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Commentary

Mechanisms of Cytokine-mediated Localized Immunoprotection

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The actions of proinflammatory or regulatory cytokines have been quite unpredictable when expressed individually within tissues such as in transgenic mice. Cytokines that have been considered proinflammatory in the classical sense have been shown to have immunosuppressive effects; likewise, cytokines that have demonstrated immunosuppressive activity have proven to be proinflammatory in these transgenic experimental systems (1). In this issue, studies on elicitation of the cytokine tumor necrosis factor-α (TNF-α) within the context of pancreatic islets elegantly illustrate this paradox (2). The proinflammatory cytokine TNF-α, long associated with phenomena such as autoimmunity, cell death, and cachexia shows quite unexpected characteristics in this transgenic system. In the current study, it was demonstrated that within the limited context of the pancreatic islets, TNF-α prevents the development of diabetes in the most widely studied animal model of the spontaneous disease: the nonobese diabetic (NOD) mouse. This work is important for several additional reasons other than the unpredicted ability of this cytokine to divert the nearly inevitable autoimmune responses that mediate disease in the NOD mouse. The data presented in this paper support a unique mechanism of disease amelioration, although a full picture will have to await additional studies.

Previous work by these authors with the identical transgene harbored by nondiabetes-prone mice has highlighted the proinflammatory nature of TNF-α because the cytokine was demonstrated to induce inflammation and leukocyte extravasation in these animals (3). This was probably mediated through the induction of adhesion molecules that support leukocyte extravasation into the pancreas. Insulitis resulted and progressed to be quite severe. Although these lymphocytes traffic in large numbers into the islet region, they are unable to promote killing of sufficient numbers of beta cells to induce any hyperglycemia despite their ability to cross into the actual islet parenchyma core and disrupt the islet architecture. Thus, on a nonsusceptible genetic background, severe infiltration does not promote disease.

TNF-α Leads to a Defect in Priming of Potentially Autoreactive T Cells

In the context of a diabetes susceptible MHC haplotype and additional genetic factors that promote a high spontaneous disease incidence, the transgene reveals the cryptic but powerful immunosuppressive ability of the TNF-α molecule (2). The mechanism of this cytokine's effect within the pancreas is still somewhat mysterious, although the current publication provides some important clues to what underlies its immunosuppression. The most striking finding in the TNF-α-protected mice is the lack of T cell responses to individual islet antigens or to whole islet extracts, to which the nontransgenic diabetes-prone NOD mice respond vigorously. The authors have ruled out generalized systemic immunosuppression through experiments demonstrating normal responses to the exogenous antigen KLH in their transgenic model. Thus, the transgene confers a specific defect in the ability to prime and activate potentially diabetogenic T cells in the pancreas or, more profoundly, can specifically and permanently inactivate these potential clones in situ. Alterations in islet antigenicity or accessibility do not explain the observed protection as emphasized by experiments where diabetogenic T cells are transferred into the transgenic mice, causing rapid beta cell loss and disease. Refreshingly, the observed protection does not appear to be mediated by a shift in functional specialization toward the Th2 phenotype, a now universally applied paradigm, since cytokine profiles to islet antigens were not found to contain a predominance of IL-4. Taken together, the evidence supports an alteration of T cell priming within the pancreatic vicinity of TNF-α transgenic mice. Supporting this notion are the related experiments performed in double-transgenic nondiabetes-prone mice showing that coexpression of the B7-1 costimulatory molecule by TNF-α-producing β cells leads to their destruction (4).

A more complete understanding of the events that lead to the priming of a diabetogenic T cell in disease-susceptible individuals may be necessary to comprehend fully the mechanism of protection in the current transgenic model. The recent surge in interest in CD8 cells as initiators of β cell damage in the NOD mouse (5) could, in the current context, implicate a signaling change in the β cells themselves that is responsible for sending alternative messages to antigen-specific T cells that enter the islet, as the authors suggest. These T cells could fail to become primed or be rendered permanently inactive (anergic) by the transgenic TNF-α-producing β cell and therefore unable to perpetuate the cascade of events that result in sensitization in the CD4 compartment, determinant spreading, and the subsequent effector phase, during which the majority of the β cells are destroyed.

This paper is dedicated to Stella Sarvetnick.

