Mouse Models of Experimental Autoimmune Uveitis

Rachel R. Caspi   Phyllis B. Silver   Dror Luger   Jun Tang   Lizette M. Cortes
Giuseppina Pennesi   Mary J. Mattapallil   Chi-Chao Chan

Laboratory of Immunology, NEI, NIH, Bethesda, Md., USA

Key Words

Uveitis, mouse • Uveoretinitis • Autoimmune disease • Th1 • Th17

Abstract

The mouse model of experimental autoimmune uveitis, induced by immunization of mice with the retinal protein IRBP, was developed in our laboratory 20 years ago and published in 1988. Since that time it has been adopted by many investigators and has given rise to many studies that helped elucidate genetic influences, dissect the basic mechanisms of pathogenesis and test novel immunotherapeutic paradigms. The current overview will summarize the salient features of the experimental autoimmune uveitis model and discuss its mechanisms.

Copyright © 2008 S. Karger AG, Basel

Introduction

Human autoimmune uveitis encompasses a diverse group of ocular inflammatory diseases including sympathetic ophthalmia, birdshot retinochoroidopathy, ocular sarcoidosis, Behçet's disease and others. While uveitic diseases can have quite diverse clinical appearance and course, they have in common a lack of known infectious etiology, strong MHC associations and improvement

with T-cell-targeting agents. Patients often display lymphocyte responses to retinal antigens (Ags), particularly retinal arrestin [retinal soluble antigen (S-Ag)]. On the basis of these findings these types of uveitis are considered to have an autoimmune etiology [1].

An autoimmune etiology was supported by the early findings that immunization of guinea pigs and rats with S-Ag emulsified in complete Freund's adjuvant (CFA) induced an ocular inflammatory disease, experimental autoimmune uveitis (EAU) that was similar in many pathological features to human uveitis. However, S-Ag did not induce EAU in the commonly used strains of mice, which was a significant impediment to basic mechanistic studies, for which the mouse species is much better suited. An EAU model in mice was developed and published almost 20 years ago, with the discovery of IRBP as a uveitogenic protein [2–4]. Since that time numerous variants on the theme of IRBP-EAU have appeared, including the 'humanized' EAU model in HLA class II transgenic (Tg) mice (where S-Ag is recognized as a uveitogenic self-Ag), EAU induced by adoptive transfer of T cells from uveitic donors, EAU induced by injection of in vitro matured Ag pulsed dendritic cells (DC) and finally spontaneous uveitis developing in AIRE-deficient mice and in nude mice implanted with an embryonic rat thymus that have an altered T cell repertoire due to deficiency in central (thymic) tolerance [5–8].

Other EAU-like models, which can also be spontaneous, include Tg models in mice expressing a Tg neo-self Ag in the retina in conjunction with T cells expressing a T cell receptor specific for that Ag, and HLA-A29 Tg mice, are covered elsewhere in this volume.

Genetics of EAU Susceptibility

Inbred mouse strains are equivalent to individuals among humans. As in humans, in whom uveitis is relatively rare, only a few mouse strains are susceptible to EAU. Early studies showed that in order to develop EAU a strain has to have both a susceptible haplotype and a permissive background [9]. Highly to moderately susceptible H-2 haplotypes (in which susceptibility tentatively maps at least in part to the MHC class II, or I-A subregion) are H-2^r, H-2^k and H-2^b, in that order. Examples of strains bearing these haplotypes are B10.RIII, B10.BR and C57BL/6, respectively. If, however, a strain has a susceptible haplotype but a nonpermissive background, or a permissive background but a resistant haplotype, the disease will be attenuated or will fail to develop. Thus, both MHC and non-MHC genes determine susceptibility.

Targeted Retinal Ags and Their Epitopes

As mentioned above, patients with uveitis often respond to retinal Ags. While it is unknown whether this responsiveness is part of the etiology of their disease, or a secondary phenomenon representing autoimmunization to retinal Ag released as a result of tissue damage, it is generally accepted that these responses are disease-relevant and may help fuel its progression.

