Pathogenic Mechanisms of Uveitis

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Anatomy of the uveal tract
The two components of the anterior uvea, the iris and the ciliary body, contain heavily pigmented connective, vascular, and muscle tissue. The iris functions as a shutter that responds to prevailing light conditions, and the ciliary body produces aqueous humor through active secretion and ultrafiltration of plasma. Both the iris and ciliary body have many blood vessels within their connective tissue, and the inner aspect of both structures is lined by a double layer of epithelium. The layer of epithelium closest to the connective tissue is pigmented, and the boundary layer closest to the vitreous is nonpigmented. The choroid, or posterior part of the uvea, functions as the primary vascular supply of the retina. It lies between the sclera and the retina and contains the tapetum, the fibrous reflective layer. The uveal tract contains most of the blood supply of the eye and is in direct contact with peripheral vasculature. Therefore, diseases of the systemic circulation (e.g., septicemia and bacteremia) will also affect the uveal blood circulation.

Blood Ocular Barrier and Ocular “immune” Physiology

There is a barrier between this blood circulation and the internal aspects of the eye, called the blood-ocular barrier. The blood-ocular barrier consists of the blood-aqueous barrier (i.e., tight junctions between the nonpigmented epithelial cells of the ciliary body and nonfenestrated iridal blood vessels) and the blood-retinal barrier (i.e., tight junctions between the cells of the retinal pigmented epithelium [RPE] and nonfenestrated retinal vessels). These semipermeable barriers normally prevent large molecules and cells from entering the eye and help the intraocular fluids remain clear. The blood-ocular barrier also limits the immune response to the internal aspects of the eye, causing the eye to be considered an immune-privileged site (see more below). In cases of trauma or inflammation, these barriers can be disrupted, allowing blood products and cells to enter the eye. Flare, cell accumulations, and haze in the aqueous or vitreous are clinically observable signs of the disruption of the blood-ocular barrier that occurs in uveitis. Disruption of the barrier enables activation of various host immune responses, including production of antibodies to self-antigens not normally recognized by the animals' own immune system, as well as production of antibodies to foreign antigens inside the eye.
Physiologically, the inner eye is devoid of immune cells, and the eye maintains an immunosuppressive environment through, for example, factors expressed in the vitreous such as transforming growth factor β (TGF-β). The eye is recognized as an immune-privileged organ. Immune privilege in the eye was originally ascribed to its separation from the systemic immune system by the blood-ocular barrier, lack of lymphatics, and the presence of limited numbers of resident leukocytes. The blood-ocular barrier is a specialized endothelium with tight junctions that control cell traffic in a highly regulated fashion. Naïve T cells cannot cross the normal blood-retinal barrier because of the high shear stress in the retinal vessels and the lack of appropriate adhesion molecules. Furthermore, inner and outer blood-retinal barriers keep cells from the healthy inner eye. Retinal blood vessels maintain the inner blood-ocular barrier. This physiologic barrier comprises a single layer of nonfenestrated endothelial cells that have tight junctions. The retinal pigmented epithelium maintains the outer blood-retinal barrier.

**Induction of uveitis**

Uveitis is a clinically heterogeneous disease and initiating events of the immunopathology remain obscure. In uveitis, large amounts of leucocytes are able to enter the inner eye and cause damage to the inner ocular tissues because of the breakdown of the block-ocular barrier.

These infiltrating cells are predominantly CD4-positive T cells that secrete proinflammatory cytokines such as interleukin 2 (IL-2) and interferon γ (IFN-γ). The IFN-γ-producing phenotype is named \( T_{H1} \) helper cell. \( T_{H} \) cells are involved in activating and directing other immune cells and are particularly important in the immune system. It is this diversity in function and their role in influencing other cells that gives \( T_{H} \) cells their name.

Auto-reactive effector CD4 + T cells have been associated with the pathogenesis of
inflammatory and autoimmune disorders such as multiple sclerosis, rheumatoid arthritis, Crohn’s disease, and uveitis. Naive CD4+ T cells differentiate into effector subsets depending on the nature of the environment in which exposure to the antigen occurs. Several T cell effector phenotypes have been defined, known as T helper 1 (TH1), TH2, or TH17 and the more recently defined TH9 subset. Early studies suggested that the IFN-γ-producing TH1 subset is responsible for the pathology of uveitis, whereas the IL-4-producing TH2 subset is regulatory. More recent studies have lead to a broader paradigm, including the new subsets TH17 and TH9. Each TH subset requires particular cytokines and transcription factors for its differentiation and maintenance, and each has its own cytokine signature, appropriate to its effector function. Proinflammatory cytokines produced by non-T cells are also critical in determining the lineage choice of differentiating TH cells (Figure).

