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The Role of Cytokines in Induction and Regulation of Autoimmune Uveitis

Rachel R. Caspi

1. INTRODUCTION

Uveitis is a generic term that encompasses a variety of intraocular inflammations. Noninfectious uveitis, affecting an otherwise intact eye, is believed to have an autoimmune or immune-mediated origin. It can affect the front, middle, or back part of the eye, and is then termed anterior, intermediate, and posterior uveitis, respectively. A schematic drawing of a human eye is shown in Fig. 1. The uveitic conditions that affect the back of the eye where the photoreceptor cells are located are particularly likely to adversely affect vision. These sight-threatening diseases can be mostly confined to the eye, such as sympathetic ophthalmia or birdshot retinochoroidopathy, or they can be part of a more generalized systemic syndrome where the eye is one of a number of organs involved, such as Behçet's disease, sarcoidosis, or Vogt-Koyanagi-Harada syndrome *(1)*. It is estimated that collectively posterior uveitic diseases are responsible for about 10% of the severe visual handicap in the United States.

Experimental autoimmune uveoretinitis (EAU) is a cell-mediated autoimmune disease model that targets the neural retina and serves as an experimental equivalent to posterior uveitic diseases in the human. EAU can be induced in susceptible animal species by immunization with retinal antigens or their fragments, and in mice and rats also by infusion of retinal antigen-specific T-cell lines and clones. A number of proteins derived from the photoreceptor cell layer, among them the interphotoreceptor retinoid-binding protein (IRBP), the retinal soluble antigen (S-Ag), recoverin, rhodopsin, and its illuminated form opsin were found to be pathogenic and to cause essentially identical pathology *(2)*. Typically, these are evolutionarily highly conserved proteins involved in the visual cycle. The microanatomy of normal and uveitic retina of a mouse immunized with IRBP is shown in Fig. 2.

From: *Contemporary Clinical Neuroscience: Cytokines and Autoimmune Diseases*
Edited by: V. K. Kuchroo, et al.

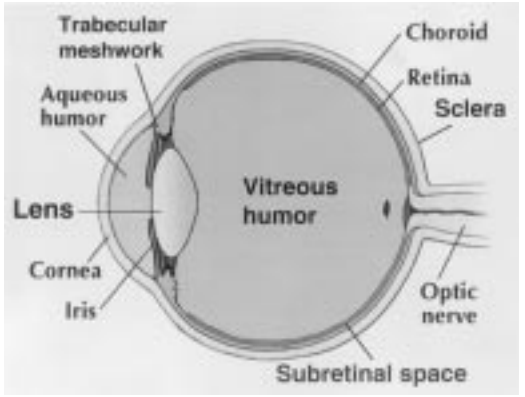

Fig. 1. Schematic of a cross-section of a human eye.

Susceptible animal species include rodents as well as primates, which makes this model useful not only for basic studies of immune mechanisms but also for preclinical testing of therapeutic modalities. Although the antigens driving human uveitis are still unknown, uveitis patients frequently display strong cellular responses to retinal antigens that are uveitogenic in animals, and it is therefore believed that the findings in animal models can be extrapolated to the human *(1–4).* A recently described autoimmune model of mostly anterior uveitis, reminiscent of Vogt-Koyanagi-Harada disease, can be induced with ocular melanin (reviewed in ref. *5*). Although cytokine-related mechanisms have not been studied extensively, they appear similar to those of EAU.

The healthy eye represents a unique, immunologically privileged environment. Immune privilege is manifested as lack of "normal" immune recognition of antigens placed in the eye. Foreign tissues placed in the eye are not rejected and foreign antigens injected into the eye elicit a deviant immune response that is characterized by suppressed cellular immunity of the delayed hypersensitivity type, and humoral immunity primarily of noncomplement-binding antibody isotypes, a phenomenon known as ACAID (anterior chamber-associated immune deviation). The processes that lead to the establishment and preservation of the immunologically privileged status of the eye are multilayered and complex. The eye becomes closed off from the immune system early in ontogeny. Like the brain, the eye resides behind blood-tissue barrier. Tight junctions between adjacent vascular endothelial cells and epithelial cells of the various ocular structures limit entry not only of cells but even of small protein molecules into the eye. Ocular fluids drain directly into the blood through a structure at the angle of

