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ELISPOT in Autoimmune Diseases

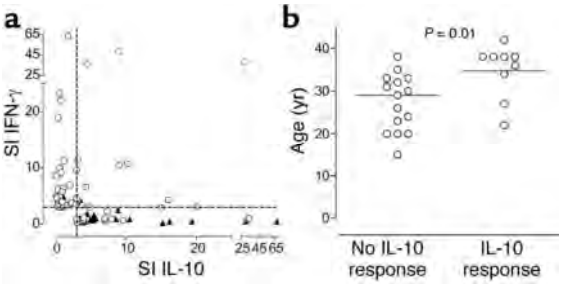
ELISPOT assays are among the most-sensitive and -specific methods available for the detection of T cell responses. Major advantages are its relatively fast and easy performance, high sensitivity (detection level of one cell out of one million), its potential for high throughput screening and no requirement for expensive instruments. The high sensitivity of the ELISPOT assay is particularly useful in autoimmune studies, as autoreactive T cell responses are typically of much lower frequency than those found for viral and tumor T cell responses. For example in human diabetes Type 1, a chronic autoimmune disease leading to selective destruction of insulin producing ß-cells, T cells play a key role, but the detection of these lymphocytes is difficult. Fortunately, cytokine secretion by autoantigen reactive T cells can be demonstrated in individual cells with the use of the ELISPOT assay, offering preclinical diagnoses and immune surrogate end points for clinical trials. Many studies have investigated cytokine production (e.g. IFN-γ, IL-5, IL-10, IL-13) by antigen-reactive peripheral blood mononuclear cells with the use of the ELISPOT assay and related T cell responses with ß-cell function.

For example, Arif *et al.* (2004) used the ELISPOT assay to examine the relationship between IL-10 and IFN-γ responses to two different islet autoantigen peptides in Type 1 Diabetes Mellitus (T1DM) and in nondiabetic, HLA-matched control subjects. They used a stimulation index (the so-called SI) (ratio of mean spot number in the presence of test peptide to mean spot number in the presence of peptide diluent) to allow comparison between patients and control subjects while taking account of background (spontaneous responsiveness). With use of a ROC plot analysis they choose a cut-off value, which provided the greatest sensitivity and specificity in the discrimination of patients from controls in the ELISPOT.

As shown in the figure below (a) the authors demonstrated a strong polarization of autoreactive T cell responses to the 2 islet autoantigen peptides in patients with T1DM (open circles) and nondiabetic control subjects (closed triangles). Each positive peptide response to IFN-γ or IL-10 has been plotted in this figure. The authors found a highly significant inverse correlation between responses represented by each of these cytokines ($P = 0.000004$), indicating strong polarization of proinflammatory and regulatory autoreactivity. Patients with T1DM are clustered close to the *y* axis, and the control subjects are distributed along the *x* axis, indicating the association of disease and tolerant states with proinflammatory and regulatory responses, respectively. In contrast, the authors found no inverse correlation between IFN-γ and IL-10 responses to tetanus toxoid ($P = 0.64$), which was included as one of the controls in the ELISPOT assay.

Additionally, the authors demonstrated that patients with T1DM who made IL-10 responses to one of the 2 islet peptides tended to be significantly older at diagnosis of disease than those who did not ($P = 0.01$; Figure part b) and suggested that this quality of IL-10 response is associated with a later disease onset.

Figure. Polarization of autoreactive T cell responses to islet autoantigen peptides in patients with Type 1 Diabetes Mellitus (open circles) and nondiabetic control subjects (closed triangles).



