erythrocytes (Moretta et al 1975, McConnel and Hurd 1976, Ferranini et al 1976, Preud'homme et al 1977). Moretta et al (1976) proposed that the failure to detect FcMR in the freshly isolated cells is attributable to binding of native circulating IgM to the FcMR in vivo. The in vitro 24 hour incubation period appears to be necessary for the T lymphocytes to shed the receptors and/or the IgM bound to them and to synthesize new receptors (Ferranini et al 1976). Support for this proposal is the finding that FcMR are readily detected on freshly isolated T lymphocytes obtained from hypogammaglobulinemic patients who have low or undetectable levels of IgM in their sera (Moretta et al 1977). On the other hand, Gmelig-Meyling (1976) observed that EAM indicator erythrocytes formed rosettes with a significant proportion of freshly isolated T lymphocytes, and concluded that overnight incubation of the T lymphocytes at 37°C was neither an essential nor a

favorable condition for optimal EAM rosette formation. A majority of investigators have confirmed Moretta's original finding of the need of T lymphocytes to be incubated for up to 24 hours at 37°C in order to generate receptors for FcM (Mingari et al 1978, Romagnini et al 1978). In contradistinction, the T lymphocytes with FcGR can be easily detected using the freshly isolated cells since the FcGR have a low affinity for native IgG. However, they can be easily detected by their ability to rosette with EAG since the FcGR have a high affinity for FcG in immune complexes. This property allows for the detection of the cells bearing FcGR by their rosetting with EAG.

During the early 1970's, there were conflicting reports regarding surface receptors for C' components on T lymphocytes. Complement receptor bearing lymphocytes (CRL), initially isolated by Bianco et al (1970), were demonstrated to have two types of C' receptors with distinct specificity. The C' receptor referred to as CR1 is specific for a single configuration composed of C'4b and C'3b, whereas the complement receptor CR2 is specific for C'3d (Ross et al 1973, Ross and Polley 1975, Weis et al 1984). Ross et al (1978) demonstrated that human circulating T lymphocytes rosette only with EAC1-3b indicator erythrocytes (EAC containing C'1, C'4, C'2 and

C'3b) thereby indicating that human T_C lymphocytes possess only CR1. The CR2 was not detected on human T_C lymphocytes. Walia et al (1979) showed that approximately 18% of mouse thymocytes bear C'3R following the administration of cortisone acetate. These cells belong to the cortisone-resistant population of T lymphocytes. Arneiz-Villeme et al (1974) demonstrated that 20% of mouse splenic T lymphocytes and 10% mouse lymph node T lymphocytes possess receptors for C'3 (C'3R) detected by immunofluorescent labelled EAC indicator erythrocytes. Chiao et al (1975) reported that 0.5-8% of the circulating human T lymphocytes possess C'3R by simultaneously rosetting the T lymphocytes with SRBC and EAC composed of sensitized pigeon RBC. Ross et al (1978) and Mendez et al (1974) rosetted T lymphocytes with EAC indicator erythrocytes and demonstrated that approximately 2% of the circulating human T lymphocytes possess C'3R. Richter et al (1980) reported that approximately 8% of freshly isolated human circulating T lymphocytes possess C'3R. The percentage of T lymphocytes which rosette with EAC increased following 24 hour incubation at 37°C (Richter et al 1980).

Richter et al (1983) identified and isolated six human T lymphocyte subpopulations on the basis of receptors for FcG, FcM and C'3. The monoreceptor-bearing

T_G, T_M and T_C lymphocytes constituted approximately 5-15%, 10-20% and 10-20% of the T lymphocytes, respectively. In addition to the T lymphocytes bearing receptors for only FcG, FcM or C'3, a significant proportion (15-25%) of the T lymphocytes bear surface receptors for both FcM and C'3 (T_{M+C}). A smaller percentage (less than 10%) of the T lymphocytes bear receptors for both FcG and C'3 (T_{G+C}). The remaining T lymphocytes (40-50%) do not bear detectable surface receptors for FcG, FcM or C'3 and are designated as T Null lymphocytes or T_N. The nature of the T_N lymphocytes can only be speculated upon at the present time. They may constitute the precursors of any or all of the receptor-bearing cells, or they may constitute a unique population of cells with roles quite distinct from those of the receptor-bearing cells.

2.1.2.3. CIRCULATING T LYMPHOCYTES CLASSIFIED ON THE BASIS OF CELL SURFACE RECEPTORS FOR FcA, FcE and FcD

T lymphocytes have been demonstrated to possess Fc receptors for the Ig isotypes IgA, IgE and IgD as well as for IgG and IgM. The identification, assessment, function and distribution of these T lymphocyte subclasses in normal and diseased individuals is currently under investigation. The receptors for the Fc of IgA (FcA) are present on 2-18% of human circulating T lymphocytes (T_A) (Lum et al 1980, Gupta and Good 1980, Lum et al 1983). The T_A lymphocytes are detected by rosette formation with either IgA sensitized ORBC or TNP-ORBC conjugates coated with a mouse IgA myeloma (MOPC-315) that has anti-TNP specificity (Endoh et al 1981). Lydyard and Fanger (1981) demonstrated that 55% of human circulating T lymphocytes expressed both FcMR and FcAR. By rosetting T_M lymphocytes simultaneously with EAA and EAM, it was demonstrated that a proportion of the T_M lymphocytes also expressed FcAR. Overnight incubation in culture medium supplemented with IgA free serum was found to be necessary for the optimal expression of FcAR on T lymphocytes. Patients with Ataxia-Telangiectasia lack T_A lymphocytes which may be related to the lack of circulating IgA. Gupta and Good

1980, Lum et al 1983, and Hoover and Lynch 1983 proposed that Ig synthesizing B lymphocytes are isotype specific cells which are regulated by specific FcAR, FcMR and FcGR subclasses of T lymphocytes.

Some human and mouse circulating T lymphocytes possess receptors for the Fc of IgE (FcE) (T_E lymphocytes) (Spiegelberg 1984). The FcE receptor is detected by rosette formation with IgE myeloma coated ORBC (Spiegelberg 1981, Jensen et al 1984). The proportion of T_E lymphocytes is increased in patients with the hyperimmunoglobulinemia E syndrome (Ricci et al 1983). Approximately 4% of the circulating T lymphocytes isolated from non-allergic (nonatopic) individuals consist of FcER (or T_E) lymphocytes. Nonatopic healthy patients having low IgE levels show transient increases in the proportion of T_E lymphocytes after exposure to allergens during the grass pollen season. Similarly, the proportion of T_E lymphocytes in grass pollen sensitive atopic patients is increased with measurable increases in total and specific IgE serum levels (Lanzavecchia et al 1983, Spiegelberg 1984). Ricci et al (1983) proposed that T_E lymphocytes are heterogeneous and may consist of at least two distinct functional subsets which regulate IgE synthesis by the B lymphocyte. Since a proportion of the T_E lymphocytes reacted with only OKT8

monoclonal antibodies, Hassner and Saxon (1984) proposed that at least some of the T_E lymphocytes may be suppressor cells.

The receptor for the Fc of IgD (FcD) has also been detected on a proportion of T lymphocytes (T_D). Latex particles coated with IgD have been used to detect this receptor. The formation of rosettes with EAD can be inhibited by the preincubation of these cells with IgD. In contrast, preincubation with IgG, IgM or IgA had no effect on the formation of EAD rosettes. The functional role of this receptor is unknown (Sjoberg 1980).

2.1.2.4. CELL SURFACE DETERMINANTS DETECTED BY XENOGENEIC MONOCLONAL ANTIBODIES

Monoclonal antibodies have been generated which are directed to specific surface configurations in human circulating T lymphocytes. These surface configurations (antigenic determinants) appear to be expressed at different stages of T lymphocyte differentiation and are therefore referred to as differentiation antigens (Reinherz and Schlossman 1980). Human T lymphocytes differentiation antigens were first described by Reinherz et al (1980) and Kung et al (1980) using the OKT (Ortho Klone Thymus) series of monoclonal antibodies. The mouse monoclonal antibodies OKT1, OKT3, OKT4, OKT8 and OKT11 identify unique cell surface constituents on circulating T lymphocytes with molecular weights of 69000, 27000, 62000, 33000 and 50000 daltons, respectively (Berger et al 1982). The T1, T3 and T11 antigens can be detected on 98-100% of the circulating T lymphocytes whereas the T4 and T8 antigens are identified on 50-60% and 30-40% of the T lymphocytes, respectively (Thomas et al 1981, van Agthoven et al 1981, van Wauwe et al 1981, Ip et al 1982, Rinnooy et al 1984). The monoclonal antibody OKT11 was found to compete with SRBC for binding sites on human T

lymphocytes. The percentage of E rosette forming cells is highly correlated with the percentage of T11+ cells. Preincubation of lymphocytes with OKT11 prevents E rosette formation (van Wauwe et al 1981, Ip et al 1982).

The T4+ subclass contains helper cells capable of inducing B lymphocyte differentiation and subsequent Ig and antibody secretion in a PWM or antigen driven cell culture. In addition, it has been proposed that the T4+ lymphocytes enhance lymphoproliferation. The T8+ lymphocytes suppress Ig and antibody synthesis in the PWM or antigen driven cell culture and appear to suppress lymphocyte proliferation (Moretta et al 1983, Reinherz et al 1980a, Thomas et al 1981). The addition of OKT1 antibodies to in vitro cutures containing both B and autologous T4+ lymphocytes enhances B lymphocyte differentiation into Ig secreting plasmablasts. Thomas et al (1984) proposed that the OKT1 antibodies react with T cell membrane determinants which are intimately involved in the execution of helper functions of the T4+ lymphocytes. The OKT1, OKT3 and OKT11 antibodies react with cell surface antigens on the majority of circulating T lymphocytes. In contrast to the enhancing role of the OKT1 antibodies, the OKT3 and OKT11 antibodies inhibit T4+ cell helper function in PWM-induced B lymphocyte differentiation and phytomitogen and antigen-induced T

lymphoproliferation (Thomas et al 1984).

The monoclonal antibodies OKT11, OKT3 and OKT1 appear to be important in the regulation of the T lymphocyte blastogenic response. At low concentrations, OKT11 significantly suppressed the proliferative response of T lymphocytes to PHA, PPD and TT stimulation (van Waume et al 1981, Palacios 1982, Thomas et al 1984). The direct interaction of OKT11 with E receptors and the inhibition of proliferation implies that the E receptor is somehow involved in the regulation or facilitation of the T lymphocyte blastogenic response.

Thomas et al (1984) demonstrated that OKT3 antibodies in low concentration induce proliferation of T lymphocytes. More importantly, it induces T lymphocytes to produce IL2 and to express IL2 receptors. Palacios and Martinez-Maza (1982) demonstrated that OKT11 inhibits the production of IL-2 in phytomitogen and antigen stimulated T lymphocytes and their becoming responsive to IL-2.

2.1.2.5. CIRCULATING T LYMPHOCYTES IDENTIFIED ON THE BASIS OF CELL SURFACE MEMBRANE IMMUNOGLOBULINS

Kuritani and Cooper (1982) and Stashenko et al (1984) have confirmed that B lymphocytes are the only cell which possess detectable Ig on the surface of the cell which constitute integral constituents of the cell membrane.

2.1.2.6. LIMITATIONS OF CELL SURFACE MARKERS

Although cell surface markers have proven useful for a clearer understanding of the interactions occurring between lymphocytes, there are several limitations in their application. The assumption that functional capabilities assigned to a given lymphocyte subpopulation in vitro are also operating in vivo may be incorrect. Also, there is a poor correlation between an antigenically defined subpopulation and the function of the T lymphocytes contained within that subpopulation. For example, Thomas et al (1981) demonstrated the existence of suppressor cells in the "pure" helper T4+ population using the monoclonal antibody OKT17 (T4+/T17+). This T17 surface antigen is expressed on activated T4+ lymphocytes. The T4+/T17+ cells suppressed PWM-induced B lymphocyte differentiation and Ig synthesis. Therefore, these cells function as potent suppressor cells. On the other hand, the T4+/T17- cells are devoid of suppressor cell activity and can function only as helper cells in the same system. Hayward et al (1978) also demonstrated that T_M lymphocytes incubated with Con-A were effective suppressor cells in the Con-A induced blastogenic response of T lymphocytes.

Reinherz et al (1982) have described a monoclonal antibody, TQ1, which identifies a different surface antigen on the T4+ subpopulation. The T4+/TQ1+ and T4+/TQ1- subpopulations proliferate equally well upon stimulation with soluble antigens and alloantigens. The T4+/TQ1+ population responds optimally in the MLR whereas only the T4+/TQ1- population is effective in facilitating PWM induced B lymphocyte differentiation and Ig secretion.

Corte et al (1981) identified yet another antigen on T4+ lymphocytes with the monoclonal antibody 5/9. The T4+/5/9+ cells are capable of proliferating to soluble antigens and alloantigens and display helper activity in PWM induction of Ig and antibody synthesis. Analysis of the FcR phenotype of the T4+/5/9+ cells indicated that this subpopulation is not homogeneous - 25% are T_G (suppressor) lymphocytes and 50% are T_M (helper) lymphocytes.

These findings indicate the inadequacy of the OKT4 monoclonal antibodies in identifying "helper" T lymphocytes because only a minor proportion of the T4+ lymphocytes are actually responsible for the helper activity. The function of individual cell subpopulations cannot be extrapolated simply by the presence of a given

surface marker. The precise identification of functional T lymphocyte subpopulations may be possible only by the combined use of much larger panel of cell surface markers.

2.1.3. THE B LYMPHOCYTES

2.1.3.1. CIRCULATING B LYMPHOCYTES IDENTIFIED ON THE BASIS OF CELL SURFACE MEMBRANE IMMUNOGLOBULINS

Cell surface membrane immunoglobulins (smIg) on circulating lymphocytes were first demonstrated in 1961 by Moller. Ten to twenty percent of circulating mouse lymphocytes exhibited smIg detected by immunofluorescence, using fluorescein isothiocyanate (FITC) conjugated anti-mouse Ig. Abney et al (1978) detected smIg on 45% of mouse spleen and bone marrow lymphocytes and 14% of the circulating mouse lymphocytes by immunofluorescence. Sell and Gell (1965) reported that 50% of rabbit circulating and splenic lymphocytes possess smIg.

The presence or absence of the known T cell marker, the receptor for SRBC, was used to distinguish human T from non-T circulating cells into the early 1970's. Froland et al (1974) utilized two different methods to isolate circulating human non-T cells. Mononuclear cells were depleted of T lymphocytes by SRBC rosette formation. The majority of the SRBC-depleted MNC, or non-T cells, stained for smIg as detected by immunofluorescence and

were incapable of rosetting with SRBC. The positively isolated T lymphocytes did not stain for smIg. A proportion of the same MNC were separated into smIg+ and smIg- cells by passing freshly isolated mononuclear cells (MNC) through a nylon column. The effluent cells (smIg-) rosetted with SRBC and did not exhibit smIg by immunofluorescence. The eluted smIg+ cells were incapable of SRBC rosette formation. The smIg+ non-T cells were treated with pronase to remove smIg. Following culture in vitro for 48 hrs, the majority of the cultured cells stained positively for smIg by immunofluorescence, thus demonstrating the synthesis of smIg by these cells. Froland et al (1974) proposed that the absence of SRBC receptors (T cell marker) on smIg+ non-T cells place these cells in the B cell catagory. Lobo et al (1975) demonstrated that the majority of non-SRBC rosette forming human circulating cells are B lymphocytes which possess smIg detected by immunofluorescence. The addition of anti-human Ig antiserum to unfractionated MNC did not inhibit SRBC rosette formation by the T lymphocytes and the T lymphocytes did not fluoresce when incubated with FITC conjugated anti-human Ig. Lobo and Horwitz (1976) determined that the sum of smIg bearing cells (4.5-18%) and SRBC rosette forming cells (61-82%) accounted for the majority of the circulating human lymphocytes.

These investigations have been confirmed on numerous occasions with similar results. There is universal acceptance today that the circulating B lymphocytes can be distinquished from circulating T lymphocytes by the presence of smIg (Wu et al 1976, Karitani and Cooper 1982, Stashenko et al 1982).

2.1.3.2. CIRCULATING B LYMPHOCYTES IDENTIFIED ON THE BASIS OF CELL SURFACE RECEPTORS FOR C'3

In addition to smIg, B lymphocytes bear cell surface receptors for C'3 (C'3R). Approximately 10-20% of the circulating human lymphocytes bear C'3R (Shevach et al 1973, Ross 1973). These cells are B lymphocytes since, in all cases, C'3R are present on cells which also bear smIg detected by utilizing double markers - FITC anti-human Ig and EAC indicator erythrocytes (Ross 1973, Abrahamsohn et al 1974, Ehlenberger et al 1976). Circulating human B lymphocytes bear two different cell surface receptors for the cleaved products of C'3 - CR1 which is a receptor for a single configuration composed of C'4b, C'5b and C'3b, and CR2 which is a receptor for C'3d. The sequential addition of fluorescein labelled EAC1423b indicator erythrocytes (EAC containing C'1, C'4, C'2 and the b fragment of C'3) and fluorescein labelled EAC3d (EAC containing only the d fragment of C'3) revealed that CR1 and CR2 cap independently (Ross 1975, Lobo and Burge 1982, Weis et al 1984).

2.1.3.3. CELL SURFACE DETERMINANTS DETECTED BY XENOGENEIC MONOCLONAL ANTIBODIES

In addition to smIg and C'3R which characterize B lymphocytes, xenogeneic mouse monoclonal antibodies have been produced capable of detecting antigenic determinants expressed on B lymphocytes. Unfortunately at this time, there is no one available antiserum which is specific in its detection of cell surface determinants on B lymphocytes to the exclusion of all other circulating lymphocytes (Kung et al 1980, Nadler et al 1981). The antisera thus far described tend to detect overlapping cell populations including the B lymphocytes. The xenogeneic mouse monoclonal antibody OKIa1, which reacts with the MHC Class II antigens (HLA-D/Dr), react with the majority of B lymphocytes, monocytes and activated T lymphocytes (Reinherz et al 1979, Kung et al 1980, Nadler et al 1981). Brooks et al (1981) described the monoclonal antibody FMC7 which reacted with a determinant found selectively on 16-42% of the circulating human non-T cells. This antigen was not found on either T lymphocytes or monocytes. This antiserum stained different percentages of B lymphocytes depending on their mode of isolation - 36% B lymphocytes isolated by nylon wool fractionation as compared to 42% B lymphocytes

isolated by EAC rosette formation reacted with FMC7. The monoclonal antisera OKB1, OKB2, OKB4 and OKB7 detect antigens not previously described on human circulating B lymphocytes. The monoclonal antisera OKB4 and OKB7 react with the majority of smIg+ B lymphocytes. The monoclonal OKB2 antiserum reacts with the majority of smIg+ B lymphocytes; however it also reacted with all the granulocytes which also possess this antigenic determinant. In contrast, OKB1 reacts with a variable percentage (70-95%) of B lymphocytes (Mittler et al 1983). Most recently, the Ortho company has made available a monoclonal antiserum, OKB6, which appears to stain only B lymphocytes and not monocytes, T or Null lymphocytes (Richter, unpublished results).

2.1.4. THE NULL LYMPHOCYTES

2.1.4.1. CIRCULATING NULL LYMPHOCYTES IDENTIFIED ON THE BASIS OF CELL SURFACE RECEPTORS FOR FcG

This class of lymphocytes was first described by Basten et al (1972) and Paraskevas et al (1972) who demonstrated the presence of mouse lymphocytes with surface receptors for FcG (FcGR). The FcGR bearing lymphocytes were originally included in the family of B lymphocytes in view of the absence on these cells of the SRBC receptor, a T cell marker, and the belief that the circulating lymphocytes were either B or T lymphocytes. Froland et al (1974) rosetted human circulating MNC with SRBC and passed the non-rosetted, non-T cells through nylon wool columns to deplete the cells of the nylon wool adherent smIg+ B lymphocytes. Approximately 73% of the effluent cells (smIg- non-T cells) rosetted with EAG indicator erythrocytes, thus demonstrating the presence of detectable FcG receptors on the majority of these cells. Froland et al (1974) proposed that the FcGR+ circulating human lymphocytes detected by EAG rosette formation represent a subpopulation of B lymphocytes which lack smIg.

Chess et al (1974), Chess et al (1975) and MacDermott et al (1975) fractionated freshly-isolated circulating human MNC into smIg+ and smIg- cells by passage through a Sephadex anti-Fab column. The effluent cells, consisting of Null and T lymphocytes, were rosetted with SRBC to remove the T lymphocytes, leaving behind pure Null lymphocytes. Approximately 1% of these cells were smIg+ and 93% of the Null lymphocytes were demonstrated to rosette with EAC (not EAG) indicator erythrocytes. The eluted smIg+ cells were B lymphocytes since ~99% of these cells stained for smIg by immunofluorescence and a majority rosetted with EAC indicator erythrocytes. Both the Null lymphocytes and B lymphocytes mediated ADCC cytotoxicity and responded with blastogenesis and mitosis upon stimulation with PHA, PWM and Con-A. Chess et al (1975) demonstrated that the freshly-isolated Null lymphocytes were initially smIg negative and that >60% of the cultured Null lymphocytes developed smIg detected by immunofluorescence after 6 days in culture as compared to almost 100% of the cultured B lymphocytes. They also showed that the Ig content of the Null lymphocytes as well as their capacity to secrete Ig in culture was greater than that produced by equal numbers of B lymphocytes following 6 days in culture.

Evidence that B lymphocytes are not in fact Null lymphocytes eminates from the findings of Horwitz and Garrett (1975) and Lobo (1981), who demonstrated that the freshly-isolated circulating human FcGR+ non-T cells (obtained by first eliminating the SRBC rosette-forming T lymphocytes and rosetting the remaining cells with EAG indicator erythrocytes) possess surface membrane smIg which, however, is shed upon incubation of the cells at 37°C for 10 to 30 minutes. These cells were capable of binding Ig molecules from normal human serum at 4°C. In contrast, the FcGR- non-T lymphocytes, the B lymphocytes, did not shed their smIg following incubation at 37°C for 30 minutes. Therefore, the FcGR+ lymphocytes were operationally named L lymphocytes because of their temperature-labile cell-surface IgG. In view of the fact that these human L lymphocytes were shown to lack temperature stable smIg (which, by convention, characterizes B lymphocytes) and the receptor for SRBC (which, by convention, characterizes T lymphocytes), these cells were considered to be a new class of lymphocytes referred to as non-T and non-B Null lymphocytes (Lobo and Horwitz 1976, Lobo 1981). Further support for the Null lymphocytes as a distinct class of circulating lymphocytes is the fact that only the FcGR+ cells mediate ADCC cytotoxicity (Ziegler and Henney 1977, Herberman et al 1979, Ng et al 1982). Null cells

constitute 10-20% of human circulating lymphocytes.

2.1.4.2. THE RELATIONSHIP BETWEEN B LYMPHOCYTES AND NULL LYMPHOCYTES

Until the early 1970's, the circulating human lymphocytes were considered to consist of only T lymphocytes and B lymphocytes. Approximately 60-75% of the circulating human cells were T lymphocytes as they rosette with SRBC and are immunofluorecent positive when stained with the anti-T cell monoclonal antiserum, OKT3. The remaining cells were considered to be B lymphocytes since they bear smIg as detected by immunofluorescence using FITC-conjugated anti-human Ig. Basten et al (1972), Paraskevas et al (1972) and Froland et al (1974) demonstrated that a proportion of lymphocytes possess FcG. These cells were included in the B lymphocyte population since, all freshly isolated FcGR- cells exhibited smIg (Chess et al 1975, MacDermott et al 1975).

It was not until the mid-1970's, when specific surface markers and the functional properties for the B lymphocytes and Null lymphocytes were established, that it was accepted that these two cell types were stable and non-transitional cells. The controversy as to whether Null lymphocytes and B lymphocytes are in fact two distinct stable cell classes generated by stem cells of

two different lineages or whether a single transitional cell may at different times possess surface markers characteristic of B and Null lymphocytes may be attributed to different methodology and the sensitivity of the technique used to detect and isolate these cells. Depending on the methods used to isolate B and Null lymphocytes investigators have arrived to different conclusions.

Haegert et al (1978) utilized the direct antiglobulin rosetting reaction (DARR) and the mixed antiglobulin rosetting reaction (MARR) in their investigation of Null and B lymphocytes. These rosettes consist of only lymphocytes with intrinsic or membrane-incorporated non-labile Ig, and not cells with absorbed temperature labile Ig. Null lymphocytes were isolated from circulating human MNC depleted of monocytes, T and B lymphocytes. The negatively isolated Null lymphocytes were incubated at 37°C for 30 minutes to remove their temperature-labile Ig. Approximately 83% of these Null lymphocytes rosetted with anti-$F(ab)_2$ sensitized SRBC indicator erythrocytes (DARR). Approximately 93% of the Null lymphocytes incubated with anti-$F(ab)_2$ antibodies formed conjugates which rosetted with $F(ab)_2$ sensitized SRBC indicator erythrocytes (MARR). It must be concluded that the DARR and MARR

rosetting procedures are capable of detecting very small quantities of Ig molecules on Null lymphocytes not detected by immunofluorescence (Haegert and Coombs 1979, Haegert 1979). However, if Ig molecules are not detected by immunofluorescence but by MARR and DARR, how relevant are the Ig molecules? Every cell may contain small quantities of Ig on their cell surface. It must be determined if the Ig molecules detected by the MARR and DARR are functionally relevant on the Null lymphocyte.

Chess et al (1974), Chess et al (1975) and MacDermott et al (1975) isolated circulating human Null lymphocytes by passing MNC through a Sephadex anti-Fab column (thereby removing smIg+ B lymphocytes) followed by the removal of T lymphocytes by SRBC rosette formation. They demonstrated that the freshly isolated Null lymphocytes rosetted with EAC (not EAG) indicator erythrocytes and were initially smIg-. Following 6 days in culture >60% of the Null lymphocytes were shown to develop smIg and secrete Ig. Both the B and Null lymphocytes mediated ADCC cytotoxicity and responded with blastogenesis and mitosis upon stimulation with PHA, PWM and Con-A.

Horwitz and Garrett (1977) and Lobo (1981) isolated circulating human Null lymphocytes by rosetting MNC,

depleted of T lymphocytes and monocytes with EAG indicator erythrocytes. They incubated the Null lymphocytes for 30 minutes at 37°C. These positively isolated Null lymphocytes did not stain for smIg, as detected by immunofluorescence, nor did they rosette with EAC indicator erythrocytes.

On the basis of the above mentioned investigations the non-T lymphocytes can be classified into two seperate lineages, the true B lymphocytes which display stable smIg and are FcGR-, and the Null lymphocytes which possess temperature labile smIg, which cannot synthesize smIg, and are FcGR+ (Lobo 1981). Several functional properties also distinguish the Null lymphocytes from the T and B lymphocytes: only the Null lymphocytes participate in the ADCC reaction. The B lymphocytes and the T lymphocytes cannot mediate the ADCC reaction (Herberman et al 1979, Ng et al 1981). Furthermore, Null lymphocytes do not undergo blastogenesis and mitosis upon stimulation with phytomitogens (PHA, PWM, Con-A and STA) and antigens in culture whereas T lymphocytes and B lymphocytes do (Sakane and Green 1977, Horwitz and Garrett 1977, Caraux et al 1982).

2.1.4.3. CELL SURFACE DETERMINANTS DETECTED BY XENOGENEIC MONOCLONAL ANTIBODIES

Unfortunately, there is no available antiserum which is specific in its detection of Null lymphocytes. The antisera thus far described tend to detect overlapping cell populations including the Null lymphocytes (Zaarling and Kung 1980, Ortaldo et al 1981). Null lymphocytes appear to share an antigenic determinant recognized by the xenogeneic mouse monoclonal antibodies OKM1 and OKIa1 (Reinherz et al 1979, Kung et al 1980, Breard et al 1980, Nadler et al 1981). The monocyte monoclonal antiserum OKM1 and the B lymphocyte monoclone OKIa1 detect 12% and 20% of the Null lymphocytes, respectively (Kay and Horowitz 1980, Lohrmann-Matthes et al 1979, Breard et al 1980). The monoclonal antibody HNK-1 detects the majority of Null lymphocytes and 60% of the T_G lymphocytes. The monoclone B73.1 reacts with a 50000 dalton surface antigen on the majority of Null lymphocytes. The cells detected by B73.1 are the effector cells in the ADCC assay (Abo and Balch 1982, Tilden et al 1983), thus attesting to their being Null lymphocytes.

2.1.5. THE MONOCYTES

2.1.5.1. CIRCULATING MONOCYTES IDENTIFIED ON THE BASIS OF CELL SURFACE RECEPTORS FOR FcG AND C'3

Approximately 70-95% of human circulating monocytes express the FcR for IgG on the basis of rosetting with EAG indicator erythrocytes (Romans et al 1976, Furth et al 1979, Norris et al 1979, Barnett-Foster et al 1980, Zembala et al 1984). Approximately 65-75% of circulating human monocytes express C'3R on the basis of rosetting with EAC indicator erythrocytes. Two types of C'3R have been identified on human monocytes CR1- (a single configuration composed of C'4b and C'3b) and CR3 (the fragment referred to as C'3bi which remains after the digestion by the C'3b inactivator) (Ross et al 1978, Fearson 1980).

2.1.5.2. CELL SURFACE DETERMINANTS DETECTED BY XENOGENEIC MONOCLONAL ANTIBODIES

One antiserum which has recently become available LEU M3 (Beckton Dickinson) stains only circulating human monocytes to the exclusion of all other circulating cells. The monoclonal antisera OKM1 and 4F2 identify antigenic determinants on human circulating monocytes, Null lymphocytes, granulocytes and a small percentage of T lymphocytes (Breard et al 1980, Eisenbarth et al 1980, Reinherz et al 1980, Kay et al 1980). The antiserum OKM5 identifies only the circulating monocytes and bone marrow monocytes and does not react with T, B or Null lymphocytes as does OKM1 (Talle et al 1980, Shen et al 1983). The monoclone Mac-120 identifies only the human monocytes which have the property of adherence and does not react with any other population of cells (Hausman et al 1980).

2.2. THE INTERLEUKINS

2.2.1. INTRODUCTION

Many non-specific soluble factors have been identified which either facilitate immune reactions or are generated as a result of the immune reaction and are responsible for the sequelea of the immune reaction. Included in this broad catagory of soluble mediators are the lymphokines, leukotrienes, Interleukins, B cell growth factor (BCGF) and B cell differentiation factor (BCDF). Sensitized T lymphocytes, following their interaction with the original sensitizing antigen, secrete soluble protein or peptide mediators referred to collectively as lymphokines. The mast cells of allergic individuals, upon challange with the specific allergen, release a host of mediators among which is the slow reacting substance of anaphylaxis or SRS-A. There are a number of SRS-A's and they are referred to collectively as the leukotrienes (Parker et al 1979).

The Interleukins are synthesized and secreted by monocytes and T lymphocytes. The monocyte generated Interleukin referred to as IL-1, was originally referred

to as the lymphocyte activating factor (LAF). IL-1 has been shown to replace the monocytes in the phytomitogen-induced but not the antigen-induced blastogenic responses of the lymphoid cells in culture. It also enhances the blastogenic response to phytomitogen and antigen stimulation in the presence of limited numbers of monocytes (Gillis and Mizel 1981, Mizel 1982, Gery 1982). IL-1 does not support the proliferative response of T lymphocytes directly; it provides a signal for certain T lymphocytes to produce the T cell growth factor (TCGF) referred to as Interleukin 2 (IL-2). IL-2 has been shown to facilitate the mitogen-induced blastogenic response and to maintain T lymphocytes in long term culture (Smith 1980, Gillis and Mizel 1981, Palacios 1982). Two other mediators secreted by T lymphocytes are referred to as B cell growth factor (BCGF) and B cell differentiation factor (BCDF), both of which should also be referred to as Interleukins. BCGF and BCDF facilitate B lymphoproliferation and differentiation, respectively (Howard et al 1982, Muraguchi et al 1984, Yoshizaki et al 1983, Hirano et al 1984). Both BCGF and BCDF are secreted by T lymphocytes, they facilitate the B lymphoproliferative response and they maintain B lymphocytes in long term culture. The relationship of BCGF and BCDF is very similar to the relationship of IL-1 and IL-2 to T lymphocytes.

Interleukins differ from lymphokines and leukotrienes in one major respect - they are secreted by non-sensitized and non-committed cells in the absence of antigen challange. The lymphokines are secreted by sensitized lymphocytes upon interaction with the antigen and leukotrienes are generated by IgE-sensitized monocytes and mast cells following interaction with the allergen. The Interleukins are necessary to sustain lymphoid cells in a viable functional state, capable of undergoing blastogenesis following stimulation even after several days in culture.

2.2.2. INTERLEUKIN 1

Kasakura and Lowenstein (1965) and Gordon and MacLean (1965) working independent of each other demonstrated that cells in mixed lymphocyte cultures generated a soluble factor (which could by itself stimulate third party cells). This factor was referred to as Blastogenic Factor. Although it has been mistakenly identified as IL-1, it possesses properties quite distinct from IL-1 both functionally and biochemically. Gery and Waksman (1972) demonstrated that supernatants of PHA stimulated cultured MNC synergistically enhanced the PHA and Con-A induced blastogenic response of circulating human T lymphocytes isolated by rosetting with SRBC. These investigators were the first to detect what has since become known as Interleukin 1 (IL-1), a monocyte-derived factor which is required for a T lymphocyte blastogenic response.

Supernatants from PHA stimulated adherent monocyte cultures as well as nonadherent T lymphocytes (contaminated with monocytes to a final cell concentration of 1-5% as detected by staining for non-specific esterase) were both effective in reconstituting the blastogenic responsiveness to purified

T lymphocytes (obtained from monocyte-depleted MNC which were rosetted with SRBC) (Maizel et al 1980, Maizel et al 1981, Palacios 1982). However, supernatants of cultured lymphocyte (<0.5% monocyte contamination) were ineffective in reconstituting the blastogenic response. Maizel et al (1980) proposed that IL-1 was a soluble factor secreted from only adherent monocytes.

Monocytes appear to secrete IL-1 spontaneously after they are allowed to attach to the surface of a culture vessel. However, PHA and Con-A stimulation of monocytes increases the amount of IL-1 secreted by the cells (Unanue and Kiely 1977). Supernatants of 24 hour PHA-stimulated human monocyte cultures significantly enhanced the blastogenic response of pure T lymphocytes (deVries 1979). The 24 hour monocyte culture supernatants were more effective than 72 hour monocyte supernatants, indicating that the greatest production of IL-1 took place within the first 24 hours of the monocyte culture (deVries 1979). Supernatants from Con-A stimulated monocytes can also reconstitute the phytomitogen-induced blastogenic response of T lymphocytes (Maizel et al 1980).

The role of IL-1 and its mechanism(s) of action in the antigen-induced human T lymphocyte blastogenic

response are ill-defined. Ultraviolet (UV) irradiation interferes with the production of IL-1 by the monocyte. UV-irradiated tetanus toxoid (TT)-pulsed autologous human monocytes failed to augment the lymphoproliferative response of T lymphocytes. Both exogenous IL-1 and supernatants from Con-A stimulated human monocytes effectively reconstituted the blastogenic response of T lymphocytes in the presence of UV irradiated tetanus toxoid-pulsed monocytes. Neither IL-1 nor the supernatant from Con-A stimulated monocytes reconstituted the antigen-induced blastogenic response in the absence of the monocytes (Jakway and Shevach 1983, deFreitas et al 1983). In addition, rabbit antibody to IL-1 (anti-IL-1) inhibited the human T lymphocyte blastogenic response to TT-pulsed monocytes and reduced the capacity of exogenous IL-1 and supernatants of Con-A stimulated monocytes to reconstitute the T lymphoproliferative response in the presence of UV irradiated TT-pulsed monocytes. This suggests that IL-1 is solely responsible for the reversal of the blastogenic response abrogated by UV irradiation (deFreitas et al 1983, Jakway and Shevach et al 1983).

2.2.3. INTERLEUKIN 2

Circulating human T lymphocytes following exposure to IL-1 are induced to produce a factor referred to as IL-2 and to express functional receptors for IL-2 (IL-2R). The interaction of IL-2 with IL-2R is considered to be obligatory in the phytomitogen and antigen-induced proliferation of T lymphocytes (Andersson et al 1979, Larsson et al 1980, Ruscetti et al 1981).

Morgan et al (1976) were the first to demonstrate that supernatants from PHA-stimulated human circulating mononuclear cells could sustain long term cultures of T lymphocytes. Employing OKT monoclonal antibodies (OKT3, OKT4 and OKT8), Palacios (1982) demonstrated that human T4+ lymphocytes were solely responsible for the synthesis of IL-2 upon Con-A, PPD or TT stimulation. Recent studies have demonstrated that IL-2 can also be secreted by T8+ lymphocytes. The antigens (TT, PPD) and mitogens (PHA and OKT3) stimulate both T4+ and T8+ cells to secrete IL-2 (Palacios 1982, Meuer et al 1982, Luger et al 1982, Welte et al 1984, Reske-Kunz et al 1984, Depper et al 1984).