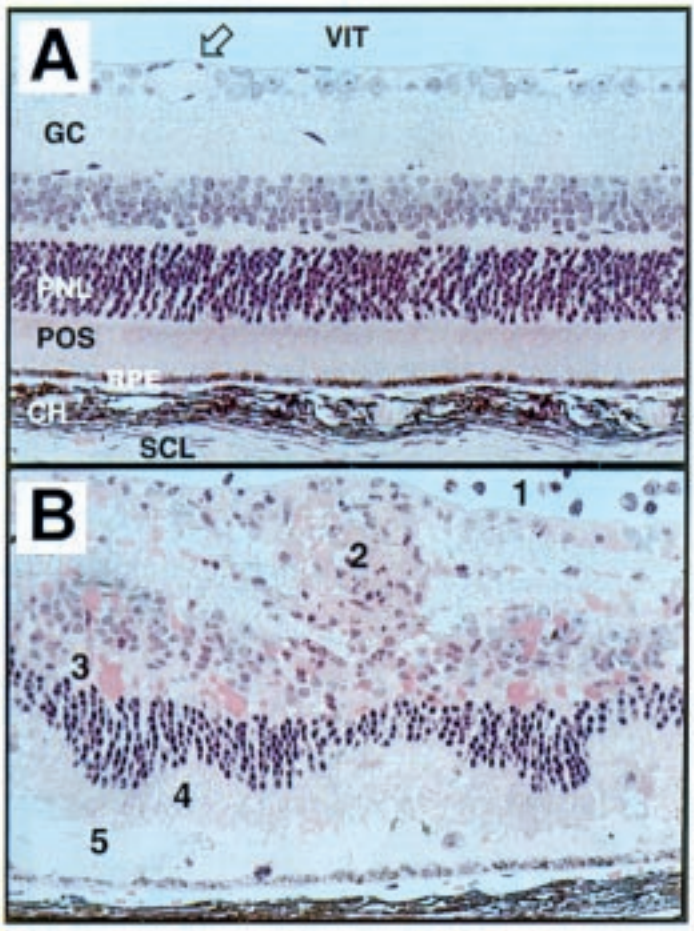

Fig. 2. Normal and uveitic eye—mouse. **(A)** Normal retina. VIT, vitreous; GC, ganglion cell layer; PNR, photoreceptor nuclear layer; POS, photoreceptor outer segments; RPE, retinal pigment epithelium; CH, choroid; SCL, sclera. Note retinal vessel (empty arrow). **(B)** Uveitic retina. Note 1, vitritis; 2, vasculitis; 3, retinal hemorrhage; 4, photoreceptor folding; 5, retinal detachment. Hematoxylin and eosin. Original magnification, ×400.

the iris and the cornea known as trabecular meshwork (*see* Fig. 1 for schematic), thus bypassing the lymphatic system and the elicitation of conventional immunity. In addition, the interior of the eye represents a profoundly immunosuppressive environment that among other things prevents T-cell activation, proliferation, and interferon-γ (IFN-γ) production, as well as the release of NO by macrophages. This is a result of the combined action of

suppressive cytokines and soluble factors, as well as contact-dependent mechanisms, that will be discussed ahead (reviewed in refs. *6–8*).

The eye is exquisitely sensitive to presence of inflammatory cytokines: inoculation of either tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, IL-8, granulocyte-macrophage colony-stimulating facotr (GM-CSF), or IFN-γ into the eye induces uveitis within hours *(9)*. It has been proposed that an ocular environment nonpermissive to the induction and expression of cellular immunity has developed during evolution in response to the need to preserve vision in the face of a variety of proinflammatory physical and microbial insults. Vision is dependent probably more than any other function of the body on the absolute integrity of the anatomical structures involved, and good vision is a most powerful selective pressure. Evolution has, therefore, favored mechanisms that preserve the integrity of the tissue by inhibiting and/or channeling destructive inflammatory processes into pathways less damaging to vision *(7,8)*.

The relative isolation of the eye from the immune system raises the question whether, and how, does the immune system "see" the ocular antigens themselves? Are they sufficiently available intrathymically during ontogeny and peripherally during postnatal life to eliminate or control autoreactive repertoires? Egwuagu et al. *(10)* presented evidence for expression of the retinal S-Ag and IRBP in the thymus of mice and rats, and correlated it to susceptibility of these strains to induction of uveitis by immunization with these antigens. However, it is not clear how effective this level of expression is in supporting negative selection, because even for antigens that are well represented in the thymus the negative selection process is not 100% efficient. Peripheral tolerance induction through presentation on "nonprofessional" antigen-presenting cells (APCs) in the absence of costimulation is probably limited, because the antigens in question are largely limited to the eye, which becomes isolated from the immune system early in ontogeny. However, retinal antigens are expressed also in the pineal gland ("third eye"), which has no blood-organ barrier. It is therefore reasonable to infer that some level of tolerance exists to retina-specific antigens. Nevertheless, the findings that retinal antigen-specific lymphocytes can be easily cultured from peripheral blood of healthy animals and humans, and that ocular autoimmunity is easily induced in experimental animals, indicate that tolerance to retinal antigens is incomplete. Recent data from our laboratory, showing that mice expressing the retinal antigen IRBP extraocularly on a class II promoter are highly resistant to EAU induced with an IRBP-derived epitope, demonstrate directly that the normally restricted expression of this retinal antigen does not support efficient self-tolerization *(11)*.This is fur-