The Ag most often recognized by patients with a variety of uveitic diseases is retinal arrestin or S-Ag. The reason for this apparent preference for S-Ag out of many possible retinal proteins is unknown. Furthermore, within the S-Ag the M and N fragments are reported to be recognized in a promiscuous fashion, even by patients with disparate MHC haplotypes [10, 11]. This apparent 'preference' for a particular Ag has puzzled researchers for a long time. However, in mice there appears to be an analogous situation with IRBP as a preferred uveitogenic target.

First, EAU in susceptible mouse strains is easily induced with IRBP, but the common laboratory strains are not significantly susceptible to EAU induced with S-Ag [9]. Second, spontaneous forms of EAU appear to target

Table 1. Uveitogenic epitopes for the 3 strains of EAU-susceptible mice

Strain	Class II haplotype	Position and sequence	Ref.
B10.RIII	r	161–180 SGIPYIISYLHPGNTILHVD	14
C57BL/6, C57BL/10	b	1–20 GPThLFQPSLVLDMAKVLLD	15
B10.A, B10.BR	k	201–216 ADKDVVVLTSRTGGV	16

IRBP as the preferred, and indeed the only, retinal auto-Ag in mice. AIRE is a transcription factor that has been shown to cause ectopic expression of many (though not all) tissue Ags in the thymus. This expression is necessary to drive negative selection of the T cell repertoire, such that T lymphocytes responding with high affinity to self are deleted or sometimes anergized (and may then become regulatory cells).

AIRE-deficient [knockout (KO)] mice fail to express many tissue Ags in the thymus, among them IRBP, S-Ag and other retinal Ags. Over time AIRE KO mice develop spontaneous Abs to IRBP and uveitis [12]. Further studies demonstrated that the specificity of these responses is to IRBP [8]. Importantly, in AIRE KO mice that have been crossed to IRBP KO and fail to express IRBP in their retina, there is no spontaneous uveitis despite the fact that they continue to express other potential target Ags for uveitis. I.e., in the absence of IRBP no other retinal Ag is recognized as a target. Another spontaneous, though little-known, model of EAU develops in a proportion of athymic (nude) mice implanted with an embryonic rat thymus. Autoimmunity is likely due to an inability of the rat thymic epithelial cells to present auto-Ag to mouse T cells, precluding negative selection. The spontaneously uveitic mice have lymphocyte responses to IRBP but not to other retinal Ags [13].

To increase the usefulness of the mouse EAU model for basic studies such as Ag recognition, epitope spreading and linked suppression, it is important to define the antigenic fragments (epitopes) within the IRBP molecule that are recognized as uveitogenic. Until recently we knew of 1 uveitogenic epitope per strain for each of 3 strains of EAU-susceptible mice: peptide 1–20 for C57BL/6, 161–180 for B10.RIII and 201–216 for B10.A (table 1). Recent studies in our laboratory using 20 amino acid overlapping peptides representing the human IRBP

Fig. 2. IL-17 vs. IFN- γ in CFA-EAU. **a** B10.RIII mice were immunized with IRBP. Lymph node cells draining the site of immunization were collected on day 21 and were stimulated in vitro with IRBP. Cytokines in 24-hour supernatants were assayed by multiplex ELISA (www.SearchLightOnline.com). **b** Eyes from uveitic mice were collected 16 days after immunization and were made into single-cell suspensions using mechanical and enzymatic dissociation of the tissues. The isolated cells were stained for CD4 and were analyzed by intracellular staining for IFN- γ and IL-17 using a published protocol. Shown are cells gated on CD4. **c, d** Mice were immunized for EAU and were treated with neutralizing antibodies to IL-17 (**c**) or IFN- γ (**d**). Eyes were collected approximately 1 week after disease onset in controls and were scored for EAU by histopathology.

