REVIEW

A modular theory of autoimmunity

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Abstract. The traditional overarching concept of disease pathogenesis entails the natural history of disease, i.e. the concept that any disease is a unified entity from beginning to termination. The concept of the natural history of disease encourages researchers and clinicians alike to conceptualize all clinical signs and symptoms in a patient as manifestations of a single disease process. Our experiences in dissecting the genetic control of autoimmune diseases and autoimmune phenotypes suggest that for many autoimmune processes, an alternative conceptual framework may be more useful. We term this approach a "modular" theory of autoimmunity. "Modules" are distinct, genetically controlled clinical or pathological phenotypes which can interact to construct a disease process. Modules may interact additively, synergistically, or antagonistically in any given individual. Multiple modules can coexist and produce unique disease phenotypes. We illustrate this concept with examples from the murine autoimmune model of type one diabetes, the nonobese diabetic (NOD) mouse. (Keio J Med 54 (3): 121–126, September 2005)

The concept of a unified disease process is deeply engrained in medical teaching and practice. The idea of the "natural history of disease" implies that all pathological manifestations in a particular patient arise as a result of a logical unfolding of biological/biochemical events in that individual's genetic and environmental makeup (Fig. 1). Of course patients may have several different diseases, but conceptually they are often non overlapping. This disease model is well suited to disease processes such as coronary artery disease. Rheumatological practice, however, is quite different. In Rheumatology a large percentage of patients are classified as "undifferentiated connective tissue disease" or "overlap syndrome". Often, however, the idea is that the true natural history of the disease in such patients simply has not yet manifested itself completely, or, that the physician has not yet understood what is in fact an underlying unified disease process. In either case the fundamental concept is that there is a natural history of disease in any given patient which fully explains the clinical manifestations over time. Another common scenario in Rheumatology involves patients who manifest more than one autoimmune disease, such as Primary Biliary Cirrhosis and type one diabetes (T1D). A related finding is extended kindreds manifesting several disparate autoimmune conditions at greater than expected rates compared to the population at large. In this scenario, the patient may have Rheumatoid Arthritis, the patient's aunt may have T1D, and a cousin may be antinuclear antibody (ANA) positive. Clearly, genetic factors play a role in such kindreds, but the nature of the genetic influences have until recently been obscure.

Our laboratory has spent several years dissecting the genetic control of spontaneous murine autoimmune phenotypes and clinical syndromes in the nonobese diabetic (NOD) mouse. These studies have revealed a different conception of disease which may better represent some autoimmune processes than a "natural history" of disease. We have found clear evidence that individual clinical and pathological phenotypes exist which are genetically controlled and may combine in multiple different ways to create different autoimmune syndromes. We term this a "modular" theory of autoimmunity (Fig. 2). The features of component "modules" in autoimmune syndromes are as follows: 1) A
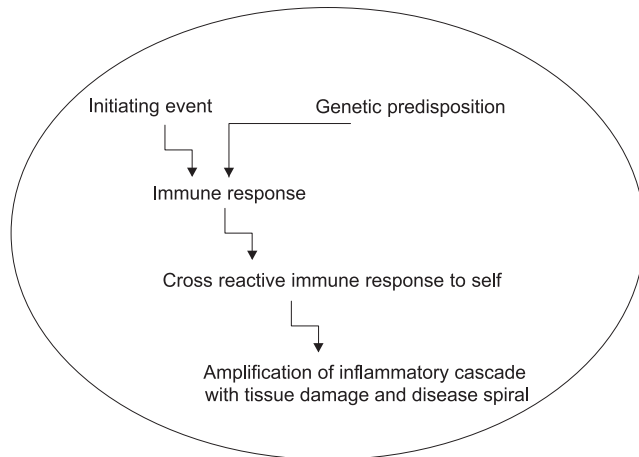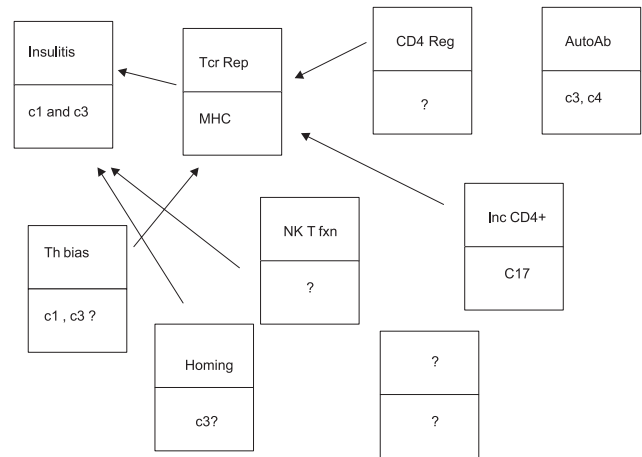

