Immune mechanisms in uveitis

Rachel R. Caspi, Ph.D., Laboratory of Immunology, NEI, NIH Bethesda, MD, USA

Running Title: Immune mechanims in uveitis

Correspondence to: Rachel R. Caspi, Ph.D.
Chief, Section on Immunoregulation
Lab. of Immunol., National Eye Inst.
NIH Bg. 10, Rm. 10N222
10 CENTER DR MSC 1857
BETHESDA MD 20892-1857
phone: 301-435-4555
fax: 301-480-6668
email: rcaspi@helix.nih.gov

EAU as a model of human uveitis

Experimental autoimmune uveoretinitis (EAU) is an animal model of 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 T cells specific to these antigens, that are isolated from immunized animals and cultured for various periods of time in the laboratory. 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 [1-3].

A number of antigens that induce uveoretinitis in laboratory animals have been identified over the years, using as starting material bovine retinal extracts. Subsequently, the genes for these antigens were cloned from a variety of animal species and characterized. Typically, uveitogenic antigens tend to represent evolutionarily conserved proteins involved in one way or another in visual functioning. They include the retinal soluble antigen (S-Ag): a 48 kilodalton intracellular protein that participates in phototransduction; the interphotoreceptor retinoid-binding binding protein (IRBP): a 140 kDa extracellular matrix protein that transports vitamin A derivatives between the photoreceptor cells and the retinal pigment epithelium; rhodopsin and its illuminated form, opsin: the 40 kDa rod visual pigment; recoverin: a 23 kDa calcium-binding protein; and phosducin: a 33 kDa cytosolic photoreceptor phosphoprotein [2]. Additional uveitogenic proteins are probably present and are yet to be described. A number of these uveitogenic antigens and their fragments are now being made as recombinant proteins in bacteria and other expression systems.

It should be stressed that the putative retinal antigen(s) involved in human uveitis have not been defined, nevertheless, the fact that a number of different retinal proteins cause very similar uveitic diseases in animals, suggests that the findings on basic mechanisms as studied in animal models can be extrapolated to the human. The notion that responses to retinal antigens are in fact involved in human uveitis is supported by the finding that many uveitis patients exhibit significant cellular responses to S-Ag [2, 3]. Although it is unknown whether these responses are involved in the etiology and/or in fueling the progression of their disease, this provides a rationale for exploring S-Ag and other retinal proteins as a basis for antigen-specific immunotherapy approaches.

The histopathological picture of EAU is reminiscent of some types of human uveitis. Lesions include serous retinal detachment, retinal folding, photoreceptor damage, vasculitis, retinitis, choroiditis and vitritis (Figure 1). Although none of the animal models by itself reproduces the full clinical and histopathological spectrum of human uveitis, each is useful for studying some specific aspects of the human disease. For example, the S-Ag-induced EAU model in the Lewis rat has an acute course; the disease is self-limited and begins to resolve spontaneously after approximately 7 days [2]. In contrast, EAU induced with IRBP in the B10.A mouse can have a chronic nature (controlled by the dose of antigen used for immunization) [4, 5]. After the first acute phase the disease goes into remission 5-6 weeks after primary immunization, but tends to relapse by week 7 or 8. In both species the disease has elements of both uveitis and retinitis, however, the rat develops an early and prominent anterior chamber involvement and strong vitritis, whereas there is minimal anterior chamber involvement in the mouse and the media usually remain sufficiently clear for fundoscopy to be possible. In addition, under some conditions of immunization the mouse, but not the rat, develops subretinal neovascularization with high frequency.