Effector Mechanisms and Cells in EAU Development

The classical model of EAU is induced by immunization with IRBP in CFA. Studies over many years indicated that there is an association between susceptibility to EAU and the genetic propensity of a given strain to mount an IFN- γ -high (Th1) response to IRBP. Furthermore, immune T cells extracted from uveitic animals that were able to transfer EAU when infused into otherwise unmanipulated recipients also produced high IFN- γ and low IL-4 in response to an in vitro stimulation with IRBP. Cells from mice immunized in incomplete Freund's adjuvant (without mycobacteria) produced little IFN- γ and did not transfer EAU but could be converted to an IFN-

γ -producing, uveitogenic phenotype by incubation with IL-12. These and similar findings from many laboratories led to the notion that Th1 cells represented the uveitogenic effector phenotype in EAU.

Recently a new effector T cell phenotype was discovered, whose hallmark cytokine is IL-17, and was aptly dubbed Th17. Th17 cells were shown to be a separate lineage, parallel to Th1 and Th2, and to function as the main uveitogenic T cell in a number of autoimmune disease models previously thought to be driven by Th1 cells [19]. Data from several laboratories indicate that this T cell type is also a uveitogenic effector in EAU [20–22].

Examination of the cytokines produced by lymph node cells of mice with EAU induced by immunization

with IRBP in CFA demonstrates that the disease is associated with a mixed Th1/Th17 cytokine profile. Both IFN- γ and IL-17 are produced, as well as TNF- α , but very little IL-4 (fig. 2a). Interestingly, IFN- γ predominates over IL-17, not only in the periphery (draining lymph node cells) but also in the target organ. Cells extracted from the eye show approximately 2-fold more cells able to produce IFN- γ than IL-17 upon triggering with phorbol myristate acetate/ionomycin (fig. 2b). However, it is IL-17 rather than IFN- γ that is critical for pathogenesis: neutralization of IL-17, but not neutralization of IFN- γ , prevents the development of the disease (fig. 2c, d). It should be emphasized that the latter finding does not negate a role for Th1 cells, which produce also TNF- α , IL-6 and possibly other cytokines that can be involved in tissue damage, but it certainly underscores a role for Th17 cells.

In view of these data, is there a role for Th1 cells in EAU? The answer is 'yes'. It has been known for many years that even small numbers of cells from a long-term IRBP-specific T cell lines, stably polarized to the Th1 phenotype and unable to produce IL-17, are highly uveitogenic when infused into otherwise unmanipulated recipients. However, it remained to be shown that recipient IL-17 does not participate in the pathogenesis of the resulting disease. Recent experiments indicated that recipients of Th1 effector cells recruit IFN- γ -producing but not Th17-producing cells into their eyes. More importantly, treatment of such recipients with neutralizing Abs to IL-17 at a dose that inhibits EAU induced with IRBP/CFA failed to affect the development of EAU, whereas neutralization of IFN- γ or TNF- α ameliorated the disease. These findings demonstrate that ocular pathology can develop without participation of Th17 effector cells and that a Th1 effector is sufficient to orchestrate the full picture of EAU [21].

Conversely, in some cases the Th17 effector response may be present but is insufficient to drive the disease. Such a situation appears to occur in the DC-EAU model mentioned above, induced in B10.RIII mice with splenic DC that are matured in vitro and pulsed with IRBP peptide 161–180, the major pathogenic epitope of IRBP for the H-2^f haplotype. Unlike CFA-EAU, DC-EAU appears to be an IFN- γ -dependent form of disease [7]. Primed lymph node cells of mice with DC-EAU produce considerably more IFN- γ but less IL-17 than mice with CFA-EAU when stimulated in vitro with IRBP peptide. However, IFN- γ is not only present in increased amounts; it also appears to be required for pathogenesis. If uveitogenic DC are injected into IFN- γ KO recipients, no dis-

ease ensues, even though the same DC induce EAU in WT mice and despite the development of a good IL-17 response in the IFN- γ KO recipients [7]. This finding leads to the conclusion that an IFN- γ -producing effector cell phenotype is needed for disease development in this model, and an IL-17-producing effector phenotype is not sufficient.

