Investigating the effects of adoptive transfer of LAK cells on the growth of B16-F10 melanoma cells in murine lungs

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I. Introduction

Immunotherapy is a promising new approach to cancer therapy that involves enhancing the body's natural immune responses to pathogens in the body. Benefits of cancer immunotherapy include the potential to provide a gentler therapy that, unlike many chemotherapeutic agents, would not have cytotoxic effects on peripheral non-cancerous cells.

The particular immunotherapeutic method of interest in this project is adoptive cell transfer, which consists of the extraction of antitumour effector cells and the subsequent expansion and activation of these cells with stimulatory cytokines before injection into the patient. This project investigates the use of natural killer (NK) cells, which take part in the initial response to defend against tumour and virus-infected cells. NK cells are able to target infected or foreign cells in tissues or the circulation and kill them by secreting cytotoxic granules containing perforin, a cytolytic protein, as well as cytotoxic proteases called granzymes.

The 2 strains of mice used in this experiment are wild-type mice that have their own NK cells, and mice that are devoid of their own natural killer cells, causing the tumour burden to be extremely high in this type of mice. Roughly half of each strain has undergone the adoptive transfer of LAK cells (lymphokine-activated NK cells) 2 days after being infected with the murine melanoma model.

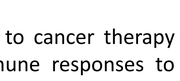
The objective is to determine the effectiveness of adoptive transfer of LAK cells in inhibiting the growth of tumours of the murine melanoma model B16-F10 in the lungs. Visual analysis and counting of the growths of metastatic tumours on the surfaces of the harvested lungs of the mice will provide insight into the immunotherapeutic effects of the LAK cells with respect to changes in tumour burden.

II. Methods

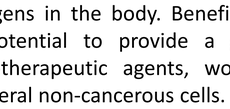
1. Enriching and culturing murine spleen–derived NK cells in RP-10 media

2. Expansion and activation of NK cells with the cytokine IL-2 (Duration: 1 week)

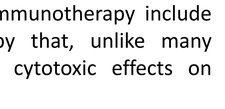
3. Culturing and harvesting of B16-F10 murine melanoma cells in 5% FBS DMEM media



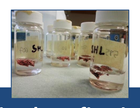
4. Day 1: Intravenous injection to the tails of all 11 mice with B16-F10 murine melanoma cells (4E5 cells/male, 2E5 cells/female)



5. Day 3: Intravenous injection to the tails of 6 of the mice (3 NK cell-deficient, 3 WT) with LAK cells (2E6 LAK cells/mouse)



6. Day 14: Euthanasia of mice and harvesting of lungs



7. Murine lung fixation with glutaraldehyde, staining with X-gal, and preservation in formalin.

8. Visual analysis of murine lung surfaces using ImageJ software

III. Results

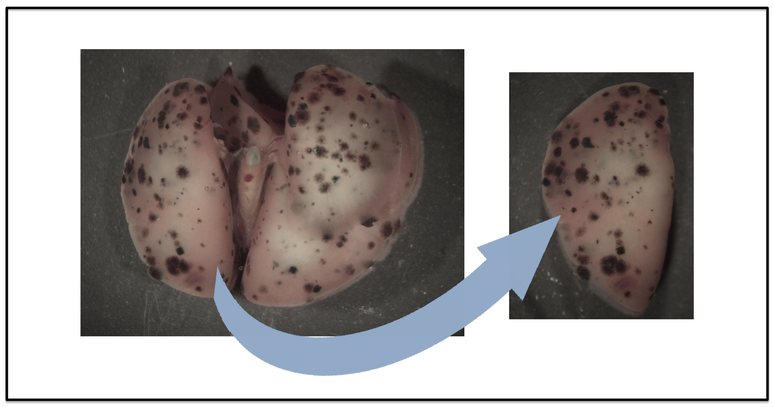


Figure 1: Full set of lungs and right lung of mouse D1, a wild-type mouse that has undergone adoptive transfer of LAK cells. For the purposes of this experiment, one side of each murine right lung was analyzed for its relative tumour burden (ie. qualitative appearance of stained lung surface and of visible tumour nodules).

