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TH1 AND TH2 RESPONSES IN PATHOGENESIS AND REGULATION OF EXPERIMENTAL AUTOIMMUNE UVEORETINITIS

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Experimental autoimmune uveoretinitis (EAU) in animals can be induced by immunization with retinal antigens or their fragments and represents human uveitis of putative autoimmune origin. The pathogenesis of EAU, and likely also of human uveitis, involves cell-mediated destruction of retinal tissues that is dependent on retinal antigen-specific T cells. Because in most cases a Th1-type response has been implicated in pathogenesis, the prevailing consensus has been that immunoregulatory manipulations designed to enhance the Th2 response at the expense of the Th1 response will be beneficial in clinical treatment of uveitis. This assumption may not always be correct. The present review will summarize the evidence that, despite a central role for Th1 response in uveitis, an unopposed Th2-like response can be equally or more destructive to the retinal tissues. Furthermore, the Th1 response itself triggers regulatory circuits that feed back and dampen further recruitment of antigen-specific T cells into the Th1 effector pool. Thus, although the Th1 effector response can and does result in retinal pathology, immunoregulatory strategies must take into account that immune deviation therapies designed to replace the Th1 with a Th2 response might result in exchanging one type of pathology for another rather than in achieving the desired therapeutic effect.

Keywords: Uveoretinitis, uveitis, Th1, Th2, autoimmunity, EAU

INTRODUCTION

Experimental autoimmune uveoretinitis (EAU) is an animal model for a series of human autoimmune uveitic diseases of a putative autoimmune nature. EAU can be induced in susceptible animal species by immunization with various retinal antigens or their fragments. Typically, within 9–14 days the eye becomes infiltrated

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with inflammatory cells and photoreceptor damage ensues. EAU can also be induced by adoptive transfer of T cells from immunized recipients to naïve, genetically compatible hosts [1–3]. The pathology resulting from adoptive transfer of primed effector cells is essentially identical to that induced by active immunization. A number of different antigens from the eye induce EAU, and in many cases the sequence of these proteins is known and the pathogenic fragments have been identified. The best-known ones are the retinal soluble antigen (arrestin, S-Ag) and the interphotoreceptor retinoid binding protein (IRBP) [2,4]. Both are pathogenic in Lewis rats, but in mice only IRBP elicits disease in common laboratory strains. Responses to retinal antigens, especially S-Ag, are frequently seen in human uveitis patients. EAU, as well as other models of uveitis that represent human diseases and that apparently have similar cellular mechanisms, are described elsewhere [1,2,5] as well as in other parts of this issue [3].

Uveitic diseases with a suspected autoimmune etiology are a significant cause of visual loss. In the United States alone, there are an estimated 70,000 cases per year of such diseases affecting various parts of the eye. It has been estimated that, collectively, uveitic diseases account for about 10 percent of severe visual handicap. More details about these diseases, including their clinical presentation, therapy, and genetics can be found elsewhere [5,6].

Accumulated evidence in animal models, with support from human studies, has indicated that the Th1 response is an essential component of autoimmune uveitic disease [7]. This is similar to other autoimmune diseases, such as multiple sclerosis, arthritis, and type 1 diabetes. In experimental models, uveitogenic T cells that can adoptively transfer EAU into naïve recipients are Th1 effector cells producing high amounts of interferon-γ (IFN-γ), considered to be a Th1 cytokine, and low amounts of IL-4 and IL-5, considered to be Th2-specific cytokines. T cell populations that have been isolated from EAU-resistant animals and produce low levels of IFN-γ can be converted to a pathogenic, IFN-γ–producing phenotype by culture in the presence of IL-12. Finally, EAU-susceptible strains are genetically programmed to be dominant Th1 responders.

The Th2 response is known to be counterregulatory to Th1. Indeed, in experimental situations it has been used successfully to counterbalance a pathogenic autoimmune Th1 response and to ameliorate Th1-driven disease [8]. In EAU, rats treated with mercuric chloride develop a Th2 response that inhibits pathology [9]. In mice, early treatment after uveitogenic challenge with a combination of IL-4 and IL-10 skews the immune response away from Th1 and reduces

pathology [10]. This has created a school of thought that has guided, and still continues to guide, clinical trials in uveitis and other tissue-specific autoimmune diseases, namely that if the immune system can be reprogrammed to mount a Th2 response instead of a Th1 response to the implicated autopathogenic antigen, we can expect a clinical benefit [11,12].