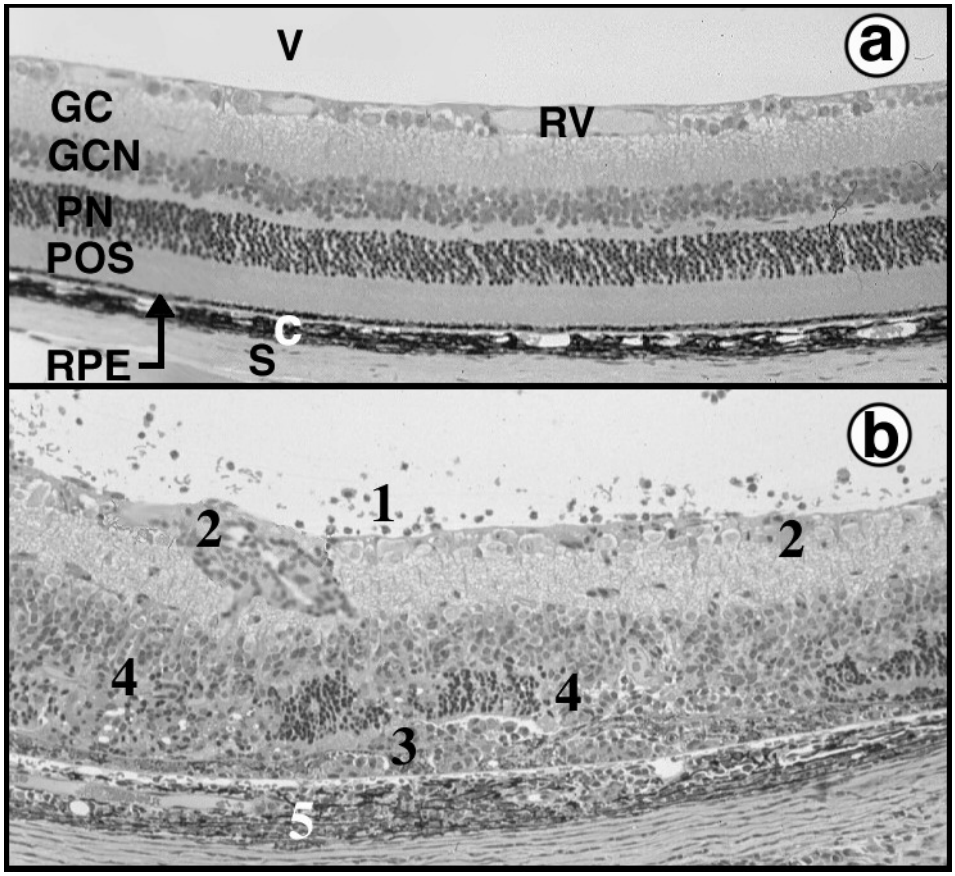

Figure 1: *EAU in the mouse. A. Normal mouse retina. V: Vitreous; RV: retinal vessel; GC: ganglion cells; GCN: ganglion cell nuclei; PN photoreceptor nuclei; POS: photoreceptor outer segments; RPE: retinal pigment epithelium; C: choroid; S: sclera. B. uveitic retina. 1: vitritis; 2: retinal vasculitis; 3: subretinal granuloma; 4: photoreceptor cell damage; 5: choroiditis.*

As in humans, susceptibility to experimental uveitis in animals is genetically determined. Previous studies have found that both a susceptible MHC type and a "permissive" genetic background are needed for susceptibility [6, 7]. Some parameters that may determine susceptibility or resistance to EAU will be discussed below.

Local vs. systemic mechanisms of immunoregulation

Self tolerance to tissue antigens is established and maintained by both central and peripheral mechanisms. Potentially dangerous lymphocytes can be physically eliminated (deletion), functionally paralyzed (anergy), kept in check by regulatory cells (active suppression), or prevented from gaining entrance to the target tissue (ignorance) [8]. All these strategies to maintain immune tolerance can be demonstrated under the appropriate circumstances, however, the precise extent and efficiency of these processes as applied to retina-specific antigens are still in question.

During ontogeny, lymphocytes undergo negative selection in the thymus if they recognize self antigens with high affinity. This reduces the number of potentially autopathogenic cells. The

efficiency of negative selection depends on the presence of the antigen in question in the thymus, and in adequate amounts, a criterion that may not always be fulfilled for tissue-specific antigens such as retinal antigens. Although Egwuagu et al. [9] presented evidence for expression of the retinal S-Ag and IRBP in the thymus of mice and rats, it is not clear to what extent that level of expression is functionally significant in terms of negative selection. Experimental data indicate that even for antigens that are represented in the thymus the negative selection process is not 100% efficient. In the periphery, tolerance is maintained by exposure of the antigen specific lymphocytes to tissue antigens presented on "non professional" antigen presenting cells, i.e., without the second (costimulation) signal. In the case of retinal antigens, however, this condition may not be fulfilled easily either, since the eye becomes a closed organ early in ontogeny. The retinal antigens reside behind a blood-organ barrier, there is no lymphatic drainage of the interior of the globe and traffic of lymphocytes in and out of the globe is restricted. 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 may exist to retina-specific antigens. Nevertheless, the findings that retina-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 interphotoreceptor retinoid binding protein (IRBP) extraocularly on a ubiquitous 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 [10].This is further supported by the report of Koevary et al. that intrathymic injection of S-Ag prevents development of EAU in Lewis rats [11].

The eye is an immunologically privileged organ. Immune privilege is a complex, multifaceted phenomenon, and involves several different processes that act in concert to maintain tissue integrity while disallowing inflammatory processes within the privileged organ [12]. Fas ligand is constitutively expressed on ocular tissues, promoting apoptotic death of lymphocytes that might find their way into the eye [13]. Other inhibitory molecules that suppress antigen-specific responses of uveitogenic lymphocytes by a contact- dependent mechanism are present on retinal glial cells and on ciliary body epithelial cells [14, 15]. Ocular fluids contain immunoinhibitory substances, such as the cytokine TGF-β2, and neuropeptides such as MSH and VIP, that suppress lymphocyte responses or direct them to a nonpathogenic pathway through a process known as anterior chamber associated immune deviation (ACAID) [12, 16]. Immune privilege of the eye and the ACAID phenomenon are more fully discussed elsewhere in this volume. That these phenomena may be relevant to ocular autoimmunity was demonstrated by Hara et al, who showed prevention, as well as cure, of EAU by induction of IRBP-specific ACAID [17]. The immune privilege and the ACAID phenomenon may thus compensate for the apparently limited effectiveness of central and peripheral tolerance mechanisms in eliminating the self-reactive repertoire capable of recognizing retinal antigens.

The afferent phase of ocular autoimmunity: pathogenic vs. nonpathogenic T cell responses

When natural mechanism of tolerance fail, autoimmunity ensues. Figure 2 depicts the sequence of events in the progression of uveitis, as deduced from numerous studies done in epxerimental models. The eliciting events in human uveitis are hypothesized to involve antigen presentation in the periphery, similarly to the process of immunization with retinal antigen in adjuvant that elicits EAU in animal models. One way to encounter retinal antigen in the periphery would follow a penetrating trauma to the eye, causing normally sequestered ocular antigens to be

released into the regional lymph node, where they might provoke an immune response [18]. A concomitant infection of the traumatized eye might provide an adjuvant effect. Another possibility is by exposure to microbes that possess structural components crossreactive with self antigens, a phenomenon known as "molecular mimicry". The immune response to epitopes that "mimic" those of the host is able to break tolerance to the crossreactive self epitope(s) [19]. Many pathogens have a built-in adjuvanticity, namely, CpG-rich DNA sequences and other components that promote Th1 responses by triggering innate immunity through production of IL-12 [20, 21]. While epitope mimicry can be shown to drive autoimmune disease in experimental models, including uveitis [19, 22, 23] , evidence that would implicate it in pathogenesis of human disease is still sparse.

