Mouse Models of Experimental Autoimmune Uveitis

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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.

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].

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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 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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Class II haplotype</th>
<th>Position and sequence</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>B10.RIII</td>
<td>r</td>
<td>161–180</td>
<td>SGIPYISSYLPOTNILHVD</td>
</tr>
<tr>
<td>C57BL/6, C57BL/10</td>
<td>b</td>
<td>1–20</td>
<td>GPTHLFQSPVLDMKVLID</td>
</tr>
<tr>
<td>B10.A, B10.BR</td>
<td>k</td>
<td>201–216</td>
<td>ADKDVVVLVSSRTGGV</td>
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molecule revealed several additional uveitogenic sites that elicit EAU upon immunization of C57BL/6 or B10. RIII mice that elicited EAU with high incidence [17]. These include peptide 51–70, 171–190 (overlaps with the previously known 161–180) and 541–560 for B10. RIII mice, and peptides 461–480 and 651–670 for C57BL/6 mice.

**Humanized Model of EAU in HLA Class II Tg Mice**

A special model of EAU develops in HLA class II Tg mice. These mice are deleted for the mouse HLA class II molecule I-A, and are made Tg for the human HLA class II molecules HLA-DR3 (DRB1*0301), –DR4 (DRB1*0401), –DQ6 (DQB1*0601) or –DQ8 (DQB1*0302) [18]. Ab blocking studies demonstrated that these mice indeed present and recognize Ags on the human class II molecules. Like the parental strain, C57BL/10, these HLA Tg mice develop EAU when immunized with IRBP. However, unlike the parental strain that is resistant to EAU induced with S-Ag, the HLA Tg mice develop varying severities of disease when immunized with S-Ag [6]. Of these 4 strains the severest disease develops in HLA-DR3 Tg mice (fig. 1). Importantly, HLA-DR3 mice immunized with S-Ag recognized peptide N, reported to be recognized by uveitis patients, and when immunized with this peptide developed severe EAU [M.J. Mattapallil, C. David and R.R. Caspi, unpublished].

The mice described above are Tg for a single human HLA molecule. However, humans have several HLA class II molecules which they coexpress. Double-Tg mice expressing DR3 + DQ6 or DR3 + DQ8 alleles were even more susceptible than single-Tg parental strains. This indicates that there are complementing influences between different HLA molecules, and it is possible that some combinations could be protective.

By demonstrating that S-Ag elicits ocular pathology when presented on human histocompatibility molecules, the humanized EAU model supports the notion that responses to S-Ag drive uveitis in humans and may help identify additional uveitogenic peptides presented by particular HLA molecules. Importantly, these findings also validate the use of the conventional EAU model induced with IRBP for the study of basic mechanisms relevant to human uveitis.

**An Alternative Model of EAU Induced with IRBP Peptide Presented by DC**

Recently we demonstrated that EAU can be induced not only by immunization with IRBP in CFA. DC are the professional Ag-presenting cells and are probably the main if not the only Ag-presenting cells capable of stimulating naive T cells. We demonstrated that EAU can be induced by infusion of DC (harvested in large numbers from spleens of mice injected with Flt3L DNA), matured in vitro with LPS + anti-CD40 Ab and pulsed with IRBP peptide 161–180 [7]. This alternative DC-EAU model differs from the ‘classical’ CFA-EAU model immunologically as well as clinically and pathologically. Mice with DC EAU have a distinct appearance of fundus lesions (punctate vs. confluent in CFA-EAU) and a distinct composition of the ocular inflammatory infiltrate (neutrophilic vs. monocytic). The course of disease is shorter and the pathology usually milder than in CFA-EAU. Importantly, the type of immune response that is induced differs as well, which will be discussed in more detail in the following section on effector mechanisms. These findings demonstrate that distinct forms of autoimmune uveitis can be induced by the same auto-Ag presented to the immune system in a different way. This model may help explain the heterogeneous nature of uveitis in humans and may provide a model for the types of uveitis that were not adequately represented by the classical EAU model.
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-\(\gamma\)-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-\(\gamma\) 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-\(\gamma\) and did not transfer EAU but could be converted to an IFN-\(\gamma\)-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 IL-17-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 DC that are matured in vitro and pulsed with IRBP mentioned above, induced in B10.RIII mice with splenic lymph node cells. Such a situation appears to occur in the DC-EAU 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.

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