Lipkovitz et al (1984) and Reske-Kunz et al (1984) examined the role(s) of monocytes, IL-1, IL-2 and IL-2R expression in the mitogen-induced human T

lymphoproliferative response. The T lymphocyte cultures containing autologous monocytes developed IL-2R as early as 2-4 hours after Con-A activation and exhibited maximal numbers of IL-2R after 18-24 hours. This early expression of IL-2R was detected by the binding of a anti-IL-2R monoclonal antibody (anti-Tac) to activated T lymphocytes. Moreover these authors demonstrated that the production of IL-2 is strictly dependent upon the secretion of IL-1 by accessory monocytes, whereas the expression of IL-2R seemed to be independent of accessory cells. The production of IL-2 by T lymphocytes appeared to require continuous mitogen stimulation since T lymphocytes did not produce IL-2 in detectable concentrations or IL-2R after removal of the mitogen and monocytes. Despite early expression of IL-2R and production of IL-2 by mitogen-stimulated T lymphocytes, these cells were not committed to proliferate in the absence of IL-2 until more than 23 hours had elapsed following incubation with monocytes and phytomitogen. After more than 40 hours of activation, the cells proliferated equally well in the presence or absence of IL-2. Proliferation of uncommitted T lymphocytes was inhibited by anti-Tac; however, the anti-Tac antiserum did not affect the proliferation of T lymphocytes which had become committed to proliferate (Lipkowitz et al 1984, Reske-Kunz et al 1984).

2.2.4. B CELL GROWTH FACTOR (BCGF)

Several soluble factors secreted by human T lymphocytes have been shown to play a role in the regulation of human B lymphocyte proliferation and differentiation. One such factor, referred to as B cell growth factor (BCGF), appears to be required for the proliferative response of B lymphocytes (Ford 1981, Howard et al 1982, Leanderson et al 1982, Lernhardt et al 1982, Yoshizaki et al 1983, Okada et al 1983, Butler et al 1983, Butler et al 1984, Muraguchi et al 1984). A second factor, referred to as B cell differentiation factor (BCDF), is involved in the terminal differentiation of proliferating B lymphocytes into Ig secreting cells (see below) (Takatsu et al 1980, Swain et al 1981, Korsmeyer et al 1983, Mingari et al 1984, Muraguchi et al 1984, Hirano et al 1984).

Supernatants obtained from PHA or PWM-stimulated human circulating MNC were effective in maintaining the proliferative response of purified circulating human B lymphocytes (Srendi et al 1981, Muraguchi et al 1982). The active factor in the supernatant was shown to be distinct from IL-1 and IL-2 and was referred to as BCGF. Muraguchi et al (1982) proposed that IL-2, BCGF and BCDF

are present in the supernatants of PHA-stimulated MNC and that BCGF is indeed a distinct soluble growth factor which augments and facilitates B lymphoproliferation in a manner analogous to the proliferation of T lymphocytes facilitated by IL-2.

In the absence of other stimulating signals, BCGF augments PWM and STA-induced B lymphoproliferation (Howard et al 1983). Similarly, $F(ab')_2$ fragments of goat anti-human IgM antibodies stimulated the proliferation of human B lymphocytes only in the presence of BCGF. Circulating human B lymphocytes failed to proliferate in response to low concentrations of anti-IgM antibodies in the absence of BCGF (Howard and Paul 1982, Hirano et al 1984). Falkoff et al (1983) demonstrated that STA and anti-IgM antibodies, in high concentrations, induced the proliferation of B lymphocytes rigorously depleted of T lymphocytes and monocytes. Since no exogenous BCGF was added or produced, STA and anti-IgM antibodies in high concentrations induced B lymphoproliferation in a BCGF-independent manner. Falkoff et al (1983) proposed that a certain degree of B lymphoproliferation is possible in the absence of exogenous BCGF in the presence of the mitogenic stimulus in high concentration, or that two populations of B lymphocytes exist which respond to mitogen stimulation in

a differential manner. Howard et al (1982) proposed that the induction of proliferation by STA or anti-IgM antibodies in high concentration in the absence of BCGF may, in fact, be dependent upon accessory cells and T lymphocytes which are in too low a concentration to be detected.

2.2.5. B CELL DIFFERENTIATION FACTOR (BCDF)

In the early 1970's, it was demonstrated that the terminal differentiation of proliferating B lymphocytes into Ig secreting cells was mediated by a T cell factor. This factor was originally designated as T cell replacing factor but is now referred to as B cell differentiation factor (BCDF). Korsmeyer et al (1983) proposed that the initial activation of B lymphocytes is associated with the expression of receptors for BCGF. Activated B lymphocytes can, in turn, respond to BCDF by the binding of this factor to B cell membrane receptors (Korsmeyer et al 1983). In the absence of BCDF, these cells continue to proliferate without terminal differentiation. The addition of exogenous BCDF to proliferating B lymphocytes stimulate the cells to differentiate and secrete Ig (Muraguchi et al 1984, Mingari et al 1984).

2.3. THE CIRCULATING MONONUCLEAR CELLS WHICH PARTICIPATE IN THE IN VITRO BLASTOGENIC RESPONSE

2.3.1. INTRODUCTION

Since the report in 1960 by Nowell that PHA can induce mitosis and blastogenesis of human and animal lymphocytes in vitro, a vast literature has accumulated on the activation of lymphocytes by mitogens (reviewed by Lis and Sharon 1981). The phytomitogens PHA, PWM and Con-A are polyclonal mitogens as they can activate lymphocytes indiscriminately, irrespective of the functional role or status of the lymphocytes. Other mitogenic agents such as the immunizing antigen can activate specifically-induced memory lymphocytes (August et al 1970, Clot et al 1975). Controversy has evolved with respect to the precise identification of the responder cells and the accessory cells in the phytomitogen and antigen-induced blastogenic responses. In the following chapter, the evidence for (i) the T lymphocyte as the sole responder cell the 3 day phytomitogen-induced and the 6 day antigen-induced blastogenic responses, (ii) the B lymphocyte as the sole responder cell to PWM stimulation in the 7 day blastogenic response, (iii) the non-participation of the Null lymphocyte in the phytomitogen and antigen-induced

blastogenic response, (iv) the monocyte as the accessory cell in the phytomitogen and antigen-induced blastogenic response, will be presented.

2.3.2. THE ROLE OF T LYMPHOCYTES IN THE BLASTOGENIC RESPONSE

The objective of investigations conducted over the past 10-15 years has been to determine whether one or more of the lymphocyte classes (T, B and Null lymphocytes) respond with blastogenesis and mitosis upon stimulation with mitogenic agents (phytomitogens and antigens) in vitro. Geha and Merler (1974), Geha et al (1974), Greaves et al (1974), Lohrman et al (1974), Janossy and Doenhoff (1975), Weksler and Kuntz (1976), van Oers et al (1979) and Dosch et al (1980) demonstrated that purified circulating human T lymphocytes isolated following rosetting with SRBC responded to PHA and Con-A. The negatively purified B lymphocytes contaminated with <3% T lymphocytes responded minimally to PHA and Con-A stimulation (Geha and Merler 1974, Geha et al 1974, Greaves et al 1974, Lohrmann et al 1974, Dosch et al 1980). Pure B lymphocytes (<1% contaminating T lymphocytes) were unresponsive to PHA (Janossy and Doenhoff 1975). On the other hand, Andersson et al 1972, Phillips and Roitt (1973), Phillips and Weisrose (1974), Epstein et al (1974) and Chess et al (1975) have reported that enriched circulating human B lymphocytes isolated by

passage through and elution from anti-Fab columns responded to PHA and Con-A. These results are most likely explained by differences in the techniques used to isolate the human circulating B lymphocytes and the purity of the cell populations obtained. However, even the "pure" smIg+ B lymphocytes isolated in all of the above studies, were contaminated with T lymphocytes to the extent of 3-15% (determined by E rosette formation).

Circulating human B and T lymphocytes have been reported to respond with a considerable blastogenic response to PWM stimulation following 3 days in culture (Greaves et al 1972, Chess et al 1975, Geha et al 1974, Mellstedt 1975, Brochier et al 1976, Keightly et al 1976). The B lymphocytes were isolated from T cell depleted (by SRBC rosette formation) MNC and were contaminated with 1-3% T lymphocytes as detected by E rosetting. Whereas, enriched circulating human non-T cells or B lymphocytes obtained following rosetting with SRBC twice responded minimally to PWM (Janossy and Doenhoff 1975, Weksler and Kuntz 1976). Circulating human B lymphocytes isolated from MNC first depleted of T lymphocytes by rosetting with SRBC followed by treatment with OKT3 antiserum and C′ (to eliminate residual contaminating T lymphocytes) were unresponsive to PWM stimulation. The T lymphocytes were the only cells

capable of responding to PWM stimulation following 3 and 5 days of culture (Stevenson et al 1983). In an attempt to identify the specific human T lymphocyte subclass which responded to PWM stimulation, Puck et al (1984) isolated T4+ lymphocytes (by lysing T8+ lymphocytes in the presence of OKT8 antiserum and C') and T8+ lymphocytes (by lysing T4+ lymphocytes in the presence of OKT4 antiserum and C'). Enriched T4+ lymphocytes responded vigorously to PWM stimulation whereas T8+ lymphocytes responded insignificantly following 3 and 5 days of culture.

Both T_M and T_G lymphocytes respond to PHA and Con-A; however T_M lymphocytes have been shown to consistently respond better than T_G lymphocytes (Moretta et al 1976, Fauci et al 1980, Gomez-Reino and Habicht 1980, Victorino et al 1980, Kay et al 1983). Victorino et al (1980) investigated the relationship between the proportion of the T_M and T_G lymphocytes present in the circulation and their blastogenic responsiveness to PHA, PWM and Con-A stimulation. A positive correlation was found between the proportion of T_M lymphocytes and the magnitude of the lymphoproliferative response. This relationship between the actual T_M lymphocyte number and increased responsiveness to phytomitogen may be due to increased

numbers of helper cells or may be a reflection of a functional property of the responding cells. In addition, Victorino (1980) demonstrated a decrease in blastogenic responsiveness to PHA, PWM and Con-A stimulation as the proportion of T_G lymphocytes was increased. The phytomitogen-induced blastogenic response of T_G lymphocytes was less than that observed with unfractionated T lymphocytes. Kay et al (1983) also showed a similar negative correlation between the proportion of T_G lymphocytes and PHA responsiveness. The decreased blastogenic response may be due to a large number of cells which are less sensitive to phytomitogen stimulation or due to the immunoregulatory suppressor function of the T_G lymphocytes.

Controversy has evolved with respect to the precise identification of the responder cell in the antigen-induced blastogenic response. Geha et al (1973) demonstrated that circulating human B lymphocytes, cultured in supernatants from cultures of PPD-stimulated sensitized T lymphocytes, proliferated in response to PPD stimulation. Chess et al (1974) were unable to demonstrate B lymphoproliferation in response to PPD, TT and DT stimulation. Only T lymphocytes of immunized donors responded to TT, DT, PPD, SKSD, mumps and rubella stimulation (Geha 1979, van Oers et al 1979, Geha et al

1981, Alpert et al 1981, Chu et al 1984).

Buckley et al (1977) reported that MNC from normal non-allergic humans isolated from either peripheral or cord blood could be induced to undergo blastogenesis by purified ragweed Antigen E (AgE). In contrast, circulating MNC obtained from only ragweed hayfever allergic individuals evoked a considerable blastogenic response to AgE following 6 days in culture. Circulating MNC obtained from normal non-allergic individuals did not respond with blastogenesis to AgE stimulation (Richter et al 1968, Brostoff and Roitt 1969, Gatien et al 1975, Rocklin et al 1976, Rocklin et al 1980). Gatien et al (1975) and Black and Marsh (1980) demonstrated that only the T lymphocytes obtained from ragweed hayfever allergic individuals responded with blastogenesis to AgE or whole ragweed pollen extract. Moreover, they proposed that a correlation exists between an increased AgE-induced blastogenic response and increases in both skin sensitivity and mast cell histamine release in vitro.

2.3.3. THE ROLE OF MONOCYTES IN THE BLASTOGENIC RESPONSE

The role of monocytes in the activation of lymphocytes by mitogens is still controversial. The opinions vary from that of monocyte independence of the proliferative response (Stobo et al 1972, Mackler et al 1972, Jones et al 1973) to a potentiating effect (Lohrmann et al 1974, Hedfors et al 1975, Schmidtke and Hatfield 1976) to absolute monocyte dependency (Rosenstreich et al 1976, Raff et al 1976). Stobo et al (1972), Mackler et al (1972) and Jones et al (1972) proposed that PHA was capable of initiating blast transformation by direct mitogen-lymphocyte interaction independent of monocyte participation. Oppenheim et al (1968) and Stobo et al (1972) demonstrated that monocyte-depleted circulating human MNC (purified lymphocytes) responded to the same extent as unfractionated MNC upon stimulation with an optimal concentration of PHA.

Levis and Robins (1970) were the first to demonstrate that the phytomitogen-induced blastogenic response was monocyte dependent. Human circulating non-adherent cells eluted from a nylon wool columns

responded minimally to PHA stimulation. Similarly, a markedly diminished proliferative response of monocyte-depleted human MNC to Con-A stimulation was demonstrated by Hedfors et al (1974).

Autologous or allogeneic glass-adherent monocytes were capable of reconstituting the proliferative response of enriched human T lymphocytes (Arala-Chaves et al 1978, de Vries et al 1979, Maizel et al 1981, Bruszewski et al 1984). Hedfors et al (1975) demonstrated that the blastogenic response of purified circulating human T lymphocytes could be potentiated by the addition of autologous monocytes (to a final cell concentration of 50%) upon stimulation with 20 ug Con-A. Schmidtke and Hatfield (1976) proposed that the potentiating effect of autologous or allogeneic monocytes was dependent on the concentration of the mitogen used. In the presence of 10 ug Con-A, the addition of autologous monocytes to purified circulating human T lymphocytes (final concentration of 15% monocytes) resulted in a blastogenic response which was 75% of the optimal Con-A - induced blastogenic response of the unfractionated MNC. However, the addition of autologous monocytes to purified circulating human T lymphocytes (final concentration of 15% monocytes) in the presence of 1 ug Con-A resulted in a blastogenic response that was 150% compared to the

optimal blastogenic response of the unfractionated MNC.

Potter and Moore[4] (1977) demonstrated that the addition of only small numbers of adherent monocytes to purified T lymphocytes were required for an optimal PHA-induced blastogenic response of the circulating human T lymphocytes. Human circulating MNC were depleted of monocytes by treatment with carbonyl iron and nylon column filtration followed by SRBC rosette formation. The monocyte contamination of these T lymphocytes was <1%. These circulating human T lymphocytes gave an insignificant PHA-induced blastogenic response. The addition of a small number of autologous monocytes (final concentration 2-5%), which were obtained by adherence to glass, reconstituted the phytomitogen-induced blastogenic response of the T lymphocytes. Maizel et al (1979) and de Vries (1979) demonstrated that the addition of <1% autologous monocytes resulted in a significant enhancement of the T lymphocyte blastogenic response whereas 4% monocytes were sufficient to totally reconstitute the PHA-induced blastogenic response of the T lymphocytes. Approximately 14% monocytes were required to fully restore the blastogenic response to Con-A stimulation.

Rosenstreich et al (1976) demonstrated that the phytomitogen-induced blastogenic response of guinea pig lymph node lymphocytes is absolutely monocyte-dependent. The blastogenic responsiveness to PHA stimulation was not affected by removing monocytes by a single passage of the guinea pig lymphocytes through columns containing nylon wool and glass beads, although the same cells failed to respond to the antigen DNP-ovalbumin to which the guinea pigs had been immunized. A second passage of the cells through the nylon wool column resulted in a significant loss of responsiveness to PHA by the residual cells. Addition of purified autologous peritoneal macrophages fully reconstituted the response (Habu and Raff 1977, Rosenwasser and Rosenthal 1976, Andersson et al 1979). Similarly, guinea pig lymph node cells passed through glass bead columns lost the capacity to respond to PPD stimulation; however the addition of autologous peritoneal macrophages reconstituted the antigen-induced blastogenic response (Hersh and Harris 1968, Seeger and Oppenheim 1970; Waldron et al 1973). The degree of blastogenic responsiveness was proportional to the number of monocytes added.

Oppenheim et al (1968) demonstrated that circulating human lymphocytes could not undergo blast transformation upon exposure to antigens such as streptolysin-O, PPD or

vaccinia vaccine after removal of glass-bead adherent monocytes. Autologous monocytes obtained by adherence to plastic reconstituted the responsiveness of the monocyte-depleted MNC (purified lymphocytes) to the antigens. In addition, Oppenheim et al (1968) demonstrated that contact between monocytes and lymphocytes was also required since monocytes separated from lymphocytes by a Millipore filter could not restore responsiveness to the purified lymphocytes. Similarly, circulating human T lymphocytes essentially free of monocytes (<0.5% monocytes) were unresponsive to tetanus toxoid (TT) and diphtheria toxoid (DT) stimulation and only the addition of autologous monocytes was able to reconstitute the antigen-induced blastogenic response (Alpert et al 1981, de Freitas et al 1983, Chu et al 1984). Allogeneic monocytes did not reconstitute the responsiveness to the T lymphocytes (Geha et al 1980, Alpert et al 1981).

2.3.4. MONOCYTE MEDIATED SUPPRESSION IN THE BLASTOGENIC RESPONSE

The discussion in the previous subsection implicated monocytes as facilitating accessory cells in the in vitro blastogenic response of T lymphocytes. However, evidence has also been presented in support of a suppressive role for monocytes in this response. Keller et al (1975) demonstrated that the phytomitogen-induced blastogenic response of rodent lymphocytes was enhanced by the addition of freshly isolated autologous peritoneal macrophages but was inhibited by the addition of the same number of autologous monocytes cultured for 48 hrs.

Rinehart et al (1979) and Passwell et al (1982) reported that PHA-induced human T lymphoproliferation was markedly inhibited by monocytes which had been cultured for 48 hrs but was enhanced by freshly isolated monocytes. Human monocytes added in excess (>20%) to circulating human T lymphocyte cultures inhibited phytomitogen and antigen-induced lymphocyte proliferation and PWM-induced B lymphoproliferation. However as the monocyte concentration was increased, a dose dependent suppressor effect was demonstrated in the blastogenic response of the cells stimulated with PHA and PPD.

The monocyte suppressor activity for phytomitogen and antigen-induced lymphoproliferation is Prostaglandin E_2 (PGE_2) dependent (Fischer et al 1981). Human monocytes are induced by Con-A to secrete PGE_2. Webb et al (1978) demonstrated that exogenous PGE_2 inhibited mitogen-induced lymphoproliferation in the presence of T lymphocytes. Fischer et al (1981) demonstrated that the addition of excess human monocytes and the addition of exogenous PGE_2 were capable of suppressing the phytomitogen and antigen-induced blastogenic response of T lymphocytes and PWM-induced B lymphoproliferation and differentiation. Fischer et al (1981) proposed that the suppression of the phytomitogen and antigen-induced blastogenic response resulting from the addition of excess monocytes was actually to to an increased secretion of PGE_2 by increased concentration of monocytes.

2.3.5. THE ROLE OF NULL LYMPHOCYTES AS ACCESSORY CELLS IN THE IN VITRO BLASTOGENIC RESPONSE

Null lymphocytes have been implicated as facilitating accessory cells in the phytomitogen-induced blastogenic response. Evidence has been presented in support of an accessory role for Null lymphocytes in the T lymphocyte blastogenic response (Caraux et al 1978). Null cell depleted-T lymphocytes (by EAG rosette formation) respond minimally to phytomitogen stimulation. The finding that the removal of Null lymphocytes from T lymphocytes results in a diminished response does not necessarily imply that Null lymphocytes enhance or facilitate the phytomitogen-induced T lymphocyte blastogenic response. Monocytes as well as Null lymphocytes possess FcG receptors on their cell surface. Since the monocytes and Null cells are simultaneously eliminated following rosetting with EAG, the remaining lymphocytes are unable to respond. The synergy between T and Null lymphocytes is in fact due to the "contaminating" monocytes which are the true accessory cell in the mitogen-induced blastogenic response.

Null lymphocytes and supernatants from cultured Null lymphocytes have been shown to augment the

phytomitogen-induced T lymphoproliferative response (Caraux et al 1982). The addition of only a few Null lymphocytes (final concentration 2%) reconstituted the T lymphoproliferative response. However, this response cannot be attributed to Null lymphocytes since it has been shown that the circulating human Null lymphocytes are unresponsive to PHA, PWM, Con-A, STA and PPD stimulation in vitro (Caraux et al 1982, Sakane and Greene 1977, Horwitz and Garrett 1977). Furthermore, the Null lymphocytes cannot be induced to proliferate even in the presence of soluble T lymphocyte products (Caraux et al 1982).

2.4. MECHANISMS OF LYMPHOCYTE TRANSFORMATION

2.4.1. INTRODUCTION

Since the recognition, in 1960, of phytohemagglutinin as a lymphocyte mitogen, a vast literature has accumulated on polyclonal activation of lymphocytes by lectins (Nowell 1960, reviewed by Lis and Sharon, 1981). In the following section the phytomitogens and antigens, the mechanism of lymphocyte activation and the morphology and biochemical changes associated with lymphocyte transformation will be discussed.

PHYTOMITOGENS

The blastogenic response of lymphocytes to in vitro stimulation is an indication of lymphocyte responsiveness to either nonspecific phytomitogens or specific antigens (Harina et al 1979). The nonspecific phytomitogens include PHA, PWM or Con-A. Lymphocyte activation by phytomitogens is initiated by the interaction of the stimulant with specific carbohydrate moieties on cell surface glycoproteins or glycolipids (Goldstein and Hayes 1978, Lis and Sharon, 1981). The majority of the binding sites are irrelevant to mitogen activation since the interaction of the mitogen with only a small percentage of the receptors results in cell activation.

Phytohemagglutinin

Phytohemagglutinin (PHA) is extracted from the red kidney bean (Phaseolus Vulgaris) (Li and Osgood 1949, Rigas and Osgood 1955). It binds to cell surface glycoproteins with N-acetyl-D-galactosamine moieties. This lectin is a tetramer with a molecular weight of 115,000-140,000. PHA binds to both B and T lymphocytes; however, only the T lymphocytes are activated to undergo a blastogenic response (Allen et al 1969, Yachnin and Svenson 1972, Miller et al 1975).

Pokeweed Mitogen

Extracts of pokeweed (PWM) (Phytolacca Americana) leaves and roots possess mitogenic properties for T and B lymphocytes (Lis and Sharon 1977). The specific binding site has been identified on the T and B cell surface as glycoproteins or glycolipids containing the B(1-4)N-acetyl-D-glucosamine sugar moieties. PWM has been fractionated by ion exchange chromatography into five isolectins with approximate molecular weights of 19,000-31,000 (Waxdal 1974, Lis and Sharon 1981).

Concanavalin A

Concanavalin A (Con-A), prepared from the jack bean (Concanavalia ensiformis), is a pure protein with binding affinity for D-mannopyranoside and D-glucopyranoside. Con-A is a T cell mitogen. In solution at pH below 5.6, Con-A exists as a single molecule composed of two protomers with a molecular weight of approximately 51,000. Above pH 5.6, the protein forms tetramers with an approximate molecular weight of 102,000. A metalloprotein, Con-A binds Mn^{2+} and Ca^{2+} ions which are required for its carbohydrate binding properties (Becker et al 1975, Becker et al 1976, Baenziger et al 1979, Lis and Sharon 1981).

Antigens

By definition an antigen may be a protein, a glycoprotein, a lipoprotein a nucleoprotein or a microbial-derived carbohydrate. Antigens bind to antigen receptors which have genetically predetermined specificity thereby inducing proliferation. An antigen will stimulate the circulating cells of those individuals which have been actively or passively immunized to the particular antigen. Antigens do not induce blastogenesis of lymphocytes sensitized to other antigens. The specific antigens include TT, DT, CA and PPD (just to name a few) bind to receptors which have a high binding affinity for the antigen (Lis and Sharon 1977, Ashman and Raff 1973). Schwartz et al (1985) reported that the antigen receptor is a heterodimer composed of two transmembrane polypeptide chains ("immunoglobulin-like") linked together by interchain disulfide bonds.

2.4.2. BIOCHEMICAL EVENTS WHICH OCCUR DURING LYMPHOCYTE ACTIVATION

It has been proposed that the following events occur sequentially following mitogen-lymphocyte interaction and culminate in the blastogenic response. The interaction of the mitogen with the appropriate surface binding sites result in cross-linking of receptors, which leads to increased membrane fluidity and permeability (Loor 1980, Kaplan and Owens 1980), accelerated turnover of membrane phospholipids (Michell 1975), enhanced uptake of Ca^{2+} and K^{+} ions (Whitney and Sutherland 1973), redistribution of surface receptors (patching) and accumulation of patches at the poles of the cell (Loor 1980, Ashman 1981). Such perturbations on the outer surface are transmitted through the "carrier" proteins or lipids to cytoskeletal elements inside the membrane, such as the microtubules and microfilaments, which transmit a signal(s) to cytoplasmic and nuclear constituents. Intracellular events such as stimulation of histone acetylation, phosphorylation of nuclear proteins, and changes in lipid and carbohydrate metabolism result in blastogenic transformation of the cell.

Many of these changes are secondary aspects of lymphocyte transformation and are not necessarily related to cell triggering. Changes in the intracellular concentration of calcium and cyclic nucleotides have been directly implicated in lymphocyte activation (Alford 1970, Krishnaraj and Talwar 1973, Hadden et al 1975). Hadden et al (1975) demonstrated that cyclic GMP increases 10 to 50 fold in PHA and Con-A-stimulated cells and proposed that cGMP is the nuclear messenger responsible for activation and proliferation. Since calcium levels regulate cGMP, it has been proposed that increased Ca^{2+} uptake may stimulate an increase in the concentration of cGMP, resulting in lymphocyte activation (Hadden et al 1975, Coffey et al 1977). The role of cAMP in lymphocyte activation is not fully clarified, it has been proposed that it functions to limit cell division (Averdunk and Wenzel 1978).

2.4.3. METHODS USED TO DETECT THE BLASTOGENIC RESPONSE

Morphologic changes are associated with lymphocyte transformation - progressive increase in cell size, cytoplasmic basophilia and increased number of vacuoles. The small, medium and large lymphocytes and their progeny, the lymphoblasts and plasmablasts may be detected with light microscopy. However, assessment of the blastogenic response by this method is laborious and highly subjective. The proliferative capacity of the lymphocytes, as routinely assessed today, is determined by measuring the incorporation of tritiated thymidine, uridine or leucine into the DNA, RNA or protein, respectively, following suitable periods of incubation of the lymphocytes with the mitogen (Udey and Parker 1981). Thymidine is the radioactive compound of choice for the assay (Sample and Cretien 1971). The amount of thymidine incorporated into the nuclear DNA is a reflection of the degree of DNA synthesis. Thymidine is phosphorylated as it enters the cell to form thymidine-triphosphate, an immediate precursor for DNA (Buckley and Wedner 1978).

Lymphocyte activation results in DNA synthesis and transformation of the cell into a lymphoblast followed by

mitosis. Cells in the G_0 phase contain the normal diploid amount of DNA and minimum amounts of RNA. Following exposure to a mitogen, the cells begin to synthesize RNA thereby entering the G_1 phase and subsequently the S phase when DNA synthesis is initiated. In the short G_2 phase, the cells contain twice the normal diploid amount of DNA. The cell divides by mitosis in the M phase, returning the daughter cells to G_1 or G_0. The absence of DNA synthesis characterizes a resting cell in the G_0 phase (Betel et al 1979, Buckley and Wedner 1978).

2.5. SUMMARY

Although numerous investigators have demonstrated that T lymphocytes proliferate in the 3 day phytomitogen and 6 day antigen-induced blastogenic responses, it has not yet been ascertained which T lymphocyte subclass(es) respond(s) in the mitogen-induced blastogenic response. Evidence has also been presented by some investigators that B lymphocytes proliferate in the phytomitogen and antigen-induced blastogenic responses. Accessory or helper function(s) have been ascribed to B lymphocytes, Null lymphocytes and monocytes. However, these latter findings require corroboration. It must also be established whether or not the accessory or helper functions are mediated by cell-cell interaction or by soluble mediators secreted by the cells. The aims of the proposed research, as presented in the Rationale, are to unequivocally identify the responder cell(s) and the accessory cell(s) and to demonstrate the mechanism of accessory cell action, in order to better understand the phytomitogen, antigen and allergen-induced blastogenic responses.

3. MATERIALS AND METHODS

3.1. MATERIALS

REAGENTS

AB sera - obtained from the Canadian Red Cross.

α-naphthyl acetate - obtained from Sigma Chemical Co., St. Louis, Missouri, and stored at 18°C.

Ammonium chloride - obtained form British Drug Houses, Montreal, Quebec, and stored at 18°C.

Antisera - The chromatographically pure rabbit IgG and IgM antibodies to ORBC were obtained from Cappel Laboratories, Westchester, Pennsylvania, and stored at 4°C.

B Cell Growth Factor - was purchased from Cellular Products, Buffalo, New York, and stored at 4°C.

Candida albicans (CA) - obtained from Hollister-Stier, Berkley, California, and stored at 4°C.

Carbonyl iron grade SF - obtained from Dyestuffs and Chemicals, Toronto, Ontario, and stored at 18°C.

Complement - The source of C'3 was serum from a normal volunteer.

Concanavalin-A - obtained in a lyophilized powdered form in 250 mg quantities from Calbiochem, La Jolla, California, and stored at 4°C.

Culture medium - The culture medium was M199 fortified with 10% AB serum (M199-AB), penicillin (100 units/ml), streptomycin (100 ug/ml), gentamicin (50 µg/ml) and 25 mm Hepes.

Diphtheria Toxoid (DT) - (5050 Lf/ml) obtained from Connaught Laboratory, Willowdale, Ontario, and stored at -20°C.

Erythrocytes - Heparinized ORBC and SRBC were obtained fresh weekly from Qualicum Laboratories, Ottawa, and stored at 4°C until required.

FCS - was obtained in 100 ml quantities from Microbiological Associates and stored at 4°C. FCS was decomplemented (heated at 56°C, 30 min) before use.

Ficoll 400 - (500 g) obtained from Pharmacia Fine Chemicals AB, Upsala, Sweden and stored at 18°C.

Gentamicin - obtained from Schering Inc., Pointe Claire, Quebec, and stored at 4°C.

H^3-Thymidine - (μCi/ml) - was obtained from New England Nuclear, Boston, Massachusetts and stored at -20°C.

Hanks Balanced Salt Solution (HBSS) (500 ml) - was obtained from Microbiological Associates, Bethesda, Maryland, and stored until use at 4°C.

Heparin (mucosa) sodium - (1% Benzylalcohol as preservative) was obtained in concentrations of 1000 USP units/ml from Organon Canada Ltd., Organon, Ontario, and stored at 4°C.

HEPES buffer - obtained from Microbiological Associates, Bethesda, Maryland, and stored until use at 18°C.

Hypaque sodium (diatriozate) 50% w/v pH = 6.5-7.7 was obtained from Winthrop Laboratories, Aurora, Ontario. The 30 ml ampules were stored in the dark at 18°C.

Hytex Polystyrene Latex Beads (1.099 μm diameter) - obtained from Hyland Laboratories, Los Angeles, California, and stored at 4°C.

Interleukin 2 - was purchased from Cellular Products, Buffalo, New York, and stored at 4°C.

Lidocaine Hydrochloride - 20 mg/ml - obtained from Squibb, Montreal, Quebec, and stored at 4°C.

Methyl green - obtained from Sigma Chemical Co., St. Louis, Missouri, and stored at 18°C.

Mitomycin-C - obtained in 2 mg vials (powder form) from Sigma, St. Louis, Missourri, and stored in the dark at 4°C. Immediately before use the powder was dissolved in 2 ml of M199 to give a solution of 1 mg/ml.

Monoclonal antisera - The OKT3, OKT4, OKT8 monoclonal antisera were obtained from Ortho Diagnostics, Don Mills,

Ontario, and stored at -20°C.

Pararosanilin - obtained from Sigma Chemical Co., St. Louis, Missouri, and stored at 18°C.

Phytohemagglutinin-M (PHA) - obtained lyophilized from Difco Laboratories, Detroit, Michigan and stored at 4°C.

Pokeweed mitogen (PWM) - obtained lyophilized from Gibco Laboratories, Chagrin Falls, Ohio, and stored at 4°C.

Potassium - penicillin G (5000 units/ml) and streptomycin sulphate (5000 ug/ml) - were obtained pre-mixed in 100 ml quantities from Microbiological Associates, Bethesda, Maryland, and stored until use at -20°C.

Purified protein derivative (PPD) - obtained from Connaught Laboratory, Willowdale, Ontario, and stored at -20°C.

Saline solution - (0.9% NaCl) in pyrogen free sterile water was obtained in 1000 ml quantities from Abbott Laboratories Ltd. Montreal, Quebec, and stored at 18°C.

Scintillation liquid - premixed cocktail obtained from Beckman Instruments, Fullerton, California and stored at 18°C.

Sodium acetate - obtained from Fisher Scientific, Fair Lawn, New Jersey and stored at 18°C.

Sodium nitrite - obtained from Fisher Scientific, Fair Lawn, New Jersey and stored at 18°C.

Tetanus Toxoid (TT) - (2250 Lf/ml) obtained from Connaught Laboratory, Willowdale, Ontario, and stored at -20°C.

Tissue culture medium M199 and RPMI-1640 (500 ml) were obtained from Microbiological Associates, Bethesda, Maryland, and stored until use at 4°C.

Trypan blue - obtained from Grand Island Biological Co., Grand Island, New York., and stored at 18°C.

Wrights Rapid Stain - obtained from British Drug Houses, Montreal, Quebec, and stored at 18°C.

SUPPLIES

Culture tubes - were 17x100 mm plastic sterile disposable tubes were obtained from Falcon Plastics, Cockesville, Maryland.

Eppendorf repeater 4780 - automatic pipette and sterile disposable tips were obtained from Brinkman Instruments, Westbury, New York and Eppendorf, Hamburg, West Germany.

Liquid scintillation vials - these plastic vials were obtained from Fisher Scientific, Fair Lawn, New Jersey.

Microtiter plates and lids - Micro Test III flat bottom plates were obtained from Becton Dickinson Labware, Oxnard, California.

Nalgene 0.22 µm filters - obtained from Nalgene Labware, Rochester, New York.

Pipette aid (Automatic Pipette) - obtained from Drummond Scientific Co., Broomall, Pennsylvania.

Pipettes - 1, 2, 5 and 10 ml Pyrex sterile disposable serological pipettes were obtained from Corning Glass Works, Corning New York. A pro-pipette or a Pipette aid was always used to manipulate fluids.

Syringes - 1, 3, 5 and 10 ml Plastiplate sterile disposable plastic syringes obtained form Becton Dickinson Labware, Oxnard, California.

EQUIPMENT

CO_2 Incubator - cultures were maintained in a National incubator (NAPCO, Portland, Oregon).

Coulter counter - obtained from Coulter Electronics Inc., Mialeah, Florida.

Harvester - Titertek - obtained from Flow Laboratories, Rockville, Maryland.

Liquid Scintillation Coulter LS-230 - obtained from Beckman Instruments, Fullerton, California.

Multipurpose rotator - obtained from Scientific Industries, Inc. Springfield, Massachusetts.

Refrigerated centrifuge CRU 5000 - obtained from Fisher Scientific, Fair Lawn, New Jersey.

Zeiss fluorescent microscope with a mercury vapor light source (bright field, dark field and epi-illumination) - obtained from Zeiss, West Germany.

3.2. METHODS

3.2.1. Erythrocytes - Heparinized ORBC and SRBC were obtained fresh weekly from Qualicum Laboratories, Ottawa, and stored at 4°C until required. The RBC were washed three times in HBSS at 490xG for 10 min at 18°C and resuspended in M199 to the desired concentration.

3.2.2. Complement - The source of C'3 was an ambulatory and apparently healthy volunteer. A volume of this serum was absorbed with a 0.1 volume of packed ORBC at 4°C overnight, centrifuged at 490xG for 10 min at 18°C and absorbed once more with ORBC at 37°C for 60 min. The absorbed human serum did not agglutinate ORBC even in the undiluted state.

3.2.3. E indicator erythrocytes - SRBC were washed with Hanks three times at 490xG for 10 min at 18°C and resuspended in RPMI-1640 to the required cell concentration.

3.2.4. EAG Indicator Erythrocytes - ORBC were washed three times at 490xG for 10 min at 18°C. One ml of a 5% ORBC suspension was mixed with 1 ml of rabbit antibodies to human IgG (1/200, which is the maximum

subagglutinating concentration) incubated for 1 hr at 37°C and washed three times with HBSS at 490xG for 10 min at 18°C. The pelleted cells EAG were resuspended in RPMI-1640 to the desired cell concentration.

3.2.5. EAC indicator erythrocytes - One ml of 5% ORBC were mixed with 1 ml rabbit antibodies to human IgM (1/100). The cells were incubated for 30 min at 37°C, washed three times in HBSS at 490xG for 10 min at 18°C and resuspended in 1 ml of RPMI-1640. One ml of human C (1/8) was added. After 30 min of incubation, the cells were washed three times with HBSS at 490xG for 10 min at 18°C and resuspended in RPMI-1640 to the desired cell concentration. Neither the rabbit IgM antiserum nor the human serum used as a source of complement, in the concentrations used in the preparation of EAC, were capable of independently sensitizing the ORBC sufficiently to impart to them the ability to form rosettes.