ther supported by the report of Koevary et al. that intrathymic injection of S-Ag prevents development of EAU in Lewis rats *(12)*. Thus, on the one hand, evolution has taken pains to limit the access and to control the interaction of the immune system with ocular components. On the other hand, the very isolation that protects the eye from the immune system, also prevents efficient development of tolerance to ocular antigens, so that when integrity of the blood-tissue barriers is disrupted, lymphocytes from the periphery—that have not been rendered tolerant—can enter the eye, and autoimmunity may ensue.

2. CYTOKINES IN THE HEALTHY EYE AND THEIR RELATION TO IMMUNE PRIVILEGE

A number of cytokines, for the most part produced by the ocular tissues themselves, have been identified in the eye, and additional ones are probably yet to be discovered. The ocular fluid that has been best-studied, owing mainly to its accessibility, is the aqueous humor (AH). However, there is diffusion of molecules among the different compartments of the eye, so that many if not most of the substances identified in the aqueous are also present in other compartments. Indeed, immune privilege also has been shown to exist in the vitreous cavity as well as in the subretinal space *(8)*.

Perhaps the most prominent and well studied among the suppressive ocular cytokines is transforming growth factor-β2 (TGF-β2) *(13)*. The eye contains surprisingly large amounts of this cytokine, mostly in latent but also in active form, and it accounts for most of the direct immunoinhibitory activity of aqueous humor. TGF-β2 is the isoform that appears to be typical of immune privileged sites. Not only the eye, but also the cerebrospinal fluid (CSF) and the amniotic fluid contain large amounts of TGF-β2 *(13–16)*. AH-derived TGF-β2 not only inhibits lymphocyte function, but—at least in the case of responses to soluble protein Ags such as OVA—also acts to endow ocular APCs with inhibitory properties that are central to the induction of the ACAID response, the deviant immune response mentioned earlier that is elicited to antigens presented through the eye. Furthermore, TGF-β alters the properties of conventional APC (peritoneal exudate cells), that would normally be able to prime T cells towards the T-helper (Th)1 pathway. When incubated with OVA in the presence of biological fluids derived from one of these privileged sites or with purified TGF-β2, these APCs produce IL-10 instead of IL-12 during antigen processing, and promote differentiation of antigen specific T cells towards the Th2 phenotype *(17,18)*. Finally, TGF-β-altered APC induce generation of ACAID-specific CD8+ regulatory cells that suppress DTH responses to OVA by a unique mechanism involving migration of the ACAID-inducing APC into the spleen

and local production of MIP-2 by them, recruitment of CD1-restricted natural killer T(NKT) 1.1 cells into the spleen, and induction of IL-10 production by these recruited NK1.1 cells, that presumably acts to promote differentiation of ACAID regulatory T cells *(19–21)*.

Other cytokines/soluble factors found in the eye include macrophage migration inhibitory factor (MIF), IL-1 receptor antagonist (IL-1RA), alpha melanocyte stimulating hormone (α-MSH), vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP) and free cortisol *(7,8)*. The combined activities of these different cytokines and soluble factors in concert are responsible for the immunoinhibitory effects of ocular fluids on T cells. Thus, the anti-proliferative effect is mediated mainly by TGF-β2 and VIP, whereas inhibition of IFN-γ synthesis is owing to α-MSH. CGRP is primarily responsible for inhibition of nitric oxide (NO) production by macrophages, and inhibition of target-cell killing by NK cells is accomplished by TGF-β2 and MIF. In contrast, killing by cytotoxic T cells is not inhibited by AH, underscoring the selectivity of the ocular environment in favor of those effector mechanisms that do not result in widespread and nonselective tissue damage.

An integral part of the unique immunological environment of the eye, and working in part through cytokine responses, is expression of FasL on ocular tissues. The demonstration of constitutive expression of FasL as an important component of immune privilege in general has first been demonstrated in the eye, and subsequently extended to other immunologically privileged tissues *(6,22)*. Studies by Ferguson et al. *(23,24)* utilizing injection of virus-infected or TNP-derivatized syngeneic spleen cells into the anterior chamber have revealed that the role of FasL in ocular immune privilege is twofold: 1) it promotes apoptosis of infiltrating lymphocytes, and thus has a direct role in eliminating pathogenic effector cells from the eye; 2) the cells that have been signaled to die promote induction of systemic ACAID to the antigen they carry. The process of apoptosis triggers IL-10 production by the affected cells. Phagocytes that ingest the IL-10-laden apoptotic cells display an altered APC function and prime antigen-specific T cells for immune deviation. Although it is possible that the interaction between apoptotic T cells and APCs might take place in the eye, apoptotic bodies (presumably derived from the T cells injected into the eye) are subsequently found in the blood; it is thought that they reach the spleen and encounter resident APCs there. This phenomenon could be conceptualized to represent the fate of autoreactive T cells that have infiltrated the eye and have come in contact with ocular antigens.

