Immunologic Research 2003;27/2–3:1–11

Regulation, Counter-Regulation, and Immunotherapy of Autoimmune Responses to Immunologically Privileged Retinal Antigens

Rachel R. Caspi

Section on Immunoregulation, Laboratory of Immunology, National Eye Institute, NIH, Bethesda MD; and Department of Pathology and Laboratory Medicine, University of Pennsylvannia, Philadelphia, PA

Abstract

Our interests revolve around the study of biological mechanisms regulating self-tolerance to immunologically privileged retinal proteins that serve as targets in sight-threatening autoimmune uveitic disease. These studies are aimed at understanding how self-tolerance to these antigens develops during ontogeny and is maintained during adulthood, the processes involved in its pathological breakdown, the regulatory mechanisms that bring about remission and recovery, and, finally, how we can utilize knowledge of these processes for therapeutic restoration of tolerance. To answer these questions, we use the experimental autoimmune uveitis (EAU) model in rats and mice. Because of the commonality of underlying immunological mechanisms, lessons and concepts learned in experimental ocular models are applicable to other disease entities, and, conversely, data gleaned from other autoimmune diseases are applicable to the study of uveitis.

Key Words: Uveits, autoimmune disease, T cells, Th1/Th2, tolerance

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. The most dangerous to vision is uveitis that affects the back of the eye where the photoreceptor cells are located *(1)*. It is estimated that posterior uveitic

Rachel R. Caspi, PHD
Chief, Section on Immunoregulation
Laboratory of Immunology, NEI
NIH Bg. 10, Rm. 10N222
10 Center Dr. MSC 1857
Bethesda, MD 20892-1857

© 2003
Humana Press Inc.
0257–277X/03/
27/2–3:1–11/$20.00

diseases collectively are responsible for about 10% of the severe visual handicap in the United States.

EAU is a cell-mediated autoimmune disease model that 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 also in mice and rats 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 *(2,3)*. Susceptible animal species include rodents as well as primates. 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 *(1–3)*. Of the uveitogenic retinal antigens the most widely studied are IRBP that transports vitamin A derivatives within the eye, and the S-Ag, otherwise known as arrestin, which quenches photoactivated rhodopsin. Both are proteins that participate in the visual cycle and are highly conserved through evolution. Whereas both S-Ag and IRBP are uveitogenic in rats, mice are susceptible to IRBP, but are resistant to S-Ag-induced disease.

Current therapies for uveitis rely mostly on nonspecific immunosuppression. Steroids are the first line of defense, followed by cyclosporin A and other macrolides, with cytotoxic agents and antimetabolites as a last resort *(1)*. These chronic therapies carry serious side effects and, therefore, it is of utmost importance to understand what are the mechanisms that drive the pathogenesis of uveitis, so as to be able to devise better and more specific therapies, ideally targeting only the antigen-specific cells that orchestrate and perpetuate the pathogenic process. This overview will attempt to present a picture of the questions and approaches that are guiding our research in an effort to achieve this goal.

How Does Tolerance to Immunologically Privileged Antigens Develop and How Is it Maintained?

The eye is an immunologically privileged organ. Immune privilege traditionally has been understood as a lack of response to antigens placed in the eye. As an example, allogeneic tumor cells injected into the anterior chamber of the eye are not rejected. Initially, this was thought to be caused by the lack of recognition due to efficient blood–organ barriers and the lack of lymphatic drainage of the interior of the globe. However, more recent studies have indicated that contributing to ocular immune privilege are active processes, which include the presence of anti-inflammatory cytokines and other mediators in the ocular fluids (e.g.,TGF-β, α-MSH,VIP, and others), and contact-mediated inhibition by ocular cells (Fas/FasL and noncytolytic contact inhibitors yet to be characterized) *(4–7)*. This raises the question: How does the immune system see the antigens derived from the eye itself? Although exogenous antigens injected into the eye are known to elicit a stereotypic deviant immune response, known as anterior chamber-associated immune deviation (ACAID), in which cellular immunity and complement-binding antibodies are selectively inhibited (reviewed in refs. *4* and *5*), there is no convincing evidence for naturally existing ACAID