The present review seeks to reexamine the notion that a Th1 response is necessarily detrimental, and a Th2 response necessarily beneficial, in terms of tissue pathology. Evidence will be presented to show that an unopposed Th2 response can be equally or more destructive to the tissue than a Th1 response. Conversely, the Th1 response counterregulates itself, using the same mediators that also cause tissue damage to limit recruitment of further antigen-specific T cells into the effector pool or to eliminate active effectors that would otherwise persist and continue to fuel disease.

CENTRAL ROLE OF TH1 EFFECTOR RESPONSE IN PATHOGENESIS

The central role of the Th1 response in pathogenesis of EAU is undisputed. Early on, it became clear that the uveitogenic T cell lines that are capable of adoptively transferring disease have a Th1 phenotype, producing high levels of IFN-γ and low levels of IL-4. Furthermore, primary T cell populations that make IFN-γ are uveitogenic, whereas those that make little or no IFN-γ are not. However, they can be converted to a pathogenic, IFN-γ–producing phenotype by culture with IL-12 [13–15]. In general, the more highly polarized the T cell population toward Th1, the more uveitogenic it is. In keeping with this paradigm, it is perhaps not surprising that genetically EAU-susceptible mouse and rat strains are dominant Th1 responders to the uveitogenic antigen [14,16,17]. Lewis rats and B10.RIII mice, which are the most highly susceptible, also display the highest Th1 responses to the antigen. EAU can be induced in these strains without the aid of pertussis toxin, which we found to enhance the Th1 response when administered concurrently with immunization [14,18]. The cytokine response profile in genetically susceptible and resistant rodent strains indicates that, whereas the susceptible genotype is invariably a dominant Th1 responder, resistant strains fall into two distinct categories: Th2 responders such as A/J and BALB/c mice are resistant, but low Th1 responders without evidence of an overt Th2 cytokine profile such as the AKR mouse and the F344 rat are equally resistant [14,17].

IL-12 is the prototypic cytokine that promotes differentiation of naïve T cells to the IFN-γ–producing Th1 pathway. Endogenous IL-12 is necessary for EAU development. This is evidenced by the fact that neither IL-12–deficient mice, nor mice treated with neutralizing antibodies to IL-12 during the first week after uveitogenic immunization when Th1 effector cells are getting primed, are able to develop EAU [15, and Silver and Caspi, unpublished]. Thus, IL-12 is needed to generate the uveitogenic Th1 effector cell.

In addition to its role in generation of Th1 effector cells, IL-12 is also needed to sustain the functional uveitogenic effector T cells in vivo after they have been generated. This is seen from an experiment where lymph node cells from IRBP-immunized wild type (IL-12–sufficient) donor mice are transferred either into IL-12–sufficient or IL-12–deficient recipients [15]. IL-12–deficient recipients develop minimal EAU, although the wild type recipients develop full blown disease indicating that the transferred cells are competent effectors. Disease in IL-12–deficient recipients can be reconstituted if they are treated with a replacement dose of 5 ng/day of IL-12 [15]. In wild type mice, treatment with IL-12 that is started 7 days after uveitogenic immunization also inhibits subsequent EAU scores. These data inevitably lead to the conclusion that IL-12 is needed to maintain the Th1 effector function in vivo after the effector cell has been primed. Experimental manipulation of IL-12 levels in models of leishmaniasis and toxoplasmosis, where host defense is dependent on a persistent Th1 response, have confirmed this notion [19,20]. The reason why continued exposure to IL-12 in vivo is needed to maintain the function of the, Th1 effector is not fully understood. One interpretation could be that the effector Th1 cell in vivo is not fully polarized, and in the absence of IL-12 in the microenvironment it reverts to a nonpathogenic phenotype. Another explanation has been proposed by the work of Fuss et al. [21], who showed in an experimental colitis model that IL-12 is a survival factor for Th1 effector cells, and in its absence the Th1 cell undergoes apoptosis. Irrespective of the actual mechanism, these data lead to the conclusion that inhibition of IL-12 may be useful therapeutically, not only to prevent the maturation of tissue-destructive effector T cells but also to limit survival or function of the ones that have already been generated.