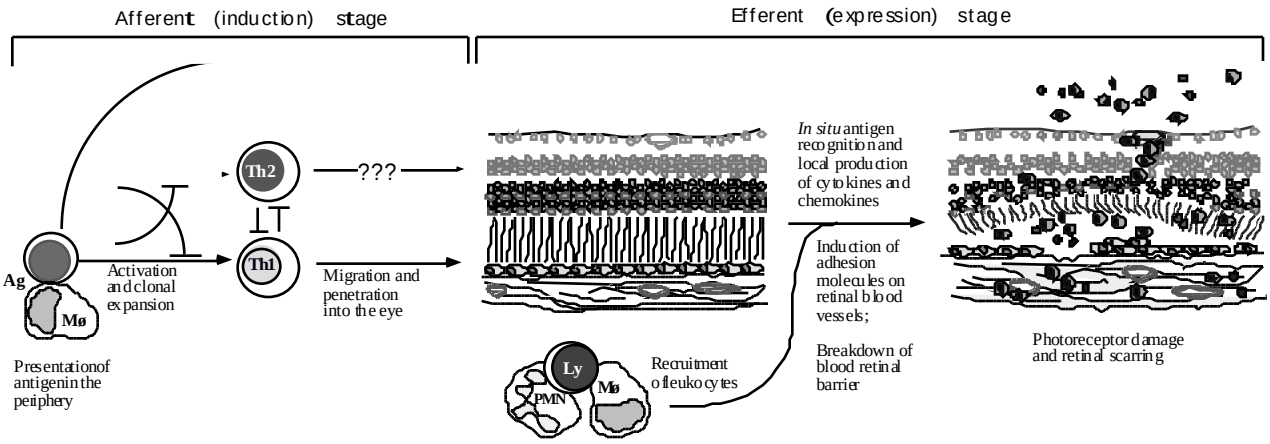

Figure 2 : *Schematic sequence of events in the progression of uveitis, as deduced from numerous studies done in experimental models. Lines ending with an arrow denote pathogenic interactions; blunt-ended lines denote inhibitory interactions.*

T cells play a central role in EAU. The disease cannot be induced in T cell-deficient animals even with repeated immunizations, is ameliorated by T cell targeting agents such as cyclosporin A, and can be transferred to genetically compatible, naive recipients with immune T cells, but not with immune serum [24, 25]. The study of the T cells involved in EAU has been facilitated by development of retinal antigen-specific long term T cell lines.

During the last several years much research has been done on T cell subsets. Mosmann and others found that antigen-specific effector T cells are heterogeneous with respect to the effector functions they mediate and the cytokine profiles that they produce [26, 27]. Effector T cells of the Th1 phenotype are associated with cellular immunity: in addition to producing lymphotoxin and IL-2 they produce large quantities of IFN-γ (but not IL-4, IL-5 or IL-10), thus stimulating the phagocyte functions, activation if iNOS, production of NO and other active oxygen intermediates, and promoting Ig switch to complement-binding and opsonizing antibody isotypes. The Type 1 response, while it can be very effective in eradicating certain types of pathogens, is also very damaging to the surrounding tissue. Effector T cells of the Th2 phenotype are associated with humoral immunity and allergic responses, produce large amounts of IL-4, IL-5 and IL-10, but not of IFN-γ, and promote Ig switch to antibodies of the non-complement binding isotypes. The two

phenotypes are mutually antagonistic, in that cells of each phenotype promote differentiation of further T cells along the same pathway, and inhibit the opposite pathway, by virtue of the cytokines they produce. Therefore, chronic immune responses may become polarized towards one of the phenotypes. It was thought that they arise from a common phenotype Th0, which produces a mixed profile of both Th1 and Th2 cytokines, but it is becoming increasingly evident that Th0 cells may in fact exist as a separate entity and do not necessarily polarize either way with continued antigenic stimulation. While discrete Th1 and Th2 phenotypes certainly exist as polarized entities under some circumstances, under normal circumstances they may in fact represent the opposite poles of a continuum rather than sharply defined subsets [28].

Uveitogenicity (understood as the ability to transfer EAU to naive recipients) of a given T cell population isolated from retinal antigen-primed rats or mice, appears to correlate tightly with its ability to produce IFN-γ in response to antigenic simulation. Among T cell populations isolated from animals primed under various conditions, those that make IFN-γ are uveitogenic, whereas that make no IFN-γ are not [29, 30]. Furthermore, by incubating a nonpathogenic population in the presence of IL-12, which primes T cells for production of IFN-γ and promotes differentiation along the Th1 pathway, the nonpathogenic population is converted into a pathogenic, IFN-γ producing phenotype [29, 30]. Uveitogenic long T cell lines and clones are typically high IFN-γ producing, Th1-like cells, or appear to become such upon adoptive transfer [30, 31]. In contrast, some suggestive evidence has been presented that if the response to a retinal antigen becomes skewed towards the Th2 phenotype, the animal is protected from EAU, suggesting the effectiveness of a nonpathogenic response to prevent development of a pathogenic one in an *in vivo* situation [32]. Similar observations have been reported in other tissue-specific autoimmune disease models.