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Examples of studies using our ELISPOT assays:

Arif S, Tree TI, Astill TP, Tremble JM, Bishop AJ, Dayan CM, Roep BO, and Peakman M.
Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health.
J Clin Invest 113:451-63 (2004). *Abstract*
U-CyTech products used in this study:
Human IFN-γ ELISPOT kit
Human IL-4 ELISPOT kit
Human IL-10 ELISPOT kit

Enee E, Martinuzzi E, Blancou P, Bach JM, Mallone R, and van Endert P.
Equivalent specificity of peripheral blood and islet-infiltrating CD8+ T lymphocytes in spontaneously diabetic HLA-A2 transgenic NOD mice.
J Immunol **180**:5430-8 (2008). *Abstract*
U-CyTech products used in this study:
Mouse IFN-γ ELISPOT antibody pair

Han G, Wang R, Chen G, Wang J, Xu R, Feng J, Yu M, Wu X, Qian J, Shen B, and Li Y.
Gene delivery GAD 500 autoantigen by AAV serotype 1 prevented diabetes in NOD mice: transduction efficiency do not play important roles.
Immunol Lett **115:**110-6 (2008). *Abstract*
U-CyTech products used in this study:
Mouse IFN-γ ELISPOT
Mouse IL-4 ELISPOT
Mouse IL-10 ELISPOT

Haanstra KG, Endell J, Estevao D, Kondova I, and Jonker M.
Blocking T cell co-stimulation using a CD80 blocking small molecule reduces delayed type hypersensitivity responses in rhesus monkeys.
Clin Exp Immunol **158:**91-8 (2009). *Abstract*
U-CyTech products used in this study:
Monkey IFN-γ ELISPOT
Monkey species: *Macaca mulatta*

Martin S, Wolf-Eichbaum D, Duinkerken G, Scherbaum WA, Kolb H, Noordzij JG, and Roep BO.
Development of type 1 diabetes despite severe hereditary B-lymphocyte deficiency.
N Engl J Med **345:**1036-40 (2001). *Abstract*
U-CyTech products used in this study:
Human IFN-γ ELISPOT kit
Human IL-4 ELISPOT kit
Human IL-5 ELISPOT kit
Human IL-10 ELISPOT kit
Human IL-13 ELISPOT kit

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Pinkse GG, Tysma OH, Bergen CA, Kester MG, Ossendorp F, van Veelen PA. Keymeulen B. Pipeleers D. Drijfhout JW, and Roep BO.
Autoreactive CD8 T cells associated with beta cell destruction in type 1 diabetes.
Proc Natl Acad Sci U S A **102:**18425-30 (2005). *Abstract*
U-CyTech products used in this study:
Human IFN-γ ELISPOT
Human Granzyme B ELISPOT
Human IL-10 ELISPOT

Raz I, Elias D, Avron A, Tamir M, Metzger M, and Cohen IR.
Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial.
Lancet **358:**1749-53 (2001). *Abstract*
U-CyTech products used in this study:
Human IFN-γ ELISPOT kit
Human IL-4 ELISPOT kit
Human IL-10 ELISPOT kit
Human IL-13 ELISPOT kit

van der Meide PH, de Labie MC, Ruuls SR, Groenestein RJ, Botman CA, Olsson T, and Dijkstra CD.
Discontinuation of treatment with IFN-beta leads to exacerbation of experimental autoimmune encephalomyelitis in Lewis rats. Rapid reversal of the antiproliferative activity of IFN-beta and excessive expansion of autoreactive T cells as disease promoting mechanisms.
J Neuroimmunol **84:**14-23 (1998). *Abstract*
U-CyTech products used in this study:
Rat IFN-γ ELISPOT kit

van Halteren AG., van Etten E, de Jong EC, Bouillon R, Roep BO, and Mathieu C.
Redirection of human autoreactive T-cells Upon interaction with dendritic cells modulated by TX527, an analog of 1,25 dihydroxyvitamin D(3).
Diabetes **51:**2119-25 (2002). *Abstract*
U-CyTech products used in this study:
Human IFN-γ ELISPOT kit
Human IL-2 ELISPOT kit
Human IL-10 ELISPOT kit
Human IL-13 ELISPOT kit

Zanone MM, Favaro E, Miceli I, Grassi G, Camussi E, Caorsi C, Amoroso A, Giovarelli M, Perin PC, and Camussi G.
Human mesenchymal stem cells modulate cellular immune response to islet antigen glutamic acid decarboxylase in type 1 diabetes.
J Clin Endocrinol Metab **95:**3788-97 (2010). *Abstract*
U-CyTech products used in this study:
Human IFN-γ ELISPOT kit
Human IL-4 ELISPOT kit
Human IL-10 ELISPOT kit

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