to endogenous retinal antigens. Because of the relative sequestration of the eye from the immune system, it is unlikely that peripheral tolerance mechanisms, which operate efficiently for tolerizing migrant lymphocytes in "open" organ systems, play a significant role in maintaining functional tolerance to retinal antigens. The findings that retinal antigen-specific lymphocytes can easily be 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. Indeed, recent data from our laboratory show that mice expressing the retinal antigen IRBP extraocularly on a class II promoter are highly resistant to EAU induced with an IRBP-derived epitope, demonstrating directly that the normally restricted expression of this retinal antigen does not support efficient self-tolerization *(8)*.

Egwuagu et al. *(9)* showed in 1997 that central tolerance mechanisms may supplement the local mechanisms described above in creating a functionally tolerant state to retinal antigens. This pioneering study showed that the retinal proteins IRBP and S-Ag (arrestin) can be detected in the thymic extracts of mice that are resistant to EAU. A more recent study by the group of Kyewski et al. *(10)* localized their expression to thymic medullary epithelial cells. However, this still leaves open the question of what is the tolerance status in mice that are susceptible to EAU in which thymic expression was not detectable, such as the highly susceptible B10.RIII strain.

Ongoing studies in our laboratory are addressing this question directly by using IRBP knockout (KO) mice *(11)* that have been bred onto the B10.RIII background. These studies revealed that, in comparison to wild-type B10.RIII mice, the IRBP KO mice have strongly enhanced cellular responses to IRBP and an expanded number of IRBP-specific T cells. Furthermore, sparse IRBP-positive can be detected in the thymic medulla of wild-type B10.RIII mice, which are absent in the KO, by using a highly sensitive immunohistochemical staining *(12)*. Importantly, IRBP message can be detected in these microdissected cells by polymerase chain reaction (PCR), suggesting that they, in fact, make the interphotoreceptor retinoid-binding protein *in situ* (unpublished data). Thus, EAU-susceptible mice, which express IRBP in what is considered to be an immunologically privileged fashion, express IRBP in the thymus and display a detectable and functionally significant tolerance to this antigen. Further studies are needed to dissect the relative contributions of the central (thymic) and peripheral (eye and pineal) contributions to this tolerance.

The precise "division of labor" that brings about this level of tolerance notwithstanding, it is apparent that the tolerance is not profound and is easily broken by a uveitogenic challenge. Thus, avoidance 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 for any reason, priming of the autoreactive T cells may ensue, overcoming the barrier of local intraocular protective mechanisms, and resulting in disease.

How Does Tolerance to Retinal Antigens Break down and What Are the Natural Mechanisms that Counterregulate Ocular Autoimmunity?

The role of adjuvants in EAU, as in other autoimmune disease models having similar cellular mechanisms, is all-important. A uveitogenic immunization must be adminis-

tered in complete Freund's adjuvant, and, in addition, many rodent strains require pertussis toxin to develop disease *(2)*. Our earlier studies have shown that in the wild-type host, the uveitogenic effector T cells must acquire a Th1 phenotype in order to induce pathology *(13,14)*. This is also supported by observations that endogenous IL-12, the prototypic Th1-inducing cytokine, is necessary for disease development because mice genetically deficient in IL-12 or treated with neutralizing antibodies to IL-12 fail to develop disease. The mycobacteria included in complete Freund's adjuvant stimulate the innate immune response that drives differentiation of T cells toward the Th1 pathway. In this context, we studied pertussis toxin, which for many years has been used to enhance a variety of autoimmune diseases in rodent models. Pertussis toxin has complex and incompletely understood effects on the immune response. One effect is promoting the breakdown of blood–tissue barriers. In addition, our data have indicated that it also strongly enhances the Th1 response, suggesting that this effect that may be important in the enhancement of autoimmunity by pertussis *(14,15)*.