It is interesting to note the different but parallel mechanisms operating in ACAID induction to soluble (OVA) and cell-bound antigen (TNP-coupled

T cells). The latter involves an obligate step of apoptotic death of antigen-bearing cells and their concomitant IL-10 production. However, in both cases cells—either APCs or apoptotic T cells—exit the eye and go to the spleen, but in the case of soluble antigen they are modified macrophages, and in the case of particulate antigen they are apoptotic T cells. In both cases tolerogenic antigen presentation is accomplished by modified APC, which in the case of soluble Ag have been affected by TGF-β2 in the eye, and in the case of particulate Ags have been affected by apoptotic T-cell-produced IL-10. In both cases production of IL-10 is required, but in the former it is accomplished by attracting IL-10 producing NKT cells, and in the latter the apoptotic T cells themselves serve as the source of the cytokine. Thus, the oculo-splenic axis, the participation of functionally altered APC, and the obligate role of IL-10 represent common themes of the two pathways.

That immune privilege and the ACAID phenomenon may be relevant to controlling ocular autoimmunity was demonstrated by Hara et al., who showed prevention, as well as reversal, of EAU by induction of IRBP-specific ACAID using injections of the soluble IRPB into the anterior chamber *(25)*. Unexpectedly, however, recent work from our laboratory has demonstrated that expression of FasL on ocular tissues does not appear to reduce susceptibility to induction of EAU (25a). This is shown by the finding that FasL deficient (lpr) mice, lethally irradiated and reconstituted with wild-type bone marrow, were just as susceptible to EAU as the FasL-competent the wild-type when challenged with a uveitogenic IRBP regimen. Thus, local mechanisms of immune privilege do not afford protection against a strong disease-inducing stimulus, but protection can be achieved by a state of systemic immune deviation through directed manipulation of these mechanisms. Given that the intensity of most natural environmental triggers probably falls short of an experimental uveitogenic regimen, the immune privilege and the ACAID phenomenon may well serve to raise the threshold of resistance, and may partly compensate for the apparently limited effectiveness of central and peripheral tolerance in eliminating self-reactive T-cell repertoires capable of recognizing retinal antigens.

3. CYTOKINES AND UVEITIS

3.1. Genetics and the Effector Response

EAU in the wild-type host is associated with a Th1 response. IL-12p40 deficient mice, that produce no functional IL-12 and are unable to mount a Th1 response, as well as wild-type mice treated with neutralizing anti-IL-12 antibodies fail to develop EAU (*26* and Silver et al., unpublished data). Genetically susceptible strains of rats and mice are dominant Th1 respond-

	Mice						Rats	
Parameter	BALB/c	A/J	AKR	B10.A	BALB/k	C57BL/10	F344	Lewis
H-2	d	a	k	a	k	b	r	l
IA	d	k	k	k	k	b	l	l
EAU Scores	–	+	–	+++	++	++	±	+++
LNC prolif.	+	+	++	++	+	++	++	++
IL-4	++	+	–	–	–	–	±	±
IFN-γ	–	–	–	+++	++	+++	±	+++
IgG2a / IgG1	1	2a	2a	2a	2a or 1	2a	ND	ND
Th1 / Th2	Th2	?	?	Th1	Th1	Th1	?	Th1

Fig. 3. EAU Susceptibility and immunological responses of genetically defined mouse and rat strains. Animals were given a uveitogenic regimen of IRBP (mice) or its major pathogenic peptide (rats). Draining lymph node cells were explanted into culture after 12–16 d and were stimulated with the immunizing antigen. Cytokines in 48 h supernatants and anti-IRBP Ig subclasses in the serum were assayed by enzyme-linked immunosorbent assay (ELISA), except for IL-4 in rats which was assessed in stimulated cells by reverse transcription polymerase chain reaction (RT-PCR). EAU was evaluated by Histopathology.

ers to the uveitogenic antigen, whereas EAU-resistant strains are likely to be low Th1 responders, or overt Th2 responders *(27,28)*. In this context, it is notable that pertussis toxin, which breaks EAU resistance in genetically nonsusceptible strains, at the same time causes polarization of their cytokine response towards Th1 *(29)*. The chart in Fig. 3 shows an analysis of the cytokine response phenotype vs susceptibility to EAU induced by IRPB or its peptide, in a series of inbred mouse and rat strains. Interestingly, the Th1-low response pattern associated with resistance does not have to be achieved through a dominant Th2 response to the uveitogen. Genotypes with a "null" response—that is, low in both Th1-type and Th2-type cytokines, such as the F344 rat and the AKR mouse—are as resistant to EAU induction as the Th2-biased BALB/c mouse. This indicates that control of the pathogenic Th1 response does not require having a response skewed towards the Th2 pathway. Additional factors (either anti-inflammatory such as TGF-β, or proinflammatory such as TNF-α, that might be superimposed on the Th1/Th2 response pattern, undoubtedly contribute to the complex nature of the regulatory mechanisms that determine susceptibility or resistance to ocular autoimmune disease.