3.2.6. EAM indicator erythrocytes - Two ml of 2% ORBC were was mixed with 2 ml of rabbit antibodies to human IgM (1/64, which is the maximum subagglutinating concentration) and incubated for 30 min at 4°C. The cells were washed three times with HBSS at 490xG for 10 min at 18°C and resuspended in RPMI-1640 to the desired

cell concentration.

3.2.7. Blood donors - The blood donors were ambulatory and apparently healthy adults (male and female) between the ages of 20-40 who had no recent history of illness nor were they taking any medication at the time of bleeding.

3.2.8. Mononuclear cell separation

The mononuclear cells (MNC) were separated from whole peripheral blood as originally described by Boyum (1968). Heparinized venous blood (50 Unit Heparin/ml blood) was diluted with an equal volume of M199, layered on 10 ml of Ficoll-Hypaque (specific gravity 1.077) and centrifuged at 400xG for 30 min 18°C. The mononuclear-rich interphase layer was removed and washed in HBSS at 314xG for 20 min at 18°C. The pellets were resuspended in HBSS, pooled and repelleted twice by centrifugation at 490xG for 10 min at 18°C. These cells were resuspended at a final ratio of 1 ml of M199 10% AB per 10 ml of whole blood. Viability was consistently >98% as determined by trypan blue exclusion.

3.2.9. Mitomycin C treatment of mononuclear cells

Mononuclear cells (10^6 cells per tube) were incubated with 75 µg mitomycin-C at 37°C for 1 hr. These cells were subsequently washed three times with HBSS at 490xG for 10 min at 18°C to remove residual mitomycin-C and resuspended in culture medium to the appropriate concentration.

3.2.10. Monocyte-depleted mononuclear cell (MNC-MO_1 and MNC-MO_2) preparation

Monocyte-depleted MNC were prepared by incubating 10^7 MNC with 5 ml of M199 containing 50% autologous serum and 10 mg of carbonyl iron. These cells were incubated on a tissue culture rotator for 45 min at 37°C. They were then layered on a Ficoll-Hypaque discontinuous gradient (SG 1.077) and centrifuged at 400xG for 30 min at 18°C. The non-phagocytic cells isolated from the interphase were washed twice in HBSS at 490xG for 10 min at 18°C and are referred to as once depleted mononuclear cells or MNC-MO_1. The MNC-MO_1 cells were cultured overnight in culture medium and subjected to monocyte depletion with carbonyl iron as described above. These twice monocyte-depleted cells are referred to as MNC-MO_2. Ninety-six to ninety-eight

percent of the MNC-MO_1 cells were lymphocytes, as identified by differential staining of the cells for non-specific esterase (NSE), whereas >99% of the MNC-MO_2 cells were identified as lymphocytes.

3.2.11. Isolation of monocytes

Mononuclear cells ($5x10^6$ cells/ml) were resuspended in warm (37°C) M199 supplemented with 20% autologous serum. Aliquots of the cells (100 µl) were transferred into sterile 5 ml Falcon tubes and incubated for 90 min at 37°C. To maximize monocyte exposure to the plastic surface of the tubes, the tubes were gently shaken every 30 min. The non-adherent cells were aspirated and the residual adherent monocytes were washed three times with warm M199 at 490xG for 10 min at 18°C. Loosely adherent cells detached during an overnight incubation at 37°C in 5% CO_2. These loosely adherent cells were aspirated. The adherent monocytes were incubated with warm 20 mM Lidocaine solution for 30 min at 37°C with frequent gentle shaking to detach the monocytes from the surface of the tubes. The monocytes from several tubes were pooled and washed with warm M199 at 490xG for 10 min at 18°C. Greater than 99% of the recovered adherent cells were identified morphologically as monocytes following staining for NSE (Yam et al 1971)

and by the capacity of the cells to ingest latex particles. The viability of the cells was >98% as determined by the trypan blue dye exclusion test.

3.2.12. Isolation of T lymphocytes

The isolation of T lymphocytes was performed by rosetting with sheep red blood cells (SRBC). Mononuclear cells ($90x10^6$ MNC in 10 ml) were mixed with 10 ml HBSS containing $3x10^9$ washed SRBC, incubated for 15 mins at 37°C and centrifuged at 140xG for 5 mins at 18°C. The cells were incubated at 4°C overnight, gently resuspended, layered over 15 ml Ficoll-Hypaque, and centrifuged at 400xG for 40 min 18°C. The non-rosetted or non-T cells at the interphase were isolated and washed three times in HBSS at 490xG for 10 min at 18°C and resuspended in M199 10% AB prior to use. The rosetted T cells in the pellet were washed once in saline at 490xG for 10 min at 18°C. The SRBC adherent to the T lymphocytes were lysed with 0.83% buffered ammonium chloride (pH 7.2). Ten ml of 0.83% buffered ammonium chloride (pH 7.2) was added to 10 ml of RBC and incubated at 4°C for 2 min with constant agitation. The reaction was stopped with 30 ml of saline. The T lymphocytes were washed three times in HBSS and resuspended in culture medium prior to use. Approximately 98% of these cells

were identified as T cells by rerosetting with SRBC.

3.2.13. Monocyte-derived culture supernatants

Monocytes (10^7 cells in 1 ml) were cultured in M199+10% AB serum in the presence or absence of PHA (1/100) for 48 hrs at 37°C and 5% CO_2. At the end of the incubation, the cells were centrifuged at 1750xG for 10 min at 18°C. The supernatants were collected and passed through a sterile 0.22 µm Nalgene filter. The monocyte-derived culture supernatants were tested immediately or frozen at -20°C.

3.2.14. T lymphocyte-derived culture supernatants

T lymphocytes (10^7 cells in 1 ml) were cultured in monocyte-derived supernatant in the presence or absence of PHA (1/100) for 48 hrs at 37°C and 5% CO_2. At the end of the incubation period, the cells were centrifuged at 2500 rpm for 10 min at 18°C. The supernatants were collected and passed through a sterile 0.22 µm Nalgene filter. The T cell-derived culture supernatants were tested immediately or frozen at -20°C.

3.2.15. Isolation of T_G and non-T_G lymphocytes

The T_G lymphocytes were isolated from T lymphocytes by incubating 90×10^6 T cells in 10 ml with 10 ml HBSS containing 5×10^9 EAG indicator erythrocytes. This cell suspension was incubated at 37°C for 15 min and centrifuged at 140xG for 5 min at 18°C. These cells were gently resuspended, layered on 15 ml Ficoll-Hypaque and centrifuged at 400xG for 40 min at 18°C. The nonrosetted (T_G-depleted) T cells were isolated from the interface and washed three times with HBSS at 490xG for 10 min at 18°C. The T_G-depleted T cells were resuspended to the desired concentration in culture medium. The T_G rosetted cells in the pellet were washed once in HBSS at 490xG for 10 min at 18°C and the ORBC were lysed with 0.83% buffered ammonium chloride (pH 7.2) for 2 min at 4°C. The T_G cells were washed three times in HBSS at 490xG for 10 min at 18°C and the ORBC were lysed with 0.83% buffered ammonium chloride (pH 7.2) for 2 min at 4°C. The T_G cells were washed three times in HBSS at 490xG for 10 min at 18°C and resuspended to the appropriate concentration in M199 10% AB. The viability of the T_G cells was >95% as determined by the trypan blue dye exclusion test.

3.2.16. Isolation of T_M and T_N lymphocytes

The isolation of T_M and T_N cells was performed by removing T_C and T_{M+C} cells from T_G-depleted T cells by rosetting these T cells with EAC indicator cells. Following overnight culture of the T_G-depleted T cells at 37°C in M199+20% FCS ($3x10^6$ cells in 3 ml), the T_G-depleted cells ($45x10^6$ cells in 5 ml) were incubated with 5 ml of $5x10^9$ EAC indicator erythrocytes for 15 min at 37°C and centrifuged at 140xG for 5 min at 18°C. The cell mixture was gently resuspended and layered over 10 ml of Ficoll-Hypaque and centrifuged at 400xG for 40 min at 18°C. The T_C-depleted (and T_{M+C}-depleted) cells at the interphase, consisting of T_M and T_N T cells, were washed three times in HBSS at 490xG for 10 min at 18°C and resuspended to the desired concentration in M199 10% AB. These cells ($10x10^6$ cells in 3 ml) were then incubated with 3 ml of $2x10^9$ EAM indicator erythocytes, centrifuged at 314xG for 5 min at 4°C and incubated on ice at 4°C for a minimum of 45 min. The cell mixture was gently resuspended and layered over 4 ml of Ficoll-Hypaque and centrifuged at 400xG for 30 min at 18°C. The T_N cells at the interface were washed three times in HBSS at 490xG for 10 min at 18°C and resuspended to the desired concentration in M199 10% AB. The T_M pelleted cells

were washed once in HBSS at 490xG for 10 min at 18°C and the OxRBC were lysed with 0.83% buffered ammonium chloride (pH 7.2) for 2 min at 4°C. The T_M cells were washed three times with HBSS at 490xG for 10 min at 18°C and resuspended to the appropriate concentration in M199 10% AB. The viability of the T_M and T_N cells, as determined by the trypan blue dye exlusion test, was greater than 92%.

3.2.17. Isolation of T_C and T_N lymphocytes

The isolation of the T_C and T_N cells was performed by removing T_M and T_{M+C} cells by rosetting the T_G-depleted cells with EAM indicator erythrocytes. Following overnight incubation the T_G-depleted T cells at 37°C, the T_G-depleted cells (10^7 cells in 3 ml) were incubated with 3 ml of $2x10^9$ EAM indicator erythrocytes, centrifuged at 314xG for 5 min at 4°C and incubated on ice at 4°C for a minimum of 45 min. The mixture was gently resuspended and layered over 4 ml of Ficoll-Hypaque and centrifuged at 400xG for 30 min at 18°C. The T_M-depleted cells at the interface, consisting of T_C and T_N T cells, were washed three times in HBSS at 490xG for 10 min at 18°C and resuspended to the desired concentration in M199 10% AB. These cells ($27x10^6$ cells in 3 ml) were incubated with

3 ml of $5x10^9$ EAC indicator erythrocytes at 37°C for 15 min and centrifuged at 140xG for 5 min at 18°C. The mixture was gently resuspended and layered over 3 ml of Ficoll-Hypaque and centrifuged at 400xG for 30 min at 18°C. The T_N cells at the interphase were washed three times with HBSS at 490xG for 10 min at 18°C and resuspended to the appropriate concentration in M199 10% AB. The pelleted T_C cells were washed once in HBSS at 490xG for 10 min at 18°C and the ORBC were lysed with 0.83% buffered ammonium chloride (pH 7.2) for 2 min at 4°C. The T_C cells were washed three times with HBSS at 490xG for 10 min at 18°C and resuspended in M199 10% AB. The viability of the T_C and T_N cells, as determined by the trypan blue dye exclusion test was greater than 92%.

3.2.18. T4+ and T8+ lymphocyte depletion

T cells ($5x10^6$ cells/ml) were incubated with 100 µl of OKT4 or OKT8 monoclonal antiserum (1/20 dilution) for 1 hr at 18°C. The tubes were agitated every 15 min. Fresh rabbit or guinea pig C' was added (10% final concentration) and incubation was continued for 60 min at 37°C . The cells were washed three times in HBSS at 490xG for 10 min at 18°C and the viability was determined by trypan blue dye exclusion. To assess the

effectiveness of killing, the residual cells were stained with OKT4 and OKT8 monoclonal antisera, counterstained with FITC conjugated anti-mouse Ig, and counted using fluorescent microscopy.

3.2.19. Isolation of B lymphocytes

The B lymphocytes were isolated from non-T cells by EAC rosette formation. Non-T cells ($90x10^6$ cells per 10 ml) were incubated with 10 ml of HBSS containing $5x10^9$ EAC indicator erythrocytes for 15 min at 37°C and centrifuged at 140xG for 5 min at 18°C. The cell suspension was gently resuspended, layered on 15 ml of Ficoll-Hypaque and centrifuged at 400xG for 30 min. The non-rosetting cells (Null cells and monocytes) were washed three times in HBSS at 490xG for 10 min at 18°C and resuspended in culture medium. The pelleted B cells were washed once in HBSS at 490xG for 10 min at 18°C and the ORBC were lysed with 0.83% buffered ammonium chloride (pH 7.2) for 2 min at 4°C. The B cells were washed three times with HBSS at 490xG for 10 min at 18°C and and resuspended to the appropriate concentration. Their viability, as determined by the trypan blue dye exclusion test was approximately 98%.

3.2.20. Isolation of Null lymphocytes

The Null lymphocytes were isolated from non-T cells by EAG rosette formation. The non-T cells (90×10^6 cells in 10 ml) were incubated with 10 ml of HBSS containing 5×10^9 EAC indicator erythrocytes at 37°C for 15 min and centrifuged at 140xG for 5 min at 18°C. The cell suspension was gently resuspended and layered on 15 ml of Ficoll-Hypaque and centrifuged at 400xG for 30 min at 18°C. The non-rosetted cells (B cells and monocytes) were washed three times in HBSS at 490xG for 10 min at 18°C and resuspended in M199. The pelleted Null cells were washed with HBSS at 490xG for 10 min at 18°C and the ORBC were lysed with 0.83% buffered ammonium chloride (pH 7.2) for 2 min at 4°C. The B cells were washed three times with HBSS at 490xG for 10 min at 18°C and resuspended to the appropriate concentration. Their viability, determined by the trypan blue dye exclusion test, was approximately 95%.

3.2.21. Antigenic determinants stained by monoclonal antisera and detected by immunofluorescence

To stain the cells with monoclonal antibodies, 5 µl of the monoclonal antiserum was added to 1×10^6 cells in 0.1 ml. The cell suspension was incubated in an

ice-water bath for 30 min and agitated every 10 min. The cells were washed three times with HBSS at 490xG for 10 min at 4°C. and The pellet was gently resuspended to which was added 100 µl of fluorescein-labelled anti-mouse immunoglobulin. Following a further incubation in an ice-water bath for 30 min, the cells were washed three with HBSS at 490xG for 10 min at 4°C. One drop of mounting medium was added for counting by fluorescent microscopy, using a Zeitz microscope.

3.2.22. Assessment of cell viability

The viability of the cells was determined by the conventional trypan blue dye exclusion test.

3.2.23. Ingestion of latex particles by lymphocytes

Latex particle ingestion was determined by incubating $1x10^6$ monocytes in 0.1 ml of culture medium with 0.1 ml of a 0.01% suspension of Hytex polystyrene latex beads (1.099 µm diameter) at 37°C for 30 min. Two hundred cells were counted and the percentage of cells ingesting three or more latex particles was ascertained by light microscopy.

3.2.24. Non-specific esterase staining

The morphology of monocytes and lymphocytes was identified by staining for non-specific esterase. The staining reagent was prepared as follows: 10 ml of distilled water was added to 0.4 g sodium nitrite, 10 ml of ethylene glycol monoethyl ether (EGME) was added to 0.2 g α-naphtyl acetate, 7.5 ml of pararosanilin solution was added to 7.5 ml of 4% sodium nitrite solution following exactly 1 min, 12 ml of EGME solution was added. Following the addition of 178 ml of PBS a buff colored precipitate formed and was filtered. The pH was adjusted to 6.1 using HCl or 1N NaOH. Air dried cytocentrifuged cells were stained for 30 min with the above mentioned reagent. The slides were washed twice in distilled water, fixed in methanol for 60 seconds and dried in cold air. The slides were counterstained with 1% methyl green for 1 min, washed twice with distilled water and air dried at 18°C.

Methyl green was prepared as follows: 100 ml of distilled water was added to 1.4 g sodium acetate and 1.0 g methyl green. The pH was adjusted to 4.2 with Acetic acid.

3.2.25. Cell Culture

Cells were cultured in vitro in sterile flat-bottom microtiter plates consisting of 96 wells (each well has a capacity of 300 µl). The culture medium was M199 supplemented with decomplemented (56°C, 30 min) human AB serum (10% final concentration), penicillin (100 IU per ml) streptomycin (100 µg per ml). The cells, $5x10^4$ or $2x10^5$ in a volume of 200 µl culture medium, were incubated with the appropriate phytomitogen or antigen (25 µl), respectively. The plated were covered with sterile plastic lids and kept in a 5% CO_2 incubator at 37°C. Twenty-five µl of tritiated thymidine (Specific activity 6.7 µCi/mM) was added to mitogen cultures after 48 hrs of culture and to antigen culture after 120 hrs. Following another 24 hr incubation, the cells were harvested with a cell harvester. The filter discs containing the cells were placed in scintillation vials containing 6 ml of scintillation solution. The extent of the H^3-thymidine incorporation by the cells was determined by scintillation counting and recorded as counts per minute (cpm) per culture.

The results will be presented and discussed below in terms of the blastogenic responsiveness which is defined as the percent of the blastogenic response of the

isolated cells relative to the optimal blastogenic response of the MNC (which equals 100%R).

4. RESULTS

Each experimental protocol was carried out with the cells of at least 15 healthy donors (unless otherwise stated). The results presented in the tables are representative of the results obtained with the cells of all the donors.

4.1. OPTIMAL CONDITIONS FOR THE IN VITRO BLASTOGENIC RESPONSE OF THE CIRCULATING MNC TO STIMULATION WITH THE PHYTOMITOGENS PHA, PWM AND CON-A

Objective

The objective was to establish the optimal conditions for the phytomitogen-induced blastogenic responses in cell cultures in this laboratory.

Protocol

Healthy volunteers were bled by venipuncture. The MNC were obtained from the interphase of Ficoll-Hypaque separated whole blood as described in Chapter 3.2.8. The MNC were cultured at varying cell concentrations and for

varying periods of time with varying quantities of the phytomitogens as described in Chapter 3.2.25. The cells were harvested and the blastogenic responses, as defined by the degree of incorporation of H^3-Thymidine by the cell cultures (cpm), were determined as described in Chapter 3.2.25.

Results

As can be seen in Table I and Figures I and II, the MNC consistently generated a biphasic blastogenic response, with peaks at day 3 and day 5, to all three of the phytomitogens irrespective of the concentration of the phytomitogen in the culture. The concentrations of the phytomitogens in the cultures which induced maximal blastogenesis (the optimal phytomitogen concentrations) were PHA 1/100, PWM 1/100 and Con-A 1/1000.

The blastogenic responses were consistently greater at day 3 than at day 5 (at optimal phytomitogen and cell concentration). Since at no time was a blastogenic response generated at day 5 and not at day 3, it was decided to limit all subsequent cultures to 3 days.

The relationship between the 3 day blastogenic response and the MNC cell concentration is presented in

Figure III. It can be seen that optimal blastogenic responses are given by $5x10^4$, $10x10^4$ and $20x10^4$ MNC per culture. In view of the frequent limitations in the acquisition of large numbers of MNC, it was therefore decided to carry out all subsequent cultures with $5x10^4$ cells per culture.

Discussion

The MNC of all the healthy volunteers responded markedly to phytomitogen stimulation. The blastogenic responses generated by the MNC were consistently bi-phasic with respect to phytomitogen stimulation in vitro. Etheridge et al (1980), Bernhard et al (1980), Maizel et al (1981), Yen and Lewis (1981), and Miller (1983) observed optimal phytomitogen-induced blastogenic responses at days 3, 4 and 5 of culture. However, these investigators have not offered any explanation for this prolonged optimal blastogenic response. It has been suggested that the cells in the circulation are not in synchrony as to their state of readiness to enter the mitotic cycle (Sasaki and Norman 1966). The resting cells (G_0) would be in second division metaphase by day 3 of phytomitogen stimulation. Thus the initial blastogenic response is reflected at day 3. The subsequent incorporation of H^3-Thymidine reflects the

stimulation of cells which were not at the resting state at the initiation of culture (Mizel et al 1981, Sasaki and Norman 1966). In view of the fact that the phytomitogen-induced blastogenic responses were consistently greater on day 3 than on day 5 and since at no time were responses observed explicitly at day 5 and not at day 3, it was therefore decided to limit all cell cultures to 3 days in all subsequent protocols.

TABLE 1

THE PHYTOMITOGEN-INDUCED BLASTOGENIC RESPONSE OF THE MNC AS A FUNCTION OF THE PHYTOMITOGEN CONCENTRATION AND TIME IN CULTURE

		Control cpm	day 3 cpm	day 4 cpm	day 5 cpm	day 6 cpm	day 7 cpm
PHA	1/250	398	40382	30978	23543	22509	20674
	1/100	593	166961	97911	135082	30982	24536
	1/50	287	126862	82614	111444	27830	11673
	1/25	446	97345	32038	53369	24570	7035
	1/10	202	41787	8658	20227	22094	257
PWM	1/250	904	5379	4680	6272	3870	3799
	1/100	264	69425	25641	52404	7893	20587
	1/50	573	69984	25492	50404	9090	19847
	1/25	442	64662	24853	42229	14256	19802
	1/10	696	62984	22463	40767	15673	17825
CON-A	1/2500	893	48357	25727	36353	20162	11038
	1/1000	756	104920	51545	86650	38904	25668
	1/500	732	98810	62756	96916	27831	23429
*	1/250	223	3469	1122	2121	7369	5378
*	1/10	690	1061	1284	1120	4790	2435

* These cpm reflect cpm due to Con-A precipitation not mitosis.

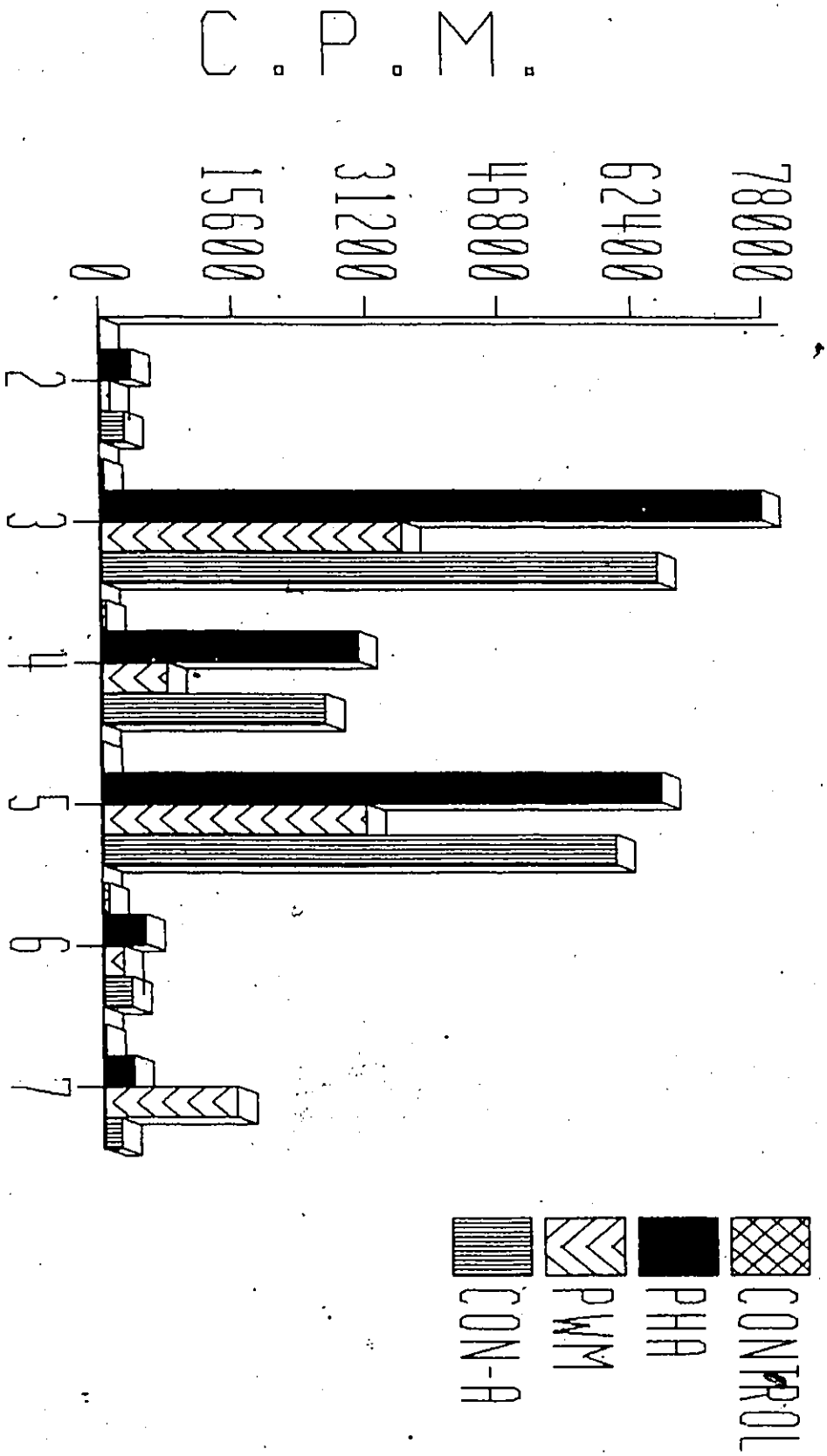

FIGURE 1. The phytomitogen-induced blastogenic response of MNC as a function of time in culture.

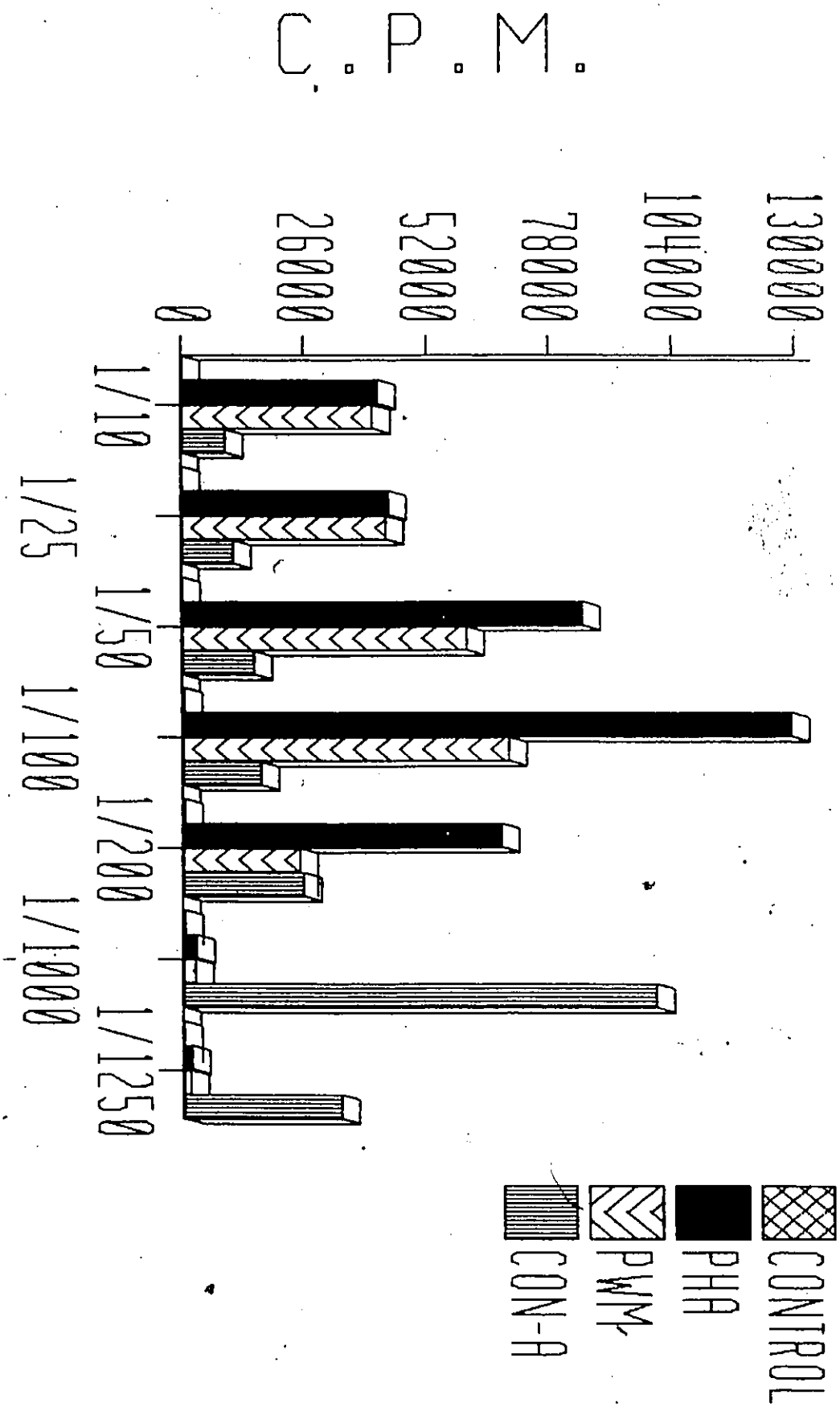

FIGURE 11. The phytomitogen-induced blastogenic response of MNC as a function of phytomitogen concentration.

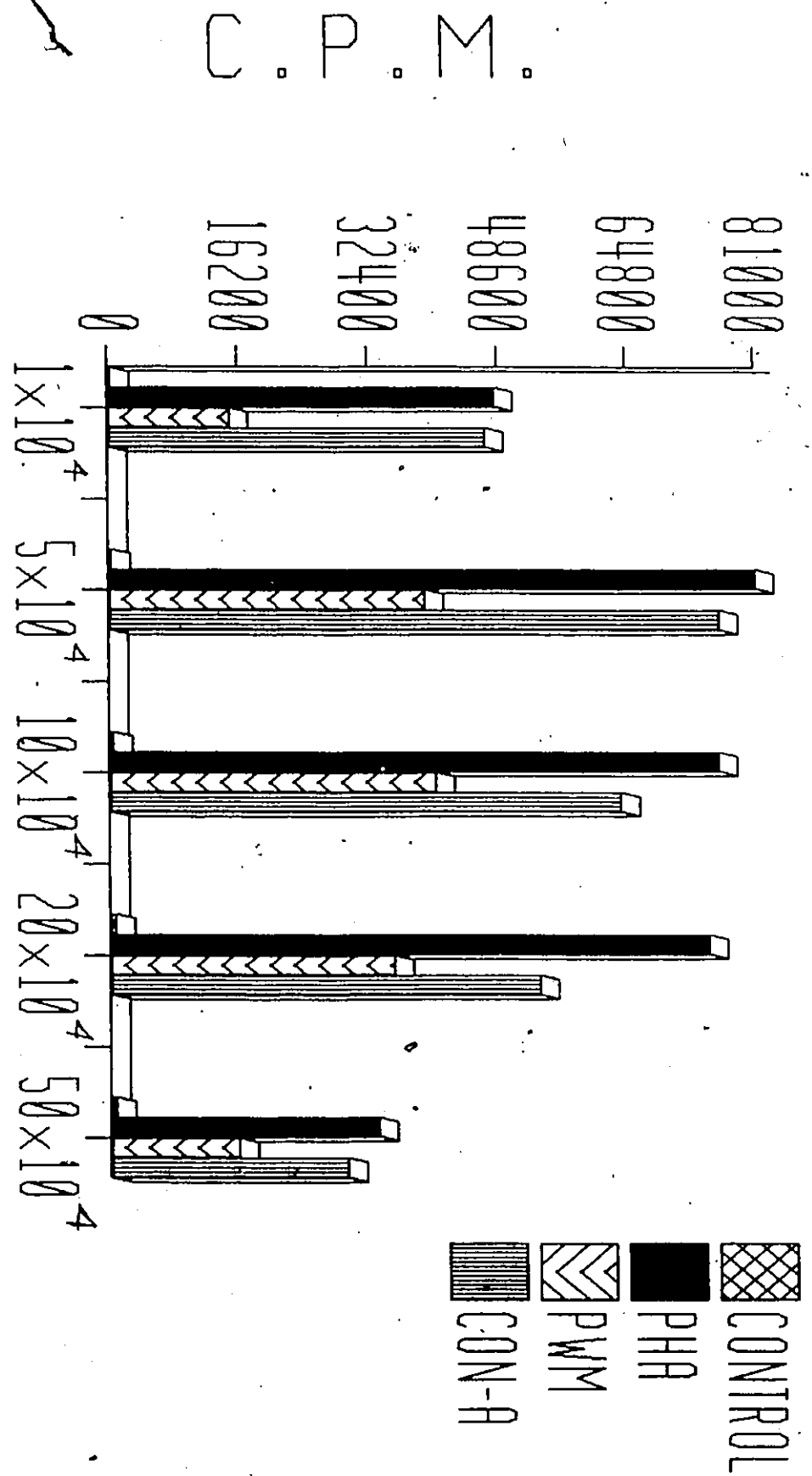

FIGURE III. The phytomitogen-induced blastogenic response of MNC as a function of cells per culture.

4.2. THE DEMONSTRATION OF T LYMPHOCYTES AS THE SOLE RESPONDER CELLS IN THE 3 DAY PHYTOMITOGEN-INDUCED BLASTOGENIC RESPONSE

Objective

The objective of the following experiments was to determine the identity of the responder cell(s) in the 3 day phytomitogen-induced blastogenic response.

Protocol

Blood donors were bled by venipuncture. The MNC were obtained from the interphase of Ficoll-Hypaque separated whole blood as described in Chapter 3.2.8. The MNC were depleted of T, B or Null lymphocytes by rosetting procedures as described in Chapters 3.2.12., 3.2.19. and 3.2.20., respectively. The B and Null lymphocytes were positively isolated from the non-T lymphocytes by rosetting procedures with EAC and EAG indicator erythrocytes, respectively, as described in Chapters 3.2.19. and 3.2.20. The B and Null lymphocytes were also negatively isolated from the non-T lymphocytes by the removal of EAG and EAC rosetting cells, respectively, as described in Chapters 3.2.20. and 3.2.19. The blastogenic responses in the cell cultures

were assessed as described in Chapter 3.2.25.

Results

As can be seen in Table 2, only the unfractionated MNC and the T lymphocytes gave marked proliferative responses to phytomitogen stimulation and the responses were comparable. Neither the positively nor the negatively selected B and the Null lymphocytes responded significantly (Table 2). The MNC-B lymphocytes and the MNC-Null lymphocytes gave highly significant blastogenic responses. The blastogenic responses of the MNC-B lymphocytes were consistently greater than those given by the MNC-Null lymphocytes. The MNC-T lymphocytes gave minimal to insignificant blastogenic responses (Table 2).

Discussion

The results obtained demonstrate that the T lymphocytes are the sole responder cells in the 3 day phytomitogen-induced blastogenic response since only these cells in culture gave blastogenic responses. None of the cell cultures depleted of T lymphocytes generated significant blastogenic responses in the 3 day phytomitogen-induced blastogenic response. On the other hand, all of the cell cultures containing T lymphocytes,

irrespective of the presence or absence of the B and Null lymphocytes, responded.

The blastogenic responses generated by the MNC-B lymphocytes were consistently greater than those given by the MNC-Null lymphocytes since T lymphocytes were not removed by the rosetting procedures to deplete the MNC of B and Null lymphocytes. The explanation for this finding would not appear obvious. An explanation for this finding is provided by the experiments in Chapter 4.3. which demonstrate that monocytes are essential participants in the T lymphocyte blastogenic response. Since monocytes as well as Null lymphocytes possess the FcG receptor, it is obvious that the MNC depleted of Null lymphocytes following rosetting with EAG would be depleted of monocytes as well. On the other hand, the monocytes would still be present in the MNC depleted of B, lymphocytes following removal of the B lymphocytes by rosetting with EAC.

It may therefore be concluded that, within the terms of reference of the culture conditions, only the T lymphocytes undergo blastogenesis and mitosis following 3 days in culture with the conventional phytomitogens (PHA, PWM and Con-A) providing monocytes are present in the cultures. The B and Null lymphocytes do not respond to

phytomitogen stimulation, either individually or if cocultured for 3 days.