Fig. 1 The traditional concept of the "Natural history of auto-immune disease". All manifestations of pathology in a patient are regarded as explained by a single disease concept, represented by the circular limit around the process. The disease "unfolds" in orderly fashion over time, as represented by the downward direction of the arrows. A classical representation of the autoimmune disease process is shown.

Understanding autoimmunity in the NOD mice
by component phenotypes: a modular concept

Fig. 2 A "Modular" theory of autoimmunity in the nonobese diabetic (NOD) mice. This figure shows a "modular" description of the type one diabetes (T1D) disease process in the NOD mice. Each module, represented by a two-tiered rectangle, is an independent, genetically controlled clinical or pathological phenotype. The top tier of each rectangle describes the phenotype, the bottom tier describes the genetic control (when known). Modules may be independent, additive, or antagonistic (see text). Arrows represent the scientific hypotheses "connecting" the modules, however modules exist in patients independent of any known theory connecting them into a coherent "disease process" (these modules, for example "Autoab", aren't connected to other ones with arrows, yet are nonetheless present in the disease process). Modules can be identified and studied in any patient regardless of a "disease diagnosis".

Abbreviations: "c1", "c3" etc. describe the chromosomal location known to control the phenotypes. "Tcr rep" = the T cell receptor repertoire in a patient. "CD4 Reg" = CD4+ regulatory cell development. "Autoab" = autoantibody production. "Th bias" = intrinsic property of CD4+ T helper cells (Th cells) to produce a skewed cytokine response (for example see reference 31). "NK T fxn" = intrinsic cytokine response of NK T cells after stimulation. "Inc CD4" refers to an increased CD4:CD8 T cell ratio in the peripheral blood T cell repertoire (see reference 32). "Homing" refers to the capability of pathological T cells to enter a target organ; for example NOD.Idd3/5 mice show decreased insulitis which may be the result of changes in T cell homing (see text). "?" = refers to an unknown phenotype or an unknown genetic location.

module is a distinct clinical or pathological phenotype which, in relation to other modular phenotypes, may be additive, independent, or antagonistic. 2) While modules are objective clinical/pathological features of a disease process; the characterization of how modules relate to each other, on the other hand, is the intellectual creation of the scientist. This means that modules can be detected and quantitated in patients even without an overarching concept of the disease process in that patient. 3) Each module is in principal genetically controlled. In other words, environmental triggers of autoimmunity are not considered in the model; therefore a modular characterization of a process involves outlining the complete genetically mediated predisposition of an individual to disease. While genetically controlled responses to environmental stimuli are likely modular, the discovery of such triggers is not a part of the modular characterization of the autoimmune process. 4) No disease related module is both necessary and sufficient for a disease process. In fact, some modules can be described which are neither necessary nor sufficient for the disease process. 5) Modules which are neither necessary nor sufficient for a disease process are not therefore irrelevant, but act as disease severity modifiers.

The last two properties of modules, for medical scientists raised on the concept of "one gene, one disease" (e.g. sickle cell anemia[1]) or experienced in the characterization of knock-out phenotypes, requires some elaboration. The familiar Mendelian concept of disease traces the entire disease pathogenesis to deletions or mutations in a single gene. Such a characterization of the hemoglobin gene in sickle cell anemia ushered in the era of molecular medicine. Many common diseases, and in particular many common autoimmune diseases, however, do not follow simple Mendelian genetics and are not the result of "knock out" mutations in single genes.[2,3] Rather, many autoimmune diseases are characterized by multigenicity in which causative genes may actually be functional, common allelic variants in a deleterious mixture. In the examples we will be demonstrating, multigenic origin precludes a singular "modular" phenotype from explaining the disease process. Hence, in contrast to many genetic studies, in the ge-

netic study of modules no single module will be both necessary and sufficient. Even harder to accept is the notion that some modules may be neither necessary nor sufficient to the disease process. The immediate tendency is to conclude that such pathological processes would therefore be irrelevant. Many examples exist, however, to demonstrate that modules which are neither necessary nor sufficient for a disease can act as disease severity modifiers. We will illustrate these concepts in the NOD mouse model of T1D.

