Organ-specific Autoimmune Disease: A Deficiency of Tolerogenic Stimulation

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It is well appreciated that organ-specific autoimmune diseases run in families, but that within a family one member may have type 1 diabetes, another autoimmune thyroid disease, and another multiple sclerosis (1). What causes clustering of different autoimmune diseases along genetic lines, and what logic causes the immune system to take aim at different organ targets? In this issue, Salomon et al. make the paradoxical finding that interference with a single costimulatory molecule, B7-2, shifts the aim of autoimmunity in the NOD mouse strain away from the pancreatic islet β cell and onto the peripheral nerves (2). The finding adds an important molecular clue to the pathogenesis of organ-specific autoimmune disease, and illustrates the caution that will be needed as new immunological interventions are applied in the clinic.

The inheritance of susceptibility to organ-specific autoimmunity is extraordinarily complex. Particular haplotypes of the major histocompatibility complex, such as HLA-DR3-DQB1*0201, are strongly associated with human susceptibility to multiple organ-specific autoimmune disorders (3). HLA-type nevertheless accounts for only a fraction of inherited susceptibility, and heterozygosity for different HLA alleles can either raise or lower the risk. Independent of HLA-type, mutations in a non-MHC gene, AIRE, cause a Mendelian syndrome, APECED, characterized by autoimmune manifestations ranging from Addison’s, thyroid, and parathyroid disease to type 1 diabetes (4, 5). The AIRE gene product appears to be a nucleic acid binding protein present in some thymic epithelial and dendritic cells. Its connection to organ-specific autoimmunity can only be speculated, perhaps related to presentation of organ specific antigens for tolerance induction in the thymus.

The unpredictable interactions between MHC and non-MHC genetic loci have been most clearly illuminated by the genetic analysis of organ-specific autoimmunity in the NOD mouse, where it has been possible to create numerous congenic substrains differing at one or more of the 20 or so loci currently shown to contribute to susceptibility (6). The NOD mouse is an inbred strain derived in Japan from a mixture of outbred strains (7). A high proportion of NOD mice spontaneously develop type 1 diabetes due to destruction of pancreatic islets by T cells. NOD carries a unique MHC class II allele, I-A\(^g\), which makes a major contribution to diabetes susceptibility (Table I). The I-A\(^g\) protein shares a unique amino acid residue in its peptide-binding clef with human diabetes–susceptible HLA-DQ chains. The NOD H-2 haplotype is not sufficient for diabetes, however, because when it is placed onto a different strain background, congenic B6.H-2\(^b\) mice, there is no insulitis or diabetes (6). Strikingly, when a NOD congenic substrate, NOD.H-2\(^b\)4, was produced that retained all of the non-MHC NOD genes but substituted the MHC region with another MHC haplotype, the congenic animals were now protected from insulitis and diabetes but became susceptible to spontaneous autoimmune thyroiditis (8–10).

The Salomon results here document a similar shift from diabetes to autoimmune peripheral neuropathy, precipitated not by a change in the MHC haplotype but by a defined genetic lesion in a single T cell costimulatory ligand, B7-2 (CD86) (2).

Immunogenic Role of B7 Ligands. B7-2 and B7-1 (CD80) are ligands for one of the best characterized T cell costimulatory receptors, CD28, and for the related inhibitory receptor on T cells, cytotoxic T lymphocyte antigen (CTLA)-4 (CD152). An important immunogenic role of B7 ligands for in vivo immune responses has been clearly shown by treating mice, primates, and humans with blocking agents such as anti-B7 mAbs or CTLA-4Ig and showing interference with immune responses to immunization or transplants (11). Similarly, B7-1–deficient mice showed a 70% reduction in the stimulation of allogenic mixed lymphocyte reactions (12). Immune responses were also impaired in B7-2–deficient mice, as no germinal centers were formed, and antibody isotype switching was reduced upon
injection of antigen in vivo (13). Surprisingly, in the B7-1/
B7-2 double knockout mice, the phenotype was similar to
that observed in B7-2–deficient mice (13). However, if an-
tigen was injected with adjuvant, then immune responses
in B7-2–deficient mice appeared normal, whereas those in
the double knockout were still impaired. These results sug-
gest that B7-1 and B7-2 have partially overlapping immu-
nogenic roles, whereby an increase in B7-1 expression me-
diated by adjuvant can substitute for B7-2.