TABLE 2

THE DEMONSTRATION OF THE T LYMPHOCYTES AS THE PRIMARY RESPONDING CELLS IN THE 3 DAY BLASTOGENIC RESPONSE TO PHYTOMITOGEN STIMULATION

	Control	PHA	%R(j)	PWM	%R	Con-A	%R
Cells of donor							
donor 1	cpm	cpm		cpm		cpm	
MNC(a)	312	98824	100	44681	100	91222	100
T(b)	204	81432	82	40411	90	88219	97
B(c)	265	1202	1	821	2	1000	1
NULL(d)	300	1508	2	1214	3	1821	2
NON-T-B(e)	452	1283	1	742	2	982	1
NON-T-NULL(f)	243	1003	1	465	1	948	1
MNC-T(g)	521	2550	3	1679	4	2013	2
MNC-B(h)	320	71428	71	34882	78	68421	75
MNC-NULL(i)	488	56821	57	24130	54	50218	55
donor 2							
MNC	948	98008	100	48572	100	95026	100
T	612	84216	83	36241	75	90412	95
B	751	1918	2	2358	5	2168	2
NULL	554	1520	2	1458	3	1783	2
NON-T-B	293	1823	2	938	1	1983	2
NON-T-NULL	100	1037	1	892	1	1563	1
MNC-T	200	2334	2	1663	3	1977	2
MNC-B	621	77131	79	33757	69	72188	76
MNC-NULL	688	41281	42	20190	42	40211	42
donor 3							
MNC	739	149255	100	57652	100	100789	100
T	651	80789	54	49198	85	79288	79
B	320	1282	1	1146	2	1275	1
NULL	301	1204	1	901	2	1588	2
NON-T-B	716	1193	1	783	1	1124	1
NON-T-NULL	312	942	1	465	1	1043	1
MNC-T	300	2025	1	1988	3	2685	3
MNC-B	381	78812	53	38121	66	77112	77
MNC-NULL	258	59959	40	21265	37	56579	56

a	MNC	unfractionated mononuclear cells
b	T	MNC which rosette with SRBC
c	B	Non-T cells which rosette with EAC
d	NULL	Non-T cells which rosette with EAG
e	NON-T-B	Non-T cells depleted of B cells
f	NON-T-NULL	Non-T cells depleted of Null cells
g	MNC-T	MNC depleted of T cells
h	MNC-B	MNC depleted of B cells
i	MNC-NULL	MNC depleted of Null cells
j	%R	% of the maximum response given by the unfractionated MNC

4.3. THE DEMONSTRATION OF MONOCYTES AS OBLIGATORY ACCESSORY CELLS IN THE 3 DAY PHYTOMITOGEN-INDUCED T LYMPHOCYTE BLASTOGENIC RESPONSE

Objective

The objective of the following experiments was to define the role(s), if any, of the monocytes in the phytomitogen-induced T lymphocyte blastogenic response.

Protocol

The MNC were obtained from the interphase of Ficoll-Hypaque separated whole blood as described in Chapter 3.2.8. The T lymphocytes were obtained by rosetting the MNC with SRBC as described in Chapter 3.2.12. The MNC and T lymphocytes were depleted of monocytes once, and twice over a period of 24 hrs, as described in Chapter 3.2.10. The method for isolating pure monocytes is described in Chapter 3.2.11. The T-MO_2 cells were reconstituted with varying numbers of pure (>99%) autologous or allogeneic monocytes so as to establish a concentration gradient of monocytes from 0.15% to 50% of the cultured cells. The cells were cultured and the blastogenic response determined as described in Chapter 3.2.25.

Results

The blastogenic responses of the MNC-MO_1 cells to phytomitogen stimulation were markedly reduced as compared to the responses given by the unfractionated MNC while the MNC-MO_2 cells gave only minimal blastogenic responses (Table 3). Similarly, the T lymphocytes responded well to phytomitogen stimulation; however the T-MO_1 cells gave diminished responses to phytomitogen stimulation and the T-MO_2 gave only insignificant blastogenic responses (Table 4).

As can be seen in Table 4, neither pure monocytes nor the T-MO_2 cells responded in culture with blastogenesis to phytomitogen stimulation. However, the addition of autologous monocytes to T-MO_2 cells (to a final cell concentration of 10%) restored the blastogenic response to phytomitogen stimulation. These responses equalled or exceeded the blastogenic responses of the T lymphocytes. or MNC not depleted of monocytes. The blastogenic response of the T-MO_2 cells to phytomitogen stimulation could not be restored by the addition of an ultrasonicated preparation of monocytes equivalent to a final monocyte concentration of 20% (Table 4).

Monocytes in a concentration as low as 0.6% of the cultured cells were capable of imparting to the T-MO_2 cells the capacity to give highly significant although but still low blastogenic responses to phytomitogen stimulation (Table 5). However, the addition of monocytes to T-MO_2 cells to a final cell concentration of 2.5% reconstituted blastogenic responsiveness to a level of 50% of the maximum blastogenic response to phytomitogen stimulation (Table 5).

There was no evidence for a suppressive role for the monocytes irrespective of their concentration in the cultures (Table 5). The addition of autologous monocytes to the T-MO_2 cells cells to a final concentration of 20% or 50% resulted in higher blastogenic responses by the T-MO_2 cells as compared to the counterpart responses of T lymphocytes or MNC not depleted of monocytes. Therefore, monocytes even in unphysiologically high concentrations were unable to suppress the phytomitogen-induced blastogenic response of the T lymphocytes.

Autologous and allogeneic monocytes were equally capable of facilitating blastogenic responses by the T-MO_2 cells. As can be seen in Table 6, T-MO_2 cells of all the donors reconstituted with autologous or

allogeneic monocytes (final cell concentration 10%) responded optimally to phytomitogen stimulation following 3 days in culture (Table 6).

Discussion

The T lymphocytes respond with blastogenesis to phytomitogen stimulation in vitro providing a minimum number of monocytes are present in the cultures. The elimination of monocytes twice over a period of 24 hours did not have a deleterious effect on the responder T lymphocytes since they responded optimally when reconstituted with pure monocytes which, by themselves, were incapable of responding. There is no evidence of a suppressive role for the monocytes as even excessive numbers of monocytes have a stimulatory, and not a suppressive, effect in the phytomitogen-induced blastogenic response. It is essential to stress that allogeneic monocytes are as effective as autologous monocytes in the restoration of blastogenic responsiveness to the T lymphocytes in the phytomitogen-induced blastogenic response.

It may be concluded that monocytes are required in small numbers in the cell cultures to facilitate optimal blastogenic responses to phytomitogen stimulation.

TABLE 3

THE EFFECT OF MONOCYTE DEPLETION ON THE MNC AND THE T LYMPHOCYTE PHYTOMITOGEN-INDUCED BLASTOGENIC RESPONSE

Cell of donor	Control	PHA	%R	PWM	%R	CON-A	%R
donor 1	cpm	cpm		cpm		cpm	
MNC(a)	374	89263	100	23617	100	71504	100
MNC-MO1(b)	361	17320	19	3127	13	8463	12
MNC-MO2(c)	178	4065	5	1780	8	4459	6
T(d)	514	74569	84	22289	94	69196	97
T-MO1(e)	822	36939	41	11192	47	32486	46
T-MO2(f)	609	3842	4	1584	7	3796	5
donor 2							
MNC	855	95791	100	41958	100	89692	100
MNC-MO1	900	26829	28	5197	12	9975	11
MNC-MO2	283	4075	4	3329	8	5129	6
T	751	85696	89	37151	89	78875	88
T-MO1	774	25875	27	7748	18	20376	23
T-MO2	159	2373	2	1637	4	3775	4
donor 3							
MNC	336	88525	100	41814	100	84886	100
MNC-MO1	768	29003	33	11869	28	17656	21
MNC-MO2	103	4487	5	1269	3	1922	2
T	946	73936	84	36344	87	72391	85
T-MO1	552	43446	49	13253	32	34400	41
T-MO2	413	3047	3	1476	4	2172	3

a MNC unfractionated mononuclear cells
b MNC-MO1 MNC depleted of monocytes once
c MNC-MO2 MNC depleted of monocytes twice
d T MNC which rosette with SRBC
e T-MO1 T cells depleted of monocytes once
f T-MO2 T cells depleted of monocytes twice

TABLE 4

THE ROLE OF MONOCYTES IN THE T LYMPHOCYTE BLASTOGENIC RESPONSE TO PHYTOMITOGEN STIMULATION

	Control	PHA	%R	PWM	%R	CON-A	%R
Cells of donor							
donor 1	cpm	cpm		cpm		cpm	
MNC(a)	212	138827	100	75648	100	144409	100
T	943	80815	58	51168	68	76411	53
T-MO1(b)	310	51679	37	10681	14	41503	29
T-MO2(c)	444	11444	8	3300	4	7100	5
MO(d)	244	2489	2	1973	3	2237	2
T-MO2+MO(e)	185	148852	107	61191	81	122280	85
T-MO2+U(f)	368	9842	7	2689	4	7320	5
donor 2							
MNC	295	104028	100	58692	100	93339	100
T	751	65696	63	37151	63	68875	74
T-MO1	329	14257	14	6177	11	14712	16
T-MO2	812	2882	3	1632	3	1844	2
MO	212	280	0	217	0	300	0
T-MO2+MO	234	117476	113	60524	103	88527	95
T-MO2+U	298	2534	2	1532	3	1900	2
donor 3							
MNC	574	74569	100	32289	100	69196	100
T	538	42586	57	20530	64	35489	51
T-MO1	999	12539	17	9846	30	12590	18
T-MO2	320	1282	2	1275	4	1126	2
MO	839	2063	3	958	3	1826	3
T-MO2+MO	302	64959	87	21265	66	56579	82
T-MO2+U	415	1129	2	1049	3	1208	2

a MNC unfractionated mononuclear cells
b T-MO1 T cells depleted of monocytes once
c T-MO2 T cells depleted of monocytes twice
d MO pure (>99 percent) monocytes
e T-MO2+MO monocytes added to T-MO2 cells (final conc 10%)
f T-MO2+U ultrasonicate of monocytes (equivalent to 20%) added to T-MO2 cells

TABLE 5

THE PHYTOMITOGEN-INDUCED BLASTOGENIC RESPONSE OF T LYMPHOCYTES AS A FUNCTION OF THE PERCENT MONOCYTES IN CULTURE

Cell of donor		Control	PHA	%R	PWM	%R	CON-A	%R
donor 1	%MO (b)	cpm	cpm		cpm		cpm	
MNC(a)	26	212	150012	100	75648	100	154109	100
T	4	318	95633	64	48815	65	99841	65
T-MO2	<1	444	9444	6	3104	4	7134	5
MO	>99	244	2486	2	1973	3	2237	1
T-MO2+MO	50	700	184355	123	75233	99	159602	104
T-MO2+MO	20	962	123493	82	26296	35	92706	60
T-MO2+MO	5	348	84797	57	12576	17	60023	39
T-MO2+MO	2.5	510	71315	48	11314	15	57980	38
T-MO2+MO	1.3	310	51679	34	10681	14	41503	27
T-MO2+MO	0.6	211	31197	21	2245	3	22230	14
T-MO2+MO	0.15	262	10320	7	1842	2	8350	5
donor 2								
MNC	28	295	144228	100	77692	100	109332	100
T	5	245	102084	71	43219	56	82691	76
T-MO2	<1	324	8257	6	2177	3	6112	6
MO	>99	218	2180	2	301	0	3290	3
T-MO2+MO	50	234	167476	116	80524	104	118527	108
T-MO2+MO	20	481	137815	96	68182	88	104028	95
T-MO2+MO	5	209	95993	67	51909	67	86253	79
T-MO2+MO	2.5	720	76842	53	34321	44	70054	64
T-MO2+MO	1.3	298	46409	32	9706	12	40648	37
T-MO2+MO	0.6	248	27054	19	3231	4	20642	19
T-MO2+MO	0.15	272	11052	8	2777	4	8853	8

a Defined as in Table 3
b percent of monocytes in culture

TABLE 6

THE CAPACITY OF ALLOGENEIC AND AUTOLOGOUS MONOCYTES TO FACILITATE THE PHYTOMITOGEN-INDUCED BLASTOGENIC RESPONSE

Cells of donor	Control	PHA	%R	PWM	%R	CON-A	%R
donor. 1	cpm	cpm		cpm		cpm	
MNC(a)	459	97182	100	40171	100	95453	100
T	218	78461	81	24218	60	73364	77
T-MO2	643	6784	7	4363	11	6356	7
MO	452	423	0	490	1	407	0
donor 2							
MNC	446	82825	100	32402	100	72132	100
T	342	66250	80	21535	66	58115	81
T-MO2	592	5031	6	1858	6	4671	6
MO	231	673	1	336	1	405	1
donor 3							
MNC	532	86191	100	35423	100	84744	100
T	681	57190	66	20461	58	56991	67
T-MO2	857	4561	5	1909	5	3312	4
MO	312	292	0	275	1	297	0
MO + T-MO2 donor + donor							
1 + 1	622	86612	100	38642	100	74851	100
2 + 1	714	64266	74	34804	90	66365	89
3 + 1	357	73182	84	34145	88	79766	107
2 + 2	829	79218	100	31988	100	62456	100
1 + 2	955	69842	88	30764	96	67738	108
3 + 2	682	63913	81	37621	118	61074	98
3 + 3	988	70067	100	36587	100	63113	100
1 + 3	301	79028	113	31518	86	66796	106
2 + 3	920	63671	91	32298	88	68478	109

a Defined as in Table 3

4.4. THE BLASTOGENIC RESPONSE OF THE T LYMPHOCYTE SUBCLASSES IN THE 3 DAY PHYTOMITOGEN-INDUCED BLASTOGENIC RESPONSE

Objective

The objective of the following experiments was to assess the blastogenic responsiveness of the T lymphocytes in the different T lymphocyte subclasses - T_M, T_G, T_C, T_N, T4+ and T8+ lymphocytes in the 3 day phytomitogen-induced blastogenic response.

Protocol

Blood donors were bled by venipuncture. MNC were obtained from the interphase of Ficoll-Hypaque separated whole blood as described in Chapter 3.2.8. The T lymphocytes were obtained by rosetting the MNC with SRBC as described in Chapter 3.2.12. The T lymphocyte subclasses defined on the basis of rosetting procedures - the T_M, T_G, T_C and T_N lymphocytes, were isolated as described in Chapters 3.2.15., 3.2.16. and 3.2.17. The T4+ and T8+ T lymphocytes were isolated by lysis of T8 cells and T4 cells in the presence of OKT8 and OKT4 monoclonal antiserum and complement, respectively, as described in Chapter 3.2.18. The method for isolating

pure monocytes is described in Chapter 3.2.11. It should be noted that monocytes were added (to a final cell concentration of 5%) to the cultures of the T lymphocyte subclasses to ensure optimal blastogenic responses. The cells were cultured and the blastogenic responses determined as described in Chapter 3.2.25.

Results

The cells in all four T lymphocyte subclasses isolated by rosetting procedures - T_M, T_G, T_C and T_N T lymphocytes, gave highly significant blastogenic responses to phytomitogen stimulation (Table 7). The consistently best responders were the T_M lymphocytes. The blastogenic responses by the T_G lymphocytes were consistently lower than those given by the T_M lymphocytes. The blastogenic responses of the T_C and T_N lymphocytes were lower than those given by the T_G lymphocytes (Table 7).

The T4+ and T8+ T lymphocytes gave similar blastogenic responses to phytomitogen stimulation (Table 8).

Discussion

The T_M, T_G, T_N and T_C lymphocytes generated marked blastogenic responses in the phytomitogen-induced blastogenic response. However, the T_M lymphocytes consistently gave the best responses and the T_G lymphocytes consistly gave lower blastogenic responses. The phytomitogen-induced blastogenic responses of the T_C lymphocytes and the T_N lymphocytes were generally lower than those given by the T_G lymphocytes. The blastogenic responses to phytomitogen stimulation generated by the T4+ and T8+ T lymphocytes were comparable. Since the T4+ lymphocytes are presumed to consist of the helper cells, as are the T_M lymphocytes, and the T8+ lymphocytes are presumed to consist of the suppressor cells, as are the T_G lymphocytes, it is difficult to understand why the T4+ and the T8+ responded to the same degree to phytomitogen stimulation whereas the T_M lymphocytes gave markedly superior blastogenic responses to phytomitogen stimulation than did the T_G lymphocytes. Obviously, the T lymphocyte subclasses, irrespective of the mode of isolation are heterogeneous. These results indicate that these two T lymphocyte classes, which are considered to exhibit helper and suppressor activity, respectively, nevertheless cannot be distinguished on the basis of the phytomitogen-induced

blastogenic response.

It must be emphasized that the monocytes were not the limiting cells in any of the blastogenic responses as all the T lymphocytes subclasses were always cultured with optimal numbers of monocytes.

It may be concluded that lymphocytes in all the T lymphocyte subclasses are capable of responding to varying degrees in the phytomitogen-induced blastogenic response. The T_M lymphocytes are the best responders.

TABLE 7

THE PHYTOMITOGEN-INDUCED BLASTOGENIC RESPONSES OF THE T LYMPHOCYTE SUBCLASSES

Cells of donor	Control	PHA	%R	PWM	%R	CON-A	%R
donor 1	cpm	cpm		cpm		cpm	
MNC	338	91388	100	44770	100	112834	100
T	317	65088	71	15104	33	56699	50
TM(a)*	312	91647	100	40188	89	70636	62
TG(b)*	458	48357	52	25330	56	36484	32
TC(c)*	927	29776	31	10291	22	26587	23
TN(d)*	944	29003	31	11347	25	21212	18
donor 2							
MNC	636	103152	100	40416	100	101034	100
T	591	90037	87	25278	62	78974	78
TM	133	72375	70	16687	41	79872	79
TG	107	50301	48	12578	31	43012	42
TC	246	23479	22	12083	29	10450	10
TN	292	66084	64	14752	36	49431	48
donor 3							
MNC	739	149255	100	27652	100	140789	100
T	600	86206	57	13314	48	84268	59
TM	735	88548	59	22656	81	82743	58
TG	502	49360	33	7409	26	32245	22
TC	322	42061	28	9362	33	25336	17
TN	810	27643	18	14522	52	26393	18
donor 4							
MNC	366	88525	100	24886	100	70814	100
T	946	73936	83	16344	65	76344	107
TM	895	74964	84	30634	123	74621	105
TG	714	47149	53	8408	33	45200	63
TC	228	29432	33	5413	21	17054	24
TN	700	36835	41	9608	38	29429	41

a TM T cells which rosette with EAM
b TG T cells which rosette with EAG
c TC T cells which rosette with EAC
d TN T cells depleted of TG, TM and TC T lymphocytes
* All cell preparations were reconstituted with autologous monocytes to a final concentration of 5%

TABLE 8

THE PHYTOMITOGEN-INDUCED BLASTOGENIC RESPONSES OF T, T4+ AND T8+ T LYMPHOCYTES

Cells of donor	Control	PHA	%R	PWM	%R	Con-A	%R
donor 1	cpm	cpm		cpm		cpm	
MNC(a)	486	70730	100	35822	100	76266	100
T(a)	412	70095	99	33456	93	67774	88
T4+(b)*	628	55081	77	28222	78	54382	71
T8+(c)*	342	51919	73	26838	74	59673	78
donor 2							
MNC	347	106260	100	57922	100	105995	100
T	421	106111	99	53194	91	99061	93
T4+	912	67508	63	20670	35	64285	60
T8+	829	66345	62	19292	33	63787	60
donor 3							
MNC	780	99364	100	58031	100	94768	100
T	255	95164	95	55760	96	87477	92
T4+	713	55531	55	29206	50	51138	53
T8+	957	55784	56	28211	48	52648	55

a defined as in Table 1
b T4+ T cells treated with OKT8 and C'
c T8+ T cells treated with OKT4 and C'
* All cell preparations were reconstituted with autologous monocytes to a final cell concentration of 5%

4.5. THE ROLE OF SOLUBLE MEDIATORS IN THE 3 DAY PHYTOMITOGEN-INDUCED BLASTOGENIC RESPONSE

Objective

The objective of the following series of experiments was to determine the capacity of the monocytes, and the supernatants of PHA-stimulated or unstimulated monocytes, to restore blastogenic responsiveness to T-MO_2 cells in the phytomitogen-induced blastogenic response.

Protocol

The MNC and T lymphocytes were isolated as described in Chapters 3.2.8. and 3.2.12., respectively. The T lymphocytes were depleted of monocytes twice over a 24 hr period as described in Chapter 3.2.10. Monocytes (obtained as described in Chapter 3.2.11.) were incubated in the presence or absence of PHA for 0, 24, 48 and 72 hrs following which they were centrifuged and the cell-free supernatants were obtained as described in Chapter 3.2.13. The T-MO_2 cells were cultured in the presence of (i) autologous monocytes (final cell concentration 10%), (ii) supernatants from 48 hr unstimulated monocyte cultures, or (iii) supernatants from 48 hr unstimulated MNC-MO_2 cells (MNC depleted of

monocytes twice) in order to ascertain the capacity of the monocyte culture supernatants to restore blastogenic responsiveness to the T-MO_2 cells in the 3 day phytomitogen-induced blastogenic response. The cells were cultured and the blastogenic responses determined as described in Chapter 3.2.25.

Results

As can be seen in Table 9, the T-MO_2 cells cultured in the presence of supernatants from 48 hr unstimulated autologous MNC-MO_2 cultures (NMS) gave insignificant blastogenic responses to phytomitogen stimulation following 3 days in culture. In contrast, the T-MO_2 cells cultured in the presence of supernatants obtained from 48 hr unstimulated autologous monocyte cultures (MS) gave highly significant blastogenic responses. These monocyte-derived supernatants were, at best, only 50% as effective as viable autologous monocytes in restoring blastogenic responsiveness to the T-MO_2 cells.

Supernatants from cultures of autologous monocytes and MNC-MO_2 cells incubated for 24, 48 or 72 hrs in the presence or absence of PHA were compared for their ability to enhance the blastogenic responsiveness of the

T-MO_2 cells. Supernatants from 24 hr PHA-stimulated autologous monocyte cultures (MSP) restored partial blastogenic responsiveness to the T-MO_2 cells whereas the supernatants from 24 hr unstimulated autologous monocyte cultures (MS) did not (Table 10). The supernatants from 48 hr PHA-stimulated cultured autologous monocytes (MSP) reconstituted blastogenic responsiveness to the T-MO_2 cells slightly better than did the supernatants from 48 hr unstimulated cultured autologous monocytes (MS) (Table 10). Similarly, the 72 hr MSP enhanced the blastogenic responsiveness of T-MO_2 cells better than did the 72 hr MS (Table 10). The supernatants from the 24 hr PWM and Con-A-stimulated autologous monocyte cultures were also capable of restoring blastogenic responsiveness to the T-MO_2 cells (Table 11). The supernatants from 24, 48, and 72 hr PHA-stimulated MNC-MO_2 cell cultures (NMS) were unable to facilitate the blastogenic responsiveness of the T-MO_2 cells (Table 10).

Supernatants from 48 hr PHA-stimulated allogeneic monocyte cultures reconstituted blastogenic responsiveness to the T-MO_2 cells to the same extent as did the supernatants from 48 hr PHA-stimulated autologous monocyte cultures (Table 12).

The capacity of the monocytes to restore blastogenic responsiveness to the T-MO_2 cells decreased as their incubation in culture increased from 24 to 72 hours (Table 13). The addition of 24 hr incubated monocytes almost totally restored the blastogenic responsiveness to the T-MO_2 cells. The 48 hr cultured monocytes were not as effective and the 72 hr cultured monocytes were even less effective in restoring the blastogenic responsiveness to the T-MO_2 cells to phytomitogen stimulation. In contrast, the capacity of the PHA-stimulated (MSP) and unstimulated (MS) monocyte culture supernatants increased following 24, 48 and 72 hrs of culture (Table 13). The effectiveness of the 48 and 72 hour culture supernatants were comparable. As was observed in Table 10, only the 24 hr MSP and not the 24 hr MS was effective in restoring blastogenic responsiveness to the T-MO_2 cells to phytomitogen stimulation (Table 13).

The capacity of the monocyte culture supernatant to enhance the blastogenic response of T-MO_2 cells to phytomitogen stimulation diminished with serial dilution of the supernatant. The undiluted MS restored blastogenic responsiveness to the T-MO_2 cells to a degree 50-70% of the optimal response. However, the T-MO_2 cells augmented with the MS diluted even ten fold

were still capable of mounting a low but significant blastogenic response (Table 14).

Discussion

The monocytes have been shown to be obligatory participants in the phytomitogen-induced blastogenic response (Table 6). However, monocytes can be replaced by supernatants from unstimulated and from PHA, PWM or Con-A-stimulated monocyte cultures. Supernatants obtained from PHA-stimulated or unstimulated autologous MNC-MO_2 cells (MNC totally depleted of monocytes) cultured for 24, 48 or 72 hrs were at no time able to restore the blastogenic response to the T-MO_2 cells. On the other hand, supernatants obtained from unstimulated (48 or 72 hr) or PHA-stimulated (24, 48 or 72 hr) monocyte cultures were invariably able to restore blastogenic responsiveness to the T-MO_2 cells. It is therefore obvious that monocyte must be present in the cell cultures in order for the blastogenic enhancing factors to be secreted. As is discussed in depth in Chapter 2.2, it has been demonstrated that at least two mediators facilitate the phytomitogen-induced blastogenic response - IL-1 and IL-2. The monocyte generated soluble mediator is referred to as IL-1 (Gillis and Mizel 1981, Mizel 1982, Gery 1982). IL-1 does not support the

proliferative response of T lymphocytes directly; it provides a signal for certain T lymphocytes to produce IL-2 (Gillis and Mizel 1981, Palacious 1982). IL-2 has been shown to facilitate the mitogen-induced blastogenic response and to maintain T lymphocytes in a viable state in long term culture.

Although this investigation has not characterized the mediator secreted by the monocytes, it is nevertheless assumed that the active factor or mediator is IL-1, since it can replace the monocytes in the 3 day phytomitogen-induced blastogenic response.

Supernatants from 48 hr PHA-stimulated autologous and allogeneic monocyte cultures were equally effective in restoring the blastogenic responsiveness to the T lymphocytes in the phytomitogen-induced blastogenic response. The blastogenic response in the presence of the monocyte supernatant is, however, always less than that observed in the presence of autologous or allogeneic monocytes. These results suggest that factors other than monocyte-secreted mediators are essential for the maximum T lymphocyte blastogenic response and that T lymphocyte-monocyte contact may be required to facilitate maximal blastogenic responses.

TABLE 9

THE CAPACITY OF SUPERNATANTS OF 48 HR CULTURES OF UNSTIMULATED AUTOLOGOUS MONOCYTES AND MNC-MO2 TO RESTORE THE BLASTOGENIC RESPONSIVENESS TO T-MO2 CELLS

	Control	PHA	%R	PWM	%R	CON-A	%R
Cells of donor							
donor 1	cpm	cpm		cpm		cpm	
MNC(a)	309	83803	100	38654	100	72826	100
MNC-MO2(a)	785	1964	2	1240	3	1758	2
T(a)	422.	79967	95	36719	95	69950	96
T-MO2(a)	513	4880	6	2309	6	4476	6
MO(a)	185	1657	2	1477	4	1907	3
T-MO2+NMS(b)	759	3316	4	1464	4	3809	5
T-MO2+MS(c)	565	45671	54	19927	52	37270	51
T-MO2+MO(d)	514	94717	113	45110	117	85318	117
donor 2							
MNC	218	109788	100	47200	100	73559	100
MNC-MO2	667	1705	2	643	2	1943	3
T	192	77884	71	36518	77	64767	88
T-MO2	247	8176	7	2415	5	6237	8
MO	825	1099	1	980	2	1226	2
T-MO2+NMS	774	2253	2	936	2	1527	2
T-MO2+MS	414	52865	48	32520	69	47535	65
T-MO2+MO	839	104940	96	49350	105	71738	98
donor 3							
MNC	928	104909	100	50966	100	82564	100
MNC-MO2	357	1316	1	912	2	1331	2
T	297	83672	80	22188	44	58799	71
T-MO2	312	7288	7	1697	3	5092	6
MO	681	3896	4	2611	5	3084	4
T-MO2+NMS	965	4929	5	2154	4	3232	4
T-MO2+MS	693	52068	50	16212	32	47424	57
T-MO2+MO	842	108658	104	50563	99	83408	101

a defined as in Table 3

b T-MO2+NMS T-MO2 cells cultured in supernatants from 48 hr unstimulated autologous MNC-MO2 cultures

c T-MO2+MS T-MO2 cells cultured in supernatants from 48 hr unstimulated autologous monocyte cultures

d T-MO2+MO T-MO2 cells cultured with autologous monocytes (final monocyte conc 10%)

TABLE 10

THE CAPACITY OF SUPERNATANTS OF 24, 48 AND 72 HR CULTURES OF UNSTIMULATED AND PHA-STIMULATED AUTOLOGOUS MONOCYTES AND MNC-MO2 CELLS TO RESTORE BLASTOGENIC RESPONSIVENESS TO T-MO2 CELLS

Cells of donor	Control	PHA	%R	PWM	%R	CON-A	%R
donor 1	cpm	cpm		cpm		cpm	
MNC	588	127991	100	69109	100	114658	100
T	514	103235	81	66250	96	105999	92
T-MO2	513	6088	5	2309	3	4476	4
MO	185	1657	1	1477	2	1907	2
T-MO2+MO	727	118419	93	64385	93	110296	96
T-MO2+MS(0)(a)	134	1029	1	866	1	872	1
T-MO2+MS(24)(a)	159	5020	4	2340	3	4949	4
T-MO2+MS(48)(a)	316	75194	59	46118	67	66214	58
T-MO2+MS(72)(a)	422	79967	62	46719	68	67950	59
T-MO2+MSP(0)(b)	825	1099	1	980	1	1226	1
T-MO2+MSP(24)(b)	565	35671	28	9927	14	27270	24
T-MO2+MSP(48)(b)	398	81101	63	55821	81	70284	61
T-MO2+MSP(72)(b)	309	82803	65	58654	85	72826	64
T-MO2+NMSP(24)(c)	398	1268	1	638	1	1335	1
T-MO2+NMSP(48)(c)	279	1584	1	672	1	1634	1
T-MO2+NMSP(72)(c)	170	1882	1	421	1	1765	2
donor 2							
MNC	366	116254	100	78364	100	109247	100
T	327	128027	110	77864	99	108609	99
T-MO2	247	7243	6	2415	3	6238	6
MO	159	2029	2	1343	2	1979	2
T-MO2+MO	429	115329	99	75301	96	108083	99
T-MO2+MS(0)	347	1428	1	1372	2	4650	4
T-MO2+MS(24)	681	3896	3	2811	4	5084	5
T-MO2+MS(48)	218	69947	60	36018	46	66268	61
T-MO2+MS(72)	693	72068	62	36212	46	67424	62
T-MO2+MSP(0)	520	4080	4	3962	5	4233	4
T-MO2+MSP(24)	371	34068	29	15213	19	25432	23
T-MO2+MSP(48)	316	72189	62	42981	55	68717	63
T-MO2+MSP(72)	615	73120	63	44418	57	70813	65
T-MO2+NMSP(24)	256	2257	2	962	1	1673	1
T-MO2+NMSP(48)	258	1143	1	469	1	940	1
T-MO2+NMSP(72)	114	952	1	495	1	931	1

a MS(0), MS(24), MS(48), MS(72) represent supernatants of unstimulated monocytes cultured for 0 min, 24 hrs, 48 hrs and 72 hrs, respectively

b MSP(0), MSP(24), MSP(48) and MSP(72) constitute supernatants of PHA-stimulated autologous monocytes cultured for 0 min, 24 hrs, 48 hrs and 72 hrs, respectively

c NMSP(24), NMSP(48) and NMSP(72) constitute supernatants of PHA-stimulated MNC-MO2 cells cultured for 24 hrs, 48 hrs and 72 hrs, respectively

TABLE 11

THE CAPACITY OF SUPERNATANTS OF 24 HR CULTURES OF UNSTIMULATED AND PHA, PWM AND CON-A-STIMULATED AUTOLOGOUS MONOCYTES TO RESTORE BLASTOGENIC RESPONSIVENESS TO T-MO2 CELLS

	Control	PHA	%R	PWM	%R	Con-A	%R
Cells of donor							
donor 1	cpm	cpm		cpm		cpm	
MNC	295	104028	100	58692	100	93339	100
T-MO2	324	4257	4	1117	2	4712	5
MO	212	280	0	215	1	300	0
T-MO2+MO(a)	245	117476	100	60534	100	88527	95
T-MO2+MS(a)	255	3791	4	1950	3	2692	3
T-MO2+MSP(b)	970	39204	38	13948	24	35109	38
T-MO2+MSPW(c)	969	27398	26	9834	17	23698	25
T-MO2+MSC(d)	990	34963	34	10606	18	33547	36
donor 2							
MNC	514	94569	100	52289	100	109196	100
T-MO2	320	2063	2	987	2	1826	17
MO	187	282	0	136	1	420	0
T-MO2+MO	498	79301	84	32136	61	79579	73
T-MO2+MS	377	8903	9	1948	4	7008	6
T-MO2+MSP	738	41867	44	26681	51	40572	4
T-MO2+MSPW	898	30290	32	17325	33	29893	27
T-MO2+MSC	994	34038	36	17946	34	31334	29
donor 3							
MNC	738	103635	100	52106	100	108090	100
T-MO2	675	1013	1	645	1	1379	1
MO	206	845	1	224	1	725	1
T-MO2+MO	603	100757	97	56303	100	98343	91
T-MO2+MS	651	4873	5	1889	4	3429	3
T-MO2+MSP	740	41345	40	11279	22	40238	37
T-MO2+MSPW	985	25601	25	9965	19	23260	22
T-MO2+MSC	802	30106	30	11281	22	32958	30

a defined as in Table 8

b T-MO2 cells cultured in supernatants from 24 hr PHA-stimulated autologous monocyte cultures

c T-MO2 cells cultured in supernatants from 24 hr PWM-stimulated autologous monocyte cultures

d T-MO2 cells cultured in supernatants from 24 hr Con-A-stimulated autologous monocyte cultures

TABLE 12

THE CAPACITY OF SUPERNATANTS OF 48 HR CULTURES OF PHA-STIMULATED ALLOGENEIC MONOCYTES TO RESTORE BLASTOGENIC RESPONSIVENESS TO T-MO2 CELLS

Cells of donor	Control	PHA	%R	PWM	%R	Con-A	%R
donor 1	cpm	cpm		cpm		cpm	
MNC	248	109788	100	57200	100	105590	100
T-MO2	240	1976	2	1490	3	1862	2
MO	159	502	0	343	1	670	1
donor 2							
MNC	297	83672	100	42188	100	88737	100
T-MO2	312	1278	3	1097	3	1093	1
MO	681	896	1	284	0	364	0
dohor 3							
MNC	728	94206	100	40992	100	90771	100
T-MO2	721	1862	2	979	2	1850	2
MO	200	1201	1	468	1	996	1
T-MO2+MO(a)							
1+1	839	104948	96	49457	86	100730	96
1+2	671	88121	80	43514	76	87673	83
1+3	924	85225	78	40633	71	83464	79
2+2	639	70490	84	30966	74	62564	71
2+1	294	71120	85	34298	82	69813	79
2+3	687	65288	78	29843	71	65970	74
3+3	329	81966	87	36385	89	80294	88
3+1	652	73241	78	32860	80	72946	80
3+2	314	75231	80	30738	75	70293	77
T-MO2+MSP(b)							
1+1	519	42111	38	28904	51	46113	44
1+2	384	43877	38	23535	41	41608	39
1+3	563	52205	48	27389	48	40286	38
2+2	928	32203	38	16122	38	30424	34
2+1	614	22338	27	12594	30	21928	25
2+3	314	29894	36	20331	48	26583	30
3+3	218	32138	34	15925	39	31827	35
3+1	498	32901	34	19647	48	29280	32
3+2	900	28262	30	12299	30	30693	34

a T-MO2+MO T-MO2 cells cultured with monocytes (final concentration 10%)

b T-MO2+MSP T-MO2 cells cultured in supernatants from 48 hr PHA-stimulated monocyte cultures

TABLE 13

THE RELATIVE CAPACITIES OF UNSTIMULATED AND PHA-STIMULATED MONOCYTE CULTURE SUPERNATANTS (24, 48 AND 72 HR) AND THE UNSTIMULATED AND PHA-STIMULATED CULTURED MONOCYTES (24, 48 AND 72 HR) TO RESTORE THE BLASTOGENIC RESPONSIVENESS TO T-MO2 CELLS

Cells of donor donor 1	Control cpm	PHA cpm	%R	PWM cpm	%R	Con-A cpm	%R
MNC	309	102800	100	58650	100	102826	100
T	219	88129	86	34218	58	86531	84
T-MO2	513	4881	5	2314	4	4470	4
MO	185	1051	1	1077	2	1081	1
T-MO2+MO(0)(a)	466	128122	125	64620	110	114871	112
T-MO2+MO(24)(a)	514	94717	92	55110	94	85318	83
T-MO2+MO(48)(a)	565	65671	64	39272	67	64270	63
T-MO2+MO(72)(a)	575	38618	38	26851	46	36724	36
T-MO2+MS(0)(b)	612	4918	5	2422	4	4930	5
T-MO2+MS(24)(b)	825	7821	8	3621	6	6126	6
T-MO2+MS(48)(b)	422	79967	78	46719	80	67950	66
T-MO2+MS(72)(b)	410	80121	78	46912	80	68122	66
T-MO2+MO(0)(c)	499	105235	102	66260	113	116000	113
T-MO2+MO(24)(c)	618	96818	94	57143	97	108610	106
T-MO2+MO(48)(c)	412	69991	68	41618	71	68418	67
T-MO2+MO(72)(c)	816	44921	44	29919	51	42988	42
T-MO2+MSP(0)(d)	1344	4827	5	2866	5	4872	5
T-MO2+MSP(24)(d)	938	38910	38	24981	43	48226	47
T-MO2+MSP(48)(d)	565	81271	79	46922	80	72692	71
T-MO2+MSP(72)(d)	422	82967	81	46799	80	79950	78

a unstimulated MO pre-incubated in medium for 0, 24, 48 or 72 hrs
b MS denotes supernatants of MO cultured for 0, 24, 48 or 72 hrs in the absence of PHA
c MO pre-incubated with PHA for 0, 24, 48 or 72 hrs
d MSP denotes supernatants of MO cultured with PHA for 0, 24, 48, or 72 hrs.

