ified by examination of the C5a receptor knockout mice. C3b on the other hand, has been shown capable of blocking IL-12 production by its interaction with the $\alpha_M\beta_2$ integrin (also known as Mac-1 or CR3)[7,8], an action perfectly in keeping with a positive role for C3 cleavage in AHR, either *via* C3a and its receptor or through C3b and reduced levels of IL-12. However, although a potential role for IL-12 in these complement-mediated processes is intriguing, it seems likely that this is not the only point of intersection between active components of the complement system and the cytokine network.

Even if IL-12 is a major point of regulation, these observations merely shift responsibility one step down. One must now ask how IL-12 modifies the cytokine mix to prevent, or its absence so alters the cytokine mix to promote, the fundamental biochemical and biophysical processes causing airway hyperresponsiveness. Presumably C3a would be expected to act more directly on these downstream effector pathways—but where? This is still a major question. Finally, although seemingly self-evident, it may still be important to emphasize avoidance of the all too common tendency to attribute key roles to any one or two molecules in biological phenomenon as complex as asthma, or even the more limited manifestation of hyperresponsiveness. The excitement in these two studies comes from the new questions that they raise and the hope for a new understanding of the actual mechanisms underlying the process of AHR itself. They also clearly raise the possibility that the two molecules, C3a and C5a—usually thought to act in the same direction—have opposite effects. An additional set of important questions might then address the source of the C3 or C5 cleavage. Candidate proteases are present in most inflammatory reactions and would be expected in asthmatic airways. Bacteria in the upper respiratory tract and sinuses may also contribute to generation of complement fragments that then gain access to the lower airways[9]. There is also an increasing interest in the possibility that chronic asthma is associated with the presence of infectious agents such as mycoplasma or chlamydia in the lower airways. Whatever role these might play in the pathogenesis or exacerbation of the human disease, and quite separate from the possible source of C3- or C5-cleaving proteases in the murine models discussed here, complement activation by such organisms could certainly add to the mix of bioactive molecules in the airways and, as a consequence, to the balance between normal and altered responses to exposure.

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National Jewish Medical and Research Center,

Selecting killers: the line between life and death

LINDSAY B. NICHOLSON AND VIJAY K. KUCHROO

How do T cells that are specific for pancreatic islet cell antigens cause diabetes? A recent paper in *Nature* provides evidence from NOD mice that the killer T cells responsible increase their avidity as the disease progresses—removing the high avidity clones prevents disease.

In contrast to some animal models of autoimmune disease, such as experimental autoimmune encephalomyelitis (EAE), that are induced by immunization with specific self-antigens, diabetes in the nonobese diabetic (NOD) mouse arises spontaneously under the influence of more than 18 polymorphic genes[1]. The disease goes through defined checkpoints from peri-insulitis to insulitis and overt diabetes as mice age and the disease progresses[2]. Also, unlike EAE, in the NOD mouse CD8 cells as well as CD4 cells have been shown to be pathogenic effectors for the induction of diabetes[3]. Antigen recognition is central to the disease process, and identifying the antigens involved has remained an important theme in diabetes research. In a recent report in *Nature*[4], Amrani *et al.* describe studies that address this question from the perspective of a specific major histocompatibility complex (MHC) class I–restricted CD8 T cell response to islet cells. In previous work the same group exploited the observation that a majority of the CD8 T cells that infiltrate the islets of the pancreas in diabetes bear a restricted set of T cell receptor (TCR) α chains, paired with a diverse repertoire of V_β chains. With a representative TCR from these $CD8^+$ T cells, they generated the 8.3 TCR transgenic mouse whose $CD8^+$ T cells can induce diabetes, although diabetes is induced more efficiently when CD4 cells are also present[5]. The antigenic epitopes that these $CD8^+$ T cells recognize in the islets is not known. However, screening a peptide library yielded two peptides, NRP (KYNKANWFL) and NRP-A7 (KYNKANAFL), that activate the transgenic 8.3 TCR–bearing T cells in the context of H-$2K^d$ (ref. 6). Neither of these peptides is a known autoantigen from pancreatic islets. In these activation assays, it was clear that whereas the NRP peptide had agonist properties, an alanine mutant of the peptide called NRP-A7 acted as a superagonist peptide for this TCR.

