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Insulin auto-antigenicity in type 1 diabetes

Arising from: M. Nakayama *et al.* *Nature* **435**, 220–223 (2005) and S. C. Kent *et al.* *Nature* **435**, 224–228 (2005)

Spontaneous type 1 diabetes occurs when the autoimmune destruction of pancreatic β -islet cells prevents production of the hormone insulin. This causes an inability to regulate glucose metabolism, which results in dangerously raised blood glucose concentrations. It is generally accepted that thymus-derived lymphocytes (T cells) are critically involved in the onset and progression of type 1 diabetes, but the antigens that initiate and drive this destructive process remain poorly characterized — although several candidates have been considered¹. Nakayama *et al.*² and Kent *et al.*³ claim that insulin itself is the primary autoantigen that initiates spontaneous type 1 diabetes in mice and humans, respectively, a result that could have implications for more effective prevention and therapy. However, I believe that this proposed immunological role of insulin may be undermined by the atypical responses of T cells to the human insulin fragment that are described by Kent *et al.*³.

Nakayama and colleagues² eliminated the normal insulin genes in a non-obese diabetic (NOD) mouse model and replaced them with an engineered gene encoding insulin that is hormonally active but fails to stimulate T-cell responses. The mice did not develop insulin antibodies, inflammation of β -islet cells (insulinitis), or diabetes. This important and surprising finding indicates that the absence of an immune response to a single fragment of the insulin B-chain, residues 9–23, could be sufficient to prevent onset of a disease that might be expected to be mediated by immune reactivity to many other potential β -cell autoantigens.

How could this happen? T cells recognize, and are activated by, antigenic peptide fragments bound to gene products of the major histocompatibility complex (MHC). CD4⁺ T cells in the NOD mouse recognize the insulin B9–23 fragment complexed to the I-A^{B7} MHC class II molecule⁴; CD8⁺ T cells recognize a related fragment, B15–23, in association with the H-K^d class I MHC molecule⁵. The mutated insulin transgene used by Nakayama *et al.* encodes a molecule with a tyrosine-to-alanine replacement at residue 16: presumably this causes the B9–23 fragment to bind with insufficient avidity to this I-A^{B7} molecule and/or failure of the B15–23 peptide to bind to H-K^d, making these insulin fragments unrecognizable to the T-cell-receptor repertoire. Prevention of diabetes in these NOD mice therefore involved replacing an immunogenic and hormonally active insulin molecule with a non-immunogenic one.

Kent and colleagues³ recovered T-cell lymphocytes from the pancreatic-draining lymph

nodes of diabetic patients, cloned them and attempted to identify the protein antigen fragments recognized by these T cells. Remarkably, a substantial proportion (about 50%) of CD4⁺ T cells recognized the amino-terminal fragment (residues 1–15) of the insulin A-chain. More surprising, however, is that the proliferative responses by these clones to the insulin peptide were at best minimal: stimulation indices were three- to sixfold; one of three clones gave an 80-fold increase in interleukin-13 production, whereas the other two produced interleukin-13 in amounts that were only three to six times above background; and very large amounts of peptide (1 mM, or about 1.6 mg ml⁻¹) were required to elicit these responses.

These seem to be valid T-cell responses in that they can be blocked by monoclonal antibodies against MHC class II peptides, but they are notably atypical in the amount of antigen required for stimulation and in the level of the resulting response. For example, this does not compare with the functional avidity of most myelin-specific CD4⁺ T cells from patients with multiple sclerosis, which have EC₅₀ values (where EC₅₀ is the effective concentration of antigen that causes half-maximal response) in the range 1–50 μ M (ref. 6), corresponding to an antigen concentration that is 200–1,000-fold lower; also, a typical encephalitogenic CD4⁺ T-cell clone in experimental allergic

encephalomyelitis, an autoimmune murine model for multiple sclerosis, requires a concentration of a myelin basic protein peptide fragment in the 300 nM range (about 300 ng ml⁻¹) for activation⁷, which is 5,000-fold lower.

The results of Kent *et al.* may indicate either that the native ligand for these pancreatic lymph-node T-cell clones uses a sequence other than the first 15 residues of the insulin A-chain and is a different nominal antigen that may be unrelated to the autoimmune process, or that these anti-insulin clones have a very low avidity. But in the latter case, where in the body would the required concentration of insulin peptide be available to activate these cells?

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Kent *et al.* reply

Replying to: D. B. Wilson *Nature* **438**, doi:10.1038/nature04423 (2005)

Wilson discusses the avidity of the lymph-node T-cell clones we described¹ that recognize the insulin A1–15 fragment. He questions whether large enough concentrations of insulin would be available *in situ* for antigenic stimulation of T cells², given the amount of peptide required to stimulate the clones and their degree of responsiveness.

To evaluate the response of these insulin-reactive T cells generated without antigen stimulation, relative to that of human autoreactive T-cell clones generated in response to myelin basic protein (MBP), we have compared the proliferative response of the Ob.1A12 T-cell clone (which recognizes the MBP p85–99 epitope³) with that of our insulin-peptide-reactive T-cell clones (unpublished). The subject was

DR2- and DR4-positive and the clone was generated using autologous antigen-presenting cells expressing both human leukocyte antigen (HLA) class II alleles. The response with DRB1*1501 as a restricting element was stronger, requiring an EC₅₀ of 0.1–10 μ M MBP p85–99 peptide, whereas the response with DRB1*0401 required an EC₅₀ of about 100 μ M peptide; this was similar to the EC₅₀ response of insulin-peptide-reactive T cells restricted by DRB1*0401 (ref. 1). (EC₅₀ is the effective concentration of antigen that causes half-maximal response.)

It is known that human T-cell clones in long-term culture (grown for years, for example) vary in their amount of proliferation and cytokine secretion (our unpublished observa-

tions), which is influenced by their activation state: therefore, the actual proliferative response may not necessarily reflect antigen sensitivity. Perhaps more important are data we have obtained since our original report: reduction of the three cysteine residues in the insulin A-chain 1–15 peptide (to abolish its secondary and tertiary structure) markedly increases the sensitivity of the insulin-reactive CD4⁺ T cells, giving a significant response with 10 μ M insulin peptide (our unpublished observations). These insulin-reactive T-cell clones therefore behave like other autoreactive T-cell clones when recognizing denatured insulin antigen.

However, it is still not known whether the T-cell clones recognize naturally processed insulin epitopes *in vivo*. Although responses to MBP and to glutamic acid decarboxylase 65 (GAD65) are consistently detectable in peripheral blood^{3,4}, we never observe the same degree of proliferation in response to insulin as to these other, more sequestered antigens. We agree that a microbe or some other self-antigen could have been the primary trigger

for production of these insulin-reactive T-cell clones and that we may be observing a cross-reactivity that is pathogenic^{5–7}.

We next consider Wilson's question about the site in the body where the required concentration of insulin peptide would be available to activate these cells². Insulin in an islet-cell granule is present at about 240 mg ml⁻¹ (ref. 8), which is much higher than the concentrations of insulin peptide we used¹. If an insulin granule were to be phagocytosed by a dendritic cell, the concentration in the early endosomal compartment would be extremely high, although bound peptide would dissociate rapidly. Sufficiently high concentrations are therefore readily obtainable for dendritic cells carrying antigen to the draining lymph nodes, although the avidity of the T-cell clones for insulin is not known. Investigation of the receptors from these clonally expanded T cells from the draining lymph nodes of diabetic subjects should help in understanding the potential cross-reactivity that may exist in the induction of human autoimmune diseases.

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