

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^{g7}, which makes a major contribution to diabetes susceptibility (Table I). The I-A^{g7} protein shares a unique amino acid residue in its peptide-binding cleft 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^{g7} mice, there is no insulinitis or diabetes (6). Strikingly, when a NOD congenic substrain, NOD.H-2^{h4}, 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 insulinitis and diabetes but became susceptible to spontaneous autoimmune thyroiditis (8–10). This observation led to the idea that there was a general susceptibility to organ-specific autoimmune disease in NOD mice encoded outside of the MHC—an immunological gun with a faulty trigger—and that changes in the way particular antigens were presented simply pointed the gun at different target antigens and organs (6).

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

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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 ^{s7} (NOD MHC)	NOD	Insulinitis Type I diabetes	Defect in tolerance induction Aberrant target organ	7
H-2 ^{h4}	NOD	Little insulinitis No diabetes Thyroiditis	Increased immunogenic presentation of thyroid autoantigens Decreased immunogenic presentation of islet autoantigens	8–10
H-2 ^{s7}	B6	No insulinitis No diabetes	Regulation of tolerance is normal	6
CD28 ^{-/-}	NOD	Exacerbation of diabetes	Failure to induce regulatory CD4 ⁺ 25 ⁺ cells Failure to induce CTLA-4-mediated inhibition Failure to induce Th2 cytokines	15
B7-1 ^{-/-}	NOD	Exacerbation of diabetes	Failure to induce CTLA-4-mediated inhibition	11
B7-2 ^{-/-}	NOD	Little insulinitis No diabetes Autoimmune polyneuropathy	Predominant expression of B7-2 in islet APCs Predominant expression of B7-1 in neural tissue	2
B7-1/B7-2 ^{-/-}	NOD	Exacerbation of diabetes	Failure to induce regulatory CD4 ⁺ 25 ⁺ cells Failure to induce CTLA-4-mediated inhibition	16
CD28/CD40L ^{-/-}	NOD	No diabetes	A sum of costimulatory pathway are interactive: blocking both “major” pathways efficiently prevents T cell activation	11

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 antigen 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 suggest that B7-1 and B7-2 have partially overlapping immunogenic roles, whereby an increase in B7-1 expression mediated 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 development 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-deficiency 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 tolerogenic function than for immunogenicity.

Possible Explanations for Tolerogenic Roles of B7. Immunology—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 immune responses relate to the strength of an inductive stimulus, rather than to weakening of a repressive stimulus. Consequently, it is natural to try to explain the exacerbation of autoimmunity in B7-deficient NOD mice by drawing on the role of these molecules as differentially inductive stimuli for Th1 and Th2 cytokine responses to immunogenic 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 diabetes response (16). Therefore, Th1 and Th2 biases fail to provide a simple explanation for how B7 ligands prevent autoimmune responses.

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 energy 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).

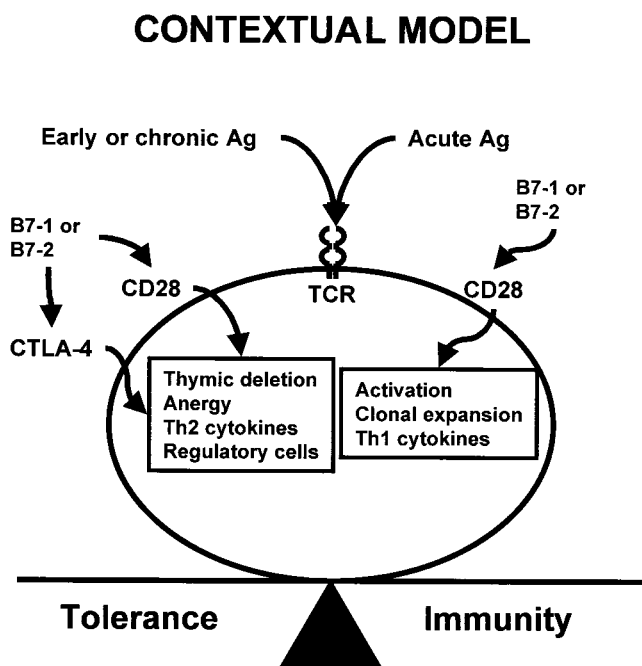


Figure 1. Immunogenic and tolerogenic roles of B7 ligands are context dependent. B7 ligands promote induction of T cell tolerance when antigens are presented chronically and/or early during thymic development. In the context of antigens presented as an acute burst—during an immune response to invading pathogens—B7 ligands play an immunogenic role promoting T cell expansion and effector functions.

