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Balancing Autoaggressive and Protective T-cell Responses

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Abstract

A finely orchestrated balance between activating and inhibitory signals is fundamental for the ability of the immune system to effectively attack and eliminate pathogenic microbes but to not react against self-antigens. Derangements of this balance underlie the pathogenesis of autoimmune diseases. Conversely, elucidating the mechanisms of this balance may provide rational strategies for manipulating it in order to enhance the efficacy of vaccines and tumor immunotherapy. One of the clearest illustrations of precise regulation is in the generation of effector and regulatory T-cells. In order to analyze the mechanisms of this regulation, we have developed a transgenic mouse model in which a single population of T-cells reacts against its known cognate antigen *in vivo*. Here we summarize our studies with this experimental model, illustrating the sequence of T-cells responses that develop and attempting to dissect the stimuli that control these responses.

Keywords

Tolerance; Autoimmunity; Cognate Antigens; Tregs

Experimental Model of T-cell Tolerance Induced by a Systemic Self Antigen

The initial goal of these studies was to explore the mechanisms of T-cell tolerance to a systemic, secreted protein antigen. To do this, we generated a transgenic mouse line in which the protein chicken ovalbumin is expressed as a soluble protein (sOva) under the control of the metallothionein promoter. These transgenic mice, called sOva-Tg, contain 10-20ng/ml of sOva in the serum, and the antigen is presumably present in all vascularized tissues. Because the protein is expressed throughout development, and in the absence of inflammation, we consider it a model “self” antigen. We transferred into these transgenic mice $1-3 \times 10^6$ CD4 T-cells from the DO11 TCR-Tg, which are specific for the Ova₃₂₃₋₃₂₉ peptide. For most of the studies the DO11 TCR was crossed on to Rag^{-/-} mice, so only a single specificity is present and all the lymphocytes have a naïve phenotype prior to exposure to antigen.

The first set of experiments showed that when the DO11 T-cells encounter sOva, the T-cells rapidly proliferate, become unresponsive to antigen stimulation within 4-5 days, and are lost (deleted) by 10-14 days (1). This sequence of proliferation – anergy – deletion upon encounter with a self-antigen mimic has previously been described in responses of T-cells to high doses of aqueous peptides (2,3) and cell-associated self antigens (4). The mechanism of anergy induced in DO11 cells appears to involve a TCR proximal signaling block, and requires

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constant exposure to the “self” antigen because the cells recover if they are removed from the sOva-Tg mice (5). The mechanism of deletion is not yet defined; it does not appear to require FasL-Fas interactions.

Lymphopenia Breaks Tolerance to Systemic Antigen

One approach for defining mechanisms of autoimmunity is to study how tolerance might be broken, and what the consequences of loss of tolerance might be. Because of the documented, and paradoxical, association of certain immunodeficiency diseases and autoimmunity (6), we asked how DO11 cells would react to the systemic antigen if the sOva-Tg mice were made lymphopenic. To do this, we crossed the sOva-Tg with Rag^{-/-} mice, and transferred DO11 T-cells into these recipients. Surprisingly, recognition of the systemic antigen in a lymphopenic environment leads to a massive and sustained expansion of the T-cells the production of effector cytokines, and a severe disease with tissue inflammation and wasting that resembles graft *vs* host disease (GVHD) (7). Endogenous T and B lymphocytes are needed to maintain systemic self-tolerance (8). Endogenous T cells prevent the initial expansion of the self-reactive DO11 cells, presumably by competing for growth factors, APCs and/or costimulators. Endogenous B cells inhibit the differentiation of self-reactive DO11 cells into potent effectors, by mechanisms that remain unclear. These functions of endogenous lymphocytes in maintaining self-tolerance and homeostasis might explain many of the known associations of lymphocyte depletion with exaggerated immune responses.

We have recently found that the critical effector cytokine responsible for tissue inflammation is IL-17, and the classical Th1 response is actually protective (9). The role of IL-17-producing “Th17” cells in inflammatory diseases, and the cross-regulation of Th1 and Th17 subsets, have been demonstrated in a number of experimental models (10).

Control of Systemic Inflammatory Disease by de novo Generation of Peripheral Regulatory T Lymphocytes

One of the most remarkable observations in the experimental model we have established is that if mice survive the acute inflammatory disease, they gradually begin to recover, and by ~4 weeks after DO11 transfer, the recipients appear entirely normal. Recovery correlates with the development of CD25⁺ Fox P3⁺ DO11 regulatory T-cells (Treg) (7). These Treg arise in the periphery without an essential role of the thymus; to the extent we can address this, the phenotype of these peripheral Treg is indistinguishable from that of thymus-derived “natural” Treg. This is the only experimental system we are aware of in which there is a sequential peripheral development of pathogenic effector cells followed by protective Treg, all from the same naïve T-cell population (Figure 1).