Conclusion

EAU models help to shed light on the mechanisms driving human disease, from genetic, through cellular, to molecular. Our ability to induce distinct clinical types of EAU on the same genetic background and with the same Ag, but presented to the immune system in a different context of innate stimulation, can explain some of the heterogeneity seen in human uveitis, frequently in the face of shared responses to S-Ag. Furthermore, although the primary site of pathology is likely to be determined by the anatomical location of the target Ag, the basic mechanisms driving the disease may be shared across diseases with different target Ags, as they will be largely determined by the conditions under which the auto-Ag is first recognized by the immune system. In humans, that critical initial event is in most cases unknown, but our data would predict that initial exposure in the context of microbial infection, but not otherwise, would drive towards involvement of Th17. EAU has also served as a useful template for novel therapeutic approaches. Examples include the use of cyclosporin, rapamycin, oral tolerance to retinal S-Ag and most recently IL-2 receptor blockade [23]. Successful immunomodulation of the experimental disease has often been predictive of clinical success, further supporting a commonality of mechanisms between experimental and human disease.

Acknowledgments

The authors thank Drs. Dan Cua and Edward P. Bowman of DNAX/Schering Plough, Palo Alto, Calif., USA, for providing anti-IL-17 antibodies, and Dr. Larry A. Donoso of the Philadelphia Retina Foundation, PN, for his gift of synthetic peptides based on the human IRBP molecule. This study was supported by NIH/NEI intramural funding.

