T cell synergy in anti-tumour responses

Principal supervisor's name: Dr **Arianne Richard**
Principal supervisor's email address: arianne.richard@babraham.ac.uk

Principal supervisor's CRUK CC theme: Cancer Immunology Programme

Department for student registration: Babraham Institute
Department or institute where research will take place:
Babraham Institute

Postgraduate scheme:

- **MRes + PhD (1 + 3-year non-clinical applicants)**
- **Part-time MRes + PhD (2 + 3-year clinical applicants)**

MRes project outline:

CD8+ T cells are a critical component of the anti-tumour immune response. Tumour-infiltrating lymphocytes have been found to include not only T cells with high affinity for tumour antigens, but also many with low or no tumour reactivity. How low-affinity T cells contribute to the anti-tumour immune response and whether they can be therapeutically leveraged remain important questions in cancer immunology.

In naïve CD8+ T cells, previous work has demonstrated that strongly stimulated cells can enhance the activation of nearby weakly stimulated cells through cytokine secretion. Effector cells also secrete and respond to cytokines and chemokines, but it is unclear whether similar synergistic behaviour occurs as they exert their cytolytic effector functions to kill tumour cells. This project will test for the presence and nature of synergistic behaviour between high- and reduced-affinity effector CD8+ T cells responding to tumour cells in vitro.

Aims:
1. Determine whether co-culture with high-affinity effector CD8+ T cells affects tumour cell killing or cytokine secretion by reduced-affinity T cells.
2. Test whether co-culture with high-affinity effector CD8+ T cells alters migration toward and tumour infiltration by reduced-affinity T cells.
3. Identify and test putative molecular mediators by which effector CD8+ T cells of different affinities interact.

This project will address whether and how high- and reduced-affinity tumour-reactive effector CD8+ T cells interact in a minimal in vitro setting. This will lay the groundwork for future experiments testing the activity of reduced-affinity tumour-infiltrating T cells in a more physiological context, allowing further study of the interplay with the tumour microenvironment and relationship to T cell exhaustion.

MRes experimental plan:

Experiments in this project will leverage TCR-transgenic effector CD8+ T cells with different affinities for a known antigen to test for cooperative behaviour between T cell populations in an in vitro setting. Specifically, tumour cell killing, cytokine secretion, infiltration among tumour cells,

and attraction/activation of other T cells will be examined. Techniques will include tissue culture, flow cytometry, and live imaging assays for cytolytic activity, infiltration, and chemotaxis.

If synergy is observed, we will leverage complementary genomic data generated for other projects in the lab, in combination with the literature, to identify putative mechanisms of communication between T cell populations. This will provide opportunities for training in bioinformatic data analysis. Specific pathways may then be tested by chemical, biological or genetic perturbation using the techniques listed above if time permits.

PhD project outline:

Therapeutic introduction of expanded or engineered T cells with high affinity receptors for tumour antigens can both directly combat cancer cells and also alter the endogenous anti-cancer response. Mechanisms of the latter are incompletely understood but involve both direct effects on other immune cells and indirect effects via the tumour microenvironment.

This PhD project will build upon its precursor MRes project, which looks for synergistic behaviour between CD8+ T cell with high and reduced affinities for tumour antigens in an in vitro model system. Based on the MRes project results, the PhD project will take two complementary directions: 1) examining the precise nature of communication between CD8+ T cells of differing affinities in vitro; 2) using in vivo models to understand how any synergistic behaviours play out in a physiological context.

Aims:
1. Test putative mechanisms of communication between high- and reduced-affinity CD8+ T cells identified during the MRes project through genetic, chemical or biological perturbation.
2. Determine how the introduction of exogenous tumour reactive CD8+ T cells impacts other T cell activities and phenotypes and the tumour microenvironment.

This project will investigate direct and indirect T cell communication in a tumour setting and examine how this communication impacts T cell phenotypes and anti-tumour function. Results have the potential to reveal means of mimicking beneficial effects of communication in a non-cellular manner.

PhD experimental plan:

This project will use in vitro and in vivo experiments to test mechanisms of direct and microenvironment-mediated interaction between tumour-specific T cells. In vitro assays of phenotype and function will examine tumour cell killing, cytokine secretion, infiltration among tumour cells, and attraction/activation of other T cells. Putative mediators of synergy between T cells with high and reduced tumour antigen affinity will be tested by antibody- or drug-based blocking experiments in the first instance, followed by genetic perturbation where appropriate. Established in vitro mechanisms may also be tested for in vivo effects where feasible.

Parallel in vivo mouse work using adoptive transfer of TCR transgenic CD8+ T cells with high and reduced affinity to a known tumour antigen will be used to test how these tumour-specific cells impact the phenotypes and functions of other tumour infiltrating lymphocytes and the tumour microenvironment. These experiments will take advantage of high-dimensional flow cytometry and imaging platforms, as well as transcriptomic profiling of sorted responding T cell populations. Training in bioinformatics and statistical analyses will be provided to take full advantage of these data. Putative mediators of T cell interactions with each other or the

microenvironment may be tested by antibody-mediated blockade, CRISPR-Cas9 deletion in adoptively transferred cells, or genetic knock-out models. If adoptively transferred T cells are found to improve anti-tumour responses by other responding T cells, we will test whether this can be recapitulated by direct administration of putative mediators without cellular transfer.

Main techniques:

• Tissue culture and in vitro assays of cytotoxic activity, cytokine secretion, chemotaxis, and infiltration
• In vivo experiments with mouse tumour models engineered to express specific known antigenic ligands (in collaboration with the de la Roche lab, CRUK-CI)
• Flow cytometry, including spectral
• Microscopy, including confocal and potentially MACSima
• RNA-seq
• Bioinformatic analysis of high-dimensional data

Key references:

From the lab:

Richard AC. (2022) Divide and conquer: temporal and phenotypic heterogeneity within cytotoxic T cell responses. Front Immunol, 13:949423.

Ma CY, Marioni JC, Griffiths GM, Richard AC. (2020) Stimulation strength controls the rate of initiation but not the molecular organisation of TCR-induced signalling. Elife, 9:e53948.

Richard AC, et al. (2018) T cell cytolytic capacity is independent of initial stimulation strength. Nat Immunol, 19:849.

Other selected references:

Barras D, et al. (2022) Tumor microenvironment cellular crosstalk predicts response to adoptive TIL therapy in melanoma. BioRxiv, doi: 10.1101/2022.12.23.519261.

Guerra E, et al. (2022) Cancer-homing CAR-T cells and endogenous immune population dynamics. Int J Mol Sci, 23:405.

Galeano Nino, et al. (2020) Cytotoxic T cells swarm by homotypic chemokine signalling. Elife, 9:e56554.

Voisinne G, et al. (2015) T cells integrate local and global cues to discriminate between structurally similar antigens. Cell Rep, 11:1208.