These results raise several important questions. First, do the Treg differentiate from naïve cells or are they expanded from a pre-existing small population of Treg in the DO11 inoculum? By flow cytometry, we find <0.1% of the naïve DO11 cells express FoxP3, but it is practically impossible at the present time to exclude a small cell population that may be “committed” to develop into Treg. Second, what is the lineage relationship of effector and regulatory cells and is the inflammation associated with the systemic GVHD-like reaction a necessary prelude for the development of FoxP3⁺ Treg? At this time, it is also impossible to answer this question, but other studies are also suggesting that Treg development in the periphery follows inflammatory reactions. Third, how do the Treg function to suppress the pathologic inflammation? We have shown that Treg inhibit proliferation of responder cells that encounter the systemic antigen (9). However, Treg appear late, after the expansion should be over, raising an obvious conundrum. It is conceivable that propagation of the inflammatory disease requires the constant recruitment of new T-cells into the reaction, but it is difficult to prove this. Finally,

is our experimental system (transfer of quite large number of antigen-specific CD4 T-cells into a recipient expressing a systemic antigen but containing no endogenous lymphocytes) relevant to other more “conventional” forms of autoimmune disease? There is, of course, no spontaneous human autoimmune disease that mimics these experimental conditions. However, the response of a single, mono-specific T-cell population allows us to dissect the mechanisms of autoimmunity and its control in a “clean” and definite way. Our approach, as with all reductionist approaches, has been to take lessons learned from highly simplified experimental systems to more complex disease situations. The first step in this strategy – a detailed analysis of the simple system – has been our focus to date.

Dual Roles of IL-2 in the Development of Effector and Regulatory T Cells

One of the great strengths of this experimental model is that it allows us to define the signals that are critical for the generation of effector and regulatory T-cells. We have found that abolishing the B7:CD28 pathway (by using CD28^{-/-} DO11 T-cells) completely abrogates the immune reaction, and neither effector cells nor Treg develop following adoptive transfer. In contrast, CD40L^{-/-} DO11 T-cells appear to go through a wild-type sequence of effector Th1 cells followed by Treg.

We have done a much more extensive series of studies looking at the role of IL-2 in the generation of effector and regulatory cells. The recent interest in the biology of IL-2 has been spurred by the surprising findings that germline knockout of IL-2 or the α or β chain of the IL-2R leads to lymphoproliferation and severe autoimmunity, rather than the predicted immunodeficiency, and IL-2 is absolutely required for the survival, and perhaps the function, of FoxP3⁺ Treg (11). To address the role of IL-2, we crossed both the DO11/Rag^{-/-} and the sOva-Tg/Rag^{-/-} mice on to an IL-2^{-/-} background, and examined the responses of DO11 T-cells transferred into the antigen transgenics. These experiments revealed two important results. First, the acute disease is delayed and less severe when IL-2 is eliminated (7). This is likely because of significantly reduced Th1 responses, because the numbers of IFN γ -producing T-cells is reduced early in the reaction but the % of skin-infiltrating Th17 DO11 cells is actually increased in the absence of IL-2 (9). More striking than the reduced acute disease is the development of a chronic, inflammatory disorder, which is associated with a failure to generate FoxP3⁺ Treg (7). Thus, the absence of IL-2 converts an acute, self-limited disease to a chronic and progressive disease.

The clinical implications of this dual role of IL-2 are obvious. There are already emerging data in humans that IL-2 antagonism may convert acute GVHD to the more intractable chronic form of GVHD, and conversely, IL-2 administration may increase functional Treg (12). In both cases, the observed effects may be the opposite of the desired outcomes. Can we predict whether, under any circumstance, the dominant effect of IL-2 will be on effector T-cell or the regulatory T-cell response. At this time, the factors that influence this balance are not defined. An appealing hypothesis is that transient, high-level IL-2 production promotes effector T-cell responses and prolonged, low levels of IL-2 enhance Treg development and maintenance. There are however, no definitive ways to test this hypothesis *in vivo*.

Conclusions

The studies reviewed in this article have exploited a simple experimental system relying on CD4 T-cells of one specificity and their cognate antigen to define the sequential development of pathogenic effector T-cell populations and protective regulatory cells. We are now well positioned to identify the stimuli and patterns of gene expression that correlate with these strikingly different responses. In fact, our results with IL-2^{-/-} mice have already shown how the dual and opposing functions of this growth factor can be analyzed *in vivo*. Ultimately, of

course, the success of such reductionist approaches will only be validated by our ability to take discoveries from these models to the more challenging situation of human inflammatory diseases.

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Figure 1. CD4 T-cell responses to a systemic antigen

The sequence of dominant responses of DO11 T-cells after transfer into sOva-Tg recipients are illustrated schematically. The signals required for the various responses and the known transcription factors involved are from various publications, and have not yet been established in the DO11 system.