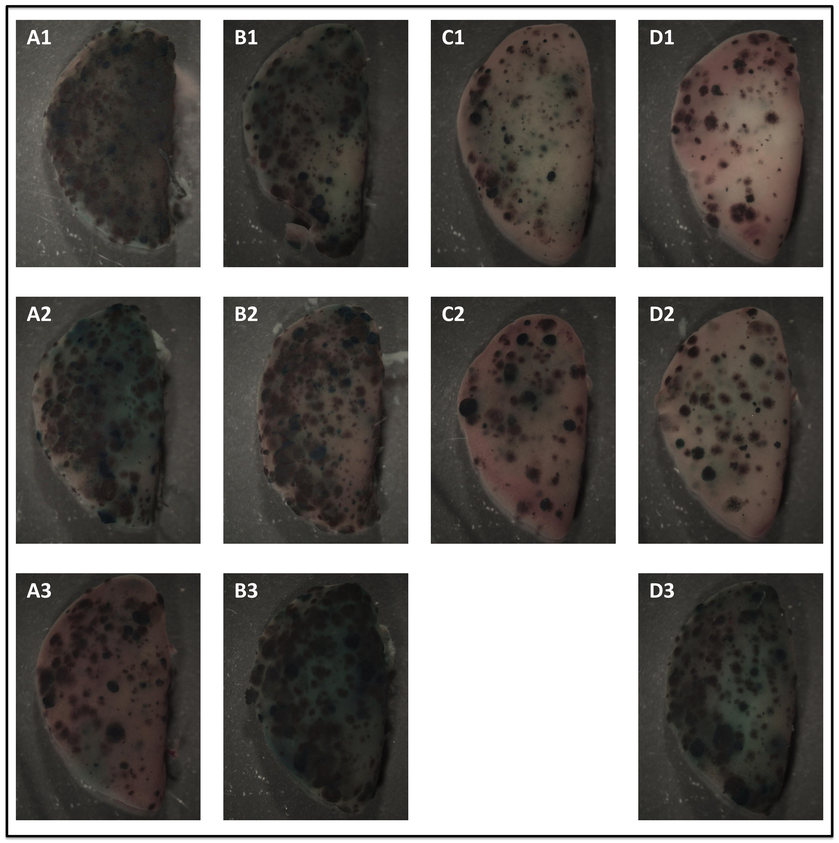


Figure 2: Right lungs of the 4 categories of mice infected with the murine melanoma model B16-F10. The lungs have been treated with an X-Gal stain, which induces the production of blue pigment in the B16-F10 tumour cells. **A1-A3:** Right lungs of NK cell-deficient strain of mice that have not undergone adoptive transfer of LAK cells. **B1-B3:** Right lungs of NK cell-deficient mice that have undergone adoptive transfer of LAK cells. **C1-C2**: Right lungs of WT mice that have not undergone adoptive transfer of LAK cells. **D1-D3**: Right lungs of WT mice that have undergone adoptive transfer of LAK cells.

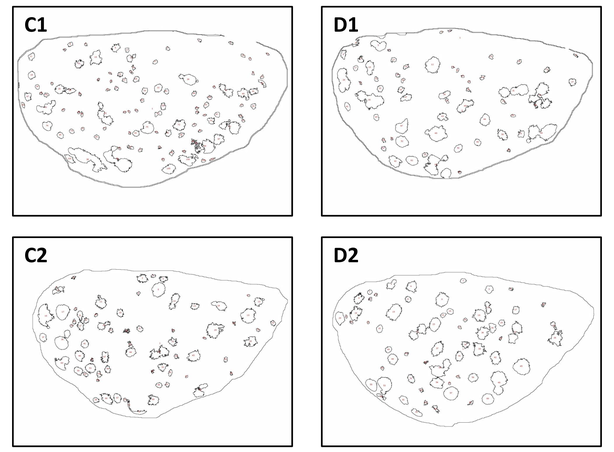


Figure 3: Comparing the tumour counts on the surfaces of the right lungs of mice C1 and C2, and D1 and D2 using ImageJ software.
Mouse **C1** was found to have a tumour nodule count of **105**, and
Mouse **C2** was found to have a tumour nodule count of **72.**
Mouse **D1** was found to have a tumour nodule count of **63**, and
Mouse **D2** was found to have a tumour nodule count of **65**.

IV. Conclusions

- The visibly decreased amount of tumour nodules as well as the decrease in blue X-Gal stain in the lungs of the B1 and B2 mice compared to those of A1 and A2 suggest that the adoptive transfer of LAK cells in NK cell-deficient mice has some effect in the inhibition of B16-F10 cell growth in the lungs.
- Quantitative analysis of the WT mice using the ImageJ software shows that the number of tumour nodules has decreased with adoptive transfer of LAK cells – this suggests that this immunotherapeutic method may also be effective in inhibiting B16-F10 cell proliferation in individuals that are not immunosuppressed.
- Staining with X-Gal caused the murine lungs with higher tumour burdens (ie. from the NK cell-deficient strain of mice) to be stained to the extent at which quantitative analysis of the lungs (which consists of using the ImageJ software to count the tumour nodules on the right lobe surfaces) became impossible. Possible future studies may include modification of the staining protocol to prevent excessively dark staining of the lung surfaces, and subsequent quantitative and statistical analysis of the results.
- 2 of the murine lungs, A3 and D3 in Figure 2, show staining patterns and tumour nodules that do not match the tentative trend identified in the others. These may have arisen due to experimental errors, or due to uncontrollable variables such as abnormalities in the mouse itself or the specific conditions of the cage it was in.
- The comparatively light tumour burden in mouse A3, in particular, may be due to experimental errors during the intravenous injection of the B16-F10 cells, as this mouse does not seem to have been infected to the extent of the other NK cell-deficient mice (A1-A3).

V. Acknowledgements

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