In keeping with the genetic predisposition towards Th1 as a factor in susceptibility, uveitogenic T-cell lines and clones are Th1-like in terms of their cytokine profile. Furthermore, primary T-cell populations that make IFN-γ are uveitogenic, whereas those that make little or no IFN-γ are not, howeever, they can be converted to a pathogenic, IFN-γ producing phenotype by culture with IL-12 *(26,27,30)*. In contrast, a Th2 response is counterregulatory, in that experimentally skewing the developing response toward Th2, by early treatment with IL4+IL-10 or by injections of mercuric chloride, can protect from development of disease *(31,32)*. However, a Th2-like effector response may also lead to severe tissue damage. An example is induction of EAU in IFN-γ knockout mice, that do not mount a normal Th1 response owing to their inability to produce the prototypic Th1 effector cytokine, IFN-γ. These mice develop EAU at least as readily as wild-type mice, but tissue damage is effected by a distinct mechanism, showing many characteristics of a deviant, Th2-like response *(33)*. IFN-γ deficient mice exhibit an antigen-specific response high in IL-5, IL-10, and IL-6, and do not upregulate iNOS (whose regulation is strongly IFN-γ driven).
Unlike the wild type, their inflammatory infiltrate is dominated by polymorphonuclear leukocytes and contains a large proportion of eosinophils, strikingly reminiscent of an immediate hypersensitivity-like reaction (Fig. 4). Data in the EAE model showed that adoptive transfer of Th2 clones to immunodeficient (SCID) mice can lead to severe tissue damage having a similar allergic-like pathology *(34)*. Thus, an unopposed Th2 response can be as destructive to the tissue as a Th1 response.

Interestingly, however, IL-12 deficient animals that also develop a Th2-like response to the uveitogenic protein, are highly resistant to EAU *(26)*. Furthermore, IFN-γ knockout mice treated with neutralizing antibodies to IL-12 fail to develop EAU. This indicates that IL-12 plays a role that is independent of IFN-γ and that IL-12, rather than IFN-γ, is a necessary cytokine for differentiation of uveitogenic effector T cells, irrespective of their particular cytokine profile.

3.2. Regulatory Cytokines in Control of EAU

Control of EAU by regulatory cytokines is accomplished at multiple levels. As an example, TGF-β and IL-10, which have a role in immune privilege, may intervene at a level that prevents the initiation of processes leading to autoimmune tissue attack. Th2 response, which under normal circumstances is counterregulatory, can prevent development of a pathogenic effector response when a perturbation has already occurred. Finally, if a pathogenic response phenotype has become established and has precipitated tissue damage, it must be downregulated to bring about remission or recovery.

