MECHANISMS OF INFLAMMATORY RESPONSE IN SYMPATHETIC OPHTHALMIA AND VKH SYNDROME

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SUMMARY

Although the inciting events in the pathogenesis of sympathetic ophthalmia and Vogt–Koyanagi–Harada (VKH) syndrome are different, these two forms of bilateral granulomatous uveitis share several clinical, histopathological and immunohistochemical features, including their association with HLA types and in their *in vitro* T-cell response to retinal antigens. These clinical and immunopathological features indicate that there is an underlying T-cell-mediated autoimmunity to uveal/retinal antigens in the development of these forms of uveitis. Both forms exhibit preservation of the choriocapillaris and retina despite extensive inflammatory cell infiltration in the choroid. Recent experimental studies suggest that this preservation of choriocapillaris could be the result of anti-inflammatory products secreted by the retinal pigment epithelium, including transforming growth factor-beta and a novel protein called retinal pigment epithelial protective protein that is known to suppress the phagocyte generation of superoxide. Such suppression of the oxidant release in the choroidal inflammation could help protect the uvea from necrotic change and preserve the choriocapillaris from inflammatory cell infiltration.

Although sympathetic ophthalmia and Vogt–Koyanagi–Harada (VKH) syndrome are two distinct clinical entities, they share virtually identical histopathological features, fluorescein angiographic findings and association with HLA antigens of DR4, DRw53 and Bw54.[1] However, whereas VKH syndrome has a predilection for darkly pigmented races - Asians, Hispanics, Native Americans and Asian Indians[2] - no racial predilection is observed in sympathetic ophthalmia. Moreover, the inciting event for the two entities is different: the development of intraocular inflammation in sympathetic ophthalmia requires a penetrating ocular injury, but no traumatic event is required for the development of VKH syndrome. The latter usually follows prodromal symptoms and signs suggestive of a systemic viral infection. In addition, although it appears that extraocular manifestations such as vitiligo, alopecia, poliosis, meningismus and dysacusis are relatively more frequent in VKH syndrome, there are studies revealing the presence of similar manifestations in sympathetic ophthalmia.[3,4]

The typical histopathological features seen in the early phases of both sympathetic ophthalmia and VKH syndrome include a granulomatous inflammation that primarily involves the choroid, with a similar albeit milder inflammatory infiltrate that involves the iris and ciliary body. However, the choroid is the site where several characteristic features are observed, including: (1) diffuse lymphocytic infiltration interrupted at multiple sites with collections of epithelioid cells and few multinucleated giant cells; (2) the presence of pigment in the epithelioid cells and giant cells in the absence of apparent choroidal necrosis; (3) sparing of the choriocapillaris from inflammatory cell infiltration; and (4) preservation of retinal pigment epithelium (RPE) and retina except at the sites of Dalen–Fuchs' nodules and other foci where RPE junctions are disrupted. These disrupted RPE cells are commonly detected by fluorescein angiography as focal leaks at the level of RPE.

Sympathetic ophthalmia and VKH syndrome also share several immunopathological features, such as infiltration of primarily T-lymphocytes in the choroid. In both entities, most of these infiltrated cells exhibit the markers of helper and suppressor/cytotoxic cells, along with class II MHC molecules.[5,6] Similar molecules are also noted on the activated

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macrophages along with various adhesion molecules, including intercellular adhesion molecule-1.[6] Moreover, in both entities the choroidal infiltrates generate various pro-inflammatory cytokines.[7] Overall, these immunohistological features suggest a delayed type of hypersensitivity (T-cell-mediated) mechanism in the induction and/or perpetuation of the uveal inflammation, possibly directed to the uveal melanocytes or other antigens in the uveal tract.[4,8] Once initiated, an immune response against a tissue target, such as in the uvea, can lead to the further release of various sequestered self-antigens, against which self-tolerance has never been established. Release of such antigens can lead to the perpetuation of uveitis, even though the initiating trigger may be directed to a restricted response to a single specific target.[9,10]