TABLE 14

THE BLASTOGENIC RESPONSE OF T-MO2 CELLS AS A FUNCTION OF THE DILUTION OF THE MONOCYTE SUPERNATANT ADDED.

	Control	PHA	%R	PWM	%R	Con-A	%R
Cells of donor							
donor 1		cpm		cpm		cpm	
MNC(a)		218	109788	100	57200	100	95559	100
T(a)		310	84981	77	39181	68	81329	85
T-MO2(a)		247	8176	7	2415	4	6237	7
MO(a)		201	918	1	741	1	899	1
T-MO2+MO(a)		519	102111	93	51906	91	91614	96
T-MO2+MS(b)	undil	192	77884	71	36189	63	64767	68
T-MO2+MS(b)	1/2	417	59549	54	24581	43	48214	50
T-MO2+MS(b)	1/5	444	44416	40	13521	24	31677	33
T-MO2+MS(b)	1/10	218	18761	17	7121	12	15230	16
donor 2								
MNC		397	93672	100	42188	100	88799	100
T		419	72492	77	24801	59	69519	78
T-MO2		312	7218	8	1597	4	5092	6
MO		218	1121	1	481	1	770	1
T-MO2+MO		628	90909	97	38960	92	82564	93
T-MO2+MS	undil	317	50052	53	18525	44	44114	50
T-MO2+MS	1/2	689	42838	46	14246	34	37521	42
T-MO2+MS	1/5	270	30374	32	7133	17	17533	20
T-MO2+MS	1/10	418	13270	14	3912	9	10788	12

a Defined as in Table 3

b MS 48 hr unstimulated monocyte supernatants diluted 2, 5 and 10 fold with culture medium

4.6. THE FAILURE OF B AND NULL LYMPHOCYTES TO GENERATE SIGNIFICANT BLASTOGENIC RESPONSES TO PHYTOMITOGEN STIMULATION IN THE ABSENCE OR PRESENCE OF MNC-SECRETED MEDIATORS FOLLOWING 3 DAYS IN CULTURE

Objective

The objective of this series of experiments was to determine the responsiveness of non-T cells (B and Null lymphocytes) to phytomitogen stimulation in the absence or presence of MNC-secreted mediators following 3 days in culture.

Protocol

The MNC and monocytes were isolated as described in Chapters 3.2.8. and 3.2.11., respectively. The non-T lymphocytes were isolated from the MNC by rosetting with SRBC as described in Chapter 3.2.12. The T, B and Null lymphocytes were positively isolated as described in Chapters 3.2.12., 3.2.19. and 3.2.20., respectively. The T lymphocytes were depleted of monocytes twice as described in Chapter 3.2.10. Unfractionated MNC or pure monocytes were cultured for 48 hours in the absence or the presence of PHA as described in Chapter 3.2.11. The supernatants obtained from these cultures were pipetted

into the microtiter wells to which were added non-T cells. Mitomycin C treated MNC (MNC_m) were prepared as described in Chapter 3.2.9. The cells were cultured and the blastogenic responses determined as described in Chapter 3.2.25.

Three and five day cultures were carried out with MNC, T-MO_2, non-T and MNC_m cells. Combinations of MNC_m and T-MO_2, MNC_m and MNC, MNC_m and non-T cells were also cultured in the presence of the phytomitogens in order to ascertain whether soluble mediators released from the MNC_m in culture could facilitate the blastogenic responses of the non-T cells.

The Null and B lymphocyte cultures (reconstituted with autologous monocytes to a final cell concentration of 10%) were also intentionally "contaminated" with autologous T lymphocytes at the initiation of the culture (final T lymphocyte concentration 1% and 5%).

Results

The T lymphocytes and not the non-T lymphocytes responded to phytomitogen stimulation following 3 and 5 days in culture (Tables 15, 16). The non-T lymphocytes failed to respond with blastogenesis in the presence of

monocytes (final monocyte concentration 10%) or supernatants of 48 hr unstimulated or PHA-stimulated monocyte or unfractionated MNC cultures (Table 15).

The MNC_m by themselves did not undergo blastogenesis to phytomitogen stimulation as expected, since Mitomycin C inhibits DNA synthesis. The $MNC+MNC_m$ and the $T-MO_2+MNC_m$ responded well following 3 and 5 days in culture (Table 16). The non-T cells and the $non-T+MNC_m$ failed to generate a blastogenic response to phytomitogen stimulation in the presence of MNC_m following either 3 days or 5 days in culture (Table 16).

The positively isolated B lymphocytes (cultured in the absence of other cells or factors) did not respond to phytomitogen stimulation following 3 days in culture (Table 17). Similar results were observed with the positively isolated Null lymphocytes. The addition of autologous monocytes (final monocyte concentration 10%) to either the B or Null lymphocytes did not result in the facilitation of the blastogenic responses by the B or Null lymphocytes (Table 17).

Normally unresponsive B lymphocytes appeared to respond following their intentional "contamination" with small numbers of T lymphocytes (final concentration 1%).

Similarly, normally unresponsive Null lymphocytes appeared to respond when intentionally "contaminated" with small numbers of T lymphocytes (final concentration 1%). Significantly greater blastogenic responses were observed when larger numbers of T lymphocytes were added to the B and Null lymphocytes (final concentration ~5% T lymphocytes (Table 17).

Discussion

Optimal blastogenic responses were observed in cultures of MNC+MNC_m and T-MO_2+MNC_m at day 3 and at day 5 in culture, thus demonstrating that the MNC_m cells did not secrete non-specific inhibitory factors during the culture period. The MNC_m were unresponsive to phytomitogen stimulation. The fact that the T-MO_2 cell cultures in the presence of the MNC_m gave marked blastogenic responses upon stimulation with the phytomitogens attests to the fact that MNC_m are, in fact, quite capable of secreting the mediator(s) which facilitates the blastogenic response. This mediator must originate from the monocytes in the MNC_m since the responding T lymphocytes were totally depleted of monocytes. Although this investigation has not characterized the mediator secreted by the monocytes, it is nevertheless assumed that the active factor is IL-1. In these studies and others the major source of human

IL-1 has been crude supernatants from autologous monocyte or MNC_m cell cultures stimulated with PHA, which are known to contain several biologically active factors, including IL-1 (Gery and Waksman 1972, Maizel et al 1980, Palacious 1982, de Vries 1979).

It is essential to note that neither the B nor the Null lymphocytes cultured individually nor in combination (non-T lymphocytes) responded to phytomitogen stimulation following 3 or 5 days of culture in the presence of optimal numbers of autologous monocytes (final monocyte concentration 10%) or MNC_m. These results unequivocally demonstrate that the B and Null lymphocytes cannot respond with blastogenesis under conditions which facilitate optimal blastogenic responses by the T lymphocytes or unfractionated MNC.

As is discussed in Chapters 2.2 and 5. a number of investigators have concluded that B and Null lymphocytes generate significant albeit minimal blastogenic responses to phytomitogen stimulation. On the basis of the results reported in this investigation whatever responses are given by the B or Null lymphocytes should be attributed to residual T lymphocytes since intentional contamination with T lymphocytes in as low a concentration as 1% is sufficient to confer apparent blastogenic responsiveness to the B and Null lymphocytes.

TABLE 15

THE FAILURE OF SUPERNATANTS OF UNSTIMULATED AND PHA-STIMULATED 48 HR CULTURES OF UNFRACTIONATED MNC OR MONOCYTES TO INDUCED A BLASTOGENIC RESPONSE BY THE CIRCULATING NON-T LYMPHOCYTES FOLLOWING 3 DAYS IN CULTURE

Cells of donor	Control	PHA	%R	PWM	%R	Con-A	%R
donor 1	cpm	cpm		cpm		cpm	
MNC	728	94206	100	60745	100	75773	100
T	200	85225	90	52953	87	73164	97
Non-T(a)	796	3854	4	2858	5	3544	5
Non-T+MNCS(b)	911	3241	3	1286	2	2946	4
Non-T+MNCSP(c)	962	4698	5	2691	4	3642	5
Non-T+MS(d)	246	1966	2	842	1	2041	3
Non-T+MSP(e)	866	2160	2	1638	3	1988	3
Non-T+MO(f)	660	3948	4	2766	5	3882	5
donor 2							
MNC	728	140981	100	81963	100	125846	100
T	212	104012	74	60351	74	92300	73
Non-T	574	3769	3	2508	3	3472	3
Non-T+MNCS	241	2138	2	1142	1	2011	2
Non-T+MNCSP	468	2920	2	2001	2	2844	2
Non-T+MS	381	1962	1	1186	1	1821	1
Non-T+MSP	946	2281	2	1348	2	2169	2
Non-T+MO	443	4053	3	2232	3	3100	2

a Non-T MNC depleted of T cells

b MNCS supernatants of unstimulated MNC cultured for 48 hrs.

c MNCSP supernatants of PHA-stimulated MNC cultured for 48 hrs.

d MS supernatants of unstimulated MO cultured for 48 hrs.

e MSP supernatants of PHA-stimulated MO cultured for 48 hrs.

f Non-T+MO non-T cells reconstituted with 10% autologous MO (final concentration 10%).

TABLE 16

THE FAILURE OF MITOMYCIN-C TREATED MONONUCLEAR CELLS TO CONFER BLASTOGENIC RESPONSIVENESS TO NULL OR B LYMPHOCYTES

	Control		PHA		PWM		CON-A	
	day 3	day 5	day 3	day 5	day 3	day 5	day 3	day 5
donor 1	cpm	cpm	cpm	cpm	cpm	cpm	cpm	cpm
MNC	588	292	127991	211623	49109	56401	114658	156965
MNCm(a)	278	193	307	829	254	147	333	257
T-MO2	667	780	3200	2929	2455	1936	2245	2923
NON-T	317	230	5559	7218	1464	2167	1977	6917
NON-T+MNCm	220	328	4712	9004	1896	3268	2605	6759
MNC+MNCm	301	691	118688	195856	49423	46144	106588	134798
T-MO2+MNCm	864	643	89696	76597	41688	43688	83897	81591
donor2								
MNC	366	676	116254	142465	51364	62746	92471	136790
MNCm	109	188	150	243	111	102	112	162
T-MO2	622	426	6970	33[illegible]	5117	2029	5972	3527
NON-T	528	342	4091	[illegible]	2966	6982	4238	6988
NON-T+MNCm	243	120	3894	[illegible]	2942	7700	3276	6143
MNC+MNCm	848	147	92090	140648	50704	62606	90728	127440
T-MO2+MNCm	829	481	72170	[illegible]2739	43509	46790	70052	72748

a	MNCm	Mitomycin C treated MNC
b	NON-T+MNCm	NON-T reconstituted with MNCm (final concentration 50%)
c	MNC+MNCm	MNC reconstituted with MNCm (final concentration 50%)
d	T-MO2+MNCm	T-MO2 reconstituted with MNCm (final concentration 50%)

TABLE 17

THE CAPACITY OF "CONTAMINATING" T LYMPHOCYTES TO CONFER BLASTOGENIC RESPONSIVENESS TO B AND NULL LYMPHOCYTES

Cells of donor	%T(a)	Control	PHA	%R	PWM	%R	CON-A	%R
donor 1		cpm	cpm		cpm		cpm	
MNC	65	211	117539	100	50081	100	106950	100
T	90	489	109328	93	46219	92	100290	93
MO	0	368	986	1	321	1	808	1
Non-T	0	455	1687	1	635	1	1371	1
T+MO	5	298	48212	41	22418	45	45916	43
NonT+T	5	213	23880	20	11824	24	23274	22
Non-T+T	2.5	299	15590	13	7128	14	14141	13
Non-T+T	1	290	11831	10	4930	10	9349	9
B	0	278	290	0	286	1	299	0
B+MO	0	145	359	0	308	1	328	0
B+MO+T	5	298	25880	22	13240	26	24555	23
B+MO+T	1	142	11021	9	4924	10	9638	9
NULL	0	178	307	0	255	1	338	0
NULL+MO	0	249	422	0	369	1	494	0
NULL+MO+T	5	383	23211	20	10645	21	22050	21
NULL+MO+T	1	140	10619	9	4045	8	8996	8
donor 2								
MNC	70	588	127901	100	69218	100	125658	100
T	84	279	100984	79	53236	77	101944	81
MO	0	108	1061	1	523	1	966	1
Non-T	0	278	976	1	832	1	947	1
T+MO	5	342	67619	53	30919	45	66618	53
NonT+T	5	230	30559	24	16768	24	29605	24
Non-T+T	2.5	190	25698	20	10423	15	23320	19
Non-T+T	1	482	14532	11	7611	11	13798	11
B	0	139	424	0	390	1	480	0
B+MO	0	323	799	1	474	1	791	1
B+MO+T	5	349	29654	23	17211	25	29004	23
B+MO+T	1	246	16901	13	7074	10	14532	12
NULL	0	153	780	1	453	1	703	1
NULL+MO	0	248	799	1	524	1	832	1
NULL+MO+T	5	419	26243	21	13274	19	24498	19
NULL+MO+T	1	310	12880	10	7282	11	12992	10

a %T Final concentration of T lymphocytes added to a final cell concentration of 0%, 1% and 5%

4.7. THE CAPACITY OF IL-1, IL-2 AND BCGF TO FACILITATE THE BLASTOGENIC RESPONSES OF THE MNC, T, B AND NULL LYMPHOCYTES TO PHYTOMITOGEN STIMULATION FOLLOWING 7 DAYS IN CULTURE

Objective

The objective of the following experiments was to determine the blastogenic responses of the MNC, T, B and Null lymphocytes to phytomitogen stimulation in the presence of IL-1, IL-2 and BCGF following 7 days in culture.

Protocol

The MNC and monocytes were isolated as described in Chapters 3.2.8. and 3.2.11., respectively. The T, B and Null lymphocytes were positively isolated as described in Chapters 3.2.12., 3.2.19. and 3.2.20., respectively. The T lymphocytes were depleted of monocytes twice as described in Chapter 3.2.10. Supernatants from 48 hr PHA-stimulated monocyte cultures were isolated as described in Chapter 3.2.13. Phytomitogens were added at the initiation and at day 4 of culture. Similarly, IL-1, IL-2 and BCGF were added at the initiation and at day 4

of culture to ascertain the capacity of these soluble factors to facilitate the blastogenic responses of the cultured lymphocytes to phytomitogen stimulation. The cells were cultured for 7 days and the degree of blastogenesis determined as described in Chapter 3.2.25.

Results

The MNC responded well on day 3 to phytomitogen stimulation; however they responded insignificantly on day 7 of culture (Table 18). They also did not respond in the presence of IL-1. In contrast, the MNC cultured in the presence of phytomitogens and IL-2 or BCGF proliferated markedly following 7 days in culture (Table 18). Neither IL-2 nor BCGF were capable of inducing blastogenesis by the MNC in the absence of the phytomitogens.

The T-MO_2 cells did not generate a significant blastogenic response to phytomitogen stimulation in the presence of IL-1 following 7 days in culture (Table 18). Both IL-2 and BCGF were however capable of facilitating the phytomitogen-induced blastogenic response of T-MO_2 cells following 7 days in culture (Table 18).

The B lymphocytes like the T-MO_2 cells failed to generate a blastogenic response to phytomitogen stimulation following 7 days in culture in the presence of IL-1. However, the B lymphocytes consistently gave marked blastogenic responses when cultured in the presence of PWM and IL-2 or BCGF (Table 18). It is important to stress that the B lymphocytes responded insignificantly to PHA and Con-A stimulation following 7 days of culture irrespective of the presence of IL-2 or BCGF (Table 18).

The Null lymphocytes cultured in the presence of IL-1, IL-2 or BCGF failed to respond to phytomitogen (PHA, PWM or Con-A) stimulation following 7 days in culture (Table 18).

Discussion

The B lymphocytes cultured for 7 days in the presence of IL-2 or BCGF gave significant blastogenic responses to PWM stimulation but not to PHA and Con-A stimulation. Pure Null lymphocytes cultured under identical conditions failed to respond. The MNC and T-MO_2 cells incubated for 7 days in the presence of IL-2 or BCGF gave marked blastogenic responses to stimulation with all three phytomitogens (PHA, PWM and

Con-A).

The optimal phytomitogen-induced blastogenic response occurs 3 days and 5 days following stimulation (Table 1 and Figure 1). However, the blastogenic responses of the MNC at day 7 of culture to phytomitogen stimulation were barely significant even though the phytomitogens and the interleukins were added at the initiation and at day 4 of culture. IL-1 was incapable of restoring the blastogenic responsiveness of MNC following 7 days of culture. However, both exogenous IL-2 (which contains BCGF/BCDF) and BCGF/BCDF (which contains IL-2) effectively facilitated the blastogenic responses of the MNC and T-MO_2 cells to phytomitogen stimulation following 7 days in culture. It may be concluded that the T lymphocytes do not generate sufficient amounts of endogenous IL-2 in the presence of IL-1 to facilitate a 7 day phytomitogen-induced blastogenic response. Since both MNC and T-MO_2 cells in the presence of IL-1 generate optimal phytomitogen-induced blastogenic responses on day 3 and day 5 of culture it must be asked why the cells respond insignificantly only 2 days later. It may be that monocytes lose their capacity to secrete IL-1 following the first few days of culture and thus the T lymphocytes cease to be stimulated to produce endogenous IL-2.

Conversly, it may be that under the constraints of the cell culture conditions which are after all artificial and unphysiological, the T lymphocytes cease to be able to secrete endogenous IL-2 after the fourth and fifth day of culture and require exogenous IL-2 to facilitate the blastogenic response following 7 days of culture.

Since the commercially obtained IL-2 containes BCGF and BCDF and the commercially obtained BCGF/BCDF contained IL-2, one cannot conclude from these experiments whether the responses of the B lymphocytes to PWM stimulation were facilitated by IL-2, BCGF or IL-2 and BCGF acting synergistically. The induction of B lymphocyte proliferation to PWM stimulation following 7 days in culture in the presence of IL-2 or BCGF, may, in fact be dependent on T lymphocytes which are in too low a concentration to de detected by SRBC rosette formation or OKT3 monoclonal antiserum. It has been shown that the B lymphocytes must undergo proliferation in vitro before they transform into Ig secreting cells. These results demonstrate that B lymphocytes do, indeed proliferate in vitro by day 7 of culture.

Null lymphocytes cultured in the presence of IL-1, IL-2 or BCGF failed to respond with blastogenesis to stimulation with any one of the phytomitogens following 7

days of culture.

TABLE 18

THE CAPACITY OF IL-1, IL-2 AND BCGF TO FACILITATE THE BLASTOGENIC RESPONSIVENESS OF MNC, T-MO2, B AND NULL LYMPHOCYTES TO PHYTOMITOGEN STIMULATION FOLLOWING 7 DAYS IN CULTURE

	Control	PHA	%R	PWM	%R	Con-A	%R
donor 1				cpm			
MNC (a)	451	94272	100	42062	100	95008	100
MNC*	340	1050	1	1982	2	1613	2
MNC+IL-1*	837	2954	2	2400	2	2749	3
MNC+IL-2*	625	75285	70	34075	53	66984	66
MNC+BCGF*	847	45150	42	29197	46	41216	40
T-MO2+IL-1*	748	2561	3	2009	5	2668	3
T-MO2+IL-2*	475	52280	55	22977	55	59235	62
T-MO2+BCGF*	708	38237	41	25975	62	32979	35
B+IL-1 *	282	1248	1	849	2	1013	1
B+IL-2 *	173	1990	2	11610	28	1953	2
B+BCGF *	378	1303	1	13218	31	1167	1
NULL+IL-1*	130	1050	1	1002	2	1613	2
NULL+IL-2*	281	931	1	466	1	908	1
NULL+BCGF *	648	969	1	349	1	834	1
donor 2							
MNC	261	92109	100	43616	100	84417	100
MNC*	259	1354	1	2183	1	1412	2
MNC+IL-1	293	1629	1	2325	1	1433	2
MNC+IL-2	339	64859	70	35729	75	66253	78
MNC+BCGF	483	61267	66	32453	74	63421	75
T-MO2+IL-1	485	1650	2	629	1	1316	2
T-MO2+IL-2	803	57784	63	29664	68	58660	69
T-MO2+BCGF	561	46027	95	31916	73	43150	51
B+IL-1	400	1916	2	923	2	1032	1
B+IL-2	512	968	1	14271	33	1009	1
B+BCGF	113	437	1	11916	27	1034	1
NULL+IL-1	327	875	1	563	1	738	1
NULL+IL-2	225	440	1	225	1	791	1
NULL+BCGF	158	972	1	412	1	441	1

a MNC cultured for 3 days

* MNC cultured for 7 days and IL-1, IL-2 or BCGF added at the initiation and at day 4 of the 7 day culture

4.8. OPTIMAL CONDITIONS FOR THE IN VITRO BLASTOGENIC RESPONSES OF THE CIRCULATING MNC TO STIMULATION WITH THE ANTIGENS (TT, DT, CA AND PPD) AND ALLERGEN (AgE)

Objective

The objective of the experiments was to establish the optimal conditions for the antigen and allergen-induced blastogenic responses in cell cultures in this laboratory.

Protocol

The subjects are all doctors, nurses and technologists employed by the Ottawa Civic Hospital. They are all between 20-45 years old and all had been routinely immunized with Diphtheria Pertussus Tetanus toxoids (DPT) on at least several occations but not in the preceeding 5 years.

The non-allergic and ragweed allergic healthy volunteers were bled by venipuncture. The MNC were obtained from the interphase of Ficoll-Hypaque separated whole blood as described in Chapter 3.2.8. The MNC were cultured at varying cell concentrations and for varying periods of time with varying guantities of the antigens

or allergen as described in Chapter 3.2.25. The cells were harvested and the blastogenic response, as defined by the degree of incorporation of H^3-Thymidine by the cell cultures (cpm), was determined as described in Chapter 3.2.25.

Results

As can be seen in Figures IV, the MNC generated the maximum blastogenic responses on day 6 of culture to antigen and allergen stimulation. The optimal concentrations for the antigens and allergen (Antigen E) which facilitate maximum blastogenic responses of the MNC following 6 days in culture were found to be as follows: TT 10 ug/ml, DT 10 ug/ml, CA 20 ug/ml, PPD 50 ug/ml and AgE 0.1 ug/ml (Figure V). The relationship between the 6 day blastogenic response and the MNC cell concentration is presented in Figure VI. It can be seen that optimal blastogenic responses are given by $20x10^4$ MNC per culture.

Discussion

In spite of the fact that the MNC were cultured under optimal conditions (cell concentration and antigen/allergen concentration) insignificant blastogenic

responses were detected on day 3 of culture to antigen and allergen stimulation. In contrast, the phytomitogen-induced blastogenic response of MNC occurs as early as day 3 and endures on day 5; however the response is insignificant by day 6 in culture (Figure I). Furthermore, it must be noted that maximum blastogenic responses obtained following antigen and allergen is much lower than that generated following phytomitogen stimulation. This is expected since the antigen binds to antigen receptors which have genetically predetermined antigen specificity thereby stimulating these cells and stimulates the antigen-specific memory cells which are the progeny of previously committed antibody-forming cells (Schwartz 1985). On the other hand, the phytomitogens are polyclonal stimulants capable of stimulating randomly all of the lymphocytes capable of proliferating when appropriately stimulated. This interpretation is supported by the fact that far fewer numbers of cells are necessary to facilitate maximum blastogenic responsiveness to phytomitogen stimulation than are required for the maximum blastogenic response to antigen and allergen stimulation. Obviously there are more cells capable of responding to the phytomitogens than to any one of the antigens in the cultured MNC.

It is not at all understood as to why the response to phytomitogen stimulation is more rapid than it is to antigen stimulation. Suffice it to say that the results are reproducible and have been verified by numerous investigators (reviewed by Lis and Sharon 1981, Goldstein and Hayes 1978, Ashman and Raff 1973, Miller et al 1975, Udey and Parker 1981).

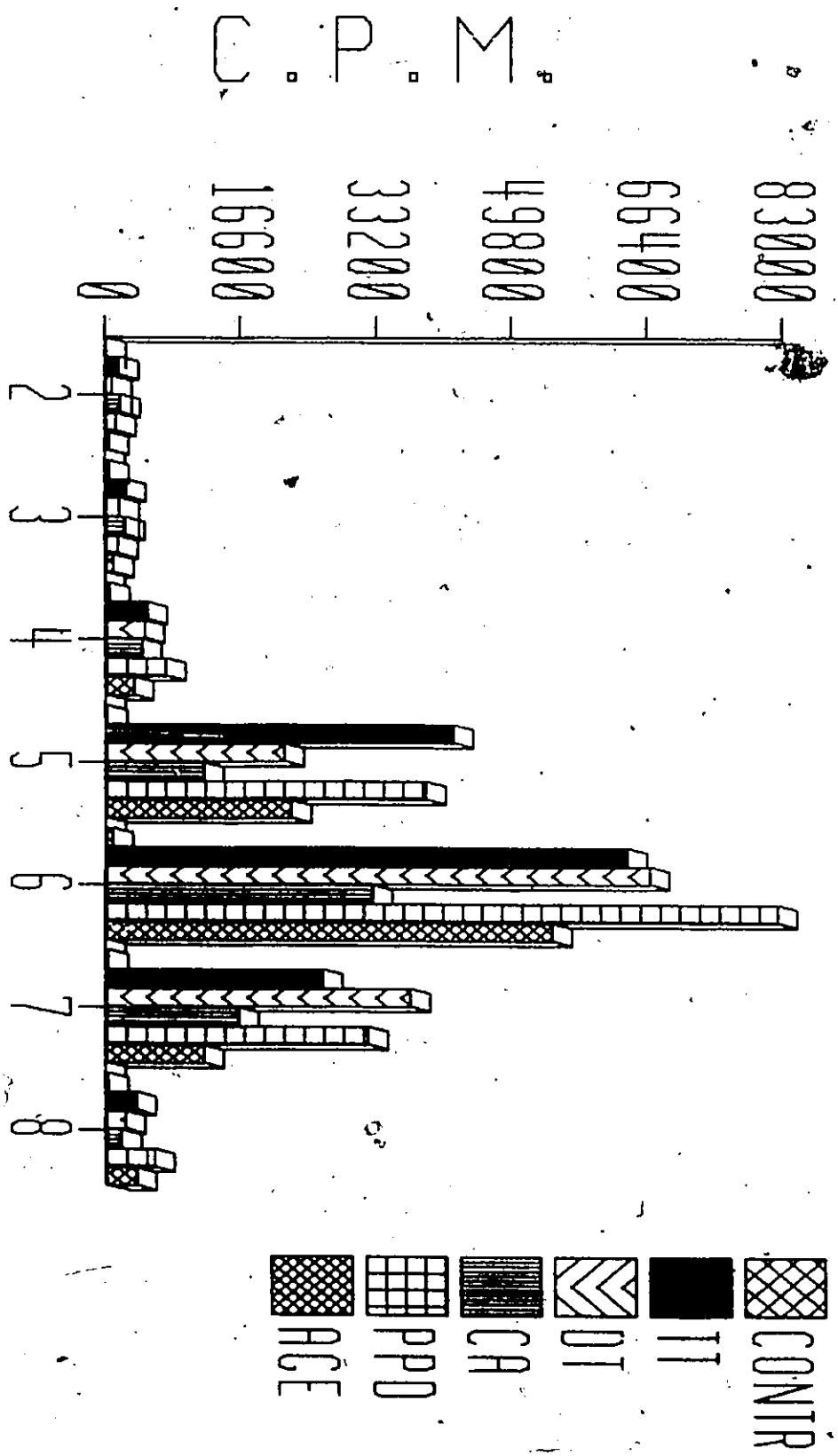

FIGURE IV. The antigen and allergen-induced blastogenic response of MNC as a function of time in culture.

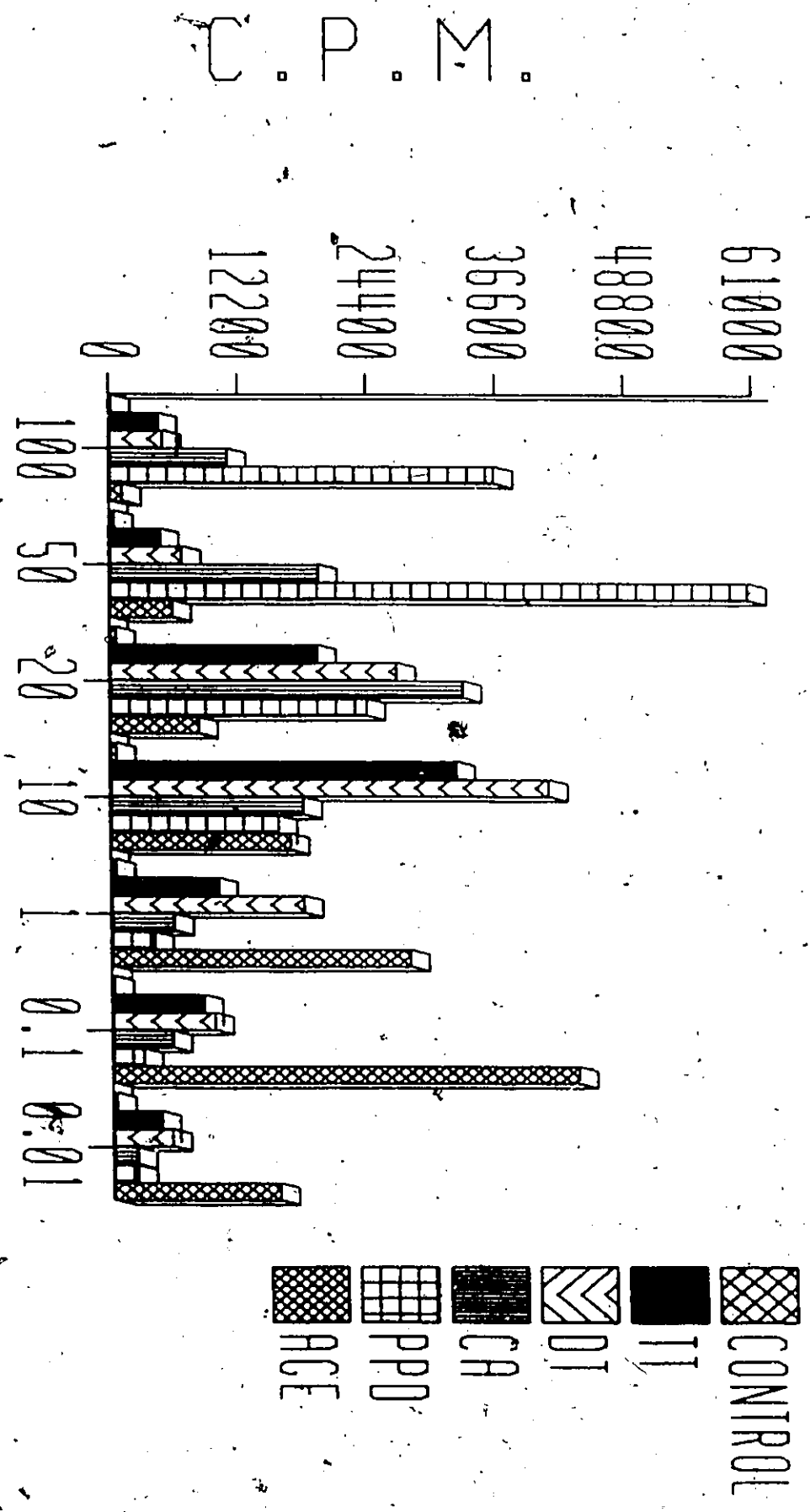

FIGURE V. The antigen and allergen-induced blastogenic response of MNC as a function of antigen and allergen concentration.

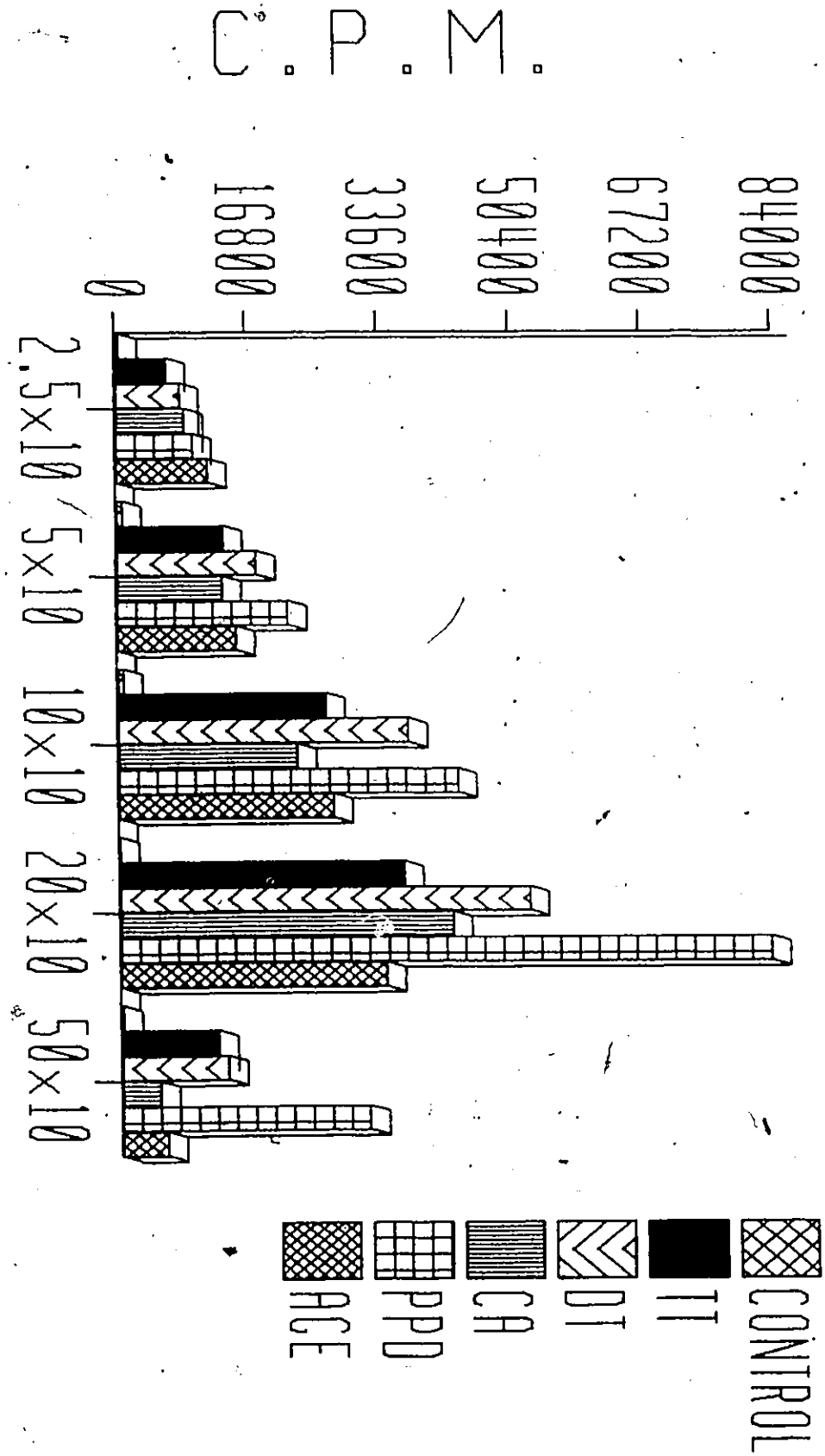

FIGURE VI. The antigen and allergen-induced blastogenic response of MNC as a function of cells per culture.

4.9. THE IDENTIFICATION OF THE RESPONDER CELLS IN THE 6 DAY ANTIGEN AND ALLERGEN-INDUCED BLASTOGENIC RESPONSES

Objective

The objective of the following experiments was to determine the identity of the responder cell(s) in the 6 day antigen and allergen-induced blastogenic responses.

Protocol

The subjects used in this part of the investigation were defined in Chapter 4.8. The non-allergic and allergic volunteers were bled by venipuncture. MNC were obtained from the interphase of Ficoll-Hypaque separated whole blood as described in Chapter 3.2.8. The MNC were depleted of T, B or Null lymphocytes by rosetting procedures as described in Chapters 3.2.12., 3.2.19. and 3.2.20., respectively. The T, B or Null lymphocytes were positively isolated as described in Chapters 3.2.12., 3.2.19. and 3.2.20., respectively. The lymphocytes of normal non-allergic and allergic individuals were tested for their blastogenic responsiveness to stimulation with conventional antigens (TT, DT, PPD, CA) and to an

allergen Antigen E (AgE) derived from ragweed pollen. The cells were cultured for 6 days and the degree of blastogenesis was determined as described in Chapter 3.2.25.

Results

Insofar as the non-allergic volunteers are concerned only the unfractionated MNC and T lymphocytes gave marked proliferative responses to the antigens (Table 19). The MNC-T lymphocytes, B lymphocytes and the Null lymphocytes gave insignificant blastogenic responses in the presence of the antigens (Table 19). The MNC-B lymphocytes and the MNC-Null lymphocytes gave marked blastogenic responses. The blastogenic responses of the MNC-B lymphocytes were consistently greater than those given by the MNC-Null lymphocytes. Not surprisingly, the lymphocytes of the non-allergic donors did not respond to stimulation with AgE (Table 19).