These observations raise the question whether genetic susceptibility and resistance to EAU might in some way be related to the natural propensity of certain genotypes to develop Th1 or Th2 dominated immune responses. Experiments done on a panel of wild type strains including both rats and mice, indicated that an EAU-susceptible individual is likely to be a high Th1 responder, whereas an EAU-resistant individual is likely to be a low Th1 responder [29, 33]. The Th1-low response pattern associated with resistance may be achieved through a dominant Th2 response to the uveitogen, however, genotypes with a “null” response that is low in both Th1-type and Th2-type cytokines are equally resistant. This indicates that control of the pathogenic Th1 response can occur through more than one mechanism, and does not require skewing of the immune response towards the Th2 pathway. Additional factors (either antiinflammatory 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.

Some data indicate, however, that a Th2-like effector response may also lead to tissue damage. An example is induction of EAU in IFN-γ knockout mice, that do not mount a normal Th1 response due 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 different mechanism than in the wild type [34]. IFN-γ deficient mice exhibit an antigen-specific effector response high in IL-5, IL-10 and IL-6, and do not upregulate iNOS in the eye. Unlike the wild type, their inflammatory infiltrate is dominated by polymorphonuclear leukocytes and contains a large proportion of eosinophils, strikingly reminiscent of an allergic-like response. Recently, data in another model showed that adoptive transfer of Th2 clones to immunodeficient (SCID) mice can lead to severe tissue damage having a similar allergic-like pathology [35]. Thus, an unopposed Th2 response can under some circumstances be as destructive to the tissue as a Th1 response.

Interestingly, IL-12 deficient animals that also develop a Th2-like response to the uveitogenic protein, are highly resistant to EAU [36]. 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.

The efferent phase of disease: the mechanisms of of tissue damage

Retinal antigen-specific T cell lines that induce EAU in a genetically compatible adoptive host that has not been otherwise manipulated, have contributed enormously to our knowledge of the pathogenic mechanisms in EAU. Such pathogenic T cell lines are isolated from immunized donors and expanded many-fold in culture by repeating cycles of stimulation with the specific antigen and antigen-presenting cells, followed by a period of growth in medium enriched with IL-2 [unpublished and 24]. These cells are typically $CD4^+$, MHC class II restricted, and produce high amounts of IFN-γ in response to stimulation with their specific antigen. Injected peripherally into a healthy animal, these uveitogenic effectors can by themselves induce the full spectrum of EAU pathology. The question arises, how can these cells find their antigen in the intact eye. Prendergast et al [37] injected S-Ag specific T cells or mitogen-stimulated nonspecific T cells labeled with a fluorescent vital dye, and studied their migration *in vivo*. Their conclusion was that initially both the specific and the nonspecific cells penetrate the eye at random in small numbers; however, only the S-Ag specific cells - probably upon recognition of their specific antigen *in situ* - are able to subsequently cause recruitment of massive numbers of inflammatory host leukocytes and induce EAU.

The phenomenon of "nonspecific" inflammatory cell recruitment is a critical step in EAU development. Lymphocytes, monocytes and neutrophils are recruited to the site, with mononuclear cells accounting for the majority of the infiltrate. Since as few as 200,000 Ag-specific T cells, of which only a minority find their way randomly into the eye, induce disease, it is clear that tremendous amplification mechanisms must exist for disease to be expressed. The amazing efficiency of this amplification process is obvious from in the study of Prendergast et al [37], where out of 5-10 million labeled T cells about 150 could be found in the entire retina at the peak of the first wave of infiltration, 24h after infusion. Cell recruitment is dependent on local production of lymphokines and chemokines by the first infiltrating T cells, that result in induction of adhesion molecules on retinal vascular endothelium and further production of chemoattractants from the tissue, and establish a chemotactic gradient for leukocytes.The recruited cells then amplify this process by contributing their own products, thus fueling an escalating inflammatory cascade. The importance of cell recruitment in pathogenesis is illustrated by the finding that preventing recruitment ameliorates or prevents disease. Our recent study has shown that athymic rats, that have no recruitable T cells, do not develop EAU readily after adoptive transfer of uveitogenic T cells, indicating that recruited "nonspecific" T cells have a role in EAU pathogenesis [38]. Similarly, blocking adhesion molecules and inhibiting the entry of leukocytes into the eye by anti-adhesion molecule antibodies, has a disease-ameliorating effect. Treatment of mice with monoclonal antibodies against ICAM-1, expressed on vascular endothelium, and LFA-1, expressed on leukocytes, inhibited EAU in mice [39].

Degranulation of mast cells that occurs at the time of disease onset [40] helps to break down the blood-retinal barrier. This facilitates the entry of cells into the eye, and the exit of tissue breakdown products and soluble mediators into the circulation, thus fueling the progression of the autoimmune process. An association between the number of ocular mast cells and susceptibility to

EAU in rodents has been observed, suggesting that the breakdown of the blood-retinal barrier may an important factor affecting disease development.

An important part of the tissue damage in the eye is mediated by active oxygen products, such as nitric oxide, peroxide, and other products [41, 42]. Inhibition of inducible nitric oxide synthase (iNOS) was shown to inhibit EAU in rats [41]. Nevertheless, iNOS knockout mice are fully susceptible to EAU (Silver et al., in preparation). This is in similarity to IFN-γ knockout mice, that also were found not to upregulate iNOS. These findings underscore the notion that oxidative and other tissue damage mechanisms are redundant, and no single pathway by itself is critical for pathogenesis.