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However, another unexplored possibility is that the suppressive activities of TNF are enacted through other intermediary cells whose function is to prime potentially diabetogenic T cells within the islet vicinity. These islet-associated antigen-presenting cells (dendritic cells, macrophages, or B cells) could be affected in a variety of subtle ways that alter the course of T cell priming. While altered chemotaxis of any individual antigen-presenting cell category was not observed by the authors, altered antigen-presenting function has yet to be assessed. This type of shift in function could be enacted through a TNF-α effect on antigen processing, transport, or presentation. Significantly, a recent elegant report demonstrates that TNF-α is extremely inhibitory to the ability of dendritic cells to present soluble antigen (6). This effect is mediated by a down regulation of the invariant chain (Ii) and the FcγRII of the dendritic cells, although surface expression of MHC class II is increased. Dendritic cells are scattered in tissues (such as pancreatic islets) and pick up and process soluble antigens; therefore, they may play a pivotal role in mediating the pathogenic priming events that precede islet recognition and autoimmune diabetes (7). When exposed to TNF-α these cells may no longer be able to prime potentially islet-autoreactive T cells. Such a mechanism may explain the lack of reactivity to islet cell antigens and extracts that was observed in the current study using TNF-α transgenic mice, and the ability of cells primed in the absence of the transgene to mediate disease when introduced by adoptive transfer into transgenic mice.

It should be mentioned that other collaborative, albeit nonimmune mechanisms could enhance the apparent immunosuppressive properties of TNF-α in the pancreas. For example, localized expression of TNF-α has been demonstrated to lead to the development of limited numbers of ductal endocrine cells (8). These ductal endocrine cells could have altered antigenic specificity and thus be unable to become targets for any remaining immune response, yet secrete enough insulin to prevent development of hyperglycemia. Additionally, TNF-α inhibits signaling through the insulin receptor, and therefore is associated with the development of a state of insulin resistance such as observed in non-insulin dependent diabetes mellitus (NIDDM) (9). In the transgenic mice, the resulting superimposition of the characteristics of NIDDM with increased insulin levels due to lack of clearance and of IDDM might lead to a state of β cell rest, which has been long associated with protection from immune-mediated destruction.

Disparities between Local and Systemic Mechanisms of Immunosuppression

The authors of the TNF-α work relate their findings to earlier interesting and enigmatic work demonstrating that diabetes and insulitis were inhibited in NOD mice by systemic treatment with TNF-α (10). The observation of these authors was that treatment of adult NOD mice with TNF-α will substantially delay spontaneous IDDM. More recent work illustrates the complexity of such systemic studies, because parallel experiments using very young NOD mice resulted in acceleration of clinical disease (11). These superficially contradictory results underscore the fact that the early and late events that occur during the progression to clinical disease in NOD mice are separable and subject to distinct regulatory mechanisms. Moreover, the mechanism of TNF-α action when studied in transgenic mice where the molecule is produced constitutively in the pancreas need not be related to the explanation of protection that was observed with systemic treatment. This is because the hierarchy of events that precedes β cell destruction in islets includes processes such as T cell development, thymic selection, and homing to peripheral lymphoid organs, which are all subject to multiple levels of counterregulation. Thus stems the truly troubling paradox that in some cases systemic treatment with either the cytokine or its neutralizing antibody lead to the equivalent net biological effect (11–13).

In the early TNF-α treatment acceleration model, the previously described action of TNF-α in the thymus (14), for example, could alter the available pool of islet-reactive T cells in these systemic studies or, alternatively, its effect on lymphoid organ development could enhance the necessary establishment of vascular structures that support lymphocyte extravasation. Prevention of disease in the adult NOD mouse by systemic administration appears to be associated with a lack of infiltration of the pancreas. This could be accounted for by lymphopenia or by altered homing properties brought about by changes in adhesion capabilities of circulating leukocytes. These mechanisms could not easily account for the observations in the localized TNF-α-expressing transgenic model.

Prospects and Perspectives on Localized Immunoprotection: TNF-α versus IL-4

The ability to modulate the responsiveness or immunological accessibility of an individual tissue has profound implications for therapy, applicable to a variety of situations. It is generally accepted that most regulatory cytokines are far too toxic for systemic use in humans. Thus, the demonstration of efficacy in animal models of localized elicitation of cytokines is an important new advance for the design and execution of gene therapy delivery protocols. Thus far in the NOD diabetes model, both IL-4 and TNF-α have shown profound efficacy in eliminating destructive autoimmune responses to islets (2, 15). Additionally, IL-4 expression by islets can induce a permanent state of tolerance to engrafted syngeneic islet cells, and block the effect of diabetogenic lymphocytes following adoptive transfer. Functional immunological tolerance as demonstrated by syngeneic islet grafts may also be induced by TNF-α, because anti-islet responses were diminished in the TNF-α transgenic mice and therefore syngeneic grafts may survive in this strain as well.