The MNC and T lymphocytes of the ragweed allergic donors responded vigorously to AgE and to all of the antigens (Table 20). As with the cells of the non-allergic individuals neither the MNC-T lymphocytes, B lymphocytes nor the Null lymphocytes of the ragweed allergic donors gave marked blastogenic responses in the

presence of the antigens and the allergen (Table 20). Furthermore the blastogenic responses of the MNC-B lymphocytes were consistently greater than those given by the MNC-Null lymphocytes.

Discussion

The circulating T lymphocytes (non-allergic and allergic) are the sole circulating lymphocytes which respond with blastogenesis and mitosis to antigen and allergen stimulation (in the presence of autologous monocytes) following 6 days in culture since neither the B nor the Null lymphocytes respond to antigen stimulation (under these culture conditions). As expected, only the circulating T lymphocytes (in the presence of autologous monocytes) of ragweed allergic individuals undergo blastogenesis upon the allergen stimulation following 6 days in culture.

The antigen and allergen-induced blastogenic responses generated by the MNC-B lymphocytes were consistently greater than those given by the MNC-Null lymphocytes. An explanation for this finding is provided by the experiments in Chapter 4.10. which demonstrated that monocytes are essential participants in the T

lymphocyte blastogenic response. Since monocytes as well as Null lymphocytes possess FcG receptors, the MNC-Null lymphocytes would be depleted of both monocytes and Null lymphocytes following the removal of the Null lymphocytes from the MNC by rosetting with EAG. Therefore, the remaining MNC-Null lymphocytes will be deficient in monocytes following rosetting with EAG and not respond well to antigenic stimulation. On the other hand, the MNC-B lymphocytes will retain the monocytes and respond well to antigen stimulation. These results are similar to those obtained in Chapter 4.3. where it was demonstrated that phytomitogen-induced blastogenic responses generated by the MNC-B lymphocytes were consistently greater than those given by the MNC-Null lymphocytes.

It may be therefore concluded that, within the terms of reference of the culture conditions, only the T lymphocytes undergo blastogenesis and mitosis following 6 days in culture with the antigens (TT, DT, CA and PPD), and allergen (AgE) in the case of the MNC from allergic individuals providing monocytes are present in the cultures. The B and Null lymphocytes do not respond to antigen and allergen stimulation either individually or if cocultured for 6 days.

Nevertheless, a major concern is that circuating T lymphocytes and not circulating B lymphocytes are the sole circulating lymphocytes which respond to antigen and allergen stimulation providing a minimal number of monocytes are present in the cultures. This consistently observed result is rather surprising since antigens like TT and DT are presumed to induce an antibody and not a cell-mediated response. Similarly, allergens are known to induce an IgE antibody response and not a cell-mediated response. Thus, one might anticipate that circulating B (memory) lymphocytes would response and not the circulating T lymphocytes. This question is addressed in depth in the General Discussion (Chapter 5).

TABLE 19

THE T RESPONDER CELL IN THE ANTIGEN-INDUCED BLASTOGENIC RESPONSES OF NON-ALLERGIC DONORS

Cells of donor	Control	PHA	%R	TT	%R	DT	%R	CA	%R	PPD	%R	AgE	%R
donor 1		cpm		cpm		cpm		cpm		cpm		cpm	
MNC(a)	248	153712	100	76519	100	60948	100	78112	100	69880	100	1587	100
T	312	124543	81	65398	85	54571	90	63307	81	67445	97	1124	71
B	348	2469	0	1139	0	1224	0	1884	0	1930	0	1191	1
NULL	321	1732	1	659	1	621	1	744	1	390	1	305	19
MNC-T	128	682	0	398	1	548	1	498	1	894	1	279	18
MNC-B	214	104268	68	58842	77	46399	76	31517	40	41269	59	1001	63
MNC-NULL	581	92930	60	31718	41	44926	74	26320	34	28948	41	1469	93
donor 2													
MNC	277	140345	100	68127	100	68568	100	72729	100	77536	100	2196	100
T	419	120378	86	54911	81	60887	89	65122	90	70487	91	1445	66
B	546	1536	0	964	0	1193	0	1421	0	1636	0	1101	1
NULL	290	1782	1	918	1	955	1	901	1	814	1	621	28
MNC-T	218	289	1	864	1	698	1	491	1	488	1	684	31
MNC-B	692	112461	80	42114	62	37729	55	31880	44	51877	67	1168	53
MNC-NULL	549	79799	57	33157	49	24437	36	22729	31	47590	61	1830	83
donor 3													
MNC	695	161543	100	77025	100	67271	100	69193	100	70055	100	2371	100
T	248	148645	92	60115	78	63909	95	62421	90	65919	94	1659	70
B	659	1546	1	1881	1	1241	1	1913	1	1136	1	1050	1
NULL	268	1991	1	659	1	648	1	797	1	702	1	271	11
MNC-T	349	734	1	561	1	750	1	380	1	499	1	512	22
MNC-B	436	141274	87	41966	54	51159	76	41321	59	41209	59	1119	47
MNC-NULL	690	112547	70	37926	49	40711	61	30136	43	30364	43	2099	89

a defined as in Table 1

* The cells were cultured with PHA for 5 days and with the antigens/allergen for 6 days

TABLE 20

THE T RESPONDER CELL IN THE ANTIGEN AND ALLERGEN-INDUCED BLASTOGENIC RESPONSES OF RAGWEED ALLERGIC DONORS

Control		PHA	%R	TT	%R	DT	%R	CA	%R	PPD	%R	AgE	%R
donor 1	cpm	cpm		cpm		cpm		cpm		cpm		cpm	
MNC(a)	209	89960	100	60101	100	49174	100	38804	100	60495	100	51401	100
T	418	77525	86	56438	94	47210	96	35277	91	55800	92	48244	94
B	348	1883	2	1839	3	1101	2	1609	4	148	0	905	2
NULL	404	1570	2	487	1	649	1	858	2	498	1	499	1
MNC-T	661	412	0	815	1	713	1	609	2	641	1	290	1
MNC-B	429	85821	95	31827	53	31524	64	31294	81	40732	67	30283	59
MNC-NULL	581	78158	87	41778	70	20236	41	22684	58	25739	43	14909	29
donor 2													
MNC	494	81497	100	55986	100	53643	100	61584	100	66954	100	57519	100
T	390	72988	90	51587	92	49699	93	55536	90	60104	90	54613	95
B	789	1788	2	1642	3	1189	2	1212	2	1359	2	969	2
NULL	226	1658	2	864	2	793	1	885	1	1271	2	587	1
MNC-T	198	861	1	801	1	721	1	698	1	540	1	812	1
MNC-B	218	72883	89	42216	75	31269	58	44282	72	31786	47	46113	80
MNC-NULL	299	65522	80	11308	20	26104	49	37594	61	26964	40	37001	64
donor 3													
MNC	695	91518	100	64315	100	63445	100	66437	100	65674	100	59442	100
T	248	82150	90	60019	93	54732	86	62408	94	61746	94	55189	93
B	659	1494	2	1621	3	1274	2	1390	2	1209	2	1455	2
NULL	268	1920	2	359	1	449	1	887	1	642	1	498	1
MNC-T	349	826	1	621	1	322	1	698	1	531	1	286	0
MNC-B	436	73621	80	44895	70	39302	62	46972	71	31866	49	41790	70
MNC-NULL	698	62334	68	24538	38	20356	32	10401	16	18672	28	23699	40

a defined as in Table 2

* The cells were cultured with PHA for 5 days and with the antigens/allergen for 6 days

4.10. THE DEMONSTRATION OF MONOCYTES AS OBLIGATORY ACCESSORY CELLS IN THE 6 DAY ANTIGEN AND ALLERGEN-INDUCED BLASTOGENIC RESPONSES

Objective

The objective of the following experiments was to define the role(s), if any, of the monocyte in the antigen and allergen-induced blastogenic responses.

Protocol

The subjects used in this part of the investigation were defined in Chapter 4.8. The MNC were obtained from the interphase of Ficoll-Hypaque separated whole blood as described in Chapter 3.2.8. The T lymphocytes were obtained by rosetting the MNC with SRBC as described in Chapter 3.2.12. The MNC and T lymphocytes were depleted of monocytes, once and twice over a period of 24 hrs, as described in Chapter 3.2.10. The method for isolating pure monocytes is described in Chapter 3.2.11. The T-MO_2 cells were cultured with varying numbers of pure (>99%) autologous and allogeneic monocytes so as to establish a concentration gradient of monocytes from 1% to 50% of the cultured cells. The lymphocytes from normal non-allergic and allergic individuals were assayed

for their blastogenic responsiveness to stimulation with the antigens TT, DT, PPD and CA and the allergen AgE. The cells were cultured and the blastogenic responses were determined as described in Chapter 3.2.25.

Results

As can be seen in Tables 21 and 22, the unfractionated MNC and the T lymphocytes from non-allergic and allergic individuals responded well to antigen stimulation. Neither the cultured pure monocytes nor the T-MO_2 cells responded with significant blastogenesis to antigen stimulation; however the addition of autologous monocytes to cultures of T-MO_2 cells (final monocyte concentration 15%) restored the antigen-induced blastogenic responses of the T lymphocytes (Table 21). These responses equalled or exceeded the blastogenic responses of the MNC or the T lymphocytes not depleted of monocytes. As demonstrated in Table 22 the unfractionated MNC and the T lymphocytes, but not the T-MO_2 cells from ragweed allergic individuals responded significantly to AgE stimulation.

Autologous monocytes in as low a concentration as 1% of the cultured cells were capable of imparting to the

$T\text{-}MO_2$ cells the capacity to give highly significant albeit low blastogenic responses to antigen stimulation. However, the addition of autologous monocytes to T-MO2 cells to a final cell concentration of 15% of the cultured cells totally restored the blastogenic responsiveness to the T lymphocytes to antigen stimulation (Table 23). It would therefore appear that minimal numbers of "contaminating" monocytes are effective in facilitating the T lymphocyte antigen-induced blastogenic response.

Autologous monocytes did not suppress the antigen-induced blastogenic response even when added to $T\text{-}MO_2$ cells in large numbers . In the presence of an equal number of monocytes (50% of the cultured cells), the $T\text{-}MO_2$ cells gave blastogenic responses which were equal or greater than those given by the unfractionated MNC and the T lymphocytes (Table 23).

Allogeneic monocytes (irrespective of their numbers) added to the cultures of the $T\text{-}MO_2$ cells failed to facilitate significant blastogenic responses by the T-MO2 cells to antigen stimulation although they invariably reconstituted the blastogenic responsiveness of the $T\text{-}MO_2$ cells to PHA stimulation (Table 24).

Discussion

The T lymphocytes respond with blastogenesis and mitosis to antigen and allergen stimulation in vitro providing a minimal number of autologous monocytes are present in the cultures. The elimination of monocytes on two separate occasions over a 24 hour period did not have a deleterious effect on the responder T lymphocytes since they responded optimally when reconstituted with pure autologous monocytes, which, by themselves, were incapable of responding to antigen and allergen stimulation. Furthermore, excessive numbers of monocytes have a stimulatory, and not a suppressive, effect in the antigen and allergen-induced blastogenic responses.

It is important to note that only the addition of autologous monocytes to cultures of $T\text{-}MO_2$ cells can effectively restore the blastogenic responsiveness of the T lymphocytes in the antigen-induced blastogenic response. The addition of allogeneic monocytes failed to restore the antigen-induced blastogenic responsiveness to the $T\text{-}MO_2$ cells although they could very effectively restore the phytomitogen-induced blastogenic response.

It may be concluded that autologous monocytes are required in small numbers in the MNC or the T lymphocyte

cultures to facilitate optimal blastogenic responses to antigen and allergen stimulation. Allogeneic monocytes (irrespective of their numbers) added to cultures containing T-MO_2 cells do not restore the blastogenic responses to antigen and allergen stimulation following 6 days in culture.

TABLE 21

THE ROLE OF MONOCYTES IN THE ANTIGEN-INDUCED BLASTOGENIC RESPONSE OF NON-ALLERGIC DONORS

Cells of donor	Control	PHA	%R	TT	%R	DT	%R	CA	%R	PPD	%R	AgE	%R
donor 1	cpm	cpm		cpm		cpm		cpm		cpm		cpm	
MNC(a)	387	89972	100	50480	100	37642	100	26085	100	39568	100	204	100
T	316	72690	81	45218	90	30101	80	20218	78	30669	78	298	146
T-MO2	769	1835	2	1516	3	1219	3	1342	5	1121	3	716	351
MO	126	324	0	298	1	390	1	429	2	618	2	121	59
T-MO2+MO	492	95891	107	46319	92	35210	94	26698	102	36618	93	392	192
donor 2													
MNC	942	92650	100	39568	100	26085	100	33462	100	35296	100	764	100
T	404	76292	82	30419	77	19294	74	28618	86	29910	85	297	39
T-MO2	394	2547	3	2932	7	1912	7	1724	5	2148	6	692	91
MO	198	636	1	518	1	476	2	397	1	258	1	218	29
T-MO2+MO	619	100913	109	36214	92	24298	93	27914	83	30018	85	399	52
donor 3													
MNC	468	82352	100	55298	100	42523	100	13751	100	42385	100	246	100
T	210	62698	76	46212	84	36088	85	10216	74	39180	92	399	162
T-MO2	316	2852	3	2241	4	1929	5	2187	16	2441	6	426	173
MO	291	628	1	710	1	214	1	319	2	201	0	110	45
T-MO2+MO	497	95415	116	51654	93	39910	94	12692	92	40912	97	432	176

a defined as in Table 2

* The cells were cultured with PHA for 5 days and with the antigens/allergen for 6 days

TABLE 22

THE ROLE OF MONOCYTES IN THE ANTIGEN AND ALLERGEN-INDUCED BLASTOGENIC RESPONSES OF RAGWEED ALLERGIC DONORS

Cells of donor	Control	PHA	%R	TT	%R	DT	%R	CA	%R	PPD	%R	AgE	%R
donor 1	cpm	cpm		cpm		cpm		cpm		cpm		cpm	
MNC(a)	183	91112	100	46253	100	41084	100	14425	100	17215	100	39117	100
T	247	89745	99	48766	100	38700	94	11297	78	20970	100	32140	82
T-MO2	430	4527	5	2838	6	1361	3	961	7	1421	8	2157	6
MO	194	549	1	685	1	575	1	738	5	820	5	126	0
T-MO2+MO	485	85380	94	42119	91	40108	98	12275	85	16643	97	45938	117
donor 2													
MNC	761	123466	100	51439	100	57005	100	15480	100	15326	100	42620	100
T	300	97335	79	42331	82	49521	87	10435	67	17977	100	40321	95
T-MO2	876	3679	3	1505	3	1479	3	2739	18	1864	12	3809	9
MO	625	572	0	403	1	117	0	312	2	733	5	116	0
T-MO2+MO	410	104436	85	48373	94	54662	96	15958	103	14245	93	52753	124
donor 3													
MNC	219	97825	100	34436	100	20796	100	39346	100	26683	100	21839	100
T		90652	93	30874	90	19052	92	35874	94	20542	77	16298	75
T-MO2	684	2498	3	1991	6	1271	6	1613	4	1254	5	1286	6
MO	385	100	0	750	2	418	2	352	1	604	2	403	2
T-MO2+MO	909	99791	102	37999	110	18352	88	33750	86	28352	106	24768	113

a defined as in Table 2

* The cells were cultured with PHA for 5 days and with the antigens/allergen for 6 days

TABLE 23

THE ANTIGEN-INDUCED BLASTOGENIC RESPONSES OF T LYMPHOCYTES AS A FUNCTION OF THE PERCENT OF MONOCYTES IN CULTURE

	%MO	Control	PHA	%R	TT	%R	DT	%R	CA	%R	PPD	%R
donor 1		cpm	cpm		cpm		cpm		cpm		cpm	
MNC	20	215	130701	100	46865	100	48572	100	23433	100	26632	100
T-MO2	1	423	4218	3	1353	3	2185	4	1121	5	1618	6
MO	99	210	321	0	842	2	212	0	921	4	684	3
T-MO2+MO	50	879	148865	114	55037	117	50246	103	37765	161	34585	130
T-MO2+MO	20	239	135003	103	43480	93	33003	68	25339	108	28854	108
T-MO2+MO	10	592	107826	82	29543	63	13931	29	11694	50	20001	75
T-MO2+MO	5	489	87738	67	9177	20	8713	18	6378	27	10073	38
T-MO2+MO	1	621	46831	36	2960	6	1146	2	2073	9	2984	11
donor 2												
MNC	22	561	128422	100	46253	100	41049	100	32111	100	40194	100
T-MO2	1	585	3750	3	1229	3	1613	4	1559	5	1624	4
MO	99	384	963	1	814	2	621	2	122	0	367	1
T-MO2+MO	50	924	120038	93	51127	111	45884	112	35210	110	49074	122
T-MO2+MO	20	302	105487	82	49006	106	44167	108	22784	71	36652	91
T-MO2+MO	10	285	75150	59	34881	75	27695	67	21005	65	25816	64
T-MO2+MO	5	551	50475	39	15629	34	12238	30	16733	52	12362	31
T-MO2+MO	1	684	38562	30	5898	13	6495	16	4629	14	6613	16
donor 3												
MNC	24	322	92691	100	70487	100	51022	100	43043	100	67308	100
T-MO2	1	200	1268	1	2198	3	1501	3	1145	3	6281	9
MO	99	195	882	1	923	1	354	1	519	1	800	1
T-MO2+MO	50	889	124892	135	72803	103	58677	115	51884	121	77611	115
T-MO2+MO	20	410	108561	117	65636	93	41253	81	45129	105	66256	98
T-MO2+MO	10	538	83620	90	48692	69	39842	78	22436	52	49811	74
T-MO2+MO	5	118	56471	61	27834	39	24453	48	19887	46	29489	44
T-MO2+MO	1	204	32296	35	12102	17	10571	21	4769	11	18339	27

a defined as in Table 2

* The cells were cultured with PHA for 5 days and with the antigen for 6 days

TABLE 24

RECONSTITUTION OF THE ANTIGEN-INDUCED BLASTOGENIC RESPONSE WITH ALLOGENEIC MONOCYTES OF NON-ALLERGIC DONORS

	Control	PHA	%R	TT	%R	DT	%R	CA	%R	PPD	%R
donor 1	cpm	cpm		cpm		cpm		cpm		cpm	
MNC(a)	368	94425	100	57215	100	52277	100	35439	100	42647	100
TMO2	814	2698	3	1226	2	1932	4	2001	6	1542	4
MO	122	780	1	245	0	368	1	748	2	300	1
donor 2											
MNC	248	102688	100	76253	100	61084	100	42180	100	56321	100
TMO2	404	3621	4	1466	2	1721	3	2112	5	1469	3
MO	113	769	1	542	1	649	1	428	1	672	1
donor 3											
MNC	219	95791	100	32537	100	22821	100	29846	100	50821	100
TMO2	690	2173	2	867	3	1203	5	681	2	1011	2
MO	265	840	1	322	1	571	3	423	1	738	1
MO+TMO2											
1 + 1	619	90218	100	48312	100	40618	100	26332	100	35031	100
2 + 1	440	56382	62	4336	9	1962	5	1420	5	1984	6
3 + 1	204	74257	82	6177	13	4033	10	2174	8	3040	9
2 + 2	222	90218	100	60782	100	51385	100	30986	100	38398	100
1 + 2	401	56382	62	1678	3	957	2	901	3	1124	3
3 + 2	908	74257	82	1063	2	1134	2	1698	5	699	2
3 + 3	348	89963	100	22821	100	19846	100	20265	100	22467	100
1 + 3	318	79963	89	1958	9	2179	11	1077	5	2574	11
2 + 3	216	64028	71	2908	13	1425	7	1980	10	1553	7

a defined as in Table 2

* The cells were cultured with PHA for 5 days and with the antigens for 6 days

4.11. THE BLASTOGENIC RESPONSES OF THE T LYMPHOCYTE SUBCLASSES TO ANTIGEN AND ALLERGEN STIMULATION FOLLOWING 6 DAYS IN CULTURE

Objective

The objective of the following experiments was to assess the blastogenic responsiveness of the T lymphocyte subclasses - T_M, T_G, T_C, T_N, T4+ and T8+ lymphocytes in the 6 day antigen and allergen-induced blastogenic response.

Protocol

The subjects used in this part of the investigation have been defined in Chapter 4.8. The non-allergic and allergic volunteers were bled by venipuncture. The MNC were obtained from the interphase of Ficoll-Hypaque separated whole blood as described in Chapter 3.2.8. The T lymphocytes were obtained by rosetting the MNC with SRBC as described in Chapter 3.2.12. The T lymphocyte subclasses defined on the basis of rosetting with the EAM, EAG and EAC indicator erythrocytes - T_M, T_G, T_C and T_N T lymphocytes, were isolated as described in Chapters 3.2.15., 3.2.16. and 3.2.17., respectively. The T4+ and T8+ T lymphocytes were isolated by lysis of

T8+ lymphocytes and T4+ lymphocytes in the presence of OKT8 and OKT4 monoclonal antiserum and complement, respectively, as described in Chapter 3.2.18.

Monocytes were isolated as described in Chapter 3.2.11. It should be noted that the final concentration of monocytes was adjusted to 10% of the cultured cells to ensure the presence of sufficient numbers of monocytes for optimal blastogenic responses.

The T lymphocyte subclasses of normal non-allergic and allergic individuals were assayed for their blastogenic responses to stimulation with conventional antigens (TT, DT, PPD, CA) and to an allergen AgE. The cells were cultured for 6 days and the blastogenic responses were determined as described in Chapter 3.2.25.

Results

All four T lymphocyte subclasses isolated by rosetting procedures (T_M, T_G, T_C and T_N T lymphocytes), of the non-allergic and allergic individuals gave highly significant blastogenic responses to antigen stimulation (Tables 25, 26). Furthermore, all four of the T lymphocyte subclasses of the allergic individuals responded to allergen-AgE stimulation (Table

26). The consistently best responders were the T_M lymphocytes. The blastogenic responses by the T_G lymphocytes were consistently lower than those generated by the T_M lymphocytes. The T_C and the T_N lymphocytes consistently gave much lower blastogenic responses than the T_G lymphocytes (Tables 25, 26).

The T4+ and the T8+ T lymphocytes generated similar blastogenic responses to stimulation with all the antigens and allergen used (Tables 27, 28).

Discussion

The T_G, T_M, T_N and T_C T lymphocytes from non-allergic and allergic individuals are all capable of generating significant blastogenic responses to antigen stimulation. The T_M lymphocytes consistently gave better responses than did the T_G lymphocytes with all the antigens and allergen tested while the T_C and the T_N lymphocytes consistently gave poorer blastogenic responses. The question why the T_M lymphocytes are the best responders to antigen stimulation is addressed in Chapter 5.

The blastogenic responses generated by the T4+ and T8+ T lymphocytes to antigen stimulation were comparable.

Since the T4+ lymphocytes are presumed to consist of the helper cells, as are the T_M lymphocytes, and the T8+ lymphocytes are presumed to consist of the suppressor cells, as are the T_G lymphocytes, it is difficult to understand why the T4+ and the T8+ lymphocytes responded to the same degree to antigen and allergen stimulation whereas the T_M lymphocytes gave markedly superior blastogenic responses to antigen and allergen stimulation than did the T_G lymphocytes. Obviously, the T lymphocyte subclasses, irrespective of the mode of their isolation are heterogeneous. These results indicate that these two T lymphocyte classes, which are considered to exhibit helper and suppressor activity, respectively, nevertheless cannot be distinquished on the basis of the antigen and allergen-induced blastogenic responses.

It must be emphasized that the monocytes were not the limiting cells in any of the blastogenic responses as all the T lymphocytes subclasses were always cultured with optimal numbers of monocytes.

It may be concluded that lymphocytes in all the T lymphocyte subclasses are capable of responding to varying degrees in the antigen and allergen-induced blastogenic response. The T_M lymphocytes are the best responders.

TABLE 25

THE ANTIGEN-INDUCED BLASTOGENIC RESPONSES OF T CELL SUBCLASSES OF NON-ALLERGIC DONORS

Cells of donor / donor 1	Control	PHA cpm	%R	TT cpm	%R	DT cpm	%R	CA cpm	%R	PPD cpm	%R	AgE cpm
MNC(a)	415	110580	100	68355	100	63015	100	56630	100	62266	100	1298
T(a)	408	106170	96	61956	91	58376	93	48168	85	60250	97	1732
TM(a)*	374	86210	78	40358	59	32639	52	45811	81	57838	93	1989
TG(a)*	410	42428	38	10336	15	14908	24	29560	52	31901	51	1211
TC(a)*	318	13249	12	4362	6	3809	6	5760	10	4936	8	1724
TN(a)*	212	28701	26	3439	5	3615	6	3916	7	4672	8	1181
donor 2												
MNC	515	128462	100	63938	100	68124	100	62976	100	67472	100	2080
T	596	103450	81	60923	95	54175	80	56711	90	59593	88	1838
TM	636	92648	72	32001	50	38213	56	49421	78	48711	72	1432
TG	440	49621	39	11289	18	12436	18	31574	50	10858	16	1118
TC	725	29234	23	4076	6	5014	7	4243	7	6634	10	1249
TN	738	38300	30	3261	5	4508	7	3590	6	5417	8	1628
donor 3												
MNC	489	137991	100	68428	100	67304	100	58702	100	65782	100	1062
T	462	114686	83	56703	83	62948	94	56681	97	62658	95	1059
TM	223	69089	50	35635	52	32619	48	45088	77	54935	84	900
TG	568	31603	23	11920	17	18849	28	24593	42	22494	34	542
TC	122	21499	16	4629	7	3689	5	3505	6	6853	10	841
TN	248	28636	21	2490	4	4417	7	2086	4	6591	10	985

a defined as in Table 6

* all cell preparations were reconstituted with autologous monocytes to a final cell concentration of 10%

** The cells were cultured with PHA for 5 days and antigens/allergen for 6 days

TABLE 26

THE ANTIGEN AND ALLERGEN-INDUCED BLASTOGENIC RESPONSE
OF T CELL SUBCLASSES OF RAGWEED ALLERGIC DONORS

	Control	PHA	%R	TT	%R	DT	%R	Ca	%R	PPD	%R	AgE	%R
donor 1	cpm	cpm		cpm		cpm		cpm		cpm		cpm	
MNC(a)	275	95050	100	49964	100	49546	100	48815	100	51600	100	42402	100
T(a)	347	89592	94	42328	85	41493	84	47540	97	48028	93	40599	96
TM(a)*	426	71143	75	30683	61	37966	77	32838	67	39852	77	37786	89
TG(a)*	673	37120	39	14438	29	12866	26	19808	41	7302	14	10987	26
TC(a)*	400	11914	13	2439	5	3516	7	3369	7	2797	5	2825	7
TN(a)*	541	15662	16	1529	3	1498	3	1852	4	2314	4	1918	5
donor 2													
MNC	618	109974	100	49408	100	51509	100	64383	100	63374	100	44872	100
T	327	100542	91	42251	86	48075	93	60305	94	57649	91	39661	88
TM	514	80943	74	29831	60	31742	62	34995	54	38021	60	35903	80
TG	492	44864	41	10487	21	14349	28	19404	30	14101	22	12281	27
TC	218	27734	25	2824	6	2305	4	2839	4	3215	5	3609	8
TN	320	25662	23	2191	4	2714	5	3018	5	2498	4	1319	3
donor 3													
MNC	671	101470	100	42609	100	41404	100	59429	100	61332	100	55398	100
T	239	82543	81	39571	93	38519	93	55902	94	56011	91	53307	96
TM	399	75662	75	30112	71	36310	88	48845	82	44803	73	48244	87
TG	242	39696	39	19404	46	10547	25	26461	45	29846	49	8050	15
TC	428	30963	31	2189	5	3087	7	1359	2	2981	5	2981	5
TN	514	29888	29	1642	4	2988	7	1433	2	4606	8	2004	4

a defined as in Table 6
* all cell preparations were reconstituted with autologous monocytes to a final cell concentration of 10%
** The cells were cultured with PHA for 5 days and antigens/allergen for 6 days

TABLE 27

THE ANTIGEN-INDUCED BLASTOGENIC RESPONSES OF T, T4+ AND T8+ LYMPHOCYTES OF NON-ALLERGIC DONORS

Cells of donor	Control	PHA	%R	TT	%R	DT	%R	CA	%R	PPD	%R
donor 1	cpm	cpm		cpm		cpm		cpm		cpm	
MNC(a)	468	101357	100	76340	100	33180	100	23920	100	64243	100
T(a)	321	99572	98	54183	71	34183	103	20301	85	61024	95
T4+(a)*	901	79349	78	33180	43	28920	87	18381	77	53784	84
T8+(a)*	686	78631	78	31892	42	28475	86	15750	66	52285	81
donor 2											
MNC	521	96224	100	55560	100	53847	100	29390	100	41020	100
T	300	90315	94	51030	92	50666	94	20952	71	37861	92
T4+	483	66238	69	41132	74	34123	63	21977	75	30531	74
T8+	911	65396	68	47286	85	31455	58	21367	73	32174	78
donor 3											
MNC	681	99763	100	51525	100	42320	100	37308	100	52839	100
T	386	81619	82	49883	97	40905	97	31045	83	47894	91
T4+	291	60318	60	40475	79	34464	81	28875	77	46209	87
T8+	604	56790	57	39309	76	32760	77	29546	79	48920	93

a as difined in Table 7

* all T cell preparations were reconstituted with autologous monocytes to a final concentration of 10%

** The cells were cultured with PHA for 5 days and with the antigens for 6 days

4.12. THE CAPACITY OF SUPERNATANTS OF PHA AND TT-STIMULATED MONOCYTES (IL-1) TO RESTORE THE 6 DAY ANTIGEN-INDUCED BLASTOGENIC RESPONSES TO THE T-MO_2 LYMPHOCYTES

Objective

The objective of the following series of experiments was to determine the capacity of autologous monocytes and the supernatants from PHA or TT-stimulated autologous and allogeneic monocyte cultures to restore the blastogenic responsiveness of T-MO_2 cells to antigen stimulation.

Protocol

The subjects used in this part of the investigation have been defined in Chapter 4.8. The MNC were obtained from the interphase of Ficoll-Hypaque separated whole blood as described in Chapter 3.2.8. The T lymphocytes were obtained by rosetting the MNC with SRBC as described in Chapter 3.2.12. The T lymphocytes were depleted of monocytes twice as described in Chapter 3.2.10. Monocytes were obtained as described in Chapter 3.2.11. and were incubated in the presence of PHA or TT for 48 hours as described in Chapter 3.2.13.

The T-MO_2 cells were reconstituted with autologous monocytes (final concentration 1%) and/or cell free monocyte culture supernatants to ascertain the capacity of the supernatants to facilitate the blastogenic response by the T lymphocytes. The T lymphocytes were cultured in the presence of the antigens TT, DT, PPD and CA for 6 days and the blastogenic response was determined as described in Chapter 3.2.25.

Results

Supernatants from 48 hr PHA-stimulated and TT-stimulated cultures of allogeneic monocytes were compared for their ability to enhance the blastogenic responsiveness of the T-MO_2 cells. Supernatants obtained from 48 hr PHA-stimulated allogeneic monocyte cultures (MSP) restored the blastogenic responsiveness of the T-MO_2 cells to PHA stimulation but failed to restore the blastogenic responsiveness to antigenic stimulation to any significant degree (Table 28). Similarly, supernatants obtained from 48 hr TT-stimulated cultures of allogeneic monocytes (MST) restored the blastogenic responsiveness of T-MO_2 cells to PHA stimulation but failed to restore the blastogenic responsiveness to antigenic stimulation (Table 28). The MSP and MST restored the blastogenic responsiveness of

the T-MO_2 cells to antigen stimulation only when threshold concentration of autologous monocytes (final monocyte concentration of 1%). were added to the T lymphocyte cultures.

Discussion

Autologous monocytes have been shown to be obligatory participants in the antigen and allergen-induced blastogenic responses. Supernatants from 48 hour PHA-stimulated or TT-stimulated autologous and allogeneic monocyte cultures were effective in restoring the blastogenic responsiveness to the T lymphocytes only in the presence of threshold numbers of autologous monocytes in the antigen-induced blastogenic response. It is therefore necessary to assume that the blastogenic responses of the T lymphocytes to antigenic stimulation require direct contact with monocytes. These results should be contrasted with those obtained in Tables 10-12 which demonstrated that monocyte culture supernatants were effective in restoring the T lymphocyte blastogenic response to phytomitogen stimulation in the absence of intact monocytes. This matter is further elaborated on in the General Discussion (Chapter 5.).

TABLE 28

THE CAPACITY OF SUPERNATANTS OF PHA-STIMULATED AND TT-STIMULATED 48 HR CULTURED AUTOLOGOUS MONOCYTES TO RESTORE BLASTOGENIC RESPONSIVENESS TO T-MO2 CELLS

donor 1	Control	PHA	%R	TT	%R	DT	%R	CA	%R	PPD	%R
MNC	384	98364	100	48591	100	42743	100	16864	100	25875	100
T-MO2	368	7373	7	1480	3	1760	4	1843	11	1937	7
MO	284	486	0	207	0	136	0	177	1	137	1
T-MO2+MSP(a)	854	56221	57	3432	7	4268	10	3980	24	4011	16
T-MO2+MO+MSP(b)	672	89740	91	34070	70	23633	55	10199	60	19691	76
T-MO2+MST(c)	649	25263	25	4342	9	3326	8	2661	16	1278	5
T-MO2+MO+MST(c)	546	82183	84	21743	45	11869	28	6545	39	18740	72
T-MO2+MO(d)	411	97753	99	45365	93	49719	116	23679	140	43679	169
donor 2											
MNC	426	101837	100	40894	100	41159	100	26945	100	40702	100
T-MO2	839	9871	10	1220	3	1379	3	1360	5	1168	3
MO	604	263	0	147	0	386	1	405	2	200	0
T-MO2+MSP	116	64218	63	4709	12	3166	8	4211	16	1962	5
T-MO2+MO+MSP	752	95739	94	26216	64	29293	71	11067	41	12101	30
T-MO2+MST	283	31020	30	1515	4	3568	9	2168	8	4218	10
T-MO2+MO+MST	552	22734	22	17780	43	15906	39	13322	49	16884	41
T-MO2+MO	381	92874	91	39000	95	39838	97	29811	111	37492	92
donor 3											
MNC	891	100726	100	50626	100	48922	100	22029	100	56160	100
T-MO2	432	5283	5	1021	2	1269	3	773	4	2102	4
MO	694	1002	1	149	0	455	1	428	2	125	0
T-MO2+MSP	819	67298	67	2946	6	3128	6	1190	5	3842	7
T-MO2+MO+MSP	308	106728	106	25803	51	24279	50	13981	63	38872	69
T-MO2+MST	345	25957	26	1927	4	1661	3	1834	8	821	1
T-MO2+MO+MST	219	24736	25	16292	32	17144	35	13255	60	16730	30
T+MO	800	97391	97	44279	87	40279	82	21622	98	49387	88

a T-MO2 cells cultured in MSP

b T-MO2 cells reconstituted with autologous MO (final conc 10%) and MSP

c T-MO cells reconstituted with autologous MO (final conc 10%) and MST

d T-MO cells reconstituted with autologous MO (final conc 15%)

4.13. THE CAPACITY OF IL-1, IL-2 AND BCGF TO FACILITATE THE BLASTOGENIC RESPONSIVENESS OF THE T, B AND NULL LYMPHOCYTES TO ANTIGEN STIMULATION FOLLOWING 6 DAYS IN CULTURE

Objective

The objective of the following experiments was to determine the blastogenic responsiveness of MNC, T, B and Null lymphocytes to antigen stimulation in the presence of IL-1, IL-2 and BCGF following 6 days in culture.

Protocol

The subjects used in this part of the investigation have been defined in Chapter 4.8. The MNC and monocytes were isolated as described in Chapters 3.2.8. and 3.2.11., respectively. The T, B and Null lymphocytes were positively isolated as described in Chapters 3.2.12., 3.2.19. and 3.2.20., respectively. T lymphocytes were depleted of monocytes twice as described in Chapter 3.2.10. Supernatants from 48 hr PHA-stimulated monocyte cultures were prepared as described in Chapter 3.2.13. and is referred to as IL-1.

The antigens (TT, DT, PPD and CA) were added with or without the Interlukins (IL-1, IL-2 or BCGF) to the lymphocytes at the initiation of culture to ascertain the capacity of the Interlukins to facilitate the blastogenic response of the T, B and Null lymphocytes to antigen stimulation (TT, DT, PPD and CA) following 6 days in culture. The degree of blastogenesis was determined as described in Chapter 3.2.25.

Results

The T-MO_2 cells, in the absence of monocytes, did not generate a significant blastogenic response to antigen stimulation following 6 days in culture (Table 29). The addition of IL-1, IL-2 or BCGF did not exert any additional stimulatory effect. However, the addition of threshold numbers of autologous monocytes (final monocyte concentration 1%) to the T-MO_2 cells cultured in the presence of IL-1, IL-2 or BCGF, now gave significant blastogenic responses to antigen stimulation following 6 days in culture. Neither IL-1, IL-2 nor BCGF in the absence of antigens were capable of inducing blastogenesis of the cells (Table 29).

Neither the B nor the Null lymphocytes, in the presence of monocytes and in the presence of IL-1, IL-2

or BCGF were capable of generating a blastogenic response to antigen stimulation following 6 days in culture (Table 29).