TH17 cells are a subset of CD4+ T-helper cells that produce IL-17. They are found at interfaces between the external environment and the internal environment, such as the skin and lining of the GI tract. Numerous immune regulatory functions have been reported for the IL-17 family of cytokines, presumably due to their induction of many immune signaling molecules. Most notably, IL-17 is involved in inducing and mediating proinflammatory responses. IL-17 induces the production of many other cytokines, chemokines, and prostaglandins from many cell types. The increased expression of chemokines attracts other cells, including neutrophils but not eosinophils. TH17 lymphocytes are implicated in a variety of immune-related diseases, including
rheumatoid arthritis. Novel data from experimental uveitis models in rodents also point to a significant role of IL-17 in uveitis. In ERU eyes, strong immunoreactivity for IL-17 in the non-pigmented ciliary epithelium and in mononuclear cells infiltrating the iris and ciliary body suggested that IL-17-secreting TH17 cells play a role in the pathogenesis of ERU.

In many autoimmune disorders, infections have been discussed as triggering events, either by antigenic mimicry with a pathogen’s antigen or as a bystander effect due to the general systemic or local immune stimulation by the pathogen. For example, the historical association between leptospiral infection and development of ERU suggests this pathogen is a potent activator of the immunopathology. Uveitogenic retinal proteins document in experimental animals include retinal arrestin (soluble Ag), interphotoreceptor retinoid-binding protein (IRBP), rhodopsin, recoverin, phosducin, and retinal pigment epithelium derived RPE-65. Irrespective of the eliciting Ag, available experimental evidence suggests that the immunological mechanisms driving the resultant disease are similar (Caspi 2014). An immune response to cellular retinaldehyde-binding protein (CRALBP) was detectable in a large percentage of ERU cases.

**The efferent phase of Uveitis: the mechanisms of tissue damage**

It is likely that specific and the nonspecific cells penetrate the eye at random in small numbers; however, only autoantigen 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 uveitis.

The phenomenon of "nonspecific" inflammatory cell recruitment is a critical step in uveitis development. Lymphocytes, monocytes and neutrophils are recruited to the site, with mononuclear cells accounting for the majority of the infiltrate. 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 chemo-attractants 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. Degranulation of mast cells that occurs at the time of disease onset 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 important part of the tissue damage in the eye is mediated by active oxygen products, such as nitric oxide, peroxide, and other products. The oxidative and other tissue damage mechanisms are redundant, and no single pathway by itself is critical for pathogenesis.

**Recurrence of uveitis**

Current concepts to explain the origin and perpetuation of autoimmune diseases include molecular mimicry, bystander activation, and epitope spreading. These mechanisms do not exclude each other but could appear together and even interact. *Epitope spreading* is defined as the diversification of epitope specificity from the initial focused, dominant, epitope-specific immune response, directed against a self or foreign protein to cryptic epitopes on that protein (intramolecular spreading) or other proteins (intermolecular spreading).

The immune response consists of an initial magnification phase and a later downregulatory phase to return the immune system to homeostasis. In most autoimmune diseases, several autoantigens participate in the pathogenesis, as mentioned above, and epitope spreading is most likely accountable for disease induction, progression, and inflammatory relapses. The shifts in immunoreactivity could account for the remitting/relapsing character of ERU.
Different target antigens may be important for a given individual, depending on their genetic background. Genetic background and antigens encountered influence the direction and extent of epitope reactivity and probably play an important role in the heterogeneous clinical manifestations of disease. In many autoimmune diseases, epitope spreading is suspected to occur as a result of an immune response against endogenous target antigens first and secondary to the release of self-antigen during the chronic autoimmune response. The formation of new antibodies may determine a different clinical picture. For example, the transformation of anterior uveitis to posterior uveitis may be an excellent example of the effects of epitope spreading. Through tissue damage, cryptic or hidden epitopes on the same molecule will be suddenly presented to the immune system. The end result is that every target antigen generally contains several epitopes, each of which reacts with a T cell or antibody response of different specificity and affinity. Thus, epitope spreading in autoimmune diseases results in the detection of an increasing array of autoantibodies against various target antigens. Studies have confirmed epitope spreading in a high percentage of cases of ERU.

**Resolution of Uveitis**

Resolution of uveitis is dependent on the presence of T regulatory cells (Tregs) that are labeled as CD4⁺Foxp3⁺ cells. For uveitis to develop, the T effector cells must be the first cells to enter the eye characterized by a low percentage of CD4⁺Foxp3⁺ to CD4⁺Foxp3⁻ cells among infiltrating CD4⁺ T cells (Silver, et al 2015). However, throughout the disease process, Foxp3⁺ T cells percentage increases to approximately 10% of the total CD4⁺ cells; after which, the acute inflammation rapidly resolves. What regulates the presence of Treg cells is not entirely known, but in the uveitic eye, Foxp3⁺ Tregs are instrumental in bringing about spontaneous resolution and in maintaining remission (Silver, et al 2015). Although presence of a resident Foxp3⁺ Treg population in the healthy eye has been suggested, any protective effects of such Tregs are clearly overcome by retina-specific Teffs that had been activated in the periphery and can enter the eye actively through the blood-retinal barrier. Thus, although Tregs are unable to prevent uveitis, they are nevertheless instrumental in bringing about resolution of the disease and in maintaining a state of remission. This appears to be achieved, at least in part, by Tregs acting locally within the eye to dampen acute inflammation and prevent its recurrence (Silver, et al 2015).
Role of Innate Immunity in Uveitis