In human uveitis, the etiologic causes/agents that bring about a functional breakdown of tolerance are largely unknown. An exception is sympathetic ophthalmia, where the eliciting event can be pinpointed exactly: a puncture wound to one eye can be followed after several weeks or months by a destructive inflammation in the other, "sympathizing," eye *(1)*. This is thought to be due to antigens released from the damaged eye that drain to the regional lymph node and elicit systemic immunity to the retinal antigens. It has been noted that sympathetic ophthalmia occurs more frequently if an infection develops in the damaged eye, which may provide the needed adjuvant effect. In the majority of uveitis cases, where there is no preceding physical damage to the eye, it is thought that a peripheral immunization event of some sort (e.g.,infection with a pathogen that has molecular mimicry with a retinal protein) might provide the initial antigenic stimulus. A number of molecular mimics that induce EAU in susceptible animals have been identified *(16)*.

The autoreactive effector T cells that have been activated in the periphery must find their way to the eye. This is akin to the situation in adoptive transfer, when a cultured T cell line or clone is infused into the animal and induces disease in an initially healthy eye. How d the T cells "know" to find their target organ? On the basis of experiments in which susceptible animals were infused with labeled activated T cells that were either retinal antigen-specific or nonspecific, our current thinking is that the activated cells enter into the eye by chance. This was concluded from experiments in which 10 million fluorescently labeled S-Ag-specific or nonspecific activated T cells were infused into recipient rats *(17)*. Very small but equal numbers (150 cells) of the specific or the nonspecific T cells entered the retina at 24 h after infusion, and both disappeared from the eye by 48 h. However, the animals that received the specific T cells subsequently developed a massive new wave of infiltration and uveitis, whereas no further activity was apparent in the eyes of the recipients of the nonspecific cells. This would suggest that, although the initial penetration into the eye is stochastic, recognition of specific antigen *in situ* precipitates an inflammatory cascade and recruitment of leukocytes from the blood, culminating in the development of disease. In that second amplification phase, non-antigen-specific host cells, including recruited nonspecific T cells, are important in the pathogenesis of the disease *(18)*. Thus, two distinct waves of infiltration into the eye can be resolved as part of EAU pathogenesis.

Recent experiments from our laboratory implicated Gi protein-dependent processes (most likely chemokine receptor-driven) not only in the second wave of infiltration (chemokine involvement in the recruitment of cells to existing inflammatory sites has by now been well-established), but also in the initial entry of T cells into the healthy eye, the mechanism of which is much less well elucidated. This conclusion was based on observations that pertussis toxin—which, as described above, enhances autoimmunity and the Th1 response when injected at the time of active immunization—was able to completely abrogate EAU when given at the time of effector cell migration and inflammatory cell recruitment to the target organ, corresponding to 7–10 d after active immunization with, or concurrently with, infusion of uveitogenic T cells *(19)*. The protection was accompanied by the inhibition of cell migration to chemokines and was dependent on the ADP-ribosyltransferase activity of pertussis toxin, all consistent with dependence on Gi protein-coupled receptor signaling.

There are a number of mechanisms that in concert act to limit EAU induction and/or its expression. The eye itself is a profoundly immunosuppressive environment. This is exerted at several levels and is part of the immune privilege. The ocular fluids contain immunosuppressive factors, such as TGF-β, α-MSH, VIP, and others, and the ocular resident cells sport immunosuppressive molecules on their surface, such as FasL and still uncharacterized nonlytic molecule(s) *(4,5,20)*. It is therefore remarkable that an effector lymphocyte entering such a hostile territory is still able to induce disease. As mentioned above, out of 10 million uveitogenic T cells injected into a rat only 150 are found in the retina after 24 h, and if they are retinal antigen-specific, they elicit disease *(17)*. Because as few as 100,000 cells from a T cell line are able to induce disease, theoretically one or two antigen-specific activated cells entering the retina are a productive uveitogenic stimulus. This would indicate that the immunosuppressive environment of the eye is insufficient to inactivate infiltrating effector cells before they trigger the amplification mechanisms that culminate in disease. In keeping with this, we found that in contrast to its effect on naive T cells, TGF-β does not inhibit antigen-driven proliferation of primed uveitogenic T cells in vitro *(21)* and does not prevent the acquisition of uveitogenicity by these cells (Xu et al., submitted).