Discussion

As was discussed in Chapter 2.2.2., IL-1 is secreted by monocytes whereas IL-2 and BCGF are secreted by T lymphocytes in the presence of monocytes or IL-1. Hence, IL-2 will not be secreted in the absence of monocytes or preformed IL-1. IL-2 is required for the long term blastogenic response of T lymphocytes.

The absence of a blastogenic response by the B and Null lymphocytes in the presence of the antigen may therefore be attributed to the absence of IL-2 or IL-2 secreting T lymphocytes. However, since neither the B nor the Null lymphocytes generated blastogenic responses in the presence of antigen and IL-2 or BCGF, it is obvious that the cells do not, in fact, possess the capacity to respond under the culture conditions utilized in this investigation.

It must be noted that neither IL-1 not IL-2 (nor BCGF) confered on T lymphocytes the capacity to undergo blastogenesis to antigen stimulation in the absence of

autologous monocytes.

The Interleukins, IL-1, IL-2 or BCGF facilitate the blastogenic responsiveness of T lymphocytes to antigen stimulation only in the presence of autologous monocytes following 6 days in culture. The requirement of the T lymphocytes to undergo blastogenesis in vitro to antigen stimulation and phytomitogen stimulation are quite different. The T lymphocytes do not require monocytes in the phytomitogen-induced blastogenic response provided monocyte culture supernatants (or IL-2) is added to the cell cultures. Furthermore, the IL-1 may be derived from autologous or allogeneic monocytes. On the other hand, the T lymphocytes undergo blastogenesis in vitro to antigen stimulation providing autologous, not allogeneic, monocytes are present. IL-1, IL-2 or BCGF cannot facilitate the blastogenic responses of the T lymphocytes to antigen stimulation in the absence of autologous monocytes present in threshold concentration.

TABLE 29

THE CAPACITY OF IL-1, IL-2 AND BCGF TO FACILITATE THE BLASTOGENIC RESPONSIVENESS OF T, B AND NULL LYMPHOCYTES TO ANTIGEN STIMULATION

	Control	PHA	%R	TT	%R	DT	%R	CA	%R	PPD	%R
donor 1	cpm	cpm		cpm		cpm		cpm		cpm	
MNC(a)	328	99276	100	57650	100	39815	100	29755	100	38103	100
T-MO2	241	4003	4	1154	2	1252	3	1254	5	1056	3
T-MO2+IL-1	128	1483	1	716	1	890	1	871	1	934	1
T-MO2+IL-2	354	6631	7	948	1	849	1	932	1	773	1
T-MO2+BCGF	223	5341	5	673	1	785	1	883	1	835	1
T+MO	354	85205	86	48392	84	25169	63	21048	71	32690	86
T+IL-1	487	100394	100	50938	88	27384	70	29880	100	34215	90
T+IL-2	642	106891	100	52334	91	29931	75	31249	100	40031	100
T+BCGF	485	104839	100	52001	90	31283	79	31983	100	42309	100
B+MO	374	485	1	615	1	937	1	444	1	385	1
B+IL-1	615	837	1	273	1	715	1	216	1	641	1
B+IL-2	376	716	1	491	1	514	1	537	1	356	1
B+BCGF	287	948	1	337	1	312	1	447	1	552	1
NULL+MO	100	391	1	416	1	446	1	846	1	631	1
NULL+IL-1	298	387	1	513	1	642	1	437	1	423	1
NULL+IL-2	472	188	1	225	1	735	1	539	1	635	1
NULL+BCGF	306	163	1	748	1	221	1	530	1	751	1
donor 2											
MNC	300	87602	100	58411	100	50910	100	39142	100	41404	100
T-MO2	619	3682	4	1346	2	1820	4	1542	4	1921	5
T-MO2+IL-1	116	1662	2	1153	2	1742	4	1546	4	1945	5
T-MO2+IL-2	452	7452	9	1042	2	1342	3	1341	3	853	2
T-MO2+BCGF	535	6450	7	856	2	993	2	638	2	937	2
T+MO	410	99698	100	50913	87	42871	84	26909	69	38730	94
T+IL-1	546	101564	100	51324	88	44978	88	28538	73	40577	98
T+IL-2	532	107565	100	54325	93	47682	94	29715	76	45288	100
T+BCGF	690	109331		53421	91	47591	94	30470	78	44126	100
B+MO	416	885	1	414	1	736	1	624	1	924	1
B+IL-1	205	387	1	264	1	837	1	441	1	813	1
B+IL-2	599	524	1	371	1	213	1	536	1	531	1
B+BCGF	316	537	1	425	1	537	1	576	1	590	1
NULL+MO	429	824	1	374	1	784	1	390	1	513	1
NULL+IL-1	461	926	1	300	1	567	1	571	1	638	1
NULL+IL-2	259	311	1	491	1	835	1	962	1	833	1
NULL+BCGF	552	253	1	847	1	400	1	614	1	557	1

a defined as in Table 17

* The cells were cultured with PHA for 5 days and with the antigens for 6 days

4.14. STATISTICAL ANALYSIS OF DATA

The raw counts per minute, mean and standard deviation were obtained for the stimulated and the control cultures, for each experimental protocol and representative data is presented. The data was derived from 52 individuals for the phytomitogen-induced series of experiments and from 32 individuals for the antigen and allergen-induced series of experiments so as to perform statistical analysis to delineate the relationships between the blastogenic responses generated by the MNC and those of the populations obtained following fractionation.

The various statistical procedures employed were: coefficients of variance (CV=SD/X*100 where SD is standard deviation and X is the mean) and paired T-tests. The statistical computations were performed by computer. Transformed means and square root transformed means were compared by one way and two way analysis of variance (ANOVA) and the variance ratios (F) and the probability (P) values were calculated. A linear regression model was designed to fit the data and the significance of the various cell populations were evaluated. The square root transformation of data did not change the fundamental statistical relationships derived from ANOVA of raw data.

The conclusions derived in this thesis were based on the statistically analyzed data.

TABLE 30

THE STATISTICAL ANALYSIS OF THE PHYTOMITOGEN-INDUCED BLASTOGENIC RESPONSES

	CONTROL			PHA			PWM			CON-A		
	MEAN	SD	CV	MEAN	SD	CV	MEAN	SD	CV	MEAN	SD	CV
MNC	666.3	324.2	48	109766.5	23533.1	21	51203.4	17153.6	33	93492.7	29659.4	31
T	489.9	247.5	50	86680.1	20471.3	23	35643.8	16430.4	46	78896.9	16298.1	20
B	445.5	266.1	59	1282.4	548.9	42	1512.7	867.6	57	2215.8	875.1	39
NULL	385.3	146.3	37	1463.6	522.9	35	1393.2	588.6	42	2051.8	765.6	37
MO	170.6	62.4	36	934.3	592.6	63	7544.6	576.6	7.6	641.5	564.4	87
T-MO2	525.5	266.9	50	3501.2	1927.5	55	2033.7	575.1	28	2930.9	2273.9	77
T-MO2+MO	375	188.4	50	96177.2	26498.1	27	33327.6	20411.9	61	84679.9	27536.9	32
T-MO2+MS	595.7	333.5	55	67597.3	17102.1	25	47327.4	13650.9	28	59802.8	14155.1	23
MNC-NULL	385.1	146.3	37	58050.7	25129.5	43	56294.8	11849.4	21	41884.8	13964.6	33
MNC-B	344.3	162.9	47	79273.8	15983.2	20	61459.5	14372.8	23	62982.2	15141.3	24
TM	393.3	309.1	78	80232.9	8863.3	11	48501.6	23335.1	48	79214.8	29057.8	36
TG	364.6	201.5	55	41987.4	5869.6	13	20832.4	16122.8	77	39789.4	12056.3	30
TC	295.7	176.9	59	25780.7	8426.3	32	6446.4	12161.8	188	30466.5	10983.4	36
TN	345.7	343.1	99	29137.2	7437.8	25	7989.3	5326.1	66	22482.9	10517.4	46
T4+	391	245.4	62	68264.1	13802.9	20	31760.1	3768.4	11	69276.7	12771.2	18
T8+	349.3	196.1	56	66473	11839	17	32748.4	2256	10	68475.2	11827.3	15

TABLE 31

STATISTICAL ANALYSIS OF THE ANTIGEN-INDUCED BLASTOGENIC RESPONSES

	TT			DT			CA			PPD			AgE		
	MEAN	SD	CV	MEAN	SD	CV	MEAN	SD	CV	MEAN	SD	CV	MEAN	SD	CV
MNC	49406	13902	28	52843	18657	35	50366	15980	31	56105	18025	32	41041	10932	26
T	48231	8887	18	50142	20304	52	49055	13436	27	54108	14054	25	37054	12240	33
B	1283	383.3	29	1257	657.5	91	1361	266.1	19	1192	583.6	48	1109	300.7	27
NULL	772.3	465.6	60	674.9	617.9	35	846.3	147.3	17	718.8	309.5	43	528.1	51.1	9.6
MO	594.9	407	68	658.1	234.8	41	511.7	203.9	39	605.4	184.1	30	215.4	162.9	75
T-MO2	1647	375	22	1479	616,4	42	1434	545.6	38	1547	2703	174	2417	1281	53
T-MO2+MO	48203	14921	30	41786	17564	34	38017	14050	36	45717	19201	41	41153	14593	35
T-MO2+MS	3326	2105	63	2749	952.7	42	1914	1091	57	1983	1061	53			
T-MO2+MO+MS	20759	11197	53	24759	10593	12	26344	13113	49	19552	6811	34			
MNC-NULL	8345	1345	16	11245	1357	12	13256	1456	10	13254	1435	10	25203	11122	44
MNC-B	35467	12346	34	33546	11324	33	43226	13456	31	44237	13248	29	39395	8182	20
TM	30208	321.2	1	34917	2314	47	45784	2920	6.3	44989	11865	26	40644	6648	16
TG	8109	1487	18	7430	3511	27	28586	3284	11	1934	10234	529	10439	2168	20
TC	2483	279.4	11	3584	977.2	3.4	3198	1139	35	4415	1691	38	3438	915.4	29
TN	1887	434.6	23	32992	1150	3.4	4505	900.5	19	3948	2319	58	1171	415.9	35
T4+	38262	4413	11	32502	3107	9.5	23077	5332	23	43508	11869	27			
T8+	39495	7698	19	30897	2196	7.1	22246	6991	31	44446	10771	24			

5. GENERAL DISCUSSION

The in vitro blastogenic responses of circulating mononuclear cells stimulated with polyclonal (non-specific) the phytomitogens (PHA, PWM and Con-A) and the specific antigens (TT, DT, PPD and CA) have been recognized as indicators of potential and actual immunocompetence, respectively, for the past 15 years (Chapter 2.3.2.). The antigens stimulate those lymphocytes which have genetically predetermined antigen receptors and the circulating memory lymphocytes (those lymphocytes which are the differentiated progeny of previously generated antibody-forming cells, the AFC) to undergo blastogenesis in vitro (Pike and Nossel 1984, Bradley-Mullen 1982). These committed immunocompetent lymphocytes constitute only a very small percentage of the circulating lymphocytes (probably less than 1%) (Geha 1981). On the other hand, the phytomitogens stimulate the majority of the T lymphocytes in an apparently indiscriminate, random manner to undergo blastogenic transformation in vitro (reviewed by Lis and Sharon 1981). The T lymphocytes constitute a very heterogeneous class of cells consisting of immunocompetent T lymphocytes and T lymphocytes with functions other than

immunologic ones. The former consist of helper cells, suppressor cells, naturally occuring and antigen-induced cytotoxic cells, antigen-receptor bearing cells (ARC) and sensitized (delayed hypersensitivity) cells (Moretta 1983, Richter 1982, Reinherz et al 1980).

It has been unequivocally demonstrated that the lymphocytes which respond in the three day phytomitogen-induced blastogenic response are T lymphocytes (Geha and Merler 1974, Dosch et al 1980, Moretta 1976). However, it has not been demonstrated whether all subclasses of T lymphocytes respond or whether it is a particular subclass of T lymphocytes which responds. Furthermore, the extent to which the circulating B and Null lymphocytes participate as accessory cells in this response is controversial (Chapter 2.3.5). There is also uncertainty as to the role of the monocyte in this T lymphocyte blastogenic response. The evidence so far presented has been overwhelmingly in favor of a monocyte function; however the minority of reports which have repudiated a role for the monocyte in the phytomitogen-induced blastogenic response necessitates a systematic evaluation of the role of the monocyte (Lohrmann et al 1974, Hedfors et al 1974, Schmidtke and Hatfield 1976).

The circulating T lymphocytes of normal adults have also been shown to respond with blastogenesis to antigenic stimulation in vitro (Geha 1973, Chess et al 1974). However, the roles of the other circulating cells - the B and Null lymphocytes and the monocytes, have not been established in the antigen-induced blastogenic response. The evidence that, they participate in this response is conflicting (Caraux 1982). In blastogenic response to in the assessment of immunocompetence, it is vital that the identity of the circulating cells which respond with blastogenesis to antigen stimulation be unequivocally identified. The question as to whether only the T lymphocytes respond irrespective of the nature of the immunizing antigen and the interval between immunization and testing must also be ascertained. One would expect that those antigens which induce a humoral (antibody) immune response following immunization, i.e. Tetanus toxoid and Diphtheria toxoid, would induce proliferation of the circulating memory B lymphocytes in vitro whereas those antigens which induce cell-mediated immune responses (delayed hypersensitivity) following immunization i.e. PPD and Candida, would induce proliferation of the circulating memory T lymphocytes in vitro. The failure to date to unequivocally identify the responder and participating cells in the blastogenic responses to antigen stimulation has tended to detract

from the interpretive value of the assay in spite of its wide use in the assessment of the immunologic status of the patient (Chapter 2.3).

The circulating cell(s) of allergic individuals which participate in the blastogenic response to allergens have not been identified although it has been demonstrated that circulating T lymphocytes respond (Gatien et al 1975, Black and Marsh 1980). This blastogenic response to allergens is used by many investigators to confirm the existence of an allergic state. Since the allergic reaction is attributed to the IgE antibodies secreted by the B lymphocytes, one might anticipate that the circulating B lymphocytes participate in the in vitro blastogenic responses to allergens.

The objectives of this investigation were therefore as follows: (i) to determine whether only the T circulating lymphocytes undergo blastogenesis upon stimulation with phytomitogens, antigens and allergen in vitro, (ii) to identify the subpopulations of the circulating human T lymphocytes which undergo blastogenesis upon stimulation with the phytomitogens, antigens and allergen, (iii) to delineate the role(s) of Null lymphocytes, B lymphocytes and monocytes in this T lymphocyte blastogenic response, and (iv) to define the

role of the interleukins (IL-1, IL-2 and BCGF) in the blastogenic responses to phytomitogen, antigen and allergen stimulation.

The methods utilized in this investigation are well established and have been used by many investigators over the past 20 years. Briefly, Ficoll-Hypaque is a discontinuous density gradient utilized for purifying MNC and lymphocytes in high yield and purity from peripheral blood using a simple and rapid centrifugation procedure based on the methods developed by Boyum in 1967. The MNC isolated from the blood - Ficoll-Hypaque interface consist primarily of lymphocytes (70-80%) and monocytes (10-20%) and small numbers of contaminating granulocytes (2-5%) and erythrocytes (2-5%). The viability of the cells is greater than 98%. The percent recovery of the lymphocytes is 60±20%.

The methods used to isolate monocytes from the MNC, to deplete the MNC of monocytes with carbonyl iron, and the rosetting procedures for the isolation of the T, B and Null lymphocytes and the T lymphocyte subclasses have been utilized for the last 10-15 years and have withstood the test of time. Therefore, there is no reason to question the validity of the methods and materials

utilized in this investigation.

The culture conditions utilized to assay the blastogenic responses to antigen and allergen stimulation were identical to those used to assay the blastogenic responses to phytomitogen stimulation (described in Chapter 3.2.25). The only difference is that the cultures used to assay the phytomitogen-induced blastogenic responses were terminated after three days whereas the cultures used to assay the antigen and allergen-induced blastogenic responses were extended to six days as the blastogenic responses to antigen and allergen stimulation are delayed in onset and do not peak until six days in culture.

The results presented in this thesis permit the following conclusions to be made:

1. Only the circulating T lymphocytes respond with blastogenesis following stimulation with phytomitogens (PHA, PWM and Con-A) after three days in culture. The B and Null lymphocytes do not respond.

2. Only the circulating T lymphocytes respond with blastogenesis following stimulation with antigen (TT, DT,

CA and PPD), and allergen (AgE) in the case of allergic volunteers, following six days in culture. The B and Null lymphocytes do not respond.

3. Only the circulating B lymphocytes in the presence of IL-2 or BCGF respond with blastogenesis following stimulation with the phytomitogen PWM following seven days in culture. The B lymphocytes in the presence of IL-2 or BCGF do not respond with blastogenesis following stimulation with the phytomitogens PHA and Con-A, or specific antigens with which they had been previously been immunized, following culture of the cells for seven days.

4. The monocytes are obligatory participants in the phytomitogen-induced blastogenic response. Monocyte-depleted MNC do not respond to the mitogenic stimulus. Reconstitution of the monocyte-depleted MNC with either autologous or allogeneic monocytes restores to the T lymphocytes the capacity to undergo blastogenesis following stimulation with phytomitogens in vitro.

5. The monocytes are essential participants in the antigen and allergen-induced blastogenic responses of the

T lymphocytes. Monocyte-depleted MNC do not respond in vitro to antigen or allergen stimulation. Reconstition with autologous monocytes restores to the T lymphocytes the capacity to undergo blastogenesis following stimulation with the antigens and allergen in vitro. However, in contrast to the phytomitogen response, reconstitution with allogeneic monocytes does not restore blastogenic responsiveness to antigenic stimulation.

6. Supernatants from PHA, PWM and Con-A-stimulated or unstimulated cultures of autologous or allogeneic monocytes are capable of restoring T lymphocyte blastogenic responsiveness to phytomitogen stimulation. Viable monocytes are not required for the phytomitogen-induced blastogenic response to take place. It is presumed that the mediator generated is similar to, if not identical with, IL-1.

7. Supernatants from phytomitogen (PHA) or antigen (TT)-stimulated cultures of autologous or allogeneic monocytes are capable of restoring T lymphocytes blastogenic responsiveness to antigenic stimulation provided autologous, and not allogeneic, monocytes are present in threshold numbers. Thus, for the

antigen-induced blastogenic response, IL-1 by itself cannot replace monocytes as it can in the phytomitogen (PHA, PWM, Con-A)-induced blastogenic response.

8. The T_M lymphocytes consistently give greater blastogenic responses than do the T_G lymphocytes irrespective of the mitogenic stimulus used (phytomitogen, antigen or allergen). The T_C and the T_N lymphocytes consistently give lower blastogenic responses than do the T_G lymphocytes.

9. The T4+ and T8+ T lymphocytes consistently respond to a similar extent, irrespective of the mitogenic stimulus.

The results presented demonstrate that the T lymphocytes are the only circulating cells which respond with blastogenesis and mitosis to phytomitogen stimulation in the conventional 3 day blastogenic response providing a minimum number of monocytes are present in the cultures (as demonstrated in Tables 4-6). This conclusion is based on the repeated findings that the elimination of T lymphocytes from the MNC abrogated the capacity of the remaining MNC (B and Null lymphocytes) to mount a blastogenic response to phytomitogen stimulation (Table 2). These results are in

agreement with those presented by other investigators (discussed in Chapter 2.3.2). In point of fact, optimal blastogenic responses of the T lymphocytes were observed on day 3 and 5 of culture. A very low or insignificant response was recorded after 7 days of culture. Since at no time was the phytomitogen-induced blastogenic responses observed on day 5 of culture and not on day 3 of culture, only 3 day cultures were carried out in the part of this investigation concerned with the identification of the cells which respond to phytomitogen stimulation. The reason for the absence of a blastogenic response after 7 days in culture will be elaborated upon later in the discussion (see page 245).

Although it has been unequivocally demonstrated that the T lymphocytes respond in the 3 day phytomitogen-induced blastogenic response, it has not, however, been demonstrated whether all the subclasses of T lymphocytes respond or whether it is a particular subclass of T lymphocytes which responds to phytomitogen stimulation (Moretta 1983, Geha 1973, Chess et al 1974). The results of this investigation clearly demonstrate that all of the receptor-specific T lymphocyte subclasses - T_M, T_G, T_C and T_N, respond with blastogenesis to phytomitogen stimulation (Tables 7). The T_M

lymphocytes were consistently the best responders in the phytomitogen-induced blastogenic response. The blastogenic response by the T_G lymphocytes were always less than that of the T_M lymphocytes. The phytomitogen-induced blastogenic responses of the T_C and T_N lymphocytes were consistently lower than those given by the T_G lymphocytes (Table 7). The increased responsiveness of the T_M lymphocytes to phytomitogen stimulation may be attributed to increased numbers of helper cells or may be a reflection of a functional property of these responding cell. The decreased blastogenic responsiveness of the T_G lymphocytes may reflect a population of cells which is innately less responsive to phytomitogen stimulation or is due to the immunoregulatory suppressor function attributed to T_G lymphocytes.

It has already been amply demonstrated that the T_G lymphocytes function as the suppressor cells (Heijnen et al 1979, Dosch and Gelfand 1979, Moretta et al 1979) and that the T_M lymphocytes function as the helper cells (Ballieux and Heijnen 1983, Moretta et al 1979) in the immune response in vitro. It has also been amply demonstrated that the OKT4 and the OKT8 monoclonal antisera detect the helper and suppressor cells,

respectively (Reinherz and Schlossman 1980, Reinherz et al 1980, Kung et al 1980, Thomas et al 1981). Since the T_M (helper) lymphocytes were more responsive than the T_G (suppressor) lymphocytes, it was anticipated that the T4+ (helper) lymphocytes would give a better response to phytomitogen stimulation than would the T8+ (suppressor) lymphocytes. However, the blastogenic responses of the T4+ and T8+ T lymphocytes to phytomitogen stimulation were comparable (Table 8). These results indicate that the rosetting procedures and the monoclonal antisera do not identify the same populations of T lymphocytes - that is, the T_M and T_G lymphocytes are not identical to the T4+ and the T8+ lymphocytes, respectively. This point must be stressed since it is generally assumed that the terms T_M and T4+ and T_G and T8+ lymphocytes are synonymous as they identify identical T lymphocytes, which they obviously do not.

The T_G, T_M, T_C and T_N lymphocyte subclasses isolated by rosetting procedures are not transitional cells with respect to the receptors for FcG, FcM and C'3 as has been suggested (Gomez-Reino and Habicht 1980, Pichler et al 1978). These receptors are stable and the cells are receptor-pure as they remain single

receptor-bearing cells over a period of at least three days in culture (Table 32). These T_G, T_M, T_C and T_N lymphocyte subclasses stain with both of the OKT4 and OKT8 monoclonal antisera (Table 33). Both, the T4+ and the T8+ lymphocytes, isolated by C' mediated lysis of OKT8 and OKT4-stained T lymphocytes, respectively, rosette with EAG and EAM indicator erythrocytes (Table 33). Therefore, it may be concluded that the T lymphocyte subclasses, irrespective of the mode of isolation, are heterogeneous and cannot be distinguished on the basis of their responses to phytomitogen stimulation.

As discussed above, IL-1 and IL-2 are essential for the T lymphocyte blastogenic response. Two other mediators are secreted by T lymphocytes and these are referred to as B cell growth factor (BCGF) and B cell differentiation factor (BCDF). Both BCGF and BCDF have been shown to facilitate the proliferation of B lymphocytes and their transformation into immunoglobulin secreting cells and to maintain B lymphocytes in long term culture (Howard et al 1982, Ford 1981, Leanderson et al 1982, Lernhardt et al 1982, Yoshizaki et al 1983, Okada et al 1983, Butler et al 1983, Butler et al 1984, Muraguchi et al 1984, Hirano et al 1984). The

TABLE 32
THE ROSETTING CAPACITY OF THE ISOLATED T LYMPHOCYTE SUBCLASSES FOLLOWING 1, 2 AND 3 DAYS IN CULTURE

Cells cultured	Percent of cells which rosette with		
donor 1	EAG	EAM	EAC
TG day 1	*	1	1
day 2	*	1	1
day 3	*	0	1
TM day 1	0	*	0
day 2	0	*	0
day 3	0	*	0
TC day 1	0	0	*
day 2	0	0	*
day 3	0	2	*
TN day 1	0	0	0
day 2	0	0	0
day 3	0	0	0
donor 2			
TG day 1	*	1	1
day 2	*	1	1
day 3	*	0	1
TM day 1	0	*	0
day 2	0	*	0
day 3	0	*	0
TC day 1	0	0	*
day 2	0	0	*
day 3	0	1	*
TN day 1	0	0	0
day 2	0	0	0
day 3	0	0	0
donor 3			
TG day 1	*	0	0
day 2	*	0	1
day 3	*	0	1
TM day 1	0	*	0
day 2	0	*	0
day 3	0	*	0
TC day 1	0	0	*
day 2	0	6	*
day 3	0	6	*
TN day 1	0	0	0
day 2	0	0	0
day 3	0	0	0

* Not done

TABLE 33

THE IDENTIFICATION OF THE FRESHLY ISOLATED T LYMPHOCYTE SUBCLASSES WITH OKT3, OKT4 AND OKT8 MONOCLONAL ANTISERUM AND ROSETTING WITH INDICATOR ERYTHROCYTES

	% OKT3	% OKT4	% OKT8	% EAM	% EAG	%EAC
donor 1						
MNC	73	46	37	*	*	*
T	85	58	33	0	6	5
TG	68	11	45	0	*	0
TM	89	61	11	*	0	0
TC	85	48	33	0	0	*
TN	93	42	33	0	0	0
T4+	*	99	0	20	6	3
T8+	*	0	98	9	3	3
donor 2						
MNC	65	30	30	*	*	*
T	84	63	38	0	5	4
TG	68	9	54	0	*	0
TM	89	65	26	*	0	0
TC	65	32	29	0	0	*
TN	80	46	35	0	0	0
T4+	*	96	0	18	6	1
T8+	*	0	95	11	3	2
donor 3						
MNC	74	34	29	*	*	*
T	84	51	26	0	6	6
TG	81	13	52	0	*	0
TM	92	48	15	*	0	0
TC	68	24	25	0	0	*
TN	77	45	18	0	0	0
T4+	*	98	0	15	3	4
T8+	*	0	97	8	4	3

* Not done

relationship of BCGF and BCDF to B lymphocytes is very similar to the relationship of IL-1 and IL-2 to T lymphocytes and these two mediators should therefore also be referred to as Interleukins.

The results in Tables 2 and 17 demonstrate that purified B lymphocytes respond insignificantly to phytomitogen stimulation after 3 days in culture. The addition of autologous monocytes, IL-2, BCGF and supernatants from cultures of monocytes or MNC were incapable of conferring to the B lymphocytes blastogenic responsiveness to phytomitogen stimulation during 3 days in culture (Tables 15, 17). These results do not support those of Phillips and Roitt (1973), Phillips and Weisrose (1973) and Epstein (1974), who demonstrated that circulating human B lymphocytes respond to the phytomitogens PHA and Con-A. These conflicting results may be explained by the differences in the techniques used to isolate the circulating human B lymphocytes and the purity of the cell populations obtained and cultured. The "pure" smIg+ B lymphocytes cultured by Phillips and Roitt (1973), Phillips and Weisrose (1973) and Epstein (1974) were contaminated with T lymphocytes to the extent of 3-15% as determined by E rosette formation. It may be argued, in light of the results obtained in this investigation, that the responding cells were not the B

lymphocytes but the contaminating T lymphocytes. As demonstrated in Table 17, pure B lymphocytes cultured in the presence of the optimal numbers of autologous monocytes (final concentration of 10%) did not respond with a blastogenic response to phytomitogen stimulation. However, the intentional "contamination" of the B lymphocytes with 1% T lymphocytes resulted in a significant phytomitogen-induced blastogenic response which can be totally attributed to the added T lymphocytes.

Delespesse et al (1976) and Kasahara et al (1979) demonstrated a certain synergism between T and B lymphocytes in the phytomitogen-induced blastogenic response. In this investigation, removal of the B lymphocytes by rosetting with EAC had no discernible effect on the phytomitogen-induced blastogenic responses of the remaining MNC (Table 2). It might be argued that not all of the B lymphocytes were removed by rosetting with EAC and that a sufficient number of B lymphocytes were left behind to facilitate an enhanced T lymphocyte response. However, cells with smIg were not detected by immunofluorescence among the MNC depleted of EAC-rosetting cells (Richter 1985), indicating the absence of B lymphocytes.

Caraux et al (1978) claimed that Null lymphocytes or a soluble product secreted by Null lymphocytes enhanced the T lymphocyte blastogenic response. However, their Null lymphocytes were contaminated with monocytes to an extent of 50% as demonstrated by peroxidase staining (Caraux et al 1978). In retrospect, it is probably not the Null lymphocytes nor a soluble product secreted by the Null lymphocytes which facilitated the phytomitogen-induced blastogenic response but rather the "contaminating" monocytes and the IL-1 secreted by them which augmented the blastogenic response. The results in this investigation do not provide evidence for a supportive role for Null lymphocytes nor for a soluble enhancing factor secreted by the Null lymphocytes.

The results in this investigation also demonstrate that the Null lymphocytes do not respond with a blastogenic response to phytomitogen stimulation even when augmented with optimal numbers of monocytes (Tables 2 and 17). These results support those previously reported by Horwitz and Garrett (1977), Caraux et al (1982) and Ng et al (1981) who concluded that Null lymphocytes were unable to proliferate in the presence of the phytomitogens. The intentional "contamination" of Null lymphocytes with 1% T lymphocytes resulted in a phytomitogen-induced blastogenic response which can be totally attributed to the added T lymphocytes (Table 17).

It is essential to note that neither the B nor the Null lymphocytes cultured individually or in combination (non-T lymphocytes) responded to phytomitogen stimulation following 3 or 5 days in culture in the presence of optimal numbers of autologous monocytes (final monocyte concentration 10% of the cultured cells) or Mitomycin C treated MNC (MNC_m) (Tables 15-17). Mitomycin C inhibits DNA synthesis. The fact that the T-MO_2 cell cultured in the presence of MNC_m gave marked phytomitogen-induced blastogenic responses attests to the fact that MNC_m are, in fact, quite capable of secreting the mediator(s) which facilitates the phytomitogen-induced blastogenic response. This mediator must originate from the monocytes in the MNC_m since the T lymphocytes were totally depleted of monocytes. Although this investigation has not characterized the mediator secreted by the monocytes, it is nevertheless assumed that the active factor is IL-1. In these studies and others the major source of human IL-1 has been crude supernatants from autologous monocyte cell cultures stimulated with PHA which are known to contain several biologically active factors, including IL-1 (Maizel et al 1981, deVries 1979, Mizel 1982). These results unequivocally demonstrate that the B and Null lymphocytes cannot respond with blastogenesis to phytomitogen stimulation following 3 and 5 days in culture under the conditions which facilitate optimal blastogenic responses by the T lymphocytes or the unfractionated MNC:

The phytomitogen-induced blastogenic responses of the MNC depleted of B lymphocytes (MNC-B) were always greater than those given by the MNC depleted of Null lymphocytes (MNC-Null) (Table 2), suggesting that the Null lymphocytes participate in the blastogenic response and that their removal therefore results in a diminished response by the remaining cells. Rosetting the MNC with EAC eliminates the B lymphocytes and only a few monocytes (Bianco et al 1975, Bianco 1977, Bianco and Edelson 1978). The majority of monocytes remain to facilitate the phytomitogen-induced blastogenic responses of the MNC-B lymphocytes. On the other hand, rosetting the MNC with EAG results in the removal of the Null lymphocytes and the majority of the monocytes since the latter cells possess receptors for FcG (Rabinowitch et al 1975, Schroitt et al 1976, Haegert 1979, Norris et al 1979). These results suggest that the monocytes are essential in the phytomitogen-induced blastogenic response and that there are insufficient monocytes in the MNC-Null lymphocytes to facilitate a phytomitogen-induced blastogenic response by the T lymphocytes (Tables 4-6). In point of fact, the addition of pure monocytes to the MNC-Null lymphocytes totally restored blastogenic responsiveness to the T lymphocytes, thus leaving no doubt as to the need for monocytes in this response.

A role for the monocyte as an obligatory accessory cell in the T lymphocyte blastogenic response has gradually gained support since the mid-1960's. It has been variously stated that the in vitro T lymphoproliferative response is independent of, synergized, potentiated, augmented, suppressed or is dependent upon monocytes. Maizel et al (1979), Rosenstreich et al (1976), Mookerjee and Balliard (1970), and Arala-Chares et al (1978) demonstrated an absolute monocytes dependency for the phytomitogen-induced T lymphocyte blastogenic response. On the other hand, Hersh and Harris (1966), Stobo et al (1972), Jones et al (1972), Mackler (1972) and Blaese et al (1977) claimed that this response is independent of monocytes. The results of these two groups of investigators would appear to be irreconcilable. A primary reason for the conflicting conclusions arrived at by different investigators is evident from the results presented in Tables 3-5, which show that monocytes are obligatory participants in the phytomitogen-induced blastogenic response but that the number of monocytes required in the cultures to facilitate a significant blastogenic response is very low. Monocytes in a concentration as low as 0.6%

of the cultured cells facilitates a discernable phytomitogen-induced blastogenic response whereas monocytes in a concentration of only 1% facilitate a significant phytomitogen-induced blastogenic response by the T lymphocytes (Table 5). Thus, it is essential to recognize that "contaminating" monocytes in insignificantly low numbers may nevertheless be sufficient to facilitate the blastogenic response of the T lymphocytes to phytomitogen stimulation.

Incubation of freshly-isolated MNC or T lymphocytes with carbonyl iron results in the elimination of 90 to 95% of the monocytes, ($MNC\text{-}MO_1$ and $T\text{-}MO_1$). Nevertheless immature, non-phagocytic monocytes remain behind since the circulating monocytes are not in synchrony with respect to their state of maturation (Rook and King 1978, Fernandez and MacSween 1977). Thus, immature monocytes exist in the circulation which are not detected in the freshly isolated state by staining for non-specific esterase and for the capacity to phagocytize iron or latex particles. On the assumption that these cells would mature in culture, the $MNC\text{-}MO_1$ and $T\text{-}MO_1$ cells were cultured overnight and treated with carbonyl iron a second time in an attempt to eliminate these newly matured phagocytic cells. The $MNC\text{-}MO_2$ and $T\text{-}MO_2$

matured phagocytic cells. The MNC-MO_2 and T-MO_2 cells were now found to be contaminated with less than 0.5% monocytes and they were incapable of mounting significant blastogenic responses to phytomitogen stimulation (Tables 3-5). It may be argued that subjecting the MNC to the monocyte-depletion technique twice within a short time span of 24 hr may inadvertently result in the simultaneous removal of or damage to non-monocytic cells essential for the blastogenic response. However, the addition of pure autologous or allogeneic monocytes to these MNC-MO_2 or T-MO_2 cells (to a final cell concentration of 10% for the phytomitogen-induced blastogenic response) totally restored the blastogenic responsiveness of these cells (Tables 4, 5), thus demonstrating that only the monocytes were removed by the carbonyl iron treatments. The accessory cell function of the monocytes requires viable cells since ultrasonicated monocytes failed to enhance the blastogenic responses of the T-MO_2 cells (Table 4). These results indicate that essentially all (greater than 98-99%) the monocytes must be eliminated before the blastogenic responses by the T lymphocytes or the MNC are abrogated and that failure to remove all the monocytes may result in the erroneous conclusion that monocytes are unnecessary in the phytomitogen-induced blastogenic

responses (Table 3). Hence, it is probable that Stobo et al (1972), Mackler (1972) and Jones et al (1972) erroneously concluded that the phytomitogen-induced blastogenic response is monocyte independent since it is likely that their T lymphocyte cultures were "contaminated" with minimal number of monocytes which facilitated the phytomitogen-induced blastogenic responses.

The results obtained clearly indicate that autologous and allogeneic monocytes are equally effective in restoring T lymphocyte blastogenic responsiveness to phytomitogen stimulation (Table 6). de Vries et al (1979) proposed that the accessory helper function provided by the monocytes is not genetically restricted but is mediated by a non-specific mechanism. The fact that the accessory monocytes are not MHC restricted was clearly demonstrated by Schmidtke and Hatfield (1976) who reported that mouse macrophages were able to potentiate human T lymphocyte blastogenic responses to phytomitogen stimulation.

On the other hand, Gerrard and Fauci (1982) and Passwell (1982) showed that monocytes can suppress the phytomitogen-induced blastogenic response. Passwell

(1982) and Goodwin (1979) demonstrated that the phytomitogen-induced blastogenic responses decreased as the monocyte:lymphocyte ratio was increased. Monocytes added to T lymphocytes to the point where they constituted 20% of the cultured cells inhibited the phytomitogen-induced blastogenic response. The results in this investigation failed to demonstrate a suppressor effect by monocytes even when they were added in excessive numbers to the phytomitogen stimulated cultures (20-50% of the cultured cells). In fact, the "excessive" numbers of monocytes in the cell cultures enhanced the phytomitogen-induced blastogenic responses (Table 5).