CD28 itself has a clearly established immunogenic role,
as CD28-deficient mice have impaired responses to lectins,
reduced T helper activity, and decreased isotype switching
(14). Immune clearance of potently immunogenic antigens,
such as replicating lymphocytic choriomeningitis virus, is
normal. The ability to mobilize effective immune responses
in B7 or CD28 null mice is presumably explained by the
fact that other costimulatory receptor-ligand pairs such as
CD40/CD40L can substitute for delivering immunogenic
costimuli to T cells.

**Tolerogenic Role of B7 Ligands.** Paradoxically, B7-1, B7-2,
and CD28 have also been shown to serve tolerogenic roles.
When a targeted deficiency of B7-1 is backcrossed to
the NOD strain background, this exacerbated the develop-
ment of spontaneous diabetes rather than suppressing the
autoimmune response as might be expected (11). Similar
exacerbation of autoimmune diabetes occurred in NOD
mice carrying a null mutation in CD28 or a double-defi-
ciency of B7-1 and B7-2 (15, 16). In the article by
Salomon et al. lack of B7-2 in NOD mice results in au-
toimmune neuropathy (2). Therefore, in the context of
spontaneous autoimmunity in NOD mice, B7-1, B7-2,
and CD28 all appear to be more important for a tolero-
genic function than for immunogenicity.

**Possible Explanations for Tolerogenic Roles of B7.** Immuno-
ology—from its origins in vaccinology—is preoccupied
with the responses made to acute stimulation with foreign
antigens. In this context, we assume that occurrence of im-
mune responses relate to the strength of an inductive stim-
ulus, rather than to weakening of a repressive stimulus.
Consequently, it is natural to try to explain the exacerba-
tion of autoimmunity in B7-deficient NOD mice by draw-
ing on the role of these molecules as differentially inductive
stimuli for Th1 and Th2 cytokine responses to immuno-
genic foreign antigens. It is nevertheless very difficult to
explain exacerbation of autoimmunity in this way. Because
B7-2 is suggested to be selectively needed for production
of Th2 cytokines, and Th2 cytokines can prevent diabetes
and inflammation, it might be expected that loss of B7-2
would exacerbate diabetes; yet in NOD mice the opposite
is observed. Conversely, if B7-1 preferentially promotes
Th1 cytokine production, its absence would be expected to
protect against diabetes, rather than exacerbate the disease.
In the absence of both B7-1 and B7-2, few Th1 and Th2
cytokines are produced to immunogenic foreign antigens,
but this still leads to an exacerbation of the autoimmune di-
betes response (16). Therefore, Th1 and Th2 biases fail to
provide a simple explanation for how B7 ligands prevent
autoimmune responses.

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**Table I. General Predisposition of NOD Mice to Autoimmunity and Tolerogenic Role of B7 Ligands**

| Gene variant | Strain | Gross phenotype | Suggested explanations | Reference |
|--------------|--------|-----------------|------------------------|-----------|
| H-2^+/+ (NOD MHC) | NOD | Insulitis | Defect in tolerance induction | 7 |
| H-2^+/+ | NOD | Little insulitis | Increased immunogenic presentation of thyroid autoantigens | 8–10 |
| H-2^+/+ | B6 | No insulitis | Regulation of tolerance is normal | 6 |
| CD28^−/− | NOD | Exacerbation of diabetes | Failure to induce regulatory CD4^+25^+ cells | 15 |
| B7-1^−/− | NOD | Exacerbation of diabetes | Failure to induce CTLA-4-mediated inhibition | 11 |
| B7-2^−/− | NOD | Little insulitis | Failure to induce Th2 cytokines | 2 |
| B7-1/B7-2^−/− | NOD | Exacerbation of diabetes | Predominant expression of B7-2 in islet APCs | 16 |
| CD28/CD40L^−/− | NOD | No diabetes | A sum of costimulatory pathway are interactive: blocking both “major” pathways efficiently prevents T cell activation | 11 |
In the context of tolerance to self-antigens, immune responses must be viewed inversely through the looking glass. Occurrence of an autoimmune disease may primarily reflect the weakness of tolerogenic responses to these self-antigens rather than an enhancement of immunogenic stimuli. B7 ligands appear to have an important role in co-stimulating these tolerogenic responses (Fig. 1), so that their removal might be expected to weaken tolerance and allow or exacerbate autoimmunity. For example, in the context of TCR engagement in immature thymocytes, co-stimulation by CD28 augments induction of thymic deletion (17–19). During peripheral T cell tolerance induced by anergy to poorly immunogenic antigens, recognition of B7 ligands by CTLA-4 is necessary to inhibit or terminate T cell activation (20, 21), and mice lacking CTLA-4 develop lethal T cell hyperactivation (22–24).

