Controlling the controls: GAD65 autoreactive T cells in type 1 diabetes

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Commentary

The clinical onset of autoimmune (type 1) diabetes is associated with multiple immune abnormalities. First, the islets of Langerhans are infiltrated with macrophages and CD4 and CD8 T lymphocytes. This islet inflammation, or insulitis, is related to a major loss of islet β cells. In addition, the onset is strongly associated with autoantibodies against specific islet cell autoantigens. In particular, autoantibodies against the smaller isoform of glutamic acid decarboxylase (GAD65), the islet antigen-2 (IA-2, also known as ICA512), or insulin are common at the day of clinical onset. The simultaneous presence of all three autoantibodies is highly predictive of type 1 diabetes (1). Autoantibody-based predictive tests Using now-standard islet cell autoantibody tests (2), alone or in combination with human leukocyte antigen typing, it is possible to predict type 1 diabetes not only among first-degree relatives, but also in the general population. Although autoantibodies to GAD65, IA-2, and insulin are effective markers for type 1 diabetes, it has not yet been clear whether they contribute to pathogenesis or merely reflect the destructive process of the islets of Langerhans. For example, GAD65 autoantibodies may appear or their levels increase in patients with polyendocrine autoimmune disease prior to the clinical onset (3) or in type 1 diabetes patients with transplanted human islets at the time islet function is lost (4, 5). In spite […]

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The clinical onset of autoimmune (type 1) diabetes is associated with multiple immune abnormalities. First, the islets of Langerhans are infiltrated with macrophages and CD4 and CD8 T lymphocytes. This islet inflammation, or insulitis, is related to a major loss of islet β cells. In addition, the onset is strongly associated with autoantibodies against specific islet cell autoantigens. In particular, autoantibodies against the smaller isoform of glutamic acid decarboxylase (GAD65), the islet antigen-2 (IA-2, also known as ICA512), or insulin are common at the day of clinical onset. The simultaneous presence of all three autoantibodies is highly predictive of type 1 diabetes (1).

Autoantibody-based predictive tests

Using now-standard islet cell autoantibody tests (2), alone or in combination with human leukocyte antigen typing, it is possible to predict type 1 diabetes not only among first-degree relatives, but also in the general population. Although autoantibodies to GAD65, IA-2, and insulin are effective markers for type 1 diabetes, it has not yet been clear whether they contribute to pathogenesis or merely reflect the destructive process of the islets of Langerhans. For example, GAD65 autoantibodies may appear or their levels increase in patients with polyendocrine autoimmune disease prior to the clinical onset (3) or in type 1 diabetes patients with transplanted human islets at the time islet function is lost (4, 5).

In spite of the fact that the autoantibodies predict disease and may be detectable in healthy subjects many years before the clinical onset of type 1 diabetes, the generally held notion of type 1 diabetes is that cell-mediated immunity is responsible for destroying β cells. In both clinical and experimental studies, type 1 diabetes is widely described as a T cell–mediated disease, and, indeed, studies of the spontaneously diabetic NOD mouse and BB rat strongly support this view. However, as was recently discussed in the *JCI* (1), the lack of a reliable assay for measuring cell-mediated immunity to β cell antigens prevents us from making such an unequivocal claim for human type 1 diabetes.

Over the years of type 1 diabetes research, it has become clear that the cellular immune response cannot be studied in isolation from the humoral counterpart. The rediscovery of insulitis in 1965 (6) inspired studies of antipancreatic cellular hypersensitivity (7) as well as of islet cell antibodies (8). Both types of investigation were originally hampered by the lack of defined autoantigens, but three major autoantigens, GAD65, IA-2, and insulin, are now widely recognized. These proteins are available in highly purified recombinant form for use in studying cellular and humoral immune responses in type 1 diabetes.

The elusive goal of T cell–based predictive assays

Despite the availability of these tools, the cellular response has been complicated to study, and progress has lagged behind work on humoral immune response in type 1 diabetes. There is no lack of reports of T cell proliferation studies in response to stimulation by GAD65 (9) or other antigens, but reproducibility and interlaboratory variation remain considerable problems. Thus, the Immunology of Diabetes Societies, in its first international standardization workshop in 1999, noted depressingly that "although a few laboratories [can] distinguish type 1 diabetes patients from non-diabetic controls in proliferative responses to individual islet autoantigens, in general, no differences in T cell proliferation between the two groups [can] be iden-

tified" (10). The report highlighted the inability to discriminate normal controls from new-onset type 1 diabetes patients. It warned that focusing on proliferative responses in PBMCs provides an incomplete picture of the immune response and that this approach is plagued by difficulties in identifying suitable antigens and assays for standardized use.