At least two mediators facilitate the phytomitogen-induced blastogenic response - Interleukin 1 and Interleukin 2. The monocyte-generated soluble factor is referred to as IL-1. Although the mediator secreted by the monocytes has not been characterized in this investigation, it is assumed that the active factor or mediator is IL-1 since it can functionally replace the monocytes. Maizel et al (1980) and de Vries et al (1979) both identified the active factor in their monocyte-derived supernatants as IL-1. The method used in this investigation to prepare the monocyte-derived supernatants was identical to that used by Maizel et al

(1980) and de Vries et al (1979).

The Interleukin IL-1 does not support the proliferative response of T lymphocytes directly; rather it provides a signal for certain T lymphocytes to produce IL-2 which has been shown to facilitate the phytomitogen-induced blastogenic response and to maintain T lymphocytes in a viable state in long term culture (Smith 1980, Gillis and Mizel 1981, Palacious 1982, Mizel 1982). Monocytes can be replaced by supernatants obtained from unstimulated (48 and 72 hr) and PHA, PWM or Con-A-stimulated (24, 48 and 72 hr) autologous or allogeneic monocyte cultures (Tables 10-14). Supernatants obtained from phytomitogen-stimulated and unstimulated autologous or allogeneic MNC-MO_2 cells (MNC totally depleted of monocytes) were unable to restore the blastogenic responsiveness to the T-MO_2 cells (Table 10). Hence it may be concluded that autologous or allogeneic monocytes must be present in the cell cultures in order for the IL-1 to be secreted.

It must be stressed that the supernatants of unstimulated monocytes were capable of restoring blastogenic responsiveness to the T lymphocytes. The supernatants of unstimulated autologous or allogeneic

monocytes cultured for 24 hrs were ineffective in restoring or facilitating the phytomitogen-induced blastogenic responses of the T-MO_2 cells whereas 48 or 72 hr culture supernatants restored the blastogenic responsiveness of T-MO_2 cells to a level of 40-70% of that observed with the MNC (Tables 9 and 10). In contrast, supernatants of 24 hr and 48 and 72 hr PHA, PWM and Con-A-stimulated autologous and allogeneic monocyte cultures were more effective (50-70%) in reconstituting the blastogenic responsiveness of the T-MO_2 cells (Tables 10, 11). Therefore, the secretion of IL-1 appears to be hastened by but not dependent upon the presence of the phytomitogens. These results are in agreement with those of other investigators who concluded that the secretion of IL-1 by the monocytes is not mitogen dependent (Unanue and Kelly 1977, de Vries et al 1979, Maizel et al 1981). De Vries et al (1979) demonstrated that supernatants of 24 hr unstimulated autologous monocyte cultures significantly augmented the phytomitogen-induced blastogenic responses of monocyte-depleted T lymphocytes. Moreover, 24 hr autologous and allogeneic monocyte culture supernatants were more effective that 72 hr monocyte culture supernatants. They proposed that the greatest production of IL-1 took place within the first 24 hr of culture. In

contrast, Maizel et al (1980) reported that only the 72 and 96 hr monocyte culture supernatants could effectively restore the blastogenic responsiveness to the T-MO_2 cells.

It must be noted that viable monocytes are more effective than monocyte culture supernatants in reconstituting the blastogenic responses of T-MO_2 cells. The addition of monocytes (10% of the cultured cells) reconstituted the blastogenic responsiveness of the T-MO_2 cells to 70-100% of the maximum blastogenic responses observed with the unfractionated MNC whereas monocyte supernatants reconstituted the responsiveness to only 50-70% of the optimal blastogenic responses observed with the unfractionated MNC (Tables 9 to 13). These results suggest that factors other than monocyte-secreted mediators are essential for the maximum T lymphocyte blastogenic response and that T lymphocyte-monocyte contact may be required to facilitate the maximum phytomitogen-induced blastogenic response.

The results in this investigation demonstrate that B lymphocytes cultured for 7 days in the presence of IL-2 or BCGF respond with blastogenesis to PWM stimulation but not to PHA and Con-A stimulation (Table 18). The BCGF

and IL-2, in the absence of the phytomitogen, were not mitogenic. These results are in accord with those reported by Howard et al (1983) who demonstrated that BCGF augmented PWM, anti-IgM or STA-induced B lymphocyte proliferation. The commercially obtained IL-2 used in this investigation contained both BCGF and BCDF and the commercially obtained BCGF contained IL-2 and BCDF. Therefore, one cannot conclude from these experiments whether the responses of the B lymphocytes to PWM stimulation were facilitated by IL-2 or BCGF and BCDF. It is also possible that the induction of B lymphocyte proliferation to PWM stimulation following 7 days in culture in the presence of IL-2 or BCGF may, in fact, be dependent on small numbers of T lymphocytes which are in too low a concentration to be detected by SRBC rosette formation or staining with OKT3 monoclonal antiserum.

Cooper et al (1971) demonstrated that circulating human B lymphocytes synthesize Ig following culture of MNC for 7 days in the presence of PWM. Since then, many investigators have confirmed this finding and have demonstrated that PWM induces both B lymphocyte proliferation and differentiation into Ig-secreting cells following 7 days in culture in the presence of T lymphocytes and monocytes. The interleukins, BCGF and

BCDF (secreted by a population of (T4+?) T lymphocytes) have been implicated as the mediators which facilitate Ig synthesis and secretion by the B lymphocytes stimulated with PWM. Current evidence (Jelinek and Lipsky 1983) suggests that the B lymphocytes must undergo proliferation in vitro before they can transform into Ig secreting cells. Jelinek and Lipsky (1983) treated MNC and Non-T cells (MNC depleted of T lymphocytes), the B and Null lymphocytes and monocytes, with Hydroxyurea (HU), an inhibitor of cellular DNA synthesis, prior to culture. This treatment completely inhibited the blastogenic response, and the synthesis and secretion of Ig in response to PWM stimulation in vitro. They therefore concluded that Ig secreting B cells cannot be generated in the absence of proliferation of these cells. However, it is possible that the failure to synthesize Ig may be due to other as yet undefined properties of HU quite unrelated to DNA synthesis and cell replication. In this investigation, Non-T cells (B and Null lymphocytes) were cultured for 7 days in the presence of PWM and IL-2 or BCGF (Table 34). Ig synthesis and secretion by the B cells were defined as the percent of cells which were immunofluorescent positive (IF+) after 7 days in culture and the amount of IgG and IgM secreted into the supernatant as determined by the ELISA technique, respectively. The results demonstrate that Ig

TABLE 34

THE CAPACITY OF IL-2 OR BCGF TO FACILITATE PWM-INDUCED BLASTOGENESIS AND IMMUNOGLOBULIN SYNTHESIS AND SECRETION FOLLOWING 7 DAYS IN CULTURE

	BLASTOGENIC RESPONSE FOLLOWING 7 DAYS IN CULTURE				Ig SYNTHESIS AND SECRETION FOLLOWING 7 DAYS IN CULTURE IN THE PRESENCE OF PWM		
	Control*	PHA	PWM	Con-A	% IF+ cells	IgG (ng/ml)	IgM (ng/ml)
donor 1							
MNC	302	2265	2148	2050	*	*	*
MNC+IL-2	228	48943	38890	40478	*	*	*
MNC+BCGF	578	35535	25766	30890	*	*	*
NON-T	201	317	1304	487	3.8	11	69.8
NON-T+IL-2	461	688	17342	713	4.8	50.2	63.4
NON-T+BCGF	356	754	18541	851	3.6	11	37.3
T+NON-T	218	1911	1936	1897	24.3	528.3	1622.9
T+NON-T+IL-2	325	43672	30745	35748	23.7	564.7	1090.2
T+NON-T+BCGF	637	45326	31857	36475	26.5	794.9	1539.5
donor 2							
MNC	259	1354	2183	1412	*	*	*
MNC+IL-2	339	64859	35729	66253	*	*	*
MNC+BCGF	483	61267	32453	63421	*	*	*
NON-T	412	937	1436	938	4.2	15	38.4
NON-T+IL-2	456	1093	15374	1145	5.1	22	35.2
NON-T+BCGF	631	1034	16983	1077	4.9	16	22.4
T+NON-T	425	1945	2074	1743	21.3	542.6	1748
T+NON-T+IL-2	579	59683	38573	55411	24.4	575.4	1046.2
T+NON T+BCGF	635	57680	38751	53200	25.4	682.7	1683.4
					<1.2	<11	<40

*Controls

synthesis and secretion is not a direct consequence of PWM-induced B lymphocyte proliferation. The B lymphocytes responded with blastogenesis and mitosis after 7 days in culture in the presence of PWM and IL-2 or BCGF. However, these cells were IF- for intracytoplasmic IgG or IgM; nor did they secrete significant quantities of IgG or IgM into the culture supernatants (Table 34). Furthermore, the non-T cells (B and Null lymphocytes) cultured for 7 days in the presence of T lymphocytes and PWM did not generate a blastogenic response but did synthesize and secrete IgG and IgM. These data therefore indicate that Ig synthesis and secretion may take place in the absence of a detectable blastogenic response and that Ig synthesis by the B cells is not an immediate and automatic consequence of B lymphocyte proliferation. It may be argued that these results are more valid than those presented by Jelinek and Lipsky (1983) since the B lymphocytes were not treated with a toxic agent prior to culture.

Pure Null lymphocytes cultured in the presence of IL-1, IL-2 and BCGF failed to respond with blastogenesis to stimulation with any one of the phytomitogens following 7 days of culture. These results unequivocally demonstrate that Null lymphocytes cannot respond with blastogenesis even when cultured in the presence of soluble mediators which facilitate B lymphoproliferation to PWM stimulation following 7 days in culture.

As was stated previously, the optimal phytomitogen-induced blastogenic response occurs 3 days and 5 days following stimulation (Table 1 and Figure 1). However, the blastogenic responses of the MNC at day 7 of culture to phytomitogen stimulation were barely significant even though the phytomitogens and IL-1 were added at the initiation and at day 4 of culture. However, both exogenous IL-2 (which contains BCGF and BCDF) and BCGF and BCDF (which contains IL-2) effectively facilitated the blastogenic responses of the MNC and T-MO_2 cells to phytomitogen stimulation following 7 days in culture. As demonstrated in Table 35 it may be concluded that under the constraints of the culture conditions used, the T lymphocytes do not generate sufficient amounts of endogenous IL-2 in the presence of IL-1 to facilitate a 7 day phytomitogen-induced blastogenic response. Since both MNC and T-MO_2 cells in the presence of IL-1 generate optimal phytomitogen-induced blastogenic responses on day 3 and day 5 of culture, it must be asked why the cells respond insignificantly only 2 days later. It may be that monocytes lose their capacity to secrete IL-1 following the first few days of culture and thus the T lymphocytes cease to be stimulated to produce endogenous IL-2. Conversely, it may be that under the constraints of the

cell culture conditions, which are after all artificial and unphysiological, the T lymphocytes cease to be able to secrete endogenous IL-2 after the fourth and fifth day of culture and require exogenous IL-2 to facilitate the blastogenic response following 7 days of culture (Table 35). The results in Table 35 demonstrate that this is, in point, the case. Addition of exogenous IL-2 to the MNC cultures containing the phytomitogens on day 4 resulted in near-optimal blastogenic responses on day 7 of culture.

TABLE- 35

THE CAPACITY OF IL-1 AND IL-2 TO FACILITATE THE BLASTOGENIC RESPONSIVENESS OF THE MNC AND T-MO2 TO PHYTOMITOGEN STIMULATION FOLLOWING 3, 5 AND 7 DAYS IN CULTURE

Donor 1		Control	PHA	%R	PWM	%R	CON-A	%R
	IL in culture							
day 3								
MNC		451	107390		64075		102200	
MNC+IL-1	0-3	340	115514	108	75543	118	115248	113
MNC+IL-2	0-3	436	152205	142	104118	162	151584	148
T-MO2		130	1224	1	1029	2	1468	1
T-MO2+MO(10%)	0-3	281	107849	100	60984	95	100837	99
T-MO2+IL-1	0-3	648	44261	41	29632	46	45130	44
T-MO2+IL-2	0-3	270	80732	75	42216	66	78586	77
day 5								
MNC		246	92109	86	43616	68	84416	83
MNC+IL-1	3-5	382	93919	87	46021	72	94504	92
MNC+IL-2	3-5	616	106710	99	63219	99	104325	102
T-MO2		921	1265	1	819	1	1132	1
T-MO2+MO	3-5	847	86372	80	39629	62	80221	78
T-MO2+IL-1	3-5	669	36269	34	15279	24	37924	37
T-MO2+IL-2	3-5	312	81984	76	41128	64	80216	78
day 7								
MNC		419	1354	1	2849	4	1076	1
MNC+IL-1	5-7	529	1857	2	1924	3	1302	1
MNC+IL-2	5-7	624	75675	70	45693	71	77208	76
T-MO2		113	915	1	427	1	879	1
T-MO2+MO	5-7	493	60495	56	26736	42	62738	61
T-MO2+IL-1	5-7	900	3422	3	2815	4	3360	3
T-MO2+IL-2	5-7	439	56675	53	24693	39	57208	56

It should be noted that the antigen and allergen-induced blastogenic responses of the MNC, which are maximum following 6 days of culture, were consistently lower than the blastogenic responses obtained to phytomitogen stimulation. This should be expected since, as discussed previously, the antigens are monoclonal stimulants and they stimulate only antigen-specific memory cells (clonally committed cells) which are the progeny of previously committed antibody-forming cells. On the other hand, the phytomitogens are polyclonal stimulants capable of randomly stimulating the lymphocytes capable of proliferating when appropriately stimulated. This interpretation is supported by the fact that far fewer cells are necessary to facilitate maximum blastogenic responsiveness to phytomitogen stimulation than are required for maximum antigen and allergen stimulation since there are more cells capable of responding to the phytomitogens than to any one of the antigens. It is not at all understood why the response to phytomitogen stimulation is more rapid than it is to antigen stimulation. Suffice it to say that the results are reproducible and have been verified by numerous

investigators.

On the basis of the results presented in this investigation, it may be concluded that the T lymphocytes are the only circulating cells which respond with blastogenesis and mitosis to antigen stimulation providing a minimum number of autologous monocytes are present in the culture (as demonstrated in Tables 21-24). These results are in agreement with those presented by van Oers et al (1979), Lohrman et al (1974), Geha et al (1974), Maizel et a (1979) and Geha et al (1984). They are disimilar to those obtained for the phytomitogen-induced blastogenic response discussed above in that only autologous monocytes can restore blastogenic responsiveness to the T lymphocytes to antigen stimulation whereas, autolgous or allogeneic monocytes can restore blastogenic responsiveness to phytomitogen stimulation.

The blastogenic responses of the receptor-specific T lymphocyte subclasses - T_M, T_G, T_C and T_N, to antigen and phytomitogen stimulation are comparable (Tables 25 and 26). As in the phytomitogen-induced blastogenic response, the T_M lymphocytes were consistently the best responders in the antigen-induced

blastogenic responses. The blastogenic responses by the T_G lymphocytes were always less than those of the T_M lymphocytes. The antigen-induced blastogenic responses of the T_C and T_N lymphocytes were consistently lower than those given by the T_G lymphocytes (Tables 25 and 26). The antigen-induced blastogenic responses of the T4+ and the T8+ lymphocytes were comparable. Since the T4+ lymphocytes are presumed to consist of the helper cells, as are the T_M lymphocytes, and the T8+ lymphocytes are presumed to consist of the suppressor cells, as are the T_G lymphocytes, it is difficult to understand why the T4+ and the T8+lymphocytes responded to the same degree to antigen stimulation whereas the T_M lymphocytes gave markedly superior blastogenic responses to antigen stimulation than did the T_G lymphocytes.

The antigen-induced blastogenic responses generated by the MNC-B lymphocytes were consistently greater than those given by the MNC-Null lymphocytes. These results are similar to those obtained with respect to the phytomitogen-induced blastogenic responses (Table 2). An explanation for this finding (Chapter 4.10) is that monocytes are essential participants in the T lymphocyte antigen-induced blastogenic response. As was discussed

previously in this chapter, monocytes as well as Null lymphocytes possess FcG receptors. Therefore, the MNC-Null lymphocytes would be depleted of both monocytes and Null lymphocytes following the removal of the Null lymphocytes from the MNC by rosetting with EAG. On the other hand, rosetting the MNC with EAC eliminates the majority of B lymphocytes and only a few monocytes. Thus, the MNC-B lymphocytes retain the monocytes and respond well to antigen stimulation (Tables 21 to 24).

Autologous monocytes are obligatory accessory cell in the antigen-induced T lymphocyte blastogenic responses (Tables 22 and 23) however, the number of monocytes required in the cultures to facilitate a significant blastogenic response is very low. Autologous monocytes in as low a concentration as 1% of the cultured cells were capable of imparting to the T-MO_2 cells the capacity to give highly significant, albeit low, blastogenic responses to antigen stimulation (Table 23). However, the addition of autologous monocytes to T-MO2 cells to a final cell concentration of 15% of the cultured cells totally restored antigen-stimulated blastogenic responsiveness to the T lymphocytes (Table 23). It would therefore appear that minimal numbers of "contaminating" monocytes are effective in facilitating

the T lymphocyte antigen-induced blastogenic response. The elimination of monocytes on two separate occasions over a 24 hr period resulted in a total loss of the capacity of the remaining cells to undergo blastogenesis to antigen stimulation. The procedure to eliminate the monocytes did not have a deleterious effect on the responder T lymphocytes since they responded optimally when reconstituted with pure autologous monocytes. The monocytes themselves were incapable of responding to antigen stimulation. Furthermore, autologous monocytes did not suppress the antigen-induced blastogenic response even when added to T-MO_2 cells in large numbers. In the presence of an equal number of monocytes (50% of the cultured cells), the T-MO_2 cells gave blastogenic responses which were equal or greater than those given by the unfractionated MNC and the T lymphocytes (Table 23). These results do not support those presented by Passwell (1982), Gerrard and Fauci (1982) and Goodwin (1979) who demonstrated that the antigen-induced blastogenic responses decreased as the monocyte:lymphocytes ratio was increased. As in the phytomitogen-induced blastogenic response, the "excessive" numbers (>20%) of monocytes in the cell cultures enhanced the antigen-induced blastogenic responses (Tables 5 and 23).

Allogeneic monocytes (irrespective of their numbers) added to the cultures of the $T\text{-}MO_2$ cells failed to facilitate significant blastogenic responses by the T-MO2 cells to antigen stimulation although they invariably reconstituted the blastogenic responsiveness of the $T\text{-}MO_2$ cells to phytomitogen stimulation (Table 24). These results are in support of the data presented by deVries et al (1979, Arala-Cheves et al (1978), Maizel et al (1979), Geha et al (1981) and Sonderstrup et al (1978). Jakway et al (1983) proposed that monocytes in the antigen-induced blastogenic response function to present the antigen to the T lymphocytes. The T lymphocytes recognize antigens only in association with appropriate histocompatability antigens (HLA-D/Dr) on the surface of the monocytes. It may therefore be concluded that autologous, but not allogeneic, monocytes are required in small numbers in the T lymphocyte cultures to facilitate optimal blastogenic responses to antigen stimulation.

Supernatants from 48 hour PHA-stimulated or TT-stimulated autologous and allogeneic monocyte cultures were effective in restoring T lymphocyte blastogenic responsiveness to antigen stimulation providing threshold numbers of autologous monocytes (1% of the cultured

cells) were present in the cultures. These results are in accord with those reported by Chu et al (1984) who reported that supernatants from Con-A-stimulated allogeneic monocytes could effectively reconstitute the blastogenic responsiveness of T-MO_2 cells to TT stimulation in the presence of UV-irradiated autologous monocytes. It is therefore necessary to assume that the blastogenic responses of the T lymphocytes to antigenic stimulation require direct contact with monocytes. These results should be contrasted with those results which demonstrated that monocyte culture supernatants were effective in restoring the T lymphocyte blastogenic response to phytomitogen stimulation in the absence of intact monocytes (Tables 10 to 12).

As was discussed in Chapter 2.2.2, IL-1 is secreted by monocytes whereas IL-2 and BCGF are secreted by T lymphocytes in the presence of monocytes or IL-1. In this investigation, it was demonstrated that IL-1, IL-2 or BCGF facilitate the the blastogenic responsiveness of T lymphocytes to antigen stimulation only in the presence of threshold numbers of autologous monocytes added at the initiation of the 6 day culture. The requirement of the T lymphocytes to undergo blastogenesis in vitro to antigen and phytomitogen stimulation appear to be quite

different. The T lymphocytes do not require monocytes in the phytomitogen-induced blastogenic response provided IL-1 or IL-2 is added to the cell cultures. Furthermore, the IL-1 may be derived from autologous or allogeneic monocytes. On the other hand, the T lymphocytes undergo blastogenesis in vitro to antigen stimulation providing autologous, but not allogeneic, monocytes are present. More importantly, IL-1, IL-2 or BCGF cannot facilitate the blastogenic responses of the T lymphocytes to antigen stimulation in the absence of autologous monocytes present in threshold concentration (1% of the cultured cells).

As discussed in Chapter 2.3.5, a number of investigators have demonstrated that B and Null lymphocytes generate significant, albeit minimal, antigen-induced blastogenic responses. The results in Tables 19 and 20 clearly demonstrate that purified B lymphocytes respond insignificantly to antigen stimulation. These results do not support those of Geha et al (1973) who also demonstrated that B lymphocytes cultured in supernatants from PPD-stimulated sensitized T lymphocytes proliferated to TT stimulation. These conflicting results may be explained by the differences in the techniques used to isolate the human circulating B

lymphocytes and the purity of the cell populations obtained and cultured. The "pure" smIg+ B lymphocytes cultured by Geha (1973) were contaminated with T lymphocytes to the extent of 5% as determined by E rosette formation. In this investigation, the intentional contamination of the B lymphocytes with small numbers of T lymphocytes (1% of the cultured cells) resulted in a discernible antigen-induced blastogenic response whereas the intentional contamination of the B lymphocytes with greater numbers of T lymphocytes (5% of the cultured cells) resulted in a significant antigen-induced blastogenic response which can be totally attributed to the added T lymphocytes (Table 17).

The results in this investigation also demonstrate that the Null lymphocytes do not respond with a blastogenic response to antigen stimulation. (Tables 19 and 20). These results support those previously reported by Horwitz and Garrett (1977), Caraux et al (1982) and Ng et al (1981), who concluded that Null lymphocytes were unable to proliferate in the presence of antigens. Furthermore, reconstituting the Null lymphocytes with autologous monocytes (to a final cell concentration 10%) did not facilitate their blastogenic responsiveness (Table 17). The intentional "contamination" of Null

lymphocytes with 1% T lymphocytes resulted in an antigen-induced blastogenic response which can be totally attributed to the added T lymphocytes.

The absence of blastogenic responses by the B and Null lymphocytes in the presence of antigens may conceivably be attributed to the absence of IL-2 or IL-2 secreting T lymphocytes. However, since neither the B nor the Null lymphocytes generated blastogenic responses in the presence of antigen and IL-2 or BCGF, it is obvious that these cells do not, in fact, possess the capacity to respond under the culture conditions utilized in this investigation.

It may therefore be concluded that only the T lymphocytes, and not the B and Null lymphocytes, generate blastogenic responses to antigen stimulation in culture. This T lymphocyte blastogenic response requires the presence of autologous monocytes or exogenous IL-2 in the presence of theshold numbers of autologous monocytes.

The blastogenic responses of "normal" non-allergic and ragweed allergic individuals to phytomitogen, antigen and allergen stimulation were also determined in this investigation. Not surprisingly, the phytomitogen and

antigen-induced blastogenic responses of the cells of the ragweed allergic individuals were essentially the same as those generated by the cells of the non-allergic individuals. Only the circulating MNC and T lymphocytes of ragweed allergic individuals (diagnosed by history and by the RAST test and skin testing for immediate skin reactivity) evoked a signifcant blastogenic response to the allergen AgE (Table 20). The data presented here leaves little doubt that allergic patients possess circulating T lymphocytes sensitized to AgE while the non-allergic individuals do not (Tables 19 and 20). Strannegard et al (1979) demonstrated that individuals with hayfever, asthma or atopic eczema had decreased numbers of circulating T lymphocytes which were hyporesponsive to PHA and Con-A stimulation. The results presented here do not demonstrate a decreased responsiveness of the MNC or the T lymphocytes of ragweed allergic patients to phytomitogen or antigen stimulation (Tables 19, 20, 22, 25 and 26).

A question which must be addressed is why only the circulating T lymphocytes respond to antigen and allergen stimulation (providing a minimal number of monocytes are present in the cultures) irrespective of the immunizing antigen and the interval between immunization and

testing. Certain antigens (for example TT and DT) are known to induce an antibody immune response following immunization which protects the individual. This immune response involves primarily B lymphocytes and it would be logical to expect that, following immunization, the circulating B lymphocytes would undergo proliferation in vitro upon stimulation with these antigens. On the other hand, antigens which induce cell-mediated immune responses (delayed hypersensitivity) following immunization (for example BCG, Candida and PPD) induce the generation of sensitized T lymphocytes. One would therefore expect circulating T lymphocytes to proliferate upon stimulation with these antigens in vitro. However, it is well recognized that antigens which induce a humoral immune response also induce the generation of a cell-mediated immune response (sensitized T lymphocytes) following immunization (Crowle 1960). The sensitized T lymphocytes are continually recirculating. They can be detected in the circulation by their ability to undergo blastogenesis upon stimulation with the sensitizing antigen and by their ability to transfer delayed skin reactivity. On the other hand, the antibody-forming B lymphocytes are found in the circulation only transiently at the peak of antibody formation following immunization and the memory B lymphocytes are normally sequestered to

the spleen. It is therefore logistically difficult to demonstrate circulating B lymphocytes capable of proliferating to antigen stimulation in vitro.

Geha et al (1981), Eugeum and Bona (1977) and Oers (1979) immunized individuals with TT to induced a secondary immune response in otherwise normal individuals. On a daily basis they assessed the antibody titers and the capacity of circulating T and B lymphocytes to respond to antigen stimulation in vitro. The antibody titer (anti-TT IgG) reached a peak at day 8 post-immunization. The circulating T lymphocytes responded with blastogenesis to TT stimulation in vitro irrespective of the time of testing (prior or post-immunization). The proportion of circulating T lymphocytes which proliferated to TT on day 5 was greater than prior to immunization. This increased blastogenic response was sustained until day 14 post-immunization. The circulating B lymphocytes did not respond to TT stimulation in vitro either prior to nor post-immunization. Since B lymphocytes can be detected in the circulation post-immunization which are capable of secreting antibodies in vitro, it is necessary to reconcile this finding with the failure to detect B lymphoproliferation to antigenic stimulation in vitro.

As has been demonstrated previously (Table 18), B lymphocytes proliferate to PWM stimulation on day 7 in vitro only in the presence of IL-2 or BCGF. The question is therefore not whether circulating B lymphocytes are capable of responding with blastogenesis to antigenic stimulation but rather why a significant blastogenic response is not observed when antibody forming B lymphocytes are demonstrated to be present in the circulation. The explanation would appear to be that the antibody forming B lymphocytes in the circulation are present in exceedingly low numbers, in numbers insufficient to generate a perceptable blastogenic response. In point of fact, Geha (1981) demonstrated that immunization with TT resulted in the transient appearance in the circulation of B lymphocytes which secreted IgG anti-TT antibodies. The number of antigen-reactive B lymphocytes capable of binding ^{125}-I-TT reached a peak by day 8 post-immunization (<.0001%) and subsequently declined. On the other hand, the frequency of TT reactive T lymphocytes in the circulation increased dramatically following immunization (2.5%) and peaked by day 14 post-immunization (Geha 1981, Oers 1977). The results presented in Table 36 concur with these findings. The circulating T lymphocytes, but not the B lymphocytes obtained from TT immunized

TABLE 36

THE ANTIGEN-INDUCED BLASTOGENIC RESPONSES OF T AND B LYMPHOCYTES AT DAY 4, DAY 8 AND DAY 15 POST-IMMUNIZATION WITH TT

	DAY 0			DAY 4			DAY 8			DAY 15	
Control	TT	DT	PPD	TT	DT	PPD	TT	DT	PPD	TT	DT
donor 1											
MNC	884 38452	30110	45321	497 47576	31587	44520	816 55519	32339	40268	313 49837	29862
T	167 37463	31289	40398	300 47290	32741	44284	265 52626	30261	41816	416 47470	30301
B	112 532	597	643	103 1269	738	831	141 1237	634	749	184 921	718
B+IL-2	258 638	736	534	219 1597	857	882	546 1926	841	892	254 996	724
B+BCGF	312 630	621	663	114 1440	718	845	224 1131	985	801	312 1021	816
donor 2											
MNC	909 22167	21750	36243	631 35596	22097	32553	382 42553	23107	31028	444 38510	22890
T	293 22304	23624	35821	330 32421	22752	32170	498 40486	21021	29681	385 36642	21816
B	267 818	693	1021	198 1031	964	1062	251 1125	907	864	262 942	413
B+IL-2	414 1091	790	855	381 1154	1032	1099	286 1016	832	1054	216 1021	568
B+BCGF	147 1113	802	892	216 1206	1109	1121	421 1283	1150	1120	119 1244	601
donor 3											
MNC	395 24699	26916	30248	508 43706	25700	31752	619 57063	26431	32573	816 48704	27708
T	291 26901	25249	30097	516 43475	24475	30089	457 55001	25619	31718	701 45203	25543
B	118 921	721	642	112 1467	892	721	316 1021	918	821	206 981	1094
B+IL-2	264 1000	616	801	198 1528	888	785	218 1476	1066	894	198 1127	1125
B+BCGF	218 1108	849	1008	248 1588	1021	816	201 1621	1221	916	268 1369	1296

individuals just prior to immunization and at days 4, 8 and 15 post-immunization responded significantly to TT stimulation. The addition of IL-2 or BCGF to the B lymphocytes did not facilitate antigen stimulated B lymphocyte blastogenic responsiveness. These results indicate that the small numbers of antibody-forming B lymphocytes in the circulation even at the time of peak antibody formation (10^3 per 10^6 circulating lymphocytes) are present in too low a number to generate a significant blastogenic response to TT stimulation in vitro even in the presence of IL-2 or BCGF under the conditions utilized in this laboratory.

The 3 day phytomitogen-induced and the 6 day antigen-induced blastogenic responses are used in the clinical setting to assess and monitor T lymphocyte function in patients presenting with symptoms of immunodeficiency disease and other diseases where the immune system is suspected to have a role (Dean et al 1977, Eibl et al 1982, Hutchins and Steel 1983). The findings presented in this thesis necessitate a reappraisal of the currently-accepted interpretation of the phytomitogen, antigen and allergen-induced blastogenic responses and their relationship to the immune system under investigation. It is generally

assumed that the failure to mount a normal blastogenic response is indicative of defective T lymphocyte function. However, it must now be considered that the defect is not in the T lymphocyte but in the monocyte: monocytes may either be deficient in numbers or they may be defective and be unable to secrete IL-1 which is required for the subsequent proliferation of the T lymphocytes. It must also be recognized that the blastogenic responses to what are usually considered to be "antibody-inducing" antigens i.e. TT, DT, does <u>not</u> infer intact antibody-forming capacity but rather indicates that cell-mediated sensitization took place since only the T lymphocytes, and not the B lymphocytes, responses. Therefore, the blastogenic response of the circulating lymphocytes to antigens in vitro reflects only upon the function state of the cell-mediated compartment of the immune system and not the antibody-forming compartment.

6. SUMMARY

The results of this investigation unequivocally demonstrate that only the T lymphocytes respond with blastogenesis and mitosis following stimulation with phytomitogens (PHA, PWM, Con-A) for 3 days in culture, and antigens (TT, DT, CA, PPD) and allergen (ragweed pollen AgE) in the case of allergic individuals following 6 days in culture. The T_M lymphocytes consistently generated a greater blastogenic response to phytomitogen and antigen stimulation than did the T_G (or the T_C or T_N) lymphocytes. The T4+ and T8+ lymphocytes consistently responds to the same extent, irrespective of the mitogenic stimulus used.

The monocytes are obligatory participants in the phytomitogen, antigen and allergen-induced blastogenic responses. Reconstitution of the monocyte-depleted T lymphocytes with either autologous or allogeneic monocytes restored to the T lymphocytes the capacity to undergo blastogenesis following stimulation with phytomitogens in vitro. In contradistinction, only

autologous and not allogeneic monocytes restored to the T lymphocytes, the capacity to undergo blastogenesis following stimulation with antigens and allergen in vitro.

Supernatants from phytomitogen-stimulated or unstimulated cultured monocytes (autologous or allogeneic) were capable of restoring to T lymphocytes blastogenic responsiveness to phytomitogen stimulation without the need of monocytes in the cultures. On the other hand, supernatants from phytomitogen or antigen-stimulated cultured monocytes (autologous or allogeneic) were capable of restoring to T lymphocytes blastogenic responsiveness to antigenic stimulation providing autologous monocytes are present in the culture in threshold numbers.

The B lymphocytes cultured in the presence of IL-2 or BCGF responded with blastogenesis to PWM stimulation following seven days in culture. The B lymphocytes did not respond with blastogenesis following stimulation with antigens (TT, DT, CA, PPD), allergen (AgE) or the phytomitogens PHA and Con-A. The Null lymphocytes do not respond to phytomitogen, antigen and allergen stimulation even when cultured for up to 7 days in the presence of monocytes, IL-1, IL-2 or BCGF.

It should be stressed that, although the TT and DT antigens are considered to induce primarily humoral (antibody) immune responses, nevertheless only the circulating T lymphocytes and not the circulating B lymphocytes undergo blastogenic responses in vitro when incubated with these antigens irrespective whether the circulating cells were obtained 4, 8 or 15 days or 5 to 10 years following immunization. The failure to detect B lymphoproliferative responses to antigenic stimulation in vitro may be attributed to the absence of antigen responsive B lymphocytes in the circulation or their presence in the circulation in numbers too low to generate significant blastogenic responses.

7. ORIGINAL CONTRIBUTIONS TO KNOWLEDGE

1. The applicant has demonstrated that only the circulating T lymphocytes respond with blastogenesis and mitosis following stimulation with phytomitogens (PHA, PWM and Con-A) after three days in culture. The B and Null lymphocytes do not respond nor do they participate in or contribute to the T lymphocyte blastogenic response.

2. The applicant has demonstrated that only the circulating T lymphocytes respond with blastogenesis following stimulation with antigen (TT, DT, CA and PPD), and allergen (AgE) in the case of allergic volunteers, following six days in culture. The B and Null lymphocytes do not respond nor do they participate in or contribute to the T lymphocyte blastogenic response.

3. The applicant has demonstrated that only the T lymphocytes are the sole responder cells which proliferate to antigen stimulation irrespective of the immunizing antigen and the interval of time between immunization and testing (4, 8 or 15 days or 5 to 10 years).

4. The applicant has demonstrated that the T_M lymphocytes consistently generate greater blastogenic response than do the T_G lymphocytes irrespective of the mitogenic stimulus used (phytomitogen, antigen or allergen). The T_C lymphocytes and the T_N cells consistently give lower blastogenic responses than do the T_G lymphocytes.

5. The applicant has demonstrated that the T4+ and T8+ T lymphocytes consistently respond to a similar extent irrespective of the mitogenic stimulus.

6. The applicant has demonstrated that only the circulating B lymphocytes in the presence of IL-2 or BCGF respond with blastogenesis following stimulation with the phytomitogen PWM following seven days in culture. The B lymphocytes in the presence of IL-2 or BCGF do not respond with blastogenesis following stimulation with the phytomitogens PHA and Con-A or the antigens following culture of the cells for up to seven days.

7. The applicant has demonstrated that the monocytes are obligatory participants in the phytomitogen-induced T lymphocyte blastogenic responses. Monocyte-depleted MNC do not respond to the mitogenic stimulus. Reconstitution

of the monocyte-depleted MNC with either autologous or allogeneic monocytes restores to the T lymphocytes the capacity to undergo blastogenesis following stimulation with phytomitogens in vitro.

8. The applicant has demonstrated that the monocytes are essential participants in the antigen and allergen-induced blastogenic responses of the T lymphocytes. Monocyte-depleted MNC do not respond in vitro to antigen or allergen stimulation. Reconstitution with autologous monocytes restores to the T lymphocytes the capacity to undergo blastogenesis following stimulation with the antigens and allergen in vitro. However, in contrast to the blastogenic response to phytomitogen stimulation, reconstitution with allogeneic monocytes does not restore blastogenic responsiveness to antigenic stimulation.

9. The applicant has demonstrated that supernatants from PHA, PWM and Con-A-stimulated or unstimulated cultures of autologous or allogeneic monocytes are capable of restoring to the T lymphocyte blastogenic responsiveness to phytomitogen stimulation. Viable monocytes are not essential for the phytomitogen-induced blastogenic response to take place. It is presumed that the mediator generated is similar to, if not identical with, IL-1.

10. The applicant has demonstrated that supernatants from phytomitogen (PHA) or antigen (TT)-stimulated cultures of autologous or allogeneic monocytes are capable of restoring to the T lymphocytes blastogenic responsiveness to antigenic stimulation provided autologous, and not allogeneic, monocytes are present in threshold numbers. Thus, for the antigen-induced blastogenic response, IL-1 by itself cannot replace monocytes as it can in the phytomitogen (PHA, PWM, Con-A)-induced blastogenic responses.

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