They have now taken the next step and addressed the role of the T cells whose TCRs bind these peptides in the pancreas of normal NOD mice, by using NRP and NRP-A7–K^d tetramers to identify them. To do this they expanded islet-derived T cells from nondiabetic mice in the presence of interleukin 2 (IL-2) for a week before analyzing them, which may explain why the frequency of tetramers recognizing an insulin peptide–K^d complex is lower than previously reported[7]. They have come to the arresting conclusion that the average avidity of the population of T cells that recognizes NRP-A7 increases as disease progresses and that elimination of the high avidity cells prevents diabetes.

The study raises several interesting questions for immunologists investigating the biology of autoimmune diseases. The first issue is why should increases in avidity correlate with pathogenicity and disease progression? The second is why should elimination of high avidity T cells of just one clonotype result in almost complete prevention of

diabetes? TCRs are known for the weak nature of their ligand interaction. Moreover, activation is often seen as a threshold phenomenon on one side of which T cells are dormant, and on the other side, fully activated. Consistent with the observations of P. Allen, A. Sette and R. Germain, the findings presented in this study lend support to models of T cell activation in which different effector functions are induced at incrementally higher activation signals. Therefore, perhaps higher avidity cells are more pathogenic because their killing functions are induced more efficiently. On the other hand, the monoclonal 8.3 TCR transgenic mouse still develops diabetes soon after developing insulitis[5]. Therefore avidity maturation may not only be critical for pathology to develop, but also for selectively expanding cells from a diverse pool of infiltrating cells of varying avidity, which can overcome the checkpoints to disease and mediate diabetes. To understand whether this is the case, we need to understand what drives the process of avidity maturation in immunization, infection and autoimmune disease.

For antibodies, affinity maturation is driven by limiting the amounts of antigen. One might have expected that in the pancreatic islets, where a large pool of islet tissue antigen is available, even low avidity T cells could expand and contribute to the progression of disease. There are several reasons why avidity maturation (or more precisely selection of high avidity T cells) may be necessary for the development of pathogenic $CD8^+$ effector cells in autoimmune diabetes. Cytolytic $CD8^+$ cells are believed to lyse pancreatic islet cells directly, even though these cells lack expression of costimulatory molecules (B7s and CD40) and generally express few class I molecules. By the time diabetes develops, the majority of the β-cells in the islets have been destroyed and so the availability of the pancreatic antigen recognized by the NRP-A7–reactive cells in the tissue may be limiting. The limited availability of both the ligand for the TCR (class I MHC and antigen) and a lack of expression of costimulatory molecules on the pancreatic islet cells may be critical factors leading to the expansion of cells of high avidity that have a lower requirement for costimulation[8], and which overcome the regulatory checkpoints and cause tissue destruction (**Fig. 1**). There may also be other fundamental mechanisms driving the selection of a single or a few clones from the whole population of cells that can respond to a particular antigen, as eventually only a single clone may be able to occupy a specific niche. This idea of competitive exclusion has been considered theoretically in the context of T cell repertoires[9], but there is little experimental data to assess its validity. In the future it will also be interesting to determine whether there is a need for pathogenic $CD4^+$ effector cells to also undergo avidity maturation before they induce disease, since their expansion will be driven by professional antigen presenting cells (APCs) that express many class II and costimulatory molecules.

