ELISPOT Assay: Cytokine

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Principles of the assay

The cytokine ELISPOT assay is based on the enzyme-linked immunosorbent technique and is designed to enumerate cytokine-secreting cells; it is extremely sensitive and therefore useful in detecting low frequency cytokine-secreting cells (up to 1 in 300,000). The cytokine released in response to antigen can be mapped to a single cell hence T cell responder frequencies can be calculated. Notably, the information gained from ELISPOT assays is both quantitative and qualitative, as it gives an indication of the type of cytokine response that has been elicited. The main application of the ELISPOT assay is in monitoring of immune responses in both humans and animals.

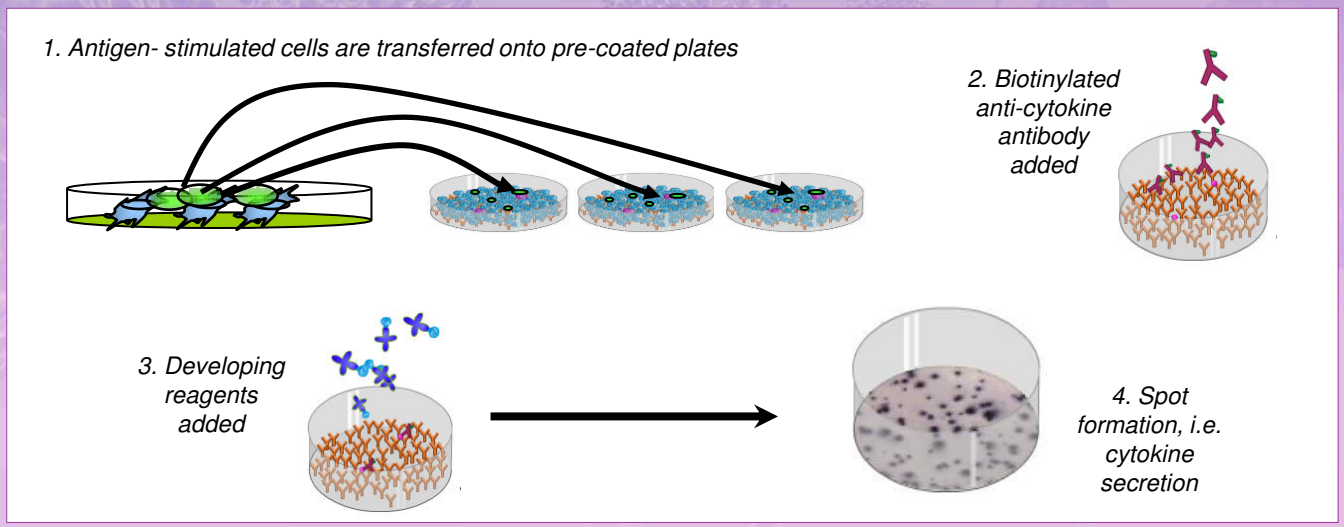

Figure 1. Overview of ELISPOT Assay
Cells are stimulated with antigen or mitogen for 48 hours and then transferred to wells of a plate pre- coated with anti-cytokine antibody for 18-22 hours. A biotinylated anti-cytokine antibody is added followed by a phi-labelled anti-biotin antibody. Developing reagents are then added and spots can be visualised.

Cytokine ELISPOT assay procedure

A high affinity monoclonal anti-cytokine antibody is coated aseptically onto a PVDF or high-protein binding polystyrene plate. Antigen or mitogen-stimulated cells are then pipetted into the wells and incubated for a specified period of time; this allows for any secreted cytokine to be promptly captured by the immobilised antibody. After a washing step to remove the cells and any unbound substances, bound cytokine is detected using biotinylated antibody and a phi-labelled anti-biotin antibody. The final step is the precipitation of, e.g. silver on phi, exposing the sites of cytokine secretion depicted as a spot. The spots can be counted with an automated ELISPOT reader or manually using a dissecting microscope. This procedure is summarized in **Figure 1**.

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Application of the cytokine ELISPOT assay

The cytokine ELISPOT has been successfully used across several disciplines of Immunology. In organ-transplantation, these assays can monitor the frequency and profile of circulating donor-reactive T cells. In cancer research, cytokine ELISPOTs have been used to measure CTL activity; in infectious diseases and vaccine development, these assays have been used to measure memory responses; and in autoimmune diseases, the sensitivity of these assays has been shown to be very useful in detecting autoreactive cells which typically occur at low frequencies (**Figure 2**).

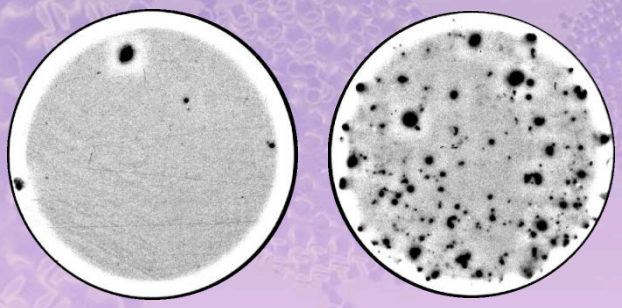

Figure 2. Interferon-γ responses in type 1 diabetes. Interferon-γ responses to a naturally processed and presented peptide of the autoantigen proinsulin (left) and the pentavaccine, Pediacel (right).

Cytokine ELISPOT assays in type 1 diabetes

In type 1 diabetes, the assay was used to show that autoreactive responses exist in both patients and in healthy controls, but in the latter these are characterised by a predominant regulatory IL-10 response, whereas in patients the response is a pro-inflammatory one characterised by the production of interferon-γ in response to stimulation with peptides of islet autoantigens (**Figure 3**). The cytokine ELISPOT has also been used in monitoring the inflammatory/regulatory T-cell balance in patients undergoing peptide trials.

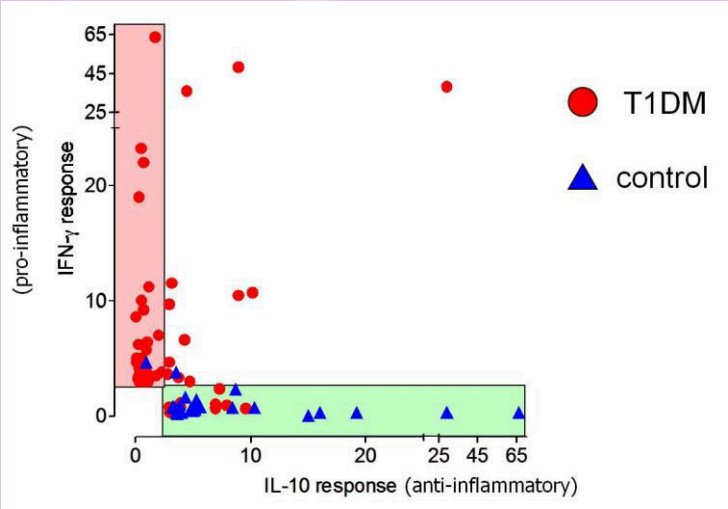

Figure 3. Autoreactive T-cell responses in type 1 diabetes
Polarisation of autoreactive T cell responses to islet autoantigen peptides in patients with type 1 diabetes (red circles) and nondiabetic control subjects (blue triangles).