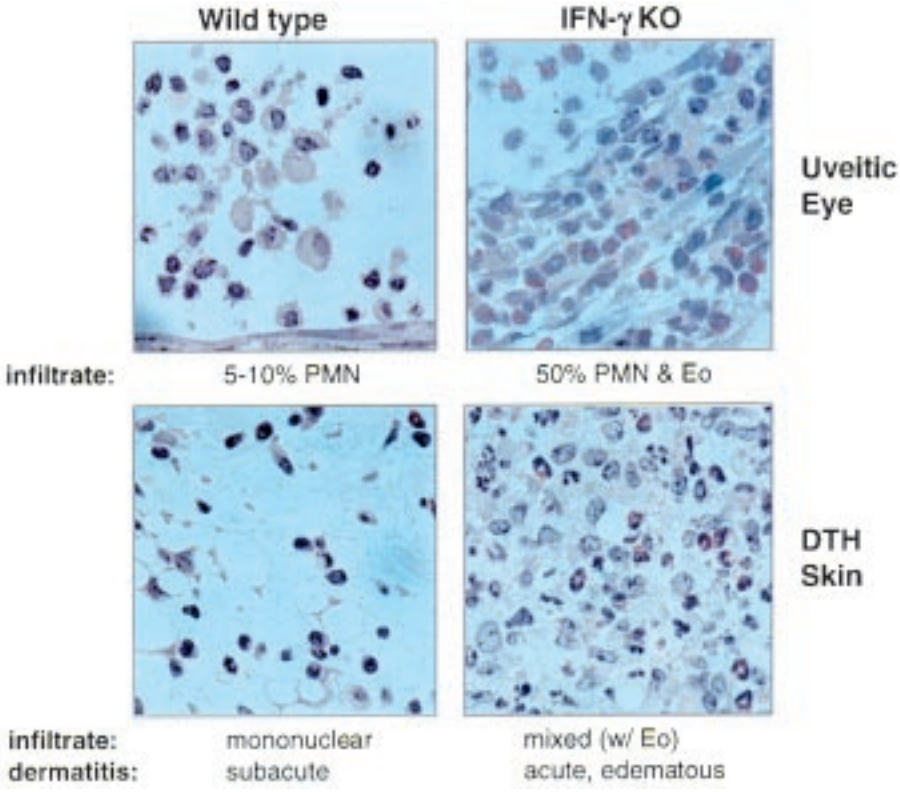

Fig. 4. Cellular composition of inflammatory infiltrate typical of EAU in IFN-γ KO mice. Shown are sections through the eye (vitreous infiltrate) and through the DTH lesion in wild-type and IFN-γ knockout mouse. Note numerous eosinophils (Eo) in IFN-γ knockout infiltrate, vs their paucity in wild-type. Hematoxylin and eosin. Original magnification ×400.

3.3. IL-4 and IL-10

Data derived from in vivo treatment of EAU-susceptible mice given a uveitogenic regimen of IRBP with recombinant IL-4 and IL-10 showed that early treatment with IL-4 alone did not affect EAU. Early treatment with IL-10 downregulated disease scores and inhibited the Th1-type cytokines, but without deviating the immune response towards Th2. However, combined IL4+IL-10 treatment shifted the cytokine profile towards Th2 and was synergistic in terms of protection from disease *(31)*. This might indicate that achieving immune deviation in a Th1-predisposed host requires a concomitant suppression of the genetically dominant Th1 response. Interestingly,

Ramanathan et al. *(35)* reported exacerbation of EAU and augmentation of the Th1 response in rats in Lewis rats, which are strongly predisposed to Th1, by treatment with IL-4.

Several lines of evidence suggest that IL-10 might constitute one of the mechanisms mediating natural recovery from EAU *(31)*. Administration of exogenous IL-10 was able to prevent EAU, whereas administration of anti-IL-10 exacerbated disease. IL-10 is one of only a few cytokines able to inhibit the function of fully differentiated uveitogenic Th1 cells in culture, and expression IL-10 mRNA in uveitic mouse eyes was seen to rise during the resolution phase of EAU. Recent data, showing higher expression of IL-10 mRNA in eyes of EAU-resistant than EAU-susceptible rat strains, suggested that basal levels of IL-10 are present in the healthy eye and can affect susceptibility to disease *(36)*. As mentioned earlier, IL-10 also appears to be one of the obligate mediators of the ACAID phenomenon, supporting the notion that endogenous IL-10 could be involved not only in recovery, but also in prevention of ocular autoimmunity *(17,24)*.

3.4. TGF-β

As discussed above, TGF-β is inextricably linked with regulation of responses in the eye, and to the eye. In addition to its central role in ocular immune privilege it may also affect systemic responses involved in EAU. Mice given a uveitogenic immunization during pregnancy develop reduced EAU scores compared to nonpregnant controls, and their antigen-specific cytokine responses indicated a selective reduction of Th1 immunity *(37)*. Interestingly, pregnant mice given an infusion of IRBP-primed lymphoid cells from nonpregnant donors also developed reduced EAU, suggesting that not only the generation, but also the function of mature uveitogenic effector T cells, is inhibited by pregnancy. Because pregnant mice have elevated serum levels of TGF-β (but not of IL-10), these effects may be secondary to supranormal systemic levels of this cytokine. Interestingly, female patients suffering from autoimmune uveitis are reported to experience a temporary remission of their symptoms during pregnancy. We hypothesize that TGF-β might cause inhibition of Th1 cells at least in part through antagonizing the effects of IL-12. This is suggested by in vitro experiments showing reduction of IL-12 driven IFN-γ production and IL-12Rβ2 mRNA accumulation in IRBP-primed lymph node cells cultured in the presence of TGF-β (Xu et al., unpublished results).