Several lines of evidence suggest that the cytokine IL-10 might constitute one of the mechanisms mediating natural recovery from EAU [43]. Administration of exogenous IL-10 was able to prevent EAU, while 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. Interestingly, IL-10 appears to be one of the 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 [44, 45].

Directed immunomodulation aimed at disrupting specific steps in the pathogenic process as an approach to therapy

The goal of immunotherapy is to halt the autoimmune process and to restore immunological balance after self tolerance has been broken. The objective is not necessarily to restore the same mechanisms that were in place before the immunologic perturbation that led to autoimmunity occurred, but to put in place new mechanisms that will be able to maintain homeostasis, ideally without need for further intervention. The ideal immunotherapy would thus ultimately "work itself out of a job" by achieving a lasting tolerance. While peripheral tolerance might not be a major mechanism that maintains ocular homeostasis under natural circumstances, due to the tissue-specific nature and relative sequestration of ocular antigens, it can still be exploited as a therapeutic approach.

The accumulating knowledge of the various steps that are involved in pathogenesis of autoimmune diseases make it possible to devise specific strategies that disrupt discrete stages in the process. Some of these strategies are in the process of being translated to the clinic. Antigen presentation to T cells by APC can be disrupted at the level of the APC by competition for binding to the MHC molecule (MHC blockade) by peptides that have a high affinity to the MHC, or at the level of the T cell, by TCR antagonist peptides that are antigenically crossreactive but that do not transduce the kind of signal that produces a pathogenic response (altered peptide ligand therapy). T cell activation and clonal expansion can be disrupted by blocking costimulatory molecules (B7, CD2, CD40L, etc.), by crosslinking the "off" signaling costimulatory molecule CTLA4, or by drugs such as cyclosporin/FK 506 or rapamycin, that block cytokine gene transcription or cytokine effects, respectively, by blocking intracellular signaling [46-48]. Activated IL-2 receptor-expressing cells can be targeted by anti-TCR therapy, through monoclonal antibodies that can be "humanized" for use in patients, i.e., most of the antibody molecule other than the active site is replaced by the human Ig sequence, thus diminishing its antigenicity [49]. Therapy with the humanized antibody has in fact been found effective in ameliorating EAU in primates [50]. The migration and penetration into the target organ can be inhibited by adhesion molecule blockade,

either by monoclonal antibodies [39] or by the natural ligands in soluble form. Finally, tissue damage within the target organ might be ameliorated by inhibitors of reactive oxygen metabolites [41, 42]. Future approaches to controlling tissue damage at the local level might consist of intraocular delivery of regulatory cytokines such as IL-10, inhibitors of peroxidation and free radical scavengers using sustained release intraocular devices, or of gene therapy using targeted gene delivery techniques.

The existence of Th1-low, nonpathogenic response phenotypes that were discussed above, raises the notion of evoking a nonpathogenic response as a means of preventing a pathogenic one. This type of strategy is known as immune deviation therapy. Although itself an attractive notion, the data indicating that a Th2-like effector response may, under some circumstances, be equally destructive to the tissue as a Th1-like response, raises a warning. The pathology seen in IFN-γ knockout mice and SCID mice infused with Th2 clones clearly indicate that abrogation of the Th1 response is not necessarily synonymous with abrogation of pathogenicity [34, 35]. Thus, the utmost caution must be used when attempting to therapeutically manipulate delicately balanced immunological systems.

One such immunotherapy approach, whose success may at least in part be dependent on elicitation of immune deviation, is oral tolerance. This approach serves as a good example how natural control mechanisms can be exploited to achieve a therapeutic effect. It has been known for a long time that oral administration of an antigen downregulates subsequent responses to the same antigen when it is administered parenterally. This is thought to represent a natural mechanism that prevents immunological responses to food antigens and bacterial flora. Oral tolerance has recently gained attention and been explored experimentally and clinically as an immunotherapeutic strategy for a number of autoimmune diseases, including uveitis [51]. Two distinct, non mutually 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 the suppressive cytokines 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 [51-53]. Tolerance by administration of antigen through mucosal surfaces of the upper respiratory tract, known as nasal or inhalational tolerance, has also been found effective in controlling autoimmune disease in experimental models, among them uveitis [54, 55].

Much has been discussed on the issue whether anergy/deletion or active suppression is the more desirable mechanism from the standpoint of achieving lasting antigen-specific tolerance. While strategies aimed at a permanent removal of the autopathogenic cells would at first appear to be the ideal ones, it must be remembered that in most cases the autoantigen driving human autoimmune disease is unknown. Furthermore, the phenomena of epitope spreading and antigen spreading that follow tissue damage and release of its component antigens, make it likely that multiple specificities would be involved in chronic disease, making successful elimination of all the involved specificities difficult [56]. Active regulation, on the other hand, may obviate the need to know what is the eliciting antigen through the phenomenon known as antigen-driven tissue specific bystander suppression, where regulatory cytokines released in response to one antigen within the tissue will suppress effector responses directed against other antigens in the same microenvironment [51, 57]. Although bystander suppression has been shown conclusively in other experimental models, several studies in the EAU model have failed to demonstrate it [52, 58]. Thus, it is still unclear whether bystander suppression is operative in the case of retinal antigens. Nevertheless, a double-masked placebo controlled trial using oral tolerance as immunotherapy for uveitis has given encouraging results, although the actual mechanism(s) of the effect have not been elucidated [59]. The possibility has been raised that an oral tolerance regimen

will "backfire", and induce pathology instead of tolerance [54, 60]. Nevertheless, in many patients who have received oral therapy to date, worsening of their disease due to such a complication has not been noted. However, it has been noted in the uveitis trial on oral tolerance that a complex mixture of antigens seemed to abrogate the therapeutic benefit of single-antigen therapy [59].