The NOD mouse is a spontaneous, genetically complex model of T1D.[4,5] Starting around 3 weeks of age, NOD mice develop lymphocytes invading the pancreatic islets ("insulitis"), although they do not immediately cause destruction of tissue.[6] Around 12–16 weeks, pancreatic islet destruction begins, with subsequent glycosuria and clinical diabetes. It has been shown that T cells are key mediators of the disease process, since CD4+ T cells can transfer the disease into naive NOD and NOD-scid recipients.[7] In addition, the NOD MHC class II molecule (I-A^{g7}) is unique, bearing a strong structural resemblance to a human MHC associated with T1D.[8] The I-A^{g7} molecule was shown to possess a non-aspartic residue at position 57 and probably acts both by selecting an autoreactive T cell repertoire and by presenting autoantigens to T cells.[9] Finally, anti-T cell therapies such as anti-CD3 can prevent diabetes and reverse established disease in NOD mice.[10] Not only T cells, but most immune cells have been implicated in the NOD autoreactive process.

The NOD mouse model is a prototype for the characterization of a genetically complex autoimmune disease. The first genome scan of NOD mice was performed almost 15 years ago, and demonstrated that multiple recessive loci, termed "insulin dependent diabetes" (*Idd*) loci, were linked to diabetes resistance or susceptibility.[11] At present at least 20 genes are thought to play a role in this complex, multigenic process.[12] The strongest genetic contribution is from the MHC class II molecule, I-A^{g7}, however it is important to note that even two copies of this powerful gene are insufficient to mediate diabetes when placed on a non-autoimmune background: the congenic mouse B6.G7, which has two copies of the NOD MHC class II molecule bred onto the non-disease associated B6 background, does not develop diabetes.[13] Therefore while substitution of other MHC class II molecules can prevent diabetes, (indicating that I-A^{g7} is necessary for disease), it is not both necessary and sufficient.

Following the genome scan identification of areas linked to disease, the next step was construction of *Idd* congenic mice, which are mice bred to contain a predominant NOD (disease related) genetic background with a B6 resistance (*Idd*) locus bred onto it. These congenic mice show decreased susceptibility to diabetes, confirming that the gene or genes whose B6 alleles were resistant to disease were "captured" in the congenic intervals.[12] The congenic genetic intervals are initially quite large (>40 megabases), so the next step to isolating the gene(s) involved is called "congenic mapping", whereby mice are progressively bred so that the genetic interval is narrowed. This approach has led to the identification of several "candidate" genes in the intervals.[14–19] An accompanying step is the identification of unique immunophenotypes associated with the unique genotypic structure of these mice. The rest of this article will describe our results with several of these mice and show how they illustrate a modular concept of autoimmunity.

Our first example concerns genetic control of autoantibody production. Antinuclear antibodies are considered a hallmark of autoimmunity, in particular connective tissue diseases such as Systemic Lupus Erythematosis (SLE). Anti-Sm antibodies, directed against the spliceosome complex, are considered very specific for human SLE.[20] In our colony, neither NOD, B6 nor B6.G7 mice develop ANA or anti-Sm antibodies. When we checked NOD.*Idd9* congenic mice, (which are mice with mostly an NOD genetic background except for B6 "resistance" *Idd* loci on chromosome 4 ("c4"); the "*Idd9*" simply means this was the ninth *Idd* locus identified in the literature) however, they developed both ANA and anti-Sm antibodies.[21] Only 2% of NOD.*Idd9* congenic mice develop diabetes.[15] Since B6 and B6.G7 mice, which have the genetic region on chromosome four found in NOD.*Idd9* mice, don't develop autoantibodies, it is clear that the c4 region must interact with the NOD genetic background to generate autoantibodies. The process is specific to the c4 congenic mice, since NOD.*Idd3/5* mice (with *Idd* loci on chromosomes 1 and 3) which also have a low (2%) prevalence of diabetes, do not generate these autoantibodies. Nor do NOD.*Idd3/10/18* mice (with a B6 genetic interval from chromosome three ("c3")). In contrast, a congenic mouse with both the c3 and c4 intervals, which we described as NOD.c3c4 mice, develop no autoimmune diabetes but also demonstrated both ANA and anti-Sm antibodies.[21]