References

- 1 Nussenblatt RB, Whitcup SM: Uveitis. Fundamentals and Clinical Practice, ed 3. Philadelphia, Mosby (Elsevier), 2004.
- 2 Gery I, Wiggert B, Redmond TM, Kuwabara T, Crawford MA, Vistica BP, Chader GJ: Uveoretinitis and pinealitis induced by immunization with interphotoreceptor retinoid-binding protein. *Invest Ophthalmol Vis Sci* 1986;27:1296–1300.
- 3 Caspi RR, Roberge FG, Chan CC, Wiggert B, Chader GJ, Rozenszajn LA, Lando Z, Nussenblatt RB: A new model of autoimmune disease: experimental autoimmune uveoretinitis induced in mice with two different retinal antigens. *J Immunol* 1988;140:1490–1495.
- 4 Caspi RR, Chan CC, Wiggert B, Chader GJ: The mouse as a model of experimental autoimmune uveoretinitis (EAU). *Curr Eye Res* 1990;9(suppl):169–174.
- 5 Agarwal RK, Caspi RR: Rodent models of experimental autoimmune uveitis. *Methods Mol Med* 2004;102:395–420.
- 6 Pennesi G, Mattapallil MJ, Sun SH, Avichezer D, Silver PB, Karabekian Z, David CS, Hargrave PA, McDowell JH, Smith WC, Wiggert B, Donoso LA, Chan CC, Caspi RR: A humanized model of experimental autoimmune uveitis in HLA class II transgenic mice. *J Clin Invest* 2003;111:1171–1180.
- 7 Tang J, Zhu W, Silver PB, Su SB, Chan CC, Caspi RR: Autoimmune uveitis elicited with antigen-pulsed dendritic cells has a distinct clinical signature and is driven by unique effector mechanisms: initial encounter with autoantigen defines disease phenotype. *J Immunol* 2007;178:5578–5587.
- 8 DeVoss J, Hou Y, Johannes K, Lu W, Liou GI, Rinn J, Chang H, Caspi RR, Fong L, Anderson MS: Spontaneous autoimmunity prevented by thymic expression of a single self-antigen. *J Exp Med* 2006;203:2727–2735.
- 9 Caspi RR, Grubbs BG, Chan CC, Chader GJ, Wiggert B: 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.
- 10 De Smet MD, Yamamoto JH, Mochizuki M, Gery I, Singh VK, Shinohara T, Wiggert B, Chader GJ, Nussenblatt RB: Cellular immune responses of patients with uveitis to retinal antigens and their fragments. *Am J Ophthalmol* 1990;110:135–142.
- 11 Yamamoto JH, Minami M, Inaba G, Masuda K, Mochizuki M: Cellular autoimmunity to retinal specific antigens in patients with Behçet's disease. *Br J Ophthalmol* 1993;77:584–589.
- 12 Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, von Boehmer H, Bronson R, Dierich A, Benoist C, Mathis D: Projection of an immunological self shadow within the thymus by the aire protein. *Science* 2002;298:1395–1401.
- 13 Ichikawa T, Taguchi O, Takahashi T, Ikeda H, Takeuchi M, Tanaka T, Usui M, Nishizuka Y: Spontaneous development of autoimmune uveoretinitis in nude mice following reconstitution with embryonic rat thymus. *Clin Exp Immunol* 1991;86:112–117.
- 14 Silver PB, Rizzo LV, Chan CC, Donoso LA, Wiggert B, Caspi RR: Identification of a major pathogenic epitope in the human IRBP molecule recognized by mice of the H-2r haplotype. *Invest Ophthalmol Vis Sci* 1995;36:946–954.
- 15 Avichezer D, Chan CC, Silver PB, Wiggert B, Caspi RR: Residues 1–20 of IRBP and whole IRBP elicit different uveitogenic and immunological responses in interferon gamma deficient mice. *Exp Eye Res* 2000;71:111–118.
- 16 Namba K, Ogasawara K, Kitaichi N, Matsuki N, Takahashi A, Sasamoto Y, Kotake S, Matsuda H, Iwabuchi K, Ohno S, Onoe K: Identification of a peptide inducing experimental autoimmune uveoretinitis (EAU) in H-2Ak-carrying mice. *Clin Exp Immunol* 1998;111:442–449.
- 17 Cortes LM, Mattapallil MJ, Silver PB, Donoso LA, Liou GI, Zhu W, Chan C-C, Caspi RR: Repertoire analysis and new pathogenic epitopes of IRBP in C57BL/6 (H-2b) and B10.RIII (H-2r) mice. *Invest Ophthalmol Vis Sci*, in press.
- 18 Taneja V, David CS: HLA class II transgenic mice as models of human diseases. *Immunol Rev* 1999;169:67–79.
- 19 Kastelein RA, Hunter CA, Cua DJ: Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. *Annu Rev Immunol* 2007;25:221–242.
- 20 Amadi-Obi A, Yu CR, Liu X, Mahdi RM, Clarke GL, Nussenblatt RB, Gery I, Lee YS, Egwuagu CE: TH17 cells contribute to uveitis and scleritis and are expanded by IL-2 and inhibited by IL-27/STAT1. *Nat Med* 2007;13:711–718.
- 21 Luger D, Silver PB, Tang J, Cua D, Chen Z, Iwakura Y, Bowman EP, Sgambellone NM, Chan C-C, Caspi RR: Either Th17 or Th1 effectors can drive autoimmunity: conditions of disease induction affect dominant effector category. *J Exp Med*, in press.
- 22 Peng Y, Han G, Shao H, Wang Y, Kaplan HJ, Sun D: Characterization of IL-17+ interphotoreceptor retinoid-binding protein-specific T cells in experimental autoimmune uveitis. *Invest Ophthalmol Vis Sci* 2007;48:4153–4161.
- 23 Nussenblatt RB: Bench to bedside: new approaches to the immunotherapy of uveitic disease. *Int Rev Immunol* 2002;21:273–289.