As a Th1-dependent response, EAU should be counterregulated by directed skewing of the response to retinal antigen toward the Th2 pathway. We were able to achieve amelioration of EAU by treatment of mice with IL-4 and IL-10 during the first days after uveitogenic immunization, which is when the response phenotype is being determined *(22)*. Whereas treatment with IL-10 alone downregulated the Th1 cytokines, combined treatment with IL-4 additionally caused the emergence of Th2 cytokines. Recent evidence from our laboratory also implicated IL-10 in limiting the severity of, and promoting spontaneous recovery from, EAU *(22)*. This is supported by data showing that IL-10 mRNA levels increase in the eye during the spontaneous recovery phase, and neutralization of endogenous IL-10 during the effector stage of disease results in higher disease scores. Moreover, IL-10 or an Il-10-like molecule appears to be expressed in the eye, at least at the mRNA level, in EAU resistant strains of rats, and we hypothesize that it may be involved in raising the threshold of resistance to the induction of the disease *(23)*.

In addition to afferent control by elicitation of a counterregulatory response,

such as Th2, and efferent control by anti-inflammatory cytokines, such as IL-10, our work suggests that the uveitogenic Th1 response can be controlled by a process akin to negative feedback inhibition, using the very same proinflammatory mediators that the effector Th1 cells produce or induce. As will be discussed in the following section, susceptibility to EAU is associated with a Th1 response. Therefore, we expected that treatment of mice immunized for EAU with IL-12 concurrently with immunization will enhance disease scores. Surprisingly, the treatment largely or completely prevented development of disease. Subsequent experiments showed that only treatment during the first week, but not the second week after immunization was protective, suggesting that it was exerted on the priming phase and not on the effector phase of disease. Protected mice had evidence of enhanced apoptosis in secondary lymphoid organs during IL-12 treatment, and 21 d later exhibited depressed immunological responses to the immunizing retinal antigen and a reduced yield of lymph node cells. In a series of experiments with knockout mice, it became apparent that IFN-γ and iNOS knockout mice were not protected, and Bcl-2^{lck} transgenic mice, expressing the antiapoptotic molecule Bcl-2 in their T cells, were less well protected than the wild-type. Based on these data and the known functions of IL-12 and its downstream mediators, we proposed a sequence of events, whereby IL-12 triggered massive production of IFN-γ (indeed, IL-12 treated mice had nanogram levels of circulating IFN-γ in their serum), which caused upregulation of inducible nitric oxide synthase (iNOS = NOS2), and production of NO, which, in turn, precipitated apoptosis of antigen-specific effector cells as they were being primed. Thus, an excess of inflammatory mediators early in the response can limit disease by preventing the recruitment of new T cells into the effector pool, through apoptotic and possibly non-apoptotic mechanisms. Other studies indicate that IFN-γ-dependent inflammatory mediators can also limit disease severity and duration by helping to eliminate "spent" effector cells *(24,25)*.

Recent studies in other autoimmune disease models have pointed out a role for natural killer (NK) T and CD4+/CD25+ regulatory T cells in homeostatic control of autoimmune responses. The possible role of such mechanisms in preventing autoimmune uveitic disease is being investigated.

Genetic Mechanisms of Susceptibility to Uveitic Disease

In humans, uveitis has been found to be associated with certain HLA haplotypes. In mice, where the existence of inbred strains permits more precise and sophisticated studies to be done, we found that expression of EAU is controlled by both major histocompatibility complex (MHC) and non-MHC genes, and similar effects were reported in the rat species *(26,27)*. We have tentatively mapped MHC control of susceptibility to the class II genes in mice, implicating antigen presentation as a possible factor *(26)*.

More recently, we have defined genetic regions associated with EAU by correlating phenotype with genotype (using a genome-wide scan for microsatellite markers) in F2 progeny of a resistant (F344) and a susceptible (Lewis) rat strain. These two strains share the same MHC class II and a closely related MHC class I, thereby largely excluding MHC effects from the analysis. We identified four

chromosomal regions that cosegregated with the disease phenotype on rat chromosomes 2, 4, 10, and 12 *(28)*. Many immunologically relevant genes are known to be located within these regions. Furthermore, the EAU susceptibility intervals overlap with genetic regions associated with other autoimmune diseases in animal models as well as in humans, suggesting that autoimmune disease markers are shared between different autoimmune diseases and across species *(29)*. These candidate regions are currently being bred into congenic lines to isolate and refine the genetic intervals.