In summary

Negative selection in the thymus by itself is insufficient to delete potentially autopathogenic cells that recognize immunologically privileged retinal antigens. Because of their sequestered nature, effective participation of peripheral mechanisms is also in question. Thus, prevention of ocular autoimmunity may depend in a large measure on maintaining an efficient separation between the eye and the immune system. When that delicately maintained balance fails, ocular autoimmunity may ensue despite the presence of local immunosuppressive mechanisms. Research into the immunopathogenic processes that are involved in ocular autoimmunity is defining critical checkpoints in the induction and effector phases of the immunopathogenic process, and is opening new possibilities of rational immunotherapeutic intervention. Nevertheless, caution must be used when devising immunotherapeutic strategies, because therapeutic benefits may be abrogated by inducing counterregulatory mechanisms that themselves may have a pathogenic potential.

References

1. Caspi, R. R., 1994, in Cohen, I. R. and Miller, A. (eds.), Animal Models for Autoimmune Diseases: A Guidebook, Academic Press p. 57-81.

2. Gery, I., Mochizuki, M. and Nussenblatt, R. B., (1986) Retinal specific antigens and immunopathogenic processes they provoke. Prog. Retinal Res. 5: 75-109.

3. Nussenblatt, R. B., Whitcup, S. M. and Palestine, A. G., *Uveitis: Fundamentals and Clinical Practice*, 2 Edn., Mosby - Year Book, Inc., St. Louis, MO 1996.

4. Caspi, R. R., Chan, C. C., Leake, W. C., Higuchi, M., Wiggert, B. and Chader, G. J., (1990) Experimental autoimmune uveoretinitis in mice. Induction by a single eliciting event and dependence on quantitative parameters of immunization. J Autoimmun. 3: 237-246.

5. Chan, C. C., Caspi, R. R., Ni, M., Leake, W. C., Wiggert, B., Chader, G. J. and Nussenblatt, R. B., (1990) Pathology of experimental autoimmune uveoretinitis in mice. J Autoimmun. 3: 247-255.

6. Caspi, R. R., (1992) Immunogenetic aspects of clinical and experimental uveitis. Reg. Immunol. 4: 321-330.

7. Caspi, R. R., Grubbs, B. G., Chan, C. C., Chader, G. J. and Wiggert, B., (1992) Genetic control of susceptibility to experimental autoimmune uveoretinitis in the mouse model: Concomitant regulation by MHC and non-MHC genes. J. Immunol. 148: 2384-2389.

8. Miller, J. F. and Basten, A., (1996) Mechanisms of tolerance to self. Curr Opin Immunol. 8: 815-21.

9. Egwuagu, C. E., Charukamnoetkanok, P. and Gery, I., (1997) Thymic expression of autoantigens correlates with resistance to autoimmune disease. J Immunol. 159: 3109-12.

10. Xu, H., Wawrousek, E. F., Redmond, T. M., Nickerson, J. M., Wiggert, B., Chan, C.-C. and Caspi, R. R., (1998) Extraocular expression of the retinal antigen reduces the susceptibility to experimental autoimmune uveoretinitis in IRBP transgenic mice. Invest Ophthalmol Vis Sci (suppl). (in press).

11. Koevary, S. B. and Caspi, R. R., (1997) Prevention of experimental autoimmune uveoretinitis by intrathymic S- antigen injection. Ocul Immunol Inflamm. 5: 165-72.

12. Streilein, J. W., Takeuchi, M. and Taylor, A. W., (1997) Immune privilege, T-cell tolerance, and tissue-restricted autoimmunity. Hum Immunol. 52: 138-43.

13. Griffith, T. S., Yu, X., Herndon, J. M., Green, D. R. and Ferguson, T. A., (1996) CD95-induced apoptosis of lymphocytes in an immune privileged site induces immunological tolerance. Immunity. 5: 7-16.

14. Helbig, H., Gurley, R. C., Palestine, A. G., Nussenblatt, R. B. and Caspi, R. R., (1990) Dual effect of ciliary body cells on T lymphocyte proliferation. Eur J Immunol. 20: 2457-2463.

15. Caspi, R. R., Roberge, F. G. and Nussenblatt, R. B., (1987) Organ-resident, nonlymphoid cells suppress proliferation of autoimmune T-helper lymphocytes. Science. 237: 1029-1032.

16. Streilein, J. W., Wilbanks, G. A., Taylor, A. and Cousins, S., (1992) Eye-derived cytokines and the immunosuppressive intraocular microenvironment: a review. Curr Eye Res. 11: 41-7.

17. Hara, Y., Caspi, R. R., Wiggert, B., Chan, C. C. and Streilein, J. W., (1992) Use of ACAID to suppress interphotoreceptor retinoid binding protein- induced experimental autoimmune uveitis. Curr Eye Res. 11: 97-100.

18. Albert, D. M. and Diaz-Rohena, R., (1989) A historical review of sympathetic ophthalmia and its epidemiology. Surv Ophthalmol. 34: 1-14.

