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

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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 insulinitis, 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 insulinitis 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 identified” (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...
when combining Fab fragment anti-
of the control T cells was observed
Overall, the most significant reduction
controls but not of the diabetic patients.
still slightly increased the proliferation and
cytokine secretion in both patients
biding 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 phe-
nomenon 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 autoanti-
todies to GAD65, IA-2, or insulin.

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 dis-
tribution and to determine whether
these tests can identify subjects pro-
gressing toward type 1 diabetes — sub-
jects whose memory cells might be on
the rise but would still be present at
lower levels than in symptomatic indi-
viduals. Whether this effect is associat-
ed 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 block-
ade 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 standard reagents. Future work-
shops may have to include these block-
ing 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 phe-
nomenon of costimulatory blockade to

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mune type 1 diabetes: resolved and unresolved
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