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SHORT ANALYTICAL REVIEW

The Th1/Th2 Dichotomy, hsp60 Autoimmunity, and Type I Diabetes

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This paper presents some questions and issues regarding the concept of the Th1/Th2 dichotomy and summarizes results using an hsp60 peptide to treat the spontaneous autoimmune process of diabetes in NOD mice.

INTRODUCTION

The Th1/Th2 dichotomy (1) is shorthand for the observation that the antigen-specific activation of CD4 T cells can lead to two seemingly opposed biological outcomes: the secretion of "proinflammatory" cytokines (the Th1 phenotype) or the secretion of "anti-inflammatory" cytokines (the Th2 phenotype). The Th1/Th2 dichotomy is only two of several response phenotypes that can be elicited by a single antigen. For example, the CD8 T-cell response can be expressed as cytotoxicity or suppression, and a single antibody specificity, expressed by the antibody's variable moiety, can mobilize a variety of biological effects, depending on the class and isotype of the constant region of the antibody. Thus, when mounting a response, the immune system has to weigh options. The Th1/Th2 cytokine option is currently popular because this dichotomy can influence, for better or worse, the fate of the individual responding to an infectious agent, to tumor cells, or to a self-antigen in an autoimmune situation. Historically, the phenomenon we now call Th1/Th2 was described long ago as "immune deviation," denoting the capacity of an immune response to be expressed primarily either by antibody production or by "cell-mediated" immunity (2). Early evidence suggested that different populations of T cells provided help for antibody production and mediated cellular reactions (3). The renaissance of interest in T-cell classes springs from the discovery of cytokines and their classification into Th1 and Th2 groupings (1). Such is the nature of the scientific enterprise; a phenomenon, such as immune deviation, will lose the attention of the research community unless study of the phenomenon leads to a molecular mechanism that explains it. The decline of interest in immune deviation could only be reversed by the discovery of cytokines, and then the phenomenon is resurrected under a different name.

Parenthetically, the phenomenon of the "T suppressor cell" has been unusually resistant to its molecular resurrection. Reputable immunologists may continue to deny the existence of antigen-specific T suppressor cells, despite their acceptance of the fact that a T cell can secrete a "suppressor" cytokine (TGFβ, IL-4, IL-10) in response to a specific antigen. There may be a good reason for this persisting dislike of a term that seems to have a molecular basis: cytokines are pleiotropic and a particular cytokine such as TGFβ may be suppressive for one target (Th1 T cells, for example) and, at the same time, stimulatory for a different target (fibroblasts, for example). Th1/Th2 terminology refers directly to packages of cytokines and only indirectly to cellular behavior. Th2 and Th1 T cells may, without offending a single immunologist, suppress the activities of the other T-cell type while enhancing the activities of their own camp. Nevertheless, the Th1/Th2 dichotomy carries the hazard of its own phenomenological oversimplification, an issue to which I shall return at the end.

TYPE I DIABETES

Type I diabetes, or insulin-dependent diabetes mellitus (IDDM), designates the diabetes that results from autoimmune destruction of the insulin-producing β cells of the pancreatic islets (4). The disease used to be called juvenile diabetes because it appeared to be limited to young persons, in contrast to adult-onset non-insulin-dependent diabetes mellitus. However, it is now clear that the incidence of type I diabetes is increasing and the disease can develop in adults into the fifth and sixth decades of life. Although the development of IDDM requires a susceptible genetic background with a strong HLA effect (5), the autoimmune process would seem to require environmental triggering. The increasing incidence of IDDM in Northern industrialized societies is compatible with environmental toxins as triggers.

It has been difficult to study the natural history of

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IDDM autoimmunity in people because the autoimmune process proceeds without symptoms or signs until the loss of β cells nears completion and the lack of insulin surfaces more or less abruptly as an endocrine disease. The autoimmune process by then is winding down and it is difficult to sort out primary and secondary processes. Particularly nagging has been the surfeit of target autoantigens to which IDDM patients manifest T- and B-cell autoimmunity (6). The NOD mouse, which features an MHC susceptibility gene very similar to the human HLA-DQ 0302 molecule (7) and develops IDDM spontaneously (8), also manifests autoimmune responses to more than one functionally important target antigen (6). Thus, there may be no single primary autoimmune target in IDDM, but the disease may involve a collective of autoreactivities (9).

THE hsp60 CONNECTION

My colleagues and I discovered, quite unexpectedly, that NOD mice, early in the process of β-cell destruction, spontaneously developed autoantibodies and autoimmune T cells reactive to the 60-kDa heat shock protein (hsp60) of the mouse (10, 11), cross-reactive with the hsp65 molecule of Mycobacteria (12). Cloning and sequencing of the mouse hsp60 molecule led to the identification of a 24-amino-acid segment of the molecule, positions 437–460, designated peptide p277 (10, 11). This peptide was recognized by a pathogenic clone of NOD T cells, and immunization to the p277 peptide conjugated to an immunogenic carrier molecule could induce transient hyperglycemia and insulitis even in mouse strains not otherwise prone to develop diabetes (13). The triggering of autoimmune diabetes in mice of the C57Bl/KsJ strain by an ultra-low dose of the toxin streptozotocine was accompanied by the appearance of antibodies to hsp60 and T cells reactive to hsp60 and its p277 peptide (14). The p277 peptide features a motif that allows binding to the MHC class II molecule IA^{g7} of the NOD mouse (7) and to the susceptible human HLA-DQ 0302 molecules (15). Indeed, newly diagnosed human IDDM patients also show T-cell responses to p277 (in preparation). Thus, T-cell reactivity to p277 is associated with autoimmune diabetes in mice and men.