Interestingly, if the primed lymph node cells are cultured in the presence of IL-12 in vitro prior to adoptive transfer they are able to induce full blown disease in IL-12–deficient recipients [15]. Such cells cultured in vitro with IL-12 produce several-fold more IFN-γ in response to Ag, indicating that they have become more polarized toward the Th1 phenotype. Thus, a more polarized effector becomes in-

dependent of continued in vivo exposure to IL-12. Even more striking in this context is the observation that lymphocytes of 12 KO mice, activated with Ag in the presence of exogenous IL-12, develop into Th1 effector cells and induce EAU in IL-12 knockout (KO) recipients, indicating that IL-12 in culture during 2° Ag exposure can stably polarize T cells that had been primed in vivo in the absence of IL-12 [15]. It is conceivable that with chronic disease there is an increased accumulation of more polarized effector cells that would no longer be dependent on IL-12 for their survival and/or function, making them less susceptible to anti-IL-12 therapy.

THE TH2 RESPONSE AS PREVENTER AND AS INDUCER OF PATHOLOGY

It has been known for a long time that the Th1 response and the Th2 response are mutually antagonistic. Th2 mediators such as IL-4, IL-13, and IL-10 are inhibitory to the Th1 response both by preventing recruitment of new cells into the pathway and by inhibiting already mature Th1 effector cells [22]. This property is directly relevant for regulation of EAU pathogenesis that is normally dependent on the Th1 response. Experimental manipulations designed to enhance the Th2 response at the expense of the Th1 response can prevent or ameliorate disease. One such manipulation is injection of rats with mercuric chloride, an inducer of Th2 response [9,23]. Such rats are protected from EAU. Similarly, treatment of mice challenged for EAU with IL-4 and IL-10 during the first days after immunization skews the response away from Th1 and ameliorates disease, while neutralization of endogenous IL-10 (but not of endogenous IL-4) exacerbates disease [10].

Because EAU is known to be so intimately linked to the Th1 response, it was initially an unexpected finding that neutralization of endogenous IFN-γ by treatment with anti-IFN-γ antibodies exacerbated disease in a number of mouse strains and, conversely, augmentation of systemic IFN-γ by administration of the recombinant cytokine ameliorated disease [24]. Thus, IFN-γ at the systemic level has a disease-limiting role in EAU. Similar observations were also reported in other autoimmune disease models [25,26]. Here it is important to distinguish between the systemic and the local effects of IFN-γ on disease. At the local level, IFN-γ is believed to have a disease-enhancing effect due to its role in induction of proinflammatory cytokines such as TNF (Tumor Necrosis Factor) and IL-6, upregulation of chemokines and their receptors, and induction of class II antigens and Fas/FasL expression in the tissue. Another observation that spoke

against a necessarily pathogenic role for IFN-γ in EAU was that IFN-γ KO mice, which lack IFN-γ altogether, not only are susceptible to EAU but in fact develop disease of equal or greater severity compared to WT mice [27]. It soon became apparent that the EAU developed by IFN-γ KO mice was different from EAU developed by WT mice in terms of the effector response phenotype. Draining lymph nodes (LN) of IFN-γ KO mice displayed enhanced Th2-like cytokine production to the immunizing antigen IRBP, with upregulated IL-5, IL-6, and IL-10 production. A Th2-like cytokine profile was also apparent in the local microenvironment of the eye by immunohistochemical staining and the cellular composition of the inflammatory infiltrate was rich in granulocytes and eosinophils. In addition, IFN-γ mice did not upregulate inducible nitric oxide synthase (iNOS = NOS2), of which IFN-γ is a major inducer.