In addition to tolerance mediated by cell-intrinsic responses of deletion and anergy, it has long been speculated that tolerance might be mediated by regulatory/suppressor cells that are induced by stimulation with self-antigens in the thymus. Emerging evidence suggests that self-reactive CD4 T cells are induced by self-antigen within the thymus to adopt an anergic and suppressive state characterized phenotypically by expression of the low affinity IL-2 receptor α chain (CD25), low levels of CD45RB, and high CD44 (25). Cells with this phenotype have been shown to be generated from the thymus and be essential for maintaining organ-specific tolerance to tissues such as the gastric mucosa, ovary, thyroid gland, and pancreatic islet (26, 27).

Costimulation by B7 ligands appears to be important for the tolerogenic responses conferred by these cells. Fewer regulatory CD4+CD25+ T cells are found in NOD mice lacking CD28 or both B7-1 and B7-2, and cells with this phenotype express CTLA-4 and appear to require this inhibitory receptor for their suppressive actions (16, 28, 29).

Why Might Loss of a Costimulator Change the Focus of Autoimmunity? In the article by Salomon et al., lack of B7-2 in NOD mice releases an autoimmune attack on cells of the peripheral nervous system (PNS) while at the same time preventing islet inflammation and B cell destruction (2). The suppression of diabetes cannot easily be explained by the role of B7-2 as an immunogenic costimulator for effector T cells. One possibility is to invoke the notion that immunogenic APCs presenting PNS antigens primarily carry B7-1, while immunogenic APCs with islet antigens are predominantly B7-2 positive (Fig. 2). Salomon et al. raise the possibility that a compensatory increase in B7-1 expression occurs in APCs from B7-2 knockout mice (2). Assuming this occurs on APCs presenting PNS antigens, the resulting increase in immunogenic stimulation could explain the development of autoimmune neuropathy. Likewise the loss of B7-2 on immunogenic APCs bearing islet antigens would explain the suppression of insulitis and diabetes. The same reasoning could explain the exacerbation of diabetes in B7-1 knockout NOD mice, where a compensatory increase in B7-2 on immunogenic islet APCs would increase stimulation of autoimmune effector T cells. This logic falls down, however, in the CD28-deficient NOD mice, as diabetes is not suppressed but exacerbated by eliminating the CD28 receptor for both B7-1 and B7-2.

As costimulators of tolerogenic responses to self-antigens, however, it is relatively straightforward to see how this shift in autoimmune target specificity might come about if one assumes that different self-antigens are presented by different subsets of tolerogenic, as opposed to immunogenic, APCs in the thymus or periphery (Fig. 2). If the deletion of PNS-reactive T cells, or the induction of PNS-specific anergic/regulatory cells, depends on APCs with primarily B7-2 expression, then loss of B7-2 would weaken tolerogenic responses to PNS antigens, allowing autoimmunity to develop when placed on an intrinsically autoimmune-susceptible NOD background. Suppression of diabetes would need to be explained by predominance of B7-2 on immunogenic APCs in the islet, as has been described (30). By contrast, if the tolerogenic thymic or peripheral APCs for islet antigens primarily express B7-1, this would explain the exacerbation of diabetes in B7-1-deficient NOD mice. To explain the exacerbation of diabetes caused by elimination of CD28 in NOD mice, the weakening of tolerogenic co-stimulation to islet antigens by B7-1 would have to be proposed to result in so great a defect in tolerance that the immunogenic stimulation of islet effector T cells can be achieved by CD28-independent costimulatory pathways.