TGF-β may also have a role in helping to downregulate inflammation in the eye that has already developed EAU *(38)*. Loss of ability of AH to inhibit T-cell proliferation coincides with onset of EAU and elevated titers of IL-6, which counteracts the suppressive effects of TGF-β. However, within

a week, the aqueous re-expressed its ability to suppress T-cell proliferation, owing to high levels of blood-derived TGF-β1 and eye-derived TGF-β2 in the absence of IL-6. It sees a reasonable assumption that the restoration of the immunosuppressive ocular microenvironment may help to turn off inflammation at the tissue level. Interestingly, high levels of IL-6 in the eye were found to be correlated with severity of endotoxin-induced uveitis implicating IL-6 as an important inflammatory mediator in the eye. Thus, a regulatory circuit that shuts off IL-6 and prevents it from counteracting the effects TGF-β, which then antagonizes the effects IL-12 and promotes IL-10 secretion, may be involved in resolution of EAU.

3.5. Negative Regulation by IL-12 and IFN-γ

The local effects of IFN-γ within the eye are unquestionably proinflammatory *(9).* However, systemic effects of this cytokine appear to be exactly opposite. Treatment of wild-type mice with neutralizing antibodies to IFN-γ exacerbates disease, whereas treatment with recombinant IFN-γ ameliorates it. Furthermore, IFN-γ knockout mice are more susceptible than wild-type to EAU induced with a uveitogenic peptide or with limiting doses of IRBP, and in contrast to the wild-type, develop a chronic relapsing disease (*39,40,* and Silver et al., unpublished results).

Insights into the mechanism underlying the protective systemic effects of IFN-γ were recently provided by a series of experiments, initially designed to confirm the role of the Th1 response in EAU. Because genetic resistance to EAU appeared connected to a low Th1 response (Fig. 3), we expected that treatment with IL-12 would permit EAU development in resistant strains by shifting their response towards Th1. Unexpectedly, treatment of mice given a uveitogenic regimen of IRBP in complete Freund's adjuvant with 100 ng/d of IL-12 for the first 5 d after immunization not only did not enhance EAU in resistant strains, but in fact completely inhibited its development in susceptible strains *(41)*. Treated mice had nanogram levels of circulating IFN-γ in the serum, evidence of enhanced apoptosis in the draining lymph nodes, and their subsequent antigen-specific responses, especially IFN-γ production, were suppressed. In a series of experiments using IFN-γ-deficient, iNOS-deficient, and Bcl-2 transgenic mice, which are all poorly protected by IL-12, it was possible to delineate a seqeuence of events whereby administration of IL-12 causes systemic hyperinduction of IFN-γ. This mediates protection at least in part by activation of iNOS and production of NO, which in turn triggers Bcl-2-related apoptotic deletion of antigen-specific T cells as they are being primed (Fig. 5). Existence of additional IFN-γ-driven mechanisms besides NO-driven apoptosis, such as TNF or Fas/FasL-driven apoptosis, as well as other antiproliferative mechanisms, are

Genotype	Wild Type	IFN-γ KO	iNOS KO	Bcl-2 TG
% protection by IL-12	100	0	35	75

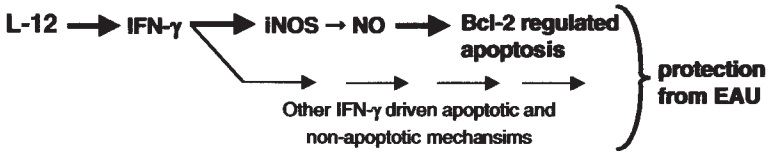

Fig. 5. Control of EAU by excess IFN-γ and the proposed pathway. Mice were immunized with a uveitogenic regimen of IRBP and were injected once daily with 100 μg of recombinant murine IL-12 for the first 5 d after immunization. EAU was evaluated by histopathology 21 d after immunization.

suggested by the progressive reduction in interference with protection at each step following IFN-γ in this proposed pathway . Interestingly, the same IL-12 treatment started on d 7 after immunization is not protective, indicating that regulation by excess systemic IFN-γ is only effective during initial priming, but is ineffective against effector cells that have already been generated. We propose that regulation of the Th1 response by excess systemic IFN-γ is a regulatory mechanism to control Th1 responses in general, by preventing recruitment of additional lymphocytes into the effector pool when a strong cellular response, signified by high levels of inflammatory cytokines such as IL-12 and IFN-γ, is already present. The in vivo relevance of this pathway is supported by the observation that IFN-γ and iNOS knockout mice, both of which cannot use this type of regulation, are highly susceptible to EAU and have elevated cellular responses to IRBP *(33,40,42)*.

3.6. Chemokines

Research on chemokines in uveitis is limited. Thus far, no chemokines have been described that are uniquely or preferentially expressed in the eye. The uninflamed eye does not express detectable chemokine mRNA species. However, chemokines such as IL-8, MCP-1, RANTES, GRO, and IP-10 are produced in the eye under inflammatory conditions. At least in some cases, they can be shown to be associated with, and presumably are produced by, the ocular tissues themselves *(43–45)*. Explanted retinal pigment epithelial cells stimulated with cytokines produce copious amounts of MCP-1 and IL-8 *(46)*.