Notably, IFN-γ KO mice had strongly exacerbated cellular responses in vivo and in vitro to IRBP. Their cellular proliferation was much higher than in the WT, and the delayed hypersensitivity response was so severe that in IFN-γ KO animals ear-tested for DTH (Delayed Type Hypersensitivity) the entire head swelled visibly [27]. Interestingly, exacerbated cellular responses are also observed in iNOS KO mice [28], which are fully susceptible to EAU, despite the known damaging effects of NO on retinal tissue [29]. Because NO is antiproliferative and proapoptotic for lymphocytes, it is an attractive notion that the disease-limiting effects of IFN-γ may in part be mediated by NO. NO would thus have not only a pathogenic but also a regulatory role in EAU, by limiting the clonal expansion of uveitogenic effector cells. This theme will be developed further later in this article.

The tissue-destructive properties of a Th2-like response are not by any means unique to IRBP-induced EAU or to IFN-γ KO mice. Lafaille et al. reported that immunodeficient mice infused with MBP-specific Th2 cells developed severe EAE (Experimental Autoimmune Encephalomyelitis) characterized by an eosinophilic infiltrate into the CNS [30]. Similarly, in a neoantigen model where HEL (Hen Egg Lysozome) is expressed in the lens of the eye, Gery et al. induced granulocytic-eosinophilic uveitis in sublethally irradiated recipients using cultured HEL-specific T cell receptor transgenic T cells polarized towards a Th2 phenotype [31]. It is interesting to note that in all three cases some form of immune deficit is present in the mice that develop Th2-driven pathology: inability to produce IFN-γ, generalized immunodeficiency, or irradiation. It follows that normal counterregulatory mechanisms in these mice are likely to be compromised or circumvented. Thus, an unopposed Th2 response can result in pathology that is at least as severe as that induced by a dominant Th1 response.

These observations underscore the possibility that excessive skewing of the response toward the Th2 phenotype to counteract Th1-driven autoimmunity even in a "normal" host has the potential to backfire by substituting one type of pathology for another. A case in point that would appear to confirm this possibility are two recent clinical trials where MS immunotherapy was attempted through altered peptide ligand (APL) therapy [32,33]. APLs are peptides based on an autopathogenic epitope with reduced binding affinity to the TCR through judicious amino acid substitution(s). Such ligands act as partial agonists and tend to promote the Th2 response. In both clinical trials complications were noted, which were compatible with induction of Th2 pathologies, that ultimately necessitated cessation of the trial.

For this reason, it may be important to examine the potential of non-Th2 immunoregulation to limit Th1-driven pathology. Th3 (TGF-β) and Tr1 (IL-10) regulatory responses have the potential to limit both Th1- and Th2-driven inflammation [34–37]. However, TGF-β can also promote fibrosis, which can be particularly detrimental for vision [38]. Thus, extreme care has to be taken when intervening in these delicately balanced biological systems.

THE TH1 RESPONSE AS ITS OWN REGULATOR

Despite the destructive pathology of an unopposed Th2 response, under normal conditions it is the Th1-driven inflammation that underpins the initiation and the progression of EAU.

Importantly, the Th1 response may have a self-limiting regulatory role. Recent evidence points to the conclusion that the Th1 response feeds back upon itself and acts to limit the recruitment of new effector cells, and/or to eliminate the ones that have already been recruited.

The first clue that the Th1 response in EAU also acts to limit itself was the observation that manipulation of systemic levels of IFN-γ in EAU-challenged mice had the opposite effect of what would be expected in a Th1-driven pathology. Neutralization of systemic IFN-γ by anti-IFN-γ antibodies resulted in enhanced disease scores, whereas augmentation of systemic IFN-γ by infusion of the recombinant cytokine ameliorated disease [24]. Similar observations reported in other autoimmune disease models indicate that this is a general phenomenon [39].

More insights into the mechanism on how IFN-γ limits EAU were obtained by the observation that IL-12 treatment given concurrently with uveitogenic immunization resulted in reduced rather than enhanced EAU scores [40]. In view of the central involvement of IL-12 in the process of induction and progression of EAU, this result seems paradoxical. Mice treated for 5 days after IRBP immunization with

100 ng/day of IL-12 displayed little or no disease and had reduced immunological responses to the uveitogenic antigen in terms of cellular proliferation and cytokine production. A detailed investigation showed that IL-12 caused massive upregulation of circulating IFN-γ levels. This was also accompanied by apoptosis of cells in secondary lymphoid organs of the treated animals. IFN-γ is a known inducer of iNOS, resulting in increased production of NO, which in turn is able to trigger lymphocyte apoptosis [41].