However, it is important to note that the available data suggests that these two cytokine transgenics reflect fundamentally different underlying mechanisms of protection (2, 15). This is best illustrated by two sets of comparative experimental results. First, splenocytes from IL-4 transgenic

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mice will respond to islet antigens, while those from TNF-α transgenics will not. This critical distinction reflects an inability to prime diabetogenic T cells in the pancreatic vicinity of TNF-α transgenics, while in the IL-4 transgenic mice T cells do become primed with diabetes relevant antigens. Second, pancreatic IL-4 will prevent diabetes when diabetogenic spleen cells from nontransgenic NOD mice are introduced, while the TNF-α transgenics succumb to disease. This implies that IL-4 elicitation mediates regulation through activated T cell specificities within natural diabetogenic repertoire, while localized TNF-α allows no such activated repertoire to be established. Therefore, IL-4 may have regulatory capability during both the initial and ongoing phases of the disease process, while TNF-α completely blocks disease initiation.

The use of the now well-established methods of gene transfer through a variety of viruses (retrovirus, adenovirus, and lentivirus) therefore should facilitate the protection of individual tissues from autoimmune-mediated damage (16, 17). Similarly, these molecules have the potential to modulate the localized immune response to the decidedly more vigorous and rapid immunity to allografts and xenografts, affording the potential for localized immunoprotection, which is highly advantageous to systemic immunosuppression. The most powerful approach, clearly, is to position the cytokine within the islets themselves, as providing IL-4-expressing T cells does not lead to any protection from immune mediated damage, possibly owing to the transience of the signal within the islets (18). Thus, such an approach could only be applied to a short-term acute situation. The ability to regulate expression of these factors using inducible elements within promoters will increase the ability to regulate the specific phases of these complex autoimmune phenomenon.

Tissue Tropisms: Additional Complexities in Localized Immunoregulation

Part of the unpredictability of the actions of individual inflammatory mediators comes from the fact that many of their properties have been characterized in using in vitro culture systems. In the body, of course, these factors are elicited within lymphoid organs and in tissues, during an inflammatory response. Recent observations suggest that the action of these factors are modulated in part by other collaborating signals present within tissues. Thus, the identical individual signal in two tissues can lead to a very different immunologic outcome. This has been observed with transgenic expression of IFN-g, which elicits cell-mediated immune destruction in the absence of humoral response in the pancreatic compartment (19, 20) compared with humoral autoimmunity and the absence of evidence of cell-mediated damage in the neuromuscular junction (21). The regulation of such distinct responses may be exerted by the category of immune cells that reside in the tissue or appear when it becomes permeablized to inflammatory cells. It is also possible that antigen-presenting cells resident within tissues have mutually distinct pathways to respond to these mediators. Some evidence for this comes from the subtly distinct response to TNF-α in Langerhans cells versus dendritic cells, the former of which downregulate MHC class II synthesis in response to TNF-α, while the latter do not (6, 22). Such subtle variation of terminally differentiated function could allow the possibility for distinct processing, presentation, or activation capacities of antigen-presenting cells within tissues when exposed to specific inflammatory mediators. This type of phenomenon may lead to vast differences in patterns of T cell activation or even altered functional specialization of activated T cells, thus diversifying responses within individual tissue compartments. A further understanding of tissue tropisms will be important for predicting the outcome of cytokine-mediated immunoprotection studies, and will eventually enhance our ability to hide individual pools of antigen that are targets in pathogenic responses to tissues from the autodestructive immune system.

Thus, the ability to segregate a tissue compartment from immune-mediated surveillance and damage, while sparing the organism from systemic immunosuppression, is increasingly within reach. Recent work utilizing transgenic technology has raised significant hopes as to the overall application of this type of approach. As additional basic information regarding tissue tropisms will emerge, subtle differences in terminal differentiation of the regional immune system will be discovered, eventually allowing for more specialized compartment appropriate regulatory strategies.

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