In their analysis of this system, the authors have concluded that the cells that bind NRP tetramer are a subset of the whole population of cells that bind the NRP-A7 tetramer, because the affinity of the 8.3 TCR is higher for the NRP-A7 tetramer than for the NRP tetramer. This seems inconsistent with the results presented in the paper for two reasons. First the NRP-A7 tetramer binds more cells than NRP tetramer because it binds the low avidity cells that the NRP tetramer cannot. Therefore if the avidity of the population is increasing, then the fraction of cells that binds NRP tetramer should increase, but in fact it decreases. Second, in cells derived from the islets of 5-week-old mice, the percentage and number of NRP-A7 tetramer–binding cells is lower than the percentage and number of NRP tetramer–binding cells. This cannot be the case if all the NRP tetramer–binding cells are contained within the set of cells that bind NRP-A7 tetramer. Therefore a more likely interpretation of the data is that cells that bind NRP-A7 tetramer and NRP tetramer are qualitatively distinct but overlapping populations, rather than one population being a subset of the other. This interpretation makes the relative ineffectiveness of NRP inhibition of disease compared with NRP-A7 much easier to understand because the NRP-A7–K^d tetramer may recognize qualitatively distinct cells and not just cells with higher avidity compared to the NRP-K^d–binding cells.

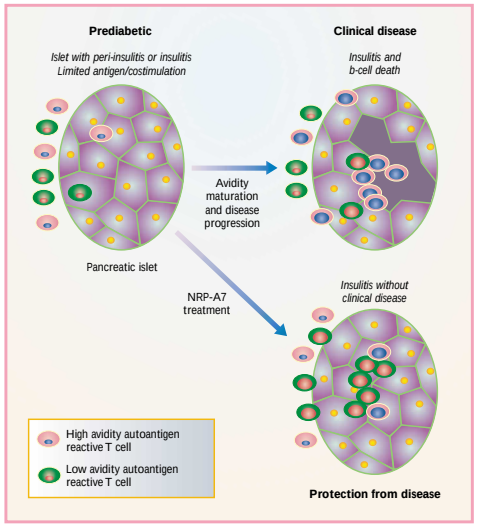

Figure 1. Preventing clinical disease. In prediabetic mice a mixture of low and high avidity autoantigen-reactive T cells accumulate outside the pancreatic islet. Under conditions of limiting antigen or costimulation high avidity cells are selected and expand to become pathogenic effectors that kill β-cells. Treatment with soluble NRP-A7 peptide favors the development of low avidity cells. Insulitis still develops but with much reduced clinical disease.

Bob Crimi

The inhibition of disease by administration of NRP-A7 peptide is very striking, and the fact that it is accompanied by an expansion of low avidity cells that recognize NRP-A7 at the expense of high avidity cells raises several important questions for regulation of immunity and autoimmunity. One question is why is the effect is so profound? Simple deletion of one pathogenic clonotype cannot easily explain this result. In fact, the number of high avidity cells may be relatively unchanged, if low avidity cells are expanding (**Fig. 1**). Although it is possible that all the NRP-A7–recognizing $CD8^+$ T cells that are high affinity and cause disease could have been eliminated, would this lead to such a profound reduction in disease in normal NOD mice? There are several other pathogenic CD4 and CD8 clonotypes available[7,10], and these clonotypes can induce profound diabetes: they should therefore be able to take over and cause disease. Blocking reactivity to one antigen would only be expected to halt disease progression if there was a sequential recognition of different antigens, and blocking recognition to one prevents progress to the next. It should also stop the development of insulitis, which is not seen in these experiments.

A more provocative interpretation of the data would be that the treatment of NOD mice by soluble NRP-A7 can itself inhibit the progression of disease. A possible mechanism could be deletion of pathogenic NRP-A7–reactive $CD8^+$ cells and simultaneous induction and/or expansion of cells that regulate diabetes by producing anti-inflammatory cytokines, such as IL-10 and transforming growth factor β (TGF-β)[11], or by other less well understood mechanisms[12]. Thus it is more plausible that the mechanism by which NRP-A7 peptide inhibits disease is by inducing regulatory T cells that can mediate bystander suppression and inhibit the patho-

genic effects of T cells of other specificities, rather than by deleting one among many pathogenic cell specificities.

It is clear from other data that there is a balance *in vivo* of pathogenic and protective repertoires with specificity for the same autoantigenic epitopes[13,14]. Treatment with altered peptide ligands and soluble peptide may shift this balance and alter the course of disease[15]. This study supports the possibility that one important characteristic of pathogenic *versus* protective repertoires may be their affinity for the peptide-MHC complex. Therefore, changes in the balance of different TCRs in the repertoire, which differ in their avidity for autoantigen, may differentiate pathogenic clonotypes from protective ones. If experiments directed at testing this hypothesis are informative, then we will have data not only for the role of high avidity interactions in the pathogenesis of autoimmune disease but also for the role of low avidity interactions in its regulation.