Because IFN-γ, iNOS, or Bcl-2^{lck} transgenic mice (in which the apoptosis-protective Bcl-2 gene is expressed in T cells) all had impaired ability to be protected by IL-12, we postulated that IL-12–induced upregulation of IFN-γ, followed by induction of iNOS and production of NO, mediated protection from EAU by causing apoptosis of IRBP-specific T cells. NO is made by a variety of cells, including monocyte/macrophages, dendritic cells, and activated T cells, in response to proinflammatory stimuli such as LPS, IFN-γ, TNF-α, and CD40/CD40L interaction. NO induces apoptosis of tissue cells as well as various lymphoid cells and sensitizes to Fas-induced apoptosis. Its effects include upregulation of p53 expression, caspase 8 activation, and changes in expression of proapoptotic and antiapoptotic Bcl-2 family members [41]. The notion of the IFN-γ-iNOS-NO pathway as a negative regulatory circuit in EAU is reinforced by the previously discussed observation that both IFN-γ KO mice and iNOS KO mice have exacerbated proliferative responses to IRBP [27,28]. TNF-α and Fas/FasL, both upregulated by IFN-γ and themselves able to trigger apoptosis, may also feed into this circuit.

Because IL-12 treatment that was delayed into the second week after immunization was not protective [40], it seems plausible that an early stage in T cell recruitment was being affected, but the effectors that had already been generated were being spared. Thus, high levels of proinflammatory mediators in this system appear to prevent recruitment of new T cells into the effector pool. These results clearly do not preclude the possibility that dendritic cells, which are efficient antigen-presenting cells to naïve T lymphocytes and whose function is crucial for efficient T cell priming, may also be among the cells undergoing apoptosis.

IFN-γ and its downstream mediators can act to limit the Th1 response at various stages. Although in the case above it appeared that the action is primarily on early stages of the response, other studies indicate the need for IFN-γ and/or other proinflammatory mediators, such as TNF, for eliminating mature effector cells and allowing recovery. Our observations in IFN-γ KO mice and iNOS KO mice of exacerbated proliferative responses [27,28] is compatible with

increased recruitment as well as decreased elimination of effector cells. Chu et al. [42] reported in IFN-γ KO mice an enhanced and more prolonged EAE disease, accompanied by accumulation of cells having an antigen-experienced phenotype and suggested that this is mostly due to reduced effector elimination. Similar observations were also reported by Kollias et al. in TNF-α deficient mice, which, although initially less susceptible to EAE than WT mice, developed a more chronic EAE due to their inability to eliminate effector cells and bring about remission of disease [43]. While regulation at the level of mature effector cells was not apparent in the IL-12–induced protection, possibly due to the relatively short timeframe of these experiments, it is likely to be relevant to recovery in the EAU model as well. The observation of Rizzo et al. that, unlike in wild type C57BL/6 mice, EAU is chronic-relapsing in IFN-γ KO mice on the same background [44], is in line with this notion.

In the aggregate, these reports underscore the property of the Th1 response to limit its own development and/or progression through feedback inhibition, utilizing the same proinflammatory mediators that bring about tissue damage.

CONCLUSIONS

Although the Th1 response is the main driver in the pathology of EAU and similar diseases, it seems clear that in the absence of a normal Th1 response severe EAU can be induced by a deviant, allergic-like effector response such as that observed in IFN-γ KO mice or in immunodeficient mice infused with Th2 cells specific for an antigen expressed in the tissue. This points out the inherent danger in attempting to promote Th2 responses to counterregulate Th1 responses as a therapeutic manipulation. Systemically produced IFN-γ and its downstream mediators serve to limit disease severity and immunological responses by limiting recruitment of new cells into the Th1 pool and by removing mature effectors at the end of the response. Thus, eliminating these Th1-driven mediators has the potential to also eliminate important negative feedback circuits. More study is needed concerning how to regulate tissue-destructive responses without also losing the benefit of the counterregulatory circuits that they represent.

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