That chemokines are necessary for expression of EAU is shown by the dependence of disease induction on chemokine-receptor signaling. Pertus-

sis toxin is known to ADP-ribosylate and inactivate Gi proteins, which are involved in chemokine signaling. Incubation of uveitogenic T cells with pertussis toxin before their infusion into recipients, or treatment of recipients themselves with pertussis toxin, completely prevents induction of adoptively transferred EAU. The effect is dependent on the ADP-ribosylating activity of pertussis toxin, and the in vitro treated cells, as well as lymphoid cells from treated animals, fail to migrate to chemokines in chemotaxis chambers *(47)*. Thus, blockade of chemokine receptor signaling aborts disease induction by disruption of cell migration and infiltration into the target organ. The effectiveness of this process in preventing disease raises the notion of blocking chemokine signaling as a potential therapeutic approach to uveitis.

4. IMMUNOTHERAPEUTIC STRATEGIES BASED ON MODULATING CYTOKINES OR CYTOKINE RECEPTORS: CLINICAL TRIALS

4.1. Immune-Deviation Strategies of Therapy and Oral Tolerance

The existence of Th1-low, nonpathogenic response phenotypes that were discussed earlier, raises the notion of evoking a nonpathogenic response as a means of preventing a pathogenic one. In animal models, deviating the response to the immunizing retinal antigen towards the Th2 phenotype by directed immunomodulation has resulted in protection from disease *(31,32)*. Although an unopposed Th2-like effector response can be as destructive to the tissue as a Th1-like response *(33,34)*, this has mostly been seen in immunologically abnormal or immunocompromised hosts. An attractive way to deviate the immune response is by oral tolerance induced by antigen feeding. Oral tolerance as an immunotherapeutic strategy has recently gained attention and been explored experimentally and clinically in a number of autoimmune diseases, including uveitis *(48)*, and is discussed more fully elsewhere in this volume.

Two distinct, nonmutually exclusive mechanisms that mediate oral tolerance have been described: clonal anergy or deletion of the antigen-specific cells and active suppression by regulatory cells secreting IL-4, IL-10, and TGF-β.Which of these two major mechanisms of tolerance will predominate is affected by the antigen dose and the feeding regimen *(48–50)*. In EAU, cytokine-mediated tolerance has been shown to require the ability to produce both IL-4 and IL-10. Mice deficient in either one fail to develop oral tolerance dependent on regulatory cytokines, although they are unimpaired in their ability to develop oral tolerance mediated by anergy/deletion *(51)*. This may indicate that tIL-4 and IL-10 are needed at different stages of

the oral-tolerance process. Based on these results, and on the effects of in vivo administration of IL-4 and IL-10 described earlier *(31)*, we have hypothesized that IL-4 is required to induce the regulatory cells, whereas IL-10 may be important as an effector cytokine in controlling the expression of EAU.

Based on the encouraging results in animals and a pilot trial in two uveitis patients given oral therapy with retinal S-Ag *(52)*, a double-masked placebo-controlled clinical trial was recently performed in which uveitis patients were fed purified bovine S-Ag, crude retinal extract, or a mixture of both *(53)*. The trial gave encouraging results, however, it has not been confirmed that the beneficial effects on disease in fact involved regulatory cytokines. Notably, however, the crude retinal extract seemed to worsen the disease compared to the placebo when fed alone, and to abrogate the therapeutic benefit of S-Ag therapy when the two were given together *(53)*. The possibility has been raised that an oral-tolerance regimen will "backfire," and induce pathology instead of tolerance *(54,55)*. Although in clinical trials of oral tolerance in rheumatoid arthritis (RA) or multiple sclerosis (MS) such a complication has not been noted, the negative effect of the crude retinal extract in the uveitis patients raises a note of caution.

4.2. Targeting IL-2 Receptor Positive Cells

The pathogenic effector T cells in EAU, and presumably in human uveitis, are Th1-type and express IL-2 receptors. Early experiments in the rat EAU model, using a chimeric protein composed of IL-2 and pseudomonas exotoxin, showed the efficacy of this type of approach *(56)*. This approach was subsequently validated in a primate model of EAU by using humanized monoclonal antibodies (MAbs) to the α-chain of the IL-2 receptor and the β-chain shared by the IL-2 and IL-15 receptors *(57)*. Finally, an open label clinical trial was performed in which 10 patients with severe sight-threatening uveitis were treated with infusions of humanized anti-IL-2 receptor α chain antibody for the period of 1 yr. This therapy prevented expression of disease in 8 of 10 patients, with noted improvements in visual acuity *(58)*. This is an excellent bench-to-bedside example how basic studies in an animal model can lead to improved understanding of the mechanisms of disease and result in a successful clinical treatment paradigm.

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