PEPTIDE INDUCTION OF A Th1 → Th2 SHIFT

Most interesting was our finding that the destruction of β cells could be arrested, even when very far advanced, by a single subcutaneous administration of peptide p277 (100 μg) emulsified in oil (10, 11, 16, 17). Treatment of the IDDM triggered by streptozotocine required a second injection of p277 (18).

Study of the immunology of p277 treatment was compatible with a shift in anti-p277 T-cell reactivity from a Th1-like phenotype to a Th2-like phenotype (19). The spleen cells of p277-treated mice responded to p277 (or to hsp60) by secreting the Th2 cytokines IL-4 and IL-10; sham-treated mice continued to manifest spontaneous T-cell reactivity to p277 expressed by the secretion the Th1 cytokines IFN-γ and IL-2 (19). The shift in cytokine production was accompanied by the appearance of antibodies to p277 of the IgG1 and IgG2b isotypes (19). IgG1 depends on IL-4 (20) and IgG2b on TGFβ (21). Thus, the induction of antibodies to p277 was also compatible with a "deviation" of the p277 response. The Th2 activity induced by p277 peptide treatment was transient and IL-4 and IL-10 secretion in response to p277 waned 1–2 months after treatment (19). However, no Th1 reactivity to p277 recurred and the diabetic process did not return. Thus, p277 treatment seemed to trigger a resetting of the cytokine profile of the autoimmune reaction following a transient wave of Th2 reactivity that "suppressed" the pathogenic Th1 reactivity.

SPREADING REGULATION

How can a shift in the immune response phenotype to a single peptide arrest a pathogenic process involving autoreactivities to a collective of self-antigens? The Th1/Th2 modulation of NOD diabetes shows both specific and nonspecific aspects that sharpen this question. Specificity was evidenced by the fact that the same population of spleen cells that showed a Th1 to Th2 shift in anti-p277 reactivity, persisted in showing a spontaneous Th1 (IL-2, IFN-γ) response to a bacterial hsp60 peptide not cross-reactive with self-hsp60 (19). Thus, it was only the anti-self-reactivity that shifted its phenotype. Spreading of regulation to other IDDM-associated antigens was seen, however, in the p277-treated mice. Th1-type T cell responses and Th1-type antibodies to other antigens such as insulin and glutamic acid decarboxylase were found to be down-regulated (19). Thus, inhibiting the Th1 response to p277 was associated with suppressed Th1 responses to other antigens in the IDDM collective, albeit not to foreign antigens.

Spreading down-regulation was most impressive in the islet infiltrating T cell population. We developed an ELISPOT system (22) for enumerating the actual numbers of islet T cells producing various cytokines upon activation with T-cell mitogenic anti-CD3 antibodies (23). Using this technique, we could document the numbers of all islet T cells capable of secreting particular cytokines before and after treatment. The progression of β-cell destruction was clearly associated with the spontaneous accumulation of IFN-γ-secreting T cells in the islets. Following p277 peptide treatment, there was an abrupt fall in the numbers of IFN-γ producers in the islets, followed by a general fall in islet

leukocytes and a relative rise in the numbers of islets, mostly insulitis-free (23). Interestingly, we could detect no T cells producing IL-4 or IL-10 in the islets associated with the fall in IFN-γ producers. Thus, a Th1 to Th2 shift could be observed in the spleen, while the islet T cells showed only the loss of Th1 cells. Perhaps we merely missed a transient Th2-type response in the islets; perhaps the idea of a Th1 to Th2 shift is an oversimplification of a more complex regulatory mechanism. Th1 T cells may be down-regulated without necessarily up-regulating Th2 T cells. We have been able to prevent NOD diabetes by introducing the mouse hsp60 gene under the direction of an MHC class II promoter (24). The transgenic mice, too, showed a loss of IFN-γ producers in response to p277, without a shift to IL-4 producers (in preparation).

SIMPLICITY

Simple ideas are more understandable and more easily taught than are complex ideas. The simple, in addition, is beautiful and beauty, as we have been taught by Plato, is the mark of truth. So we need not apologize for propounding simple ideas to explain complex biology. Nevertheless, simple ideas in biology can become hazards when we believe them to be not only useful, but true to nature. The concept of the Th1/Th2 shift is a convenient mental prop for thinking about the regulatory structure that controls autoimmunity and autoimmune disease. But let us beware of thinking that the arrest of disease is explained just because we have a name for it. We have yet to understand how administration of a target peptide in a different context (25) can shift an immune response phenotype, how a Th2 response can suppress Th1 responses to a whole collective of self antigens leaving other reactivities intact (19), how antigen collectives are mutually connected (26), how IFNγ damages β cells but not α cells in the same islet, and so forth and so on. Indeed, what appears to be a Th1 to Th2 shift in a T-cell response to myelin basic protein epitope can be induced by immunization to a T-cell antigen receptor (27). How can T cells responding to T-cell receptors change cytokine phenotypes? Unanswered questions need no apology; they generate research. Fortunately, we do not have to know all the answers to know how to use p277 effectively. Clinical trials of peptide p277 therapy in type I diabetes are in progress.

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