19. Davies, J. M., (1997) Molecular mimicry: can epitope mimicry induce autoimmune disease? Immunol Cell Biol. 75: 113-26.

20. Segal, B. M., Klinman, D. M. and Shevach, E. M., (1997) Microbial products induce autoimmune disease by an IL-12-dependent pathway. J Immunol. 158: 5087-5090.

21. Roman, M., Martin-Orozco, E., Goodman, S., Nguyen, M. D., Sato, Y., Ronaghy, A., Kornbluth, R. S., Richman, D. D., Carson, D. A. and Raz, E., (1997) Immunostimulatory DNA sequences function as T helper-1-promoting adjuvants. Nature Med. 3: 849-854.

22. Wildner, G. and Thurau, S. R., (1994) Cross-reactivity between an HLA-B27-derived peptide and a retinal autoantigen peptide: a clue to major histocompatibility complex association with autoimmune disease. Eur J Immunol. 24: 2579-85.

23. Singh, V. K., Kalra, H. K., Yamaki, K., Abe, T., Donoso, L. A. and Shinohara, T., (1990) Molecular mimicry between a uveitopathogenic site of S-antigen and viral peptides. Induction of experimental autoimmune uveitis in Lewis rats. J Immunol. 144: 1282-7.

24. Caspi, R. R., Roberge, F. G., McAllister, C. G., el Saied, M., Kuwabara, T., Gery, I., Hanna, E. and Nussenblatt, R. B., (1986) T cell lines mediating experimental autoimmune uveoretinitis (EAU) in the rat. J Immunol. 136: 928-933.

25. Gregerson, D. S., Fling, S. P., Obritsch, W. F., Merryman, C. F. and Donoso, L. A., (1989) Identification of T cell recognition sites in S-antigen: dissociation of proliferative and pathogenic sites. Cell Immunol. 123: 427-440.

26. Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A. and Coffman, R. L., (1986) Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol. 136: 2348-2357.

27. Mosmann, T. R. and Coffman, R. L., (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol. 7: 145-73.

28. Kelso, A., (1995) Th1 and Th2 subsets: paradigms lost? Immunol Today. 16: 374-379.

29. Caspi, R. R., Silver, P. B., Chan, C. C., Sun, B., Agarwal, R. K., Wells, J., Oddo, S., Fujino, Y., Najafian, F. and Wilder, R. L., (1996) Genetic susceptibility to experimental autoimmune uveoretinitis in the rat is associated with an elevated Th1 response. J. Immunol. 157: 2668-2675.

30. Xu, H., Rizzo, L. V., Silver, P. B. and Caspi, R. R., (1997) Uveitogenicity is associated with a Th1-like lymphokine profile: cytokine-dependent modulation of primary and committed T cells in EAU. Cell. Immunol. 178: 69-78.

31. Rizzo, L. V., Silver, P. B., Hakim, F., Chan, C. C., Wiggert, B. and Caspi, R. R., (1993) Establishment and characterization of an IRBP-specific T cell line that induces eau in B10.A mice. Invest. Ophthalmol. Vis. Sci. 34(suppl.): 1143.

32. Saoudi, A., Kuhn, J., Huygen, K., de Kozak, Y., Velu, T., Goldman, M., Druet, P. and Bellon, B., (1993) TH2 activated cells prevent experimental autoimmune uveoretinitis, a TH1-dependent autoimmune disease. Eur J Immunol. 23: 3096-103.

33. Sun, B., Rizzo, L. V., Sun, S. H., Chan, C. C., Wiggert, B., Wilder, R. L. and Caspi, R. R., (1997) Genetic susceptibility to experimental autoimmune uveitis involves more than a predisposition to generate a T helper-1-like or a T helper-2- like response. J Immunol. 159: 1004-11.

34. Jones, L. S., Rizzo, L. V., Agarwal, R. K., Tarrant, T. K., Chan, C. C., Wiggerrt, B. and Caspi, R. R., (1997) Interferon gamma-deficient mice develop experimental autoimmune uveitis in the context of a deviant effector response. J. Immunol. 158: 5997-6005.

35. Lafaille, J. J., Keere, F. V., Hsu, A. L., Baron, J. L., Haas, W., Raine, C. S. and Tonegawa, S., (1997) Myelin basic protein-specific T helper 2 (Th2) cells cause experimental

autoimmune encephalomyelitis in immunodeficient hosts rather than protect them from the disease. J Exp Med. 186: 307-12.

36. Tarrant, T. K., Silver, P. B., Chan, C.-C., Wiggert, B. and Caspi, R. R., (Endogenous il-12 is required for induction and expression of experimental autoimmune uveitis. J. Immunol. (in press).

37. Prendergast, R. A., Iliff, C. E., Coskuncan, N. M., Caspi, R. R., Sartani, G., Tarrant, T. K., Lutty, G. A. and McLeod, D. S., (1998) T cell traffic and the inflammatory response in experimental autoimmune uveoretinitis. Invest Ophthalmol Vis Sci. 39: 754-62.

38. Caspi, R. R., Chan, C. C., Fujino, Y., Najafian, F., Grover, S., Hansen, C. T. and Wilder, R. L., (1993) Recruitment of antigen-nonspecific cells plays a pivotal role in the pathogenesis of a T cell-mediated organ-specific autoimmune disease, experimental autoimmune uveoretinitis. J Neuroimmunol. 47: 177-188.