GRANULOMATOUS INFLAMMATIONS OF THE UVEA

The cellular and molecular mechanisms leading to development of granulomatous uveitis are poorly understood. However, the role of macrophages, T-cells and their cytokines, melanocytes, vascular endothelial cells and arachidonic acid (AA) metabolites in the initiation, maintenance and resolution of the granulomatous inflammation is becoming clear. In general the granulomatous uveitis can be classified as either hypersensitivity type or foreign body type. The latter is rarely observed and usually results from traumatic events associated with retained foreign material. The hypersensitivity granulomas usually result either from an infectious agent such as mycobacteria, or from a non-infectious aetiology, such as an autoimmune disorder, mediated by either T-cells or immune complexes.

Hypersensitivity granulomas show three characteristic microscopic patterns: zonal, sarcoidal or diffuse. These three patterns may indicate an underlying mechanism of granuloma formation. The zonal pattern of granulomatous uveitis consists of a central area of necrosis surrounded by zones of different infiltrating inflammatory cells, such as epithelioid cell/histiocytes, lymphocytes and other inflammatory cells, including fibroblasts. Such a zonal pattern is seen in tuberculosis and other infectious diseases. The sarcoidal pattern consists of a collection of epithelioid cells without necrosis. Typically such a pattern is observed in sarcoidosis. The diffuse pattern reveals collections, primarily of lymphocytes, throughout the choroid and the anterior uvea with focal collections of epithelioid cells. This pattern is commonly seen in immune-mediated granulomas such as sympathetic ophthalmia and VKH syndrome.

In granulomatous uveitis, the epithelioid cells represent modified macrophages displaying primarily the morphological features of secretory functions. It appears that the formation of these epithelioid cells requires the presence of activated T-cells in the inflammatory lesion. Moreover, the cytokines of T-cells, such as the interleukins IL-2, IL-4, IL-6, IL-8 and IL-10, interferon gamma and others, are also known to modulate epithelioid cell formation and the maintenance and resolution of the granulomatous inflammation.[11] Granuloma formation is also modulated by the level of class II MHC molecule expression by the macrophages. Adhesion molecules displayed on the macrophages and vascular endothelium may also contribute to the development of granulomatous inflammation.[6]

The metabolic products released at the site of inflammation are found either to accentuate granuloma development or to suppress formation of epithelioid cell granulomas and the associated chronic inflammatory process.[12] Studies on experimental granulomatous uveitis have revealed enhancement of the granulomatous process in the presence of lipoxygenase metabolic products of AA. In contrast cyclo-oxygenase products are noted to suppress granuloma formation.[10] Such modulation of granulomatous inflammation in the presence of AA metabolites could be the result of altered macrophage expression of MHC class II molecules. Moreover, lipoxygenase products of AA are important inflammatory mediators with pro-inflammatory functions of chemotaxis, vasodilation and induction of lysosomal enzyme release.[13]

T-cells cause pathological changes through their ability to produce cytokines, which can recruit additional T-cells, macrophages and other phagocytic cells, including eosinophilic leucocytes. All these cells are present in the uveal tracts of patients with sympathetic ophthalmia and all may cause tissue damage if not properly regulated. Additionally, a subset of T-lymphocytes, cytotoxic T-cells, may cause these pathological changes directly.

TISSUE INJURY IN UVEITIS

Several kinds of experimentally induced uveitis in animals have revealed the importance of macrophages and other phagocytic inflammatory cells in inducing tissue damage, although T-cells are required for the induction of uveitis. These phagocytes cause necrosis of the tissue and amplify the inflammation by releasing various secretory products, including proteases, AA metabolites and free radicals. The free radicals are potent cytotoxic agents that primarily cause peroxidation of lipid cell membranes at the site of their release. The free radicals that cause such tissue damage are mostly derived from the oxygen metabolite superoxide and its derived agents such as hydrogen peroxide, hypochlorous acid and hydroxyl radicals.[14] Nitric oxide, another molecule released by the activated macrophages, may also

induce tissue damage in the presence of superoxide by formation of peroxynitrites. Release or formation of all these oxidants at the site of inflammation can lead to uveal necrosis and amplification of the inflammatory process.[14,15] However, tissue necrosis is minimal or is not apparent in sympathetic ophthalmia and VKH syndrome, thus suggesting the presence of protective mechanisms in the uvea and/or in the granulomas that may minimise tissue necrosis.