The Underlying Autoimmune Defect in NOD Mice: A Gun with a Hair Trigger or a Missing Safety Lock? NOD mice spontaneously develop autoimmune diabetes, and also have

Figure 1. Immunogenic and tolerogenic roles of B7 ligands are context dependent. B7 ligands promote induction of T cell tolerance when antigens are presented chronically and/or early during thymic development. In the context of antigens presented as an acute burst—during an immune response to invading pathogens—B7 ligands play an immunogenic role promoting T cell expansion and effector functions.
lymphocytic infiltrates in the thyroid and salivary glands. Changing the MHC haplotype switches autoimmunity from diabetes to thyroiditis (8–10), injecting CFA promotes lupus (31), and removing B7-2 induces polyneuropathy. NOD mice thus seem generally predisposed to autoimmune diseases. This propensity could equally reflect an increase in immunogenic stimulation by self antigens—an immunological hair trigger—or a decrease in tolerogenic stimulation by self antigens—a missing safety lock.

One faulty trigger could be that the target organ for autoimmunity expresses a greater propensity for eliciting and succumbing to autoimmune inflammation. Higher rates of apoptosis in epithelial salivary cells have been shown to occur in NOD.SCID mice than in BALB.SCID (6, 32), and NOD β cells are hypersensitive to oxidative stress (6, 33). Subclinical cell stress or dysplasia could release “danger signals” that deliver immunogenic rather than tolerogenic signals to dendritic cells and activate autoreactive lymphocytes.

A second possibility is that tolerogenic antigen presentation is globally diminished. The NOD MHC I-A<sup>b</sup> is an unstable structure. This may reduce self-peptide presentation to developing thymocytes allowing more autoreactive effector T cells and/or fewer regulatory cells to reach the periphery. In addition to the MHC-linked susceptibility, we recently found that NOD.H-2k congenic hemopoietic precursors intrinsically reconstitute the thymic and splenic microenvironment with an excess of immature myeloid-type dendritic cells relative to B10.H-2k hemopoietic precursors (unpublished data). These results indicate that non-MHC NOD gene alleles alter dendritic cell maturation, a critical APC, and this could weaken the balance of tolerogenic and immunogenic antigen presentation to T cells.

Finally, an intrinsic defect in reception of tolerogenic stimuli by T cells, preventing adequate induction of central or peripheral tolerance could also predispose NOD mice to autoimmune diseases. For instance, induction of central tolerance is highly dependent on the induction of thymocyte apoptosis, while peripheral tolerance mechanisms rely on intracellular T cell signaling. Multiple idd loci are associated to regions containing genes involved in both of these processes. Still, finer mapping is required before we can associate genes to idd susceptibility loci. Recently, we have found evidence that NOD T cells carry an intrinsic...
defect which prevents their tolerance induction. Using a TCR transgenic model, we have shown that thymocyte deletion of islet-reactive CD4 T cells is intrinsically defective in NOD.H-2\(^k\) thymocytes compared with B10. H-2\(^k\) thymocytes (unpublished data).

Faulty triggers or safety catches could also lie in the control of T cell costimulation. Indeed, some costimulatory molecules are closely linked to diabetes susceptibility (idd) loci. For instance, CD28, CTLA-4, PD-1, and inducible costimulator (ICOS) are closely linked to idd5 and IL-2 is almost certainly idd3. A structural polymorphism has been confirmed in IL-2 between NOD and B6 strains (34). These important costimulatory molecules could certainly play a role in the induction of T cell tolerance.

Thus, a sum of faulty safety catches, diminishing tolerogenic stimulation, and responses at many different levels, most probably explains the predisposition of NOD mice to autoimmune diseases.

*Implications for Human Clinical Intervention.* Just as we do not yet fully understand the pathogenesis of autoimmune immunity in NOD mice, we are completely operating in the dark when it comes to the underlying abnormalities of immune regulation that lead to autoimmune disease in humans. If the pathogenesis stems from a relative deficiency of a tolerogenic costimulatory signal, and we block that signal inadvertently or deliberately, there is a very real possibility of worsening the patients condition. There are now many examples of molecules whose function was defined in vitro as immunogenic, but when analyzed in vivo are also tolerogenic (e.g. Lyn kinase, CD40L, IL-2, and IFN-\(\gamma\)). We need careful in vivo charting of the immunogenic and tolerogenic roles of the molecules targeted for immunosuppression. Then, we need means to measure these regulatory pathways in patients to tailor the treatment to correct rather than exacerbate the underlying deficit.

In short, understanding and predicting the role of costimulatory ligands in the development of autoimmune diseases seems more complex than initially believed. We must carefully understand the role of each costimulatory ligand before we can predict outcomes of blocking such ligands for prevention of autoimmune diseases.

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