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Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, 77 Avenue Louis Pasteur, Boston MA 02115 USA.

Modeling costimulation

P. ANTON VAN DER MERWE

T cell activation usually requires TCR engagement by antigen and another, costimulatory, signal. Modeling studies now indicate that this second signal may only slightly enhance TCR signaling, but nevertheless results in an exponential increase in cell numbers.

The two-signal or costimulation model of T cell activation was first proposed by Lafferty and colleagues[1,2] to explain why transplants of foreign tissue are not always rejected. They postulated that full activation of naïve T cells requires engagement of an antigen receptor by foreign antigen (signal one) as well as engagement of a "costimulatory" receptor by a soluble or cell surface ligand provided by the antigen presenting cell (signal two). This two-signal model is consistent with a considerable body of experimental data and is now widely accepted. Variants of the model, for which the evidence is less compelling, propose that delivery of signal one alone inactivates T cells by killing them[3] or rendering them unresponsive (anergic)[4]. In this issue of *Nature Immunology* Gett and Hodgkin investigate costimulation using modeling[5].

When the two-signal model was first proposed neither the T cell antigen receptor (TCR) nor any candidate costimulatory receptor–ligand interaction had been identified. Subsequently the CD28 molecule and its ligands B7-1 and B7-2 were shown to have the requisite properties[6], and for a time the terms CD28 and costimulatory receptor were considered by some to be synonymous. However, the demonstration that T cell antigen recognition occurs in mice deficient in CD28 showed that other receptor-ligand systems must contribute to signal two[7,8]. To date a large number of receptor-ligand interactions have been shown to enhance or inhibit T cell antigen recognition, through several different mechanisms[9]. Furthermore it is evident that signal one can vary because slightly different peptides can induce different T cell responses following peptide-MHC recognition[10]. These results suggest that it is necessary to update the two-signal model along the lines outlined in **Fig. 1a**. Instead of "costimulatory receptor" the more general term "accessory receptor" is used. This term refers to all T cell surface receptors, other than the TCR itself, that inhibit or enhance T cell antigen recognition upon binding to their soluble or cell-surface ligands. An important feature of the updated model is that the outcome of T cell antigen recognition is determined by the nature the TCR ligand.

Increasingly sophisticated experimental approaches are needed to study the complex set of accessory receptors that modulate T cell responses to antigen. The study by Gett and Hodgkin in this issue[5] is a good example of such an analysis. They used a simplified system in which T cells are stimulated by an immobilized monoclonal antibody (mAb) to CD3, and the CD28 and IL-4 receptors are engaged with soluble mAb to CD28 and interleukin 4 (IL-4), respectively. Instead of using the usual, rather crude, measures of cell proliferation, Gett and Hodgkin labeled the cells with the fluorescent dye CFSE and used flow cytometry to monitor cell division directly. They found that naïve CD4 T cells given an identical stimulus vary considerably in the delay time to the first division, but that the subsequent division rate was the same for all cells. By measuring the response to mAb to CD3 with or without anti-CD28 at different time points they showed that CD28 engagement shortened the time to the first division but had no effect on the subsequent division rate. In contrast, addition of IL-4 both shortened the time to the first division and increased the division rate. Furthermore, combining CD28 and IL-4 receptor engagement resulted in strictly additive effects on time to first division and division rate, suggesting independent mechanisms of action.

Using a simple mathematical model of proliferation, Gett and Hodgkin go on to show that the cell division parameters they measured experimentally accurately predicted the observed cell division profiles, but substantially overestimated the cell numbers. When the model was modified to take into account the spontaneous cell death that occurs in T cell cultures it accurately predicted cell numbers until ~90 h, after which time cell numbers were again overestimated, probably because the model did not account for activation-induced cell-death. This model was then used to perform a "what if" experiment, examining the effect of varying parameters such as the