Our description of autoantibody production in NOD congenic strains confirms similar data from other labs looking at anti-insulin autoantibody (IAA) production in the same set of mice.[22] This paper demonstrates that NOD.*Idd3/5* and NOD.*Idd3/10/18* mice had low prevalence of IAA production, while NOD.*Idd9* mice had a high prevalence. Remarkably, however, the NOD.*Idd9* mice, despite the high prevalence of autoantibodies, have a low prevalence of disease; conversely mice without the autoantibodies developed diabetes.[22] This therefore has the hallmark of one of the features of a modular phenotype: autoantibodies which are geneti-

cally controlled in expression, but neither necessary nor sufficient for disease onset. If the phenotype of IAA production is neither necessary nor sufficient for T1D, is it therefore irrelevant? We would argue not. Rather, we suggest that such genetically controlled "modules" can "stack" with other modules and make a disease process worse, i.e. they can act as disease modifiers.

The next module we will describe is "insulitis", i.e. the accumulation of lymphocytes in the pancreatic islets. It is hard to imagine diabetes occurring in the absolute absence of insulitis, so it is tempting to describe it as a "necessary" component of the autoimmune process. Is it also sufficient? And is it genetically controlled? These answers are known. The NOD.*Idd9* mouse described above has a very low (2%) prevalence of diabetes. Surprisingly, however, it has a very high grade insulitis.[16] Therefore, insulitis can occur without progression to diabetes, and is not therefore sufficient for diabetes. Genetic control of insulitis has also been clearly demonstrated: Loci on chromosomes one and three clearly can act to allow or prevent insulitis depending on genetic background.[16,23] NOD.*Idd3/5* mice, for example, show marked suppression of insulitis.[14] We have shown that NOD.*Idd3/10/18* mice, in contrast, not only show decreased insulitis, but they develop lymphocytic infiltrates into the liver, in the absence of ANA or anti-Sm autoantibodies. It appears that in NOD.*Idd3/10/18* mice, the trafficking of lymphocytes to the liver and pancreas is mutually exclusive or antagonistic.[21]

The description of high grade insulitis in NOD.*Idd9* mice, along with ANA and anti-Sm autoantibodies, clearly illustrates an "additive" feature of the two modules, i.e. insulitis and anti-Sm antibodies can exist in the same individual. The question arises as to what "disease" the NOD.*Idd9* mice have. We would argue they have no particular classically defined autoimmune disease, but a modular disease composed of additive autoimmune modules. A rheumatologist might diagnose these mice with SLE. Two of one hundred such mice, however, develop autoimmune diabetes, and most of them develop insulitis, not "normal" features of SLE.

Our next example of an immunomodule concerns NK ("Natural Killer") T cell function. NK T cells are lymphocytes expressing both T cell and NK T cell markers. They characteristically rapidly express immunomodulatory cytokines after anti-CD3 stimulation *in vivo*.[24] A large number of publications described a functional NK T cell defect as causative in NOD diabetes. Gombert *et al.* demonstrated decreased NK T cell numbers with decreased IL-4 production.[25] Hammond *et al.* showed that NK T cells form another strain could correct the defect and prevent diabetes.[26] NOD mice constructed with NK T cell transgenes (T cell

receptors from NK T cells were overexpressed in transgenic mice) were protected from diabetes. In addition, several groups reported that an NK T cell ligand, alpha-galactosylceramide, prevented diabetes in NOD mice.[27–29] Finally, Yang *et al.* reported that thymocytes from AKR mice (a non diabetic strain) did not reconstitute diabetes in NOD-*scid* mice, but thymocytes from B-2m (Beta-2 microglobulin, a necessary component of the MHC class I molecule. In absence of the class I molecule NK T cells cannot develop) deficient AKR mice did cause diabetes, seemingly strong evidence that an NK T cell deficiency is both necessary and sufficient for diabetes in NOD mice.[30] We examined NK T cell function in NOD congenic mice. We confirmed that NOD mice, compared to MHC class II matched B6.G7 mice, had a profoundly defective cytokine response to intravenous anti-CD3, characterized by defective IL-4 production. Surprisingly, however, NOD.*Idd3/5* mice showed the same functional defect, despite a 40 fold decreased diabetes incidence compared to NOD mice (unpublished observations, Ridgway lab). Therefore, the NOD NK T cell functional defect is neither necessary nor sufficient for diabetes, just as in the case with the autoantibodies described above. Again, we would not therefore conclude that this phenotype is irrelevant to autoimmunity. Rather, NK T cell cytokine defects may act as a disease modifying module, which could enhance an autoimmune process in the context of other autoimmune modules. It is likely that the NK T cell defect is not operative in NOD.*Idd3/5* mice due to the absence of the insulitis module described above.