In parallel, we are also employing a microarray analysis of lymph node cells from the parental susceptible and resistant strains,and, subsequently, also of the congenic lines, to identify differentially expressed genes associated with susceptibility and resistance. Preliminary analysis has yielded a number of genes that are known to be located within the genetic regions associated with EAU susceptibility, constituting an independent confirmation of the validity of these approaches.

Recently, we developed a humanized model of EAU in HLA-class II transgenic mice that are deficient for mouse class II molecules (Pennesi et al., submitted for publication). Typical EAU was induced in HLA-DR3, DR4, DQ6, and DQ8 transgenic mice by immunization with the retinal antigen IRBP. Importantly, HLA-DR3 transgenic (TG) mice developed severe EAU also with the retinal antigen arrestin, to which human uveitis patients frequently display cellular responses, but which is not uveitogenic in wild-type mice. The EAU model in HLA TG mice supports a role for the retinal antigens that are pathogenic in animals in human uveitis and offers a tool to dissect the disease-relevant epitopes presented by different HLA molecules.

Therapeutic Re-Education of the Immune System for Tolerance

Conventional therapies in autoimmune uveitis, as for other autoimmune diseases, rely on broad immunosuppressive regimens that are nonspecific and result in a global inhibition of the immune system. On the other hand, in their most refined, immunotherapy approaches seek to elicit a functional tolerance to the autoantigen, reestablishing a stable immunological balance that will ideally obviate the need for further treatment. Immunotherapeutic approaches can be either antigen-specific or nonspecific, and depending on the circumstances, the fate of the T cells may be deletion, anergy, or control by active regulatory mechanisms. Each strategy has its advantages and drawbacks.

The antigen(s) that are causally involved in human uveitis are still unidentified, although some data point to arrestin, to which many uveitis patients have cellular responses, as a candidate. Non-antigen-specific approaches have the advantage that we do not need to know the antigens causally involved in human uveitis, but their drawback is that they do not differentiate between the autoreactive T cells and those T cells that respond to microbial antigens and are important in host defense. Another consideration is that the therapy should be able to reverse, not only to prevent, pathogenesis. Reversal regimens are able to target already primed effector cells, whereas prevention regimens primarily target the activation stage of the T cell.

Examples of antigen-nonspecific therapies are monoclonal antibodies or immunotoxins directed against surface receptors, such as CD4, IL-2 receptor, or costimulatory molecules. In this context, we recently studied the

blockade of costimulation as a therapy for EAU. We showed that disruption of B7/CD28t in vivo indeed prevents EAU onset, but contrary to expectations from studies done in model antigen systems, such as OVA, does not result in lasting tolerance. Quite the contrary, immunization under cover of such costimulatory blockade permits formation of immunological memory, and disease elicited after a second challenge is more severe *(30)*.

Antigen-specific approaches represent the ideal form of immunotherapy, as they target only the T cells implicated in pathology while leaving other responses intact. However, they require knowledge of the driving antigen(s). In addition, the specificity of the T cell response may change over time (a phenomenon known as epitope-spreading and antigen-spreading). Ultimately, we believe that the antigen-specific responses in humans will be dissected (perhaps with the help of HLA-transgenic mice, as described above), and for this reason, have been devoting attention to the development of antigen-specific strategies.

As described above, we showed that by treating mice immunized for EAU with a combination of IL-4 and IL-10 it is possible to skew the response away from the Th1 pathway, and at the same time, ameliorate pathology *(22)*. These results would suggest that immune deviation strategies, seeking to promote Th2 response at the expense of the Th1 response, could be a viable approach to therapy. However, it is becoming increasingly apparent that an unopposed Th2 response can be equally or more destructive to the tissue as a Th1 response. IFN-γ knockout mice, which lack a normal Th1 response owing to their inability to make the prototypic Th1 cytokine, IFN-γ, still develop EAU, but they do so in the context of a deviant effector response, having many Th2-like elements *(31)*. 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. Similar data have been reported in another autoimmune disease model, experimental autoimmune encephalomyelitis, where polarized Th2 cells caused severe paralytic disease in SCID mice *(32)*. Thus, therapeutic paradigms seeking to replace a Th1 with a Th2 response, if carried too far, may result in replacing one type of pathology with another. A case in point are two clinical trials in multiple sclerosis, where deviation was attempted using an altered peptide ligand: both were stopped due to emergence of what looked like Th2-related pathologies *(33,34)*.