PRESERVATION OF CHORIOCAPILLARIS AND RETINA

A constant feature of sympathetic ophthalmia and VKH syndrome is the preservation of choriocapillaris and retina despite extensive uveal infiltration by mononuclear cells and other phagocytes. Although no precise reason for such preservation is known, plausible explanations include one or a combination of the following mechanisms: (1) RPE cells may release soluble and diffusible factors that down-regulate the uveal inflammation; (2) endothelial cells of the choriocapillaris could produce anti-inflammatory cytokines that could down-regulate the inflammation; (3) the choriocapillaris endothelial cells may not express adhesion molecules required for attachment and migration of leucocytes; and/or (4) immune complexes may not deposit in the capillaries. We recently made an attempt to investigate the role of RPE in the down-regulation of inflammation, particularly with regard to its role in the preservation of choriocapillaris and retina in severe uveitis as seen in sympathetic ophthalmia and VKH syndrome.

Our current *in vitro* studies on RPE have revealed that these cells synthesise and release a protein that suppresses phagocyte generation of superoxide[16] and that is known to dismutate into hydrogen peroxide, forming highly reactive hydroxyl radicals. In inflammatory conditions these highly reactive oxidants are primarily formed at the extracellular sites. Such oxidants can induce tissue damage at the site of their formation by various mechanisms, including peroxidation of lipid cell membranes. Such oxidised lipids can amplify the inflammatory process by their chemotactic property.[15]

Although the RPE is endowed with multiple antioxidant enzymes, including superoxide dismutase, such enzymes have at most a limited significance in scavenging the phagocyte-generated superoxide in the extracellular sites because they are localised to the cytosol of the RPE.[17] Moreover, scavenging of phagocyte-derived extracellular superoxide by these antioxidants has never been demonstrated. It is possible that the newly discovered RPE protein may offer protection against such extracellular superoxide formation in uveitis, since this protein is secreted into extracellular sites.[16] Primarily because of the plausibility of such a role for this novel RPE protein, we called the protein 'retinal pigment epithelial protective protein'.[16,18] There are other anti-inflammatory factors produced by the RPE, such as transforming growth factor-beta; these factors could also down-regulate uveal inflammation by altering ongoing inflammatory events, such as antigen presentation.

In sympathetic ophthalmia and VKH syndrome the histological finding of preserved choriocapillaris and retina may suggest that the RPE could offer protection against extension of inflammatory cells into the choriocapillaris and retina. However, RPE cells in culture are known to generate various pro-inflammatory cytokines such as IL-1, IL-6, IL-8, platelet-derived growth factor-like protein, granulocyte macrophage-colony stimulating factor and various adhesion molecules.[19,20] All these molecules, if operative *in vivo*, could enhance uveitis and lead to extension of inflammation into the choriocapillaris and the adjacent retina. Paradoxically, the enucleated eyes of patients with sympathetic ophthalmia and VKH show preservation of the retina and choriocapillaris at the site of intact RPE, even though the choroid is heavily infiltrated by macrophages and other inflammatory cells. Such contradictory findings suggest that similar to macrophages, RPE cells can modulate uveal inflammation, i.e. either enhance it by releasing various pro-inflammatory cytokines or suppress the inflammation by releasing transforming growth factor-beta and other soluble factors, including the novel retinal pigment epithelial protective protein.[18] However, further *in vivo* studies are required on sympathetic ophthalmia and VKH syndrome to delineate the importance of RPE and its products in the modulation of uveitis.

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