Insulin teaches a new lesson in tolerance

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The immune system must be educated to discriminate between the body's own tissues ('self') and foreign pathogenic microorganisms (non-self). This ability is acquired by T lymphocytes in the thymus (central tolerance) and it is further refined by peripheral tolerance. Errors in discrimination potentially result in autoimmune diseases, such as type 1 diabetes, in which the insulin-producing beta cells of the pancreas are destroyed by T lymphocytes. In this issue of *The EMBO Journal*, Fan *et al* (2009) describe how a failure of central tolerance to insulin causes diabetes in a mouse strain resistant to spontaneous autoimmune diabetes. Conversely, enhanced central tolerance to insulin can prevent diabetes in diabetes-prone mice (French *et al*, 1997). Inducing or preventing diabetes by controlling the immune response to insulin is consistent with autoimmunity being the cause of diabetes rather than a secondary response to tissue damage.

Developing T cells are exposed to short peptides derived from self proteins 'presented' in the antigen-binding cleft of major histocompatibility complex (MHC) proteins, highly polymorphic membrane-bound glycoproteins expressed on thymic epithelial cells. These cells transcribe and translate numerous genes into proteins otherwise expressed in extrathymic organs (Derbinski *et al*, 2001). This promiscuous expression is not for the function usually associated with the protein, for example, glucose regulation in the case of insulin, but for central tolerance—deletion from the immune repertoire of immature T cells that recognize antigen with high affinity. Mutations in the transcription factor Aire reduce thymic expression of peripheral antigens, and result in widespread autoimmunity and in autoimmune polyglandular syndrome type 1 (Mathis and Benoist, 2009). Furthermore, there is evidence that polymorphisms in the insulin promoter affect its thymic expression and increase the risk for diabetes (Pugliese *et al*, 1997; Vafiadis *et al*, 1997).

Allelic variation in the MHC has long been associated with autoimmune diseases. The association of type 1 diabetes with MHC in humans and mice is the greatest for any genetic locus with a complex disease. Although believed to be due to the ability of these MHC alleles to mediate central tolerance to pancreatic beta cell antigens, direct mechanistic evidence has proven elusive. Fan *et al* have used mouse genetic technology to eliminate the insulin gene from thymic epithelial cells using cre recombinase driven by the *Aire* promoter, but

insulin was still expressed in the pancreas. Therefore, T cells were not exposed to insulin peptides during their development leaving insulin-specific T cells untouched by negative selection. These H-2b-bearing C57BL/6 mice, that are not diabetes prone, developed diabetes by 3 weeks of age due to apparent immune-mediated beta cell destruction associated with demonstrable insulin-specific T and B cells. The data provide direct confirmation that lack of central tolerance to one individual autoantigen, insulin, can cause diabetes. They also confirm that peripheral antigen expression in thymic epithelial cells is essential because expression of insulin itself, rather than other proteins with related cross-reactive peptides, was required for sufficient negative selection to avoid autoimmunity.

There are potential confounding factors in the experimental design. As mice have two insulin genes these experiments were done in mice with global deletion of the insulin 1 gene and conditional deletion of insulin 2 gene to ensure no insulin expression in the thymic epithelium. Autoimmune reactivity against the beta cells could be transferred to unmodified recipient mice but diabetes did not occur. It is possible that deletion of insulin may have occurred in other cell types or that deletion of insulin may have reduced selection of insulin-specific regulatory T cells. These caveats need to be explored.

This experimental model represents an extreme genetic case resulting in zero exposure of the developing immune system to insulin. In non-transgenic mice (and probably also in people), it is likely that some insulin-specific T cells escape central tolerance, but to a much less dramatic extent and thus subsequent events are required, including the immune response to spread to other beta-cell antigens, for diabetes to occur. The data provide evidence that variation in insulin presentation in the thymus—whether due to insulin expression, or structural variation in MHC–autoantigen peptide affinity—can cause autoimmune diabetes. At diagnosis, patients with type 1 diabetes typically have autoantibodies against a variable set of beta cell autoantigens, including (in order of prevalence) glutamic acid decarboxylase, zinc transporter ZnT8, tyrosine phosphatase IA-2 and insulin. Increasingly, insulin autoimmunity is seen as the primary driver rather than just one widely studied example of autoimmunity against beta cell antigens. The effect of deleting other beta cell antigens from the thymus in this way would be of interest. Whether acquired variation in thymic insulin presentation could explain some of the environmental impact

on diabetes incidence or whether it is possible to influence thymic insulin expression in humans remain important questions.

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Conflict of interest

The authors declare that they have no conflict of interest.

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