Another approach, whose success may at least in part be dependent on elicitation of immune deviation, is oral tolerance, where feeding of an autoantigen subsequently down-regulates cognate responses to this antigen. Three to five feedings of 200 mg at a time of S-Ag or IRBP, or their peptides, prevented uveitis in rats and in mice *(35–37)*. Furthermore, in a relapsing model of EAU in B10.A mice, feeding during remission prevents a relapse, suggesting that oral tolerance can be effective in existing disease *(38)*. Indeed, a recent clinical trial where uveitis patients were fed retinal S-Ag, we obtained encouraging results *(39)*. Endogenously produced IL-4 and IL-10 are necessary to develop oral tolerance in the EAU model, and it can be enhanced by administration of low doses of IL-2 concurrently with the oral tolerogenic regimen *(36,40)*. Oral tolerance can elicit several non-mutually exclusive regulatory mechanisms, from active regulation at lower antigen doses to deletion at highest Ag doses. Although permanent removal of the autopathogenic cells would appear to be the ideal outcome, the complex HLA haplotypes in humans, combined

with epitope and antigen spreading, make this goal difficult to achieve because diverse epitopes and multiple antigens are likely to be involved. For this reason, active suppression by regulatory cells may be a more desirable goal, as regulatory cytokines produced in response to one antigen will act to downregulate responses to other antigens in the same tissue microenvironment.

Recently, we have begun to explore genetic strategies for induction of tolerance. We used retroviral and naked DNA approaches to introduce sequences encoding the retinal antigen into the animal, so as to achieve in vivo expression of the protein under tolerogenic conditions. In one approach, a major uveitogenic epitope of the IRBP fused in frame to the heavy chain of the mouse IgG1 molecule was inserted into peripheral B cells using a retroviral vector. Naive recipients of the transduced B cells were protected from EAU, induced by a subsequent uveitogenic challenge for at least 8 mo and exhibited immunological hyporesponsiveness to the tolerizing antigen (*41*; Agarwal et al., unpublished). Protection could not be adoptively transferred, suggesting that the mechanism may involve anergy or deletion of the uveitogenic T cells, rather than the elicitation of regulatory cells. Importantly, the retrovirally transduced B cells were effective in a reversal protocol when given to mice that been immunized 7 d earlier or had been infused with a uveitogenic T cell line, and thus already had circulating effector cells. This suggests the utility of this therapy in a clinical situation. In a second approach, a naked DNA plasmid encoding a fragment of IRBP was injected intramuscularly or intravenously. Mice vaccinated with the naked DNA were protected from disease and displayed immunological hyporesponsiveness to the antigen *(42)*. These data underscore the potential of DNA-based strategies for eliciting antigen-specific tolerance.

Summary

Negative selection in the thymus by itself is insufficient to delete potentially autopathogenic cells that recognize immunologically privileged retinal antigens. Because of the sequestered nature of these antigens, effective participation of peripheral mechanisms is also in question. When potentially pathogenic T cells—that have not been deleted or tolerized—become primed in the periphery and find their way to the eye, 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 therapeutic intervention. Reprogramming of the immune system for tolerance to establish a stable immunological balance is the goal of immunotherapeutic strategies of the future.

References

1 Nussenblatt RB, Whitcup SM, Palestine AG: Uveitis: Fundamentals and Clinical Practice, 2nd ed. St. Louis, MO, Mosby—Year Book, Inc., 1996, pp. 22–26.

2 Caspi RR: Experimental autoimmune uveoretinitis—rat and mouse; in Cohen IR, Miller A, (eds). Animal Models for Autoimmune Diseases: A Guidebook. Academic Press, 1994.