With the ACAID phenomenon in the eye, where the eye has developed a strong bias towards adaptive immune tolerance to exogenous peptides in an effort to limit potentially sight threatening effects of inflammation, how does the eye defend itself against potential pathogens? It is likely that the protection stems from the fact that the the normally sterile internal environment of the eye is exquisitely sensitive to pathogen-associated molecular patterns (PAMPs). Recent studies have explored the role of a wide range of innate immune receptors, known as pattern recognition receptors (PRRs), which recognize microbes on the basis of their PAMPs. A large group of mammalian PRRs have been identified, including at least 11 Toll-like receptors (TLRs), along with nuclear oligomerisation domain (NOD) receptors and C-type lectin receptors. The PAMPs recognized by many PRRs have been defined, and include uniquely prokaryotic, fungal and viral molecules. Unlike T and B cell receptors, innate immune receptors appear to be capable of initiating and directing both innate and adaptive immune responses.

In recent studies it has been suggested that the immune system may recognize not only “non-self” but also anything that is a danger to the host and may signal danger, such as necrotic tissue damage. These danger and damage signals can be accurately recognized and an appropriate inflammatory response can develop. These signals are called damage associated molecular patterns (DAMPs). DAMPs include any endogenous molecule which undergoes a change of state (eg, concentration or conformation) in association with tissue injury that can inform the immune system that damage has occurred (Wakefield, 2010).

Examples of intracellular molecules that can act as DAMPs include heat shock proteins (HSP), high mobility group box 1 proteins (HMGB-1), S100 proteins and ATP. Importantly, several of these molecules, including HSP and HGMB-1, appear not to be released during apoptosis, which is in keeping with the idea that programmed cell death is not a danger signal. Extracellular DAMPs may include inert matrix proteins, such as hyaluronic acid, which when it is broken down into oligosaccharides in the context of tissue damage or infection may become biologically active and activate immune receptors (Wakefield, 2010). In the eye, acute anterior uveitis results in ocular tissue damage and the release of endogenous molecules (damage associated molecular patterns), such as heat shock proteins and S100 proteins that can also activate Toll-like receptors and thus perpetuate or reactivate intraocular inflammation (Wakefield, 2010). The TLR that is activated by a specific PAMP or DAMP is listed in the table below.
<table>
<thead>
<tr>
<th>TLR</th>
<th>PAMPs</th>
<th>DAMPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR-1</td>
<td>Triacyl lipopeptides</td>
<td>HSP60, HSP70, defensins</td>
</tr>
<tr>
<td>TLR-2</td>
<td>Lipoprotein, LPS, PGN, LTA, zymosan, trypanosomal phospholipids, Pam3Cys Porins, lipoarabinomannan</td>
<td>mRNA</td>
</tr>
<tr>
<td>TLR-3</td>
<td>dsRNA, poly(I:C)</td>
<td>HSP60, HSP70, HSP90, HMGB-1, hyaluronic acid, fibrinogen, fibronectin, fx1-defensin, heparan sulphate</td>
</tr>
<tr>
<td>TLR-4</td>
<td>LPS, Pseudomonas exoenzyme S, RSV F protein, MMTV envelope protein, trepanosomal lipids, taxol</td>
<td>mRNA</td>
</tr>
<tr>
<td>TLR-5</td>
<td>Flagellin</td>
<td></td>
</tr>
<tr>
<td>TLR-6</td>
<td>Diacyl lipopeptides</td>
<td></td>
</tr>
<tr>
<td>TLR-7</td>
<td>ssRNA, imiquimod</td>
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<tr>
<td>TLR-8</td>
<td>ssRNA, resiquimod</td>
<td></td>
</tr>
<tr>
<td>TLR-9</td>
<td>Bacterial/viral DNA, CpG DNA</td>
<td>Unmethylated CpG DNA</td>
</tr>
<tr>
<td>TLR-10</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>TLR-11</td>
<td>Ureobacteria, toxoplasma LPS</td>
<td></td>
</tr>
</tbody>
</table>

DAMPs, damage associated molecular patterns; HMGB-1, high mobility group box 1 proteins; HSP, heat shock protein; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MMTV, mouse mammary tumour virus; PAMPs, pathogen associated molecule patterns; PGN, peptidoglycan; RSV, respiratory syncytial virus; TLR, Toll-like receptors.


From: http://www.rheumatologynetwork.com/
References