We have described several independent, additive, autoimmune related, genetically controlled modules. We would like to conclude by illustrating an "epistatic" effect of combining modules, i.e. the unexpected manifestation of new phenotypes when two sets of genes are combined. We have already shown that NOD.c4 (*Idd9*) mice develop ANAs, anti-Sm antibodies, and insulitis. NOD.c3 (*Idd3/10/18*) mice show suppression of insulitis and no autoantibodies, but trafficking of lymphocytes to the liver. What would happen if these genotypes were combined? The answer is dramatic. These mice, termed by us NOD.c3c4 to indicate that they had "protective" (for diabetes) loci from both chromosome three and four, were totally protected from diabetes and showed no insulitis. About 90% of these mice, however, developed an eventually fatal autoimmune biliary disease.[21] The NOD.c3c4 mice developed ANA and Anti-Sm antibodies, as did the NOD.c4 mice. In addition, however, they developed novel autoantibodies not seen in the NOD.c4 nor any other NOD congenic mice.[21] The NOD.c3 mice had shown liver infiltrating lymphocytes but no clear disease process. The NOD.c3c4 mice, however, developed progressive lymphocytic biliary infiltration with biliary epithelial disturbance and hep-

atomegaly, and the mice eventually die from biliary obstruction.

NOD.c3 mice showed liver infiltrates but no overall disease process; NOD.c4 mice showed autoantibodies but no overt disease process. Combining these genetic segments lead to a novel "emergent" liver disease process and novel autoantibody production in the NOD.c3c4 mice. The interaction of homozygous B6 c3 and c4 intervals with the rest of the NOD genome is necessary for the disease, as shown by breeding NOD.c3c4 mice to either NOD or B6 (making "F1" mice which were heterozygous at some loci). (NOD) × (NOD.c3c4)F1 mice, surprisingly, had no liver disease, but developed diabetes. Therefore heterozygous c3c4 intervals interacting with a homozygous NOD background were not sufficient to prevent diabetes nor mediate liver disease. (NOD.c3c4) × (B6.G7)F1 mice, in contrast, expressed homozygous B6 intervals across the c3c4 region, but developed no diabetes, no liver disease, no antibodies, and no insulitis; in fact these mice appeared perfectly healthy.[21] These breeding experiments clearly illustrate the importance of interactions between the disease related genes and the rest of the genome in the disease process.

The congenic mice described in this article are all remarkably genetically similar, with 90–99% NOD genetic background and small B6 genetic intervals crossed in. In a sense, therefore, they represent a kindred of genetically closely related individuals. As such they demonstrate how profoundly sensitive the expression of autoimmune phenotypes can be to small changes in genetic composition. We have shown that mice which have no disease in the classical sense of the word can manifest genetically controlled "modules" which, if combined with other modules by mating, can interact to produce novel autoimmune syndromes. Alternately, mice manifesting no disease can carry several disease related "modules" which, in the "wrong" mating cross, could erupt into profound autoimmunity. For example, NOD.*Idd9* mice show abnormal lymphocyte trafficking to the pancreas and autoantibodies; NOD.*Idd3/5* mice have profound NK T cell defects; both mice have the disease related MHC class II molecule, but neither of them develop an overt classical disease process.

Human clinical autoimmunity demonstrates characteristics which could be explained by the "modular" theory presented here. Autoimmunity of different forms in an extended family could reflect segregation of different modules in different combinations. Patients with bizarre mixtures of autoimmune features, in particular "undifferentiated" connective tissue diseases or "overlap" syndromes, likely represent patients with deleterious mixtures of autoimmune related modules. The proper approach to understanding these patients in human autoimmune disease likely is to focus on "subset-ting" of patients according to immune phenotypes (or modules) regardless of their "disease diagnosis". A modular approach to human autoimmunity will provide a more flexible paradigm for understanding the diversity of autoimmune expression and for eventually dissecting the genetic control of human autoimmunity.

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