3 Gery I, Mochizuki M, Nussenblatt RB: Retinal specific antigens and immunopathogenic processes they provoke. Prog Retinal Res 1986;5: 75–109.

4 Streilein JW: Regional immunity and ocular immune privilege. Chem Immunol 1999;73:11–38.
5 Streilein JW: Immunologic privilege of the eye. Springer Semin Immunopathol 1999;21:95–111.
6 Helbig H, Gurley RC, Palestine AG, et al.: Dual effect of ciliary body cells on T lymphocyte proliferation. Eur J Immunol 1990;20: 2457–2463.
7 Caspi RR, Roberge FG, Nussenblatt RB: Organ-resident, nonlymphoid cells suppress proliferation of autoimmune T-helper lymphocytes. Science 1987;237:-1029–1032.
8 Xu H, Wawrousek EF, Redmond TM, et al.: Transgenic expression of an immunologically privileged retinal antigen extraocularly enhances self tolerance and abrogates susceptibility to autoimmune uveitis. Eur J Immunol 2000;30: 272–278.
9 Egwuagu CE, Charukamnoetkanok P, Gery I: Thymic expression of autoantigens correlates with resistance to autoimmune disease. J Immunol 1997;159:3109–3112.
10 Derbinski J, Schulte A, Kyewski B, Klein L: Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. Nat Immunol 2001;2: 1032–1039.
11 Liou GI, Fei Y, Peachey NS, et al.: Early onset photoreceptor abnormalities induced by targeted disruption of the interphotoreceptor retinoid-binding protein gene. J Neurosci 1998;18:4511–4520.
12 Caspi RR, Avichezer D, Chan C-C, et al. Immunologically privileged expression of a sequestered ocular antigen results in tolerance. FASEB J 2002;16:A1066.
13 Sun B, Rizzo LV, Sun SH, et al.: 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 1997;159:1004–1011.
14 Caspi RR, Silver PB, Chan CC, et al.: Genetic susceptibility to experimental autoimmune uveoretinitis in the rat is associated with an elevated Th1 response. J Immunol 1996;157:2668–2675.
15 Silver PB, Chan CC, Wiggert B, Caspi RR: The requirement for pertussis to induce EAU is strain-dependent: B10.RIII, but not B10.A mice, develop EAU and Th1 responses to IRBP without pertussis treatment. Invest Ophthalmol Vis Sci 1999;40:2898–2905.
16 Forrester JV, McMenamin PG: Immunopathogenic mechanisms in intraocular inflammation. Chem Immunol 1999;73:159–185.
17 Prendergast RA, Iliff CE, Coskuncan NM, et al.: T cell traffic and the inflammatory response in experimental autoimmune uveoretinitis. Invest Ophthalmol Vis Sci 1998;39:754–762.
18 Caspi RR, Chan CC, Fujino Y, et al.: 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 1993;47:177–188.
19 Su SB, Silver PB, Zhang M, et al.: Pertussis toxin inhibits induction of tissue-specific autoimmune disease by disrupting G protein-coupled signals. J Immunol 2001; 167:250–256.
20 Caspi RR. Immune mechanisms in uveitis. Springer Semin Immunopathol 1999;21:113–124.
21 Xu H, Rizzo LV, Silver PB, Caspi RR: Uveitogenicity is associated with a Th1-like lymphokine profile: cytokine-dependent modulation of early and committed effector T cells in experimental autoimmune uveitis. Cell Immunol 1997;178:69–78.
22 Rizzo LV, Xu H, Chan CC, et al.: IL-10 has a protective role in experimental autoimmune uveoretinitis. Int Immunol 1998;10:807–814.
23 Sun B, Sun SH, Chan CC, Caspi RR:Evaluation of in vivo cytokine expression in EAU-susceptible and resistant rats: a role for IL-10 in resistance? Exp Eye Res 2000;70: 493–502.
24 Caspi RR, Chan CC, Grubbs BG, et al.: Endogenous systemic IFN-gamma has a protective role against ocular autoimmunity in mice. J Immunol 1994;152:890–899.
25 Chu CQ, Wittmer S, Dalton DK: Failure to suppress the expansion of the activated CD4 T cell population in interferon gamma-deficient mice leads to exacerbation of experimental autoimmune encephalomyelitis. J Exp Med 2000;192: 123–128.
26 Caspi RR, Grubbs BG, Chan CC, et al.: Genetic control of susceptibility to experimental autoimmune uveoretinitis in the mouse model. Concomitant regulation by MHC and non-MHC genes. J Immunol 1992;148:2384–2389.
27 Hirose S, Ogasawara K, Natori T, et al.:Regulation of experimental autoimmune uveitis in rats—separation of MHC and non-MHC gene effects. Clin Exp Immunol 1991;86: 419–425.
28 Sun SH, Silver PB, Caspi RR, et al.: Identification of genomic regions controlling experimental autoimmune uveoretinitis in rats. Int Immunol 1999;11:529–534.
29 Becker KG, Simon RM, Bailey-Wilson JE, et al.: Clustering of non-major histocompatibility complex susceptibility candidate loci in human autoimmune diseases. Proc Natl Acad Sci USA 1998;95: 9979–9984.
30 Silver PB, Hathcock KS, Chan CC, et al.: Blockade of costimulation through B7/CD28 inhibits experimental autoimmune uveoretinitis, but does not induce long-term tolerance. J Immunol 2000;165: 5041–5047.
31 Jones LS, Rizzo LV, Agarwal RK, et al.: IFN-gamma-deficient mice develop experimental autoimmune uveitis in the context of a deviant effector response. J Immunol 1997; 158:5997–6005.
32 Lafaille JJ, Keere FV, Hsu AL, et al.: 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 1997;186: 307–312.
33 Bielekova B, Goodwin B, Richert N, et al.: Encephalitogenic potential of the myelin basic protein peptide (amino acids 83-99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. Nat Med 2000;6: 1167–1175.

