

Immunofluorescence: towards a diagnostic test

A diagnostic test for tolerance, looking for regulatory T lymphocytes or their products, must be simple and convenient to perform on patients. Ideally this means using a readily obtainable sample such as urine or blood. Normal blood is a mixture of different cells types, and we need a test that can identify the rare regulatory T lymphocyte amongst all the others. This can be done by making antibodies against the regulatory gene protein product. These antibodies can be labelled with different coloured fluorescent dyes such as **phycoerythrin** or **allophycocyanin**. We can then take a blood sample, remove the red blood cells, and use such fluorescently labelled antibodies that bind to different molecules that are unique to the T lymphocyte cell surface, such as the *T cell receptor* (TCR) on all T lymphocytes, and the *CD4 molecule* on "helper" subset of T cells.

It is also possible to stain for gene products that remain inside the cell or are being transported ready for secretion, such as the molecules that T lymphocytes use to talk to each other, called *cytokines*. This is done by fixing the cells and then permeabilising the surface membrane with a mild detergent, or saponin. In this way we can further discriminate amongst the TCR positive, CD4 positive lymphocytes with **fluorescein** labelled antibodies against effector gene products and **Cy-Chrome** labelled antibodies against regulatory molecules.

Fluorescence Activated Cell Scanning of Blood Samples

The *FACS machine* injects the fluorescently labelled mixture of white blood cells into a flow cell such that the cells rapidly pass (at around 2000 per second), in single file, past a focussed laser beam. This laser light not only excites the four different coloured fluorescent dyes, but is also bent and scattered by each cell as it passes through the beam. Larger cells bend the light more, while more granules and complex nuclei increase the scatter at high angles. There are sensitive detectors that can then measure the signals from the size, complexity, and each of the four different fluorescent colours, from each cell as it passes through the laser beam. The data is all fed into a computer that accumulates and correlates the information on anything between 10,000 and 100,000 cells from the blood sample.

The accumulated data is often presented in the form of *dot plots*, where each dot plotted on a two dimensional graph represents the amount of any two selected measurements for each cell. Similar populations of cells will then appear as a *cluster* of dots. We can then select, for example, the lymphocytes based on their small cell size and low granularity and look at two further measurements on just these cells, such as the **T cell receptor** and **CD4**, on a new *dot plot*. We can then select just the CD4 positive T cells and look at the expression of the **effector** versus **regulatory** gene products, and thereby determine the proportion of regulatory T lymphocytes in the blood.