Remarkably, in 2001, the second workshop witnessed an increase in optimism. The report from this meeting (11) urged the development of islet-reactive T cell assays with specificity, sensitivity, and positive predictive value adequate for working with patients with type 1 diabetes or subjects at high risk of the disease. This work is ongoing, as researchers develop and characterize GAD65-, IA-2–, and insulin-autoreactive T cell assays that might predict type 1 diabetes better than the existing antibody tests. Meanwhile, however, the study of Viglietta et al. in the present issue of the *JCI* (12) provides an alternative functional assay that could serve the same purpose.

Monitoring memory

The concept is simple. T cells from both new-onset patients and controls proliferate in response to GAD65 stimulation ex vivo. The PBMCs are kept for almost 2 weeks in tissue culture, and their ability to proliferate or to produce IFN-γ, IL-13, or IL-5 is measured at the end. Why are T cells from controls and new-onset patients proliferating to the same extent? The authors test the hypothesis that the T cells at onset are memory cells and therefore need no second, costimulatory signal (12). This is a reasonable hypothesis since the authors document that their patients at 19–35 years of age had one or the other autoantibody. In this age group it is well known that GAD65 autoantibodies in particular might have been present for

many years before the clinical onset of type 1 diabetes. It was therefore a reasonable assumption that the patients, but not the controls, would have memory T cells in circulation.

The next question was how to distinguish naive cells that require a second costimulatory signal from memory cells that do not. The authors approached this by controlling the controls (12). First, they employed a Fab fragment of a mAb against CD28 to block the CD28-dependent costimulation at the level of the T cell. In parallel experiments, they used a Fab fragment of a mAb against B7-1, one of two known antigen-presenting cell–borne costimulatory molecules that interact with CD28. Following each of these treatments, they found, T cells from the type 1 diabetes patients continued to proliferate and to produce cytokines, whereas the proliferation and cytokine production was inhibited in the controls. The response to blockade of the other defined CD28 ligand, B7-2, was not as clear-cut, and the authors speculate that B7-1 provides the dominant costimulatory signal for antigen-specific activation of antigen-specific T cells, at least in this type of ex vivo analyses.

Other signaling mechanisms in the T cell synapse may be relevant to understanding these effects. For instance, the counter-receptor cytotoxic T lymphocyte antigen–4 (CTLA-4), which downregulates T cells, also uses B7-1 and B7-2 for its ligands. B7-1 binds CTLA-4 with the highest affinity, and B7-2 binds CD28 with the lowest affinity. A Fab mAb to CTLA-4 slightly increased the proliferation and cytokine secretion in both patients and controls. The data suggest that CTLA-4 interaction with B7 is a downregulation of the T cell response. Combining CD28 with B7-1 blockade showed an enhanced blocking of controls but not of the diabetic patients. Overall, the most significant reduction of the control T cells was observed when combining Fab fragment antibodies to CD28 and B7-1. Hence, the

optimal strategy for identifying autoreactive T cells in human blood samples may be to block the secondary costimulation. These reagents block the controls and could therefore distinguish a type 1 diabetes patient from a healthy control; other combinations of B7-1, B7-2, CD28, and CTLA-4 blockade attempted did not.

The road ahead
Future investigations will require studying a larger number of patients and controls, extending the age at onset also to younger subjects, and defining the effects of age, gender, and autoantibody status. The use of a dose-response curve for GAD65 in each experiment is important to establish a proper dose-response relationship with well-defined cutoff values around the normal range seen in controls. It should be possible to estimate to what extent the blockades show normal distribution and to determine whether these tests can identify subjects progressing toward type 1 diabetes — subjects whose memory cells might be on the rise but would still be present at lower levels than in symptomatic individuals. Whether this effect is associated with a variable response in relation to the CLTA-4 gene polymorphism or other type 1 diabetes genetic factors will also be a matter for further studies.

Will the use of CD28 or B7-1 blockade be an assay of the future and be adopted by the research community as the standard to identify subjects with GAD65-reactive T cells? This will depend on whether the Fab fragments of the CD28 and B7-1 mAb's pass the test of a standardization workshop and can be made available to investigators as standardized reagents. Future workshops may have to include these blocking agents to control the controls in order to dissect the T cell response that may predict type 1 diabetes. Additional experiments in younger subjects and in siblings at risk for type 1 diabetes should help to better define the phenomenon of costimulatory blockade to

uncover presumptive self-reactive T cells. The challenge for these future studies will be to identify whether the number of antigen-specific memory T cells predicts type 1 diabetes and whether the presence of such cells reveals higher diagnostic sensitivity and specificity and positive predictive value than are already established by the standardized tests for autoantibodies to GAD65, IA-2, or insulin.

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