39. Whitcup, S. M., DeBarge, L. R., Caspi, R. R., Harning, R., Nussenblatt, R. B. and Chan, C. C., (1993) Monoclonal antibodies against ICAM-1 (CD54) and LFA-1 (CD11a/CD18) inhibit experimental autoimmune uveitis. Clin Immunol Immunopathol. 67: 143-150.

40. Li, Q., Fujino, Y., Caspi, R. R., Najafian, F., Nussenblatt, R. B. and Chan, C. C., (1992) Association between mast cells and the development of experimental autoimmune uveitis in different rat strains. Clin. Immunol. Immunopathol. 65: 294-299.

41. Hoey, S., Grabowski, P. S., Ralston, S. H., Forrester, J. V. and Liversidge, J., (1997) Nitric oxide accelerates the onset and increases the severity of experimental autoimmune uveoretinitis through an IFN-gamma-dependent mechanism. J Immunol. 159: 5132-42.

42. Wu, G. S., Zhang, J. and Rao, N. A., (1997) Peroxynitrite and oxidative damage in experimental autoimmune uveitis. Invest Ophthalmol Vis Sci. 38: 1333-9.

43. Rizzo, L. V., Xu, H., Chan, C.-C., Wiggert, B. and Caspi, R. R., (IL-10 has a protective role in Experimental Autoimmune Uveitis. International Immunol. (in press).

44. Li, X. Y., D'Orazio, L. T. and Niederkorn, J. Y., (1996) Role of Th1 and Th2 cells in anterior chamber-associated immune deviation. Immunology. 89: 34-40.

45. D'Orazio, T. J. and Niederkorn, J. Y., (1998) A novel role for TGF-beta and IL-10 in the induction of immune privilege. J Immunol. 160: 2089-98.

46. Chambers, C. A. and Allison, J. P., (1997) Co-stimulation in T cell responses. Curr Opin Immunol. 9: 396-404.

47. Quesniaux, V. F., (1993) Immunosuppressants: tools to investigate the physiological role of cytokines. Bioessays. 15: 731-9.

48. Clardy, J., (1995) The chemistry of signal transduction. Proc Natl Acad Sci U S A. 92: 56-61.

49. Waldmann, T. A., Pastan, I. H., Gansow, O. A. and Junghans, R. P., (1992) The multichain interleukin-2 receptor: a target for immunotherapy. Ann Intern Med. 116: 148-60.

50. Guex-Crosier, Y., Raber, J., Chan, C. C., Kriete, M. S., Benichou, J., Pilson, R. S., Kerwin, J. A., Waldmann, T. A., Hakimi, J. and Roberge, F. G., (1997) Humanized antibodies against the alpha-chain of the IL-2 receptor and against the beta-chain shared by the IL-2 and IL-15 receptors in a monkey uveitis model of autoimmune diseases. J Immunol. 158: 452-8.

51. Weiner, H. L., (1997) Oral tolerance: immune mechanisms and treatment of autoimmune diseases. Immunol Today. 18: 335-43.

52. Gregerson, D. S., Obritsch, W. F. and Donoso, L. A., (1993) Oral tolerance in experimental autoimmune uveoretinitis. Distinct mechanisms of resistance are induced by low dose vs high dose feeding protocols. J Immunol. 151: 5751-61.

53. Rizzo, L. V., Miller-Rivero, N. E., Chan, C. C., Wiggert, B., Nussenblatt, R. B. and Caspi, R. R., (1994) Interleukin-2 treatment potentiates induction of oral tolerance in a murine model of autoimmunity. J Clin Invest. 94: 1668-72.

54. Xiao, B. G. and Link, H., (1997) Mucosal tolerance: a two-edged sword to prevent and treat autoimmune diseases. Clin Immunol Immunopathol. 85: 119-28.

55. Dick, A. D., Cheng, Y. F., McKinnon, A., Liversidge, J. and Forrester, J. V., (1993) Nasal administration of retinal antigens suppresses the inflammatory response in experimental allergic uveoretinitis. A preliminary report of intranasal induction of tolerance with retinal antigens. Br J Ophthalmol. 77: 171-5.

56. Miller, S. D., McRae, B. L., Vanderlugt, C. L., Nikcevich, K. M., Pope, J. G., Pope, L. and Karpus, W. J., (1995) Evolution of the T-cell repertoire during the course of experimental immune-mediated demyelinating diseases. Immunol Rev. 144: 225-44.

57. al-Sabbagh, A., Miller, A., Santos, L. M. and Weiner, H. L., (1994) Antigen-driven tissue-specific suppression following oral tolerance: orally administered myelin basic protein suppresses proteolipid protein-induced experimental autoimmune encephalomyelitis in the SJL mouse. Eur J Immunol. 24: 2104-9.

58. Wildner, G. and Thurau, S. R., (1995) Orally induced bystander suppression in experimental autoimmune uveoretinitis occurs only in the periphery and not in the eye. Eur J Immunol. 25: 1292-7.

59. Nussenblatt, R. B., Gery, I., Weiner, H. L., Ferris, F. L., Shiloach, J., Remaley, N., Perry, C., Caspi, R. R., Hafler, D. A., Foster, C. S. and Whitcup, S. M., (1997) Treatment of uveitis by oral administration of retinal antigens: results of a phase I/II randomized masked trial [see comments]. Am J Ophthalmol. 123: 583-92.

60. Blanas, E., Carbone, F. R., Allison, J., Miller, J. F. and Heath, W. R., (1996) Induction of autoimmune diabetes by oral administration of autoantigen. Science. 274: 1707-9.