34 Kappos L, Comi G, Panitch H, et al.: Induction of a non-encephalitogenic type 2 T helper-cell autoimmune response in multiple sclerosis after administration of an altered peptide ligand in a placebo-controlled, randomized phase II trial. The Altered Peptide Ligand in Relapsing MS Study Group. Nat Med 2000;6:1176–1182.
35 Nussenblatt RB, Caspi RR, Mahdi R, et al.: Inhibition of S-antigen induced experimental autoimmune uveoretinitis by oral induction of tolerance with S-antigen. J Immunol 1990;144:1689–1695.
36 Rizzo LV, Miller-Rivero NE, Chan CC, et al.: Interleukin-2 treatment potentiates induction of oral tolerance in a murine model of autoimmunity. J Clin Invest 1994;94: 1668–1672.
37 Silver PB, Rizzo LV, Sun B, et al.: Heterologous epitopes of IRBP protect against autoimmune uveitis induced by the autologous epitope. J Neuroimmunol 1998;88: 128–136.
38 Thurau SR, Chan CC, Nussenblatt RB, Caspi RR: Oral tolerance in a murine model of relapsing experimental autoimmune uveoretinitis (EAU): induction of protective tolerance in primed animals. Clin Exp Immunol 1997;109:370–376.
39 Nussenblatt RB, Gery I, Weiner HL, et al.: Treatment of uveitis by oral administration of retinal antigens: results of a phase I/II randomized masked trial. Am J Ophthalmol 1997;123: 583–592.
40 Rizzo LV, Morawetz RA, Miller-Rivero NE, et al.: IL-4 and IL-10 are both required for the induction of oral tolerance. J Immunol 1999; 162:2613–2622.
41 Agarwal RK, Kang Y, Zambidis E, et al.: Retroviral gene therapy with an immunoglobulin-antigen fusion construct protects from experimental autoimmune uveitis. J Clin Invest 2000;106:245–252.
42 Agarwal R, Silver P, Su S-B, et al.: Vaccination with a plasmid encoding retinal interphotoreceptor retinoid-binding protein (IRBP) protects mice against experimental autoimmune uveitis (EAU). FASEB J 2002;16:A1066.