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 β 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 insulinitis 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 costimulation 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

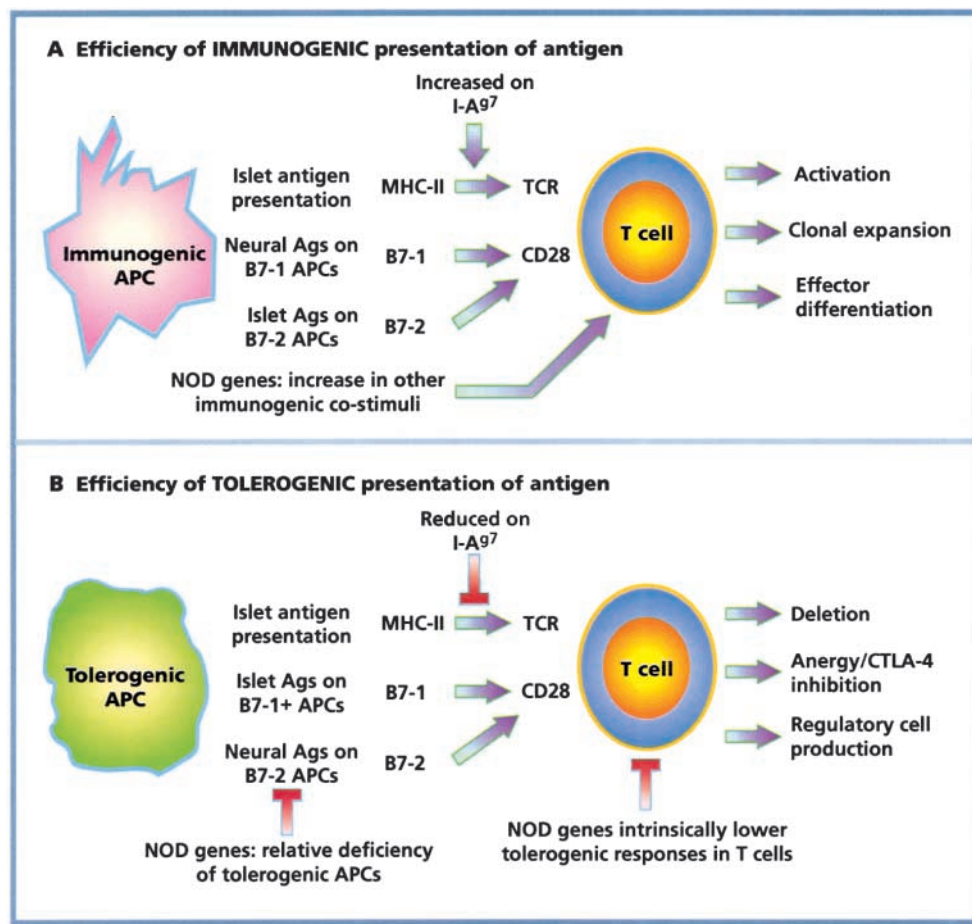


Figure 2. Models to account for autoimmunity differences among congenic NOD mouse strains. (A) Enhancement of immunogenic APCs in draining lymph nodes or within target organ. MHC II and B7-2 stimulate T cell reactivity to islet antigens, and B7-1 enhances autoimmunity to PNS antigens. In this model, deletion of B7-2 would suppress diabetes by diminishing immunogenic stimulation by islet antigens, and would trigger autoimmune neuropathy by a compensatory increase in B7-1 on immunogenic APCs. Autoimmunity is not seen in B7-2 knockout B6 mice due to a less efficient MHCII allele and absence of other immunogenic co-stimuli caused by non-MHC NOD gene alleles. It is difficult to explain the enhanced autoimmunity in NOD.CD28^{-/-} mice by this model. (B) Diminished presentation of antigens by tolerogenic APCs. In this model, islet and neural antigen presentation is reduced by NOD MHC II, diminishing induction of tolerant T cells and promoting diabetes. B7-2 is suggested to be the predominant molecule on tolerogenic APCs presenting neural antigens in the thymus or peripheral nodes, so that its elimination selectively diminishes tolerance induction to these anti-

gens. By contrast, B7-1 may be the predominant member on tolerogenic APCs bearing islet antigens, so that diabetes is exaggerated when it is inactivated, or when both costimulators or CD28 are eliminated. For spontaneous autoimmunity to occur, other NOD gene alleles are needed to further weaken tolerogenic responses at the level of tolerogenic APC numbers and by intrinsically diminishing the T cell response to tolerogenic stimuli.

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^{g7} is an unstable structure. This may reduce self-peptide presenta-

tion 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 haemopoietic precursors intrinsically reconstitute the thymic and splenic microenvironment with an excess of immature myeloid-type dendritic cells relative to B10.H-2k haemopoietic precursors (unpublished data). These results indicate that non-MHC NOD genes 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 autoimmunity 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 now CD28/B7) (6, 35). 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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