Road to Fulfilment: Taming the Immune Response to Restore Vision

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Key Words

Experimental auto-immune uveoretinitis • Auto-immune response • Inflammation • Macrophage • Angiogenesis • Auto-immunity

Abstract

While traditionally considered to be an immune privileged site, the eye, and in particular the retina, is nonetheless endowed with immune-competent cells capable of engaging powerful immune regulatory networks. By understanding the mechanisms that promote immune well-being in the eye, we are able to generate therapies which combat undue immune-mediated damage not only by revealing mechanisms that promote tissue damage, but also by an ability to restore tissue immune homeostasis by harnessing intrinsic immune-regulatory mechanisms. The result is to maintain or restore immune health as well as combat tissue damage evoked during, for example, intra-ocular inflammatory disease (uveitis), angiogenesis (age-related macular degeneration) and retinal degenerative disorders. Immune activation and regulation is a balance that is dictated by cognate and soluble factors at both a tissue and cellular level. These continuously respond to and eradicate danger and pathogenic signals whilst maintaining tissue function by controlling, and not exclusively, vascular barriers, complement activation,

macrophage activation and keeping in check local T cell proliferation. Loss of the balance between activation and inhibitory signals leads to uncontrolled tissue damage. Understanding the mechanisms has gained potential therapeutic opportunities not only to suppress on-going inflammation, but also to restore homeostasis and prevent recrudescence.

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The Clinical Need to Suppress Inflammation Goes beyond Uveitis

Inflammation and immune-mediated pathways result in much of the tissue damage and loss of function which underlie many of the major ophthalmic diseases. This is especially germane to a variety of retinal and choroidal conditions which involve loss or repression of immune regulatory control within the eye secondary to either infection or systemic activation of the immune system and present clinically as uveitis. Not surprisingly, therefore, much of the evidence of suppressing inflammation and pathways involved in immune-mediated damage has arisen from evidence and understanding of mechanisms, both clinically and experimentally, in models of uveitis [1, 2]. Although we are increasing our understanding of immunopathogenic mechanisms associated with uveitis,

including the interplay between innate and adaptive immunity and the cross-over between auto-inflammatory and auto-immune conditions, there is also a substantial involvement of inflammation in the expression of diabetic retinopathy, age-related macular degeneration and other degenerative conditions, including glaucoma, which remains relatively unexplored.

Our knowledge of effector mechanisms in the generation of auto-immunity, with the breakdown of tolerance to self-antigens, has led to the development of targeted biologic therapy including inhibition of Th1 cells, or more specifically neutralization of the signature effector cytokine TNF α [3, 4] and more recently IL-17 [5, 6]. Our understanding of individual susceptibility to disease is also increasing with understanding of gene single nucleotide polymorphisms in key cytokine genes [7–9] as well as HLA-associated genes and genes of unknown function, such as *PTPN22* [10], that contribute to whether and when tolerance to self-antigen is broken, as well as the severity of the resulting auto-immune response. Added to this are the rare yet informative genetic polymorphisms or mutations in innate detectors, such as human NOD-like receptors, which display a wide range of disease phenotypes, but characteristically all have recurrent inflammatory episodes and pyrexias, and are termed ‘hereditary periodic fevers’ [11]. These include characteristic uveitic entities, such as Blau’s syndrome, chronic infantile neurological, cutaneous and articular (CINCA) syndrome and indeed auto-inflammation may also be the drive for conditions such as Behçet’s disease. In addition, a key to such responses are the interactions which occur with the environment, not just externally but internally via our gastro-intestinal microbiome [12], and with particular reference to promotion or regulation of inflammatory responses in relation of Th17 generation [13, 14] and also in enabling a post-thymic regulation which educates the immune system to be tolerant to foreign antigen within our commensal microbiota [15].

There are several theories of what triggers an immune response. Matzinger [16] proposed the ‘Danger Theory’ where signals from injured host cells signal or set off alarms which activate antigen-presenting cells, whereas the pattern recognition theory proposes that pathogen and microbial (including as discussed above, the gut commensals) elicit activation of the innate immune system through, for example, Toll-like receptors [17]. Finally, the binary theory proposes that regulation or activation of immunity is generated via an integration of signals from cells (which include epithelial cells, platelets, neutrophils and macrophages) derived from both injury and infection [18].

So what is happening at tissue sites? Taking such concepts forward, Medzhitov [19] generated the theory of para-inflammation as a tissue-adaptive response to noxious stress or malfunction, the physiological purpose of which is an attempt to re-establish tissue homeostasis. However, the balance is that if homeostasis is not achieved then the mechanisms evoked become persistent and paradoxically promote further tissue damage. Chen et al. [20] and Xu et al. [21] have addressed this in the context of the pathology we observe in the ageing retina, including evidence of microglial activation and subretinal migration, breakdown of the blood-retinal barrier, complement activation in Bruch’s membrane and retinal pigment epithelium cells, and changes in choroidal vasculature (fibrosis) and immune cell phenotype. What breaks a ‘normal’ para-inflammatory response remains unknown, but the evidence supports a key role for innate immunity as persistent and damaging para-inflammatory responses are observed when there is altered monocyte function, such as in mice which are deficient in chemokine receptor CCR2 [20].

What remains paramount for future success is our need to gain information at the level of individual immune responses and further understanding of environmental influences on gene function to generate more successful and targeted therapies. Hence, the creation of a clear individual immunophenotype will in turn, for example, define prognosis and most importantly direct, as well as predict, response to treatment [22]. For example, the advent of genome-wide technologies to interrogate cell subset transcriptomes has already established signatures which predict prognosis [23]. With such platforms, we can create a dialogue and reciprocity of information between human and animal experimental models. By adopting this combined approach (fig. 1), we can hone our interrogation of several chorioretinal disorders to improve our future treatments of common blinding disorders, including neovascular diseases, inflammation (uveitis), diabetic retinopathy and degenerative disorders (age-related macular degeneration and glaucoma).

The Retina and Myeloid Cells: Friendly Interactions to Maintain Immune Competence

Within the retina there are immune-competent cells derived from the bone marrow [24–26] which regulate immune health within the tissue to maintain tissue (neuronal) function [27–30]. The ability to maintain neuronal homeostasis, and keep in check danger or pathogenic sig-

Fig. 1. Schematic illustrating the reciprocity between experimental models, which enable us to dissect and illuminate potential immune mechanisms, and human studies, which, with the advent of genomic and bioinformatic technologies, are generating new insights into immune phenotype and function. Both approaches inform the progress of each other.

nals, requires a fine balance as dysregulation will promote inadvertent tissue damage. Notwithstanding, the evidence is that microglia preserve a response to both Toll-like receptor ligation or cytokine stimulation (interferon- γ , IFN- γ) that promotes a predominant anti-inflammatory IL-10 response [31–33], and an ability to migrate to sites of injury/damage whilst maintaining the ability to phagocytose; a much needed scavenger response. The overarching aim and function of microglia is therefore to be able to control inflammation and reduce collateral damage.

Microglia, Turnover, Phenotype and Function

The question as to whether microglia are long-term residents of the retina and central nervous system has been resolved following recent data using GFP reporter mice that demonstrate that there is a constant turnover of these cells [26]. The question as to whether they contribute to the initiation of inflammation and/or perpetuation of inflammatory responses is also continuously under scrutiny. Whilst we show that the principal default response of retinal microglia is mostly regulatory (IL-10 production, phagocytosis and poor antigen presentation) [31, 32], others have shown that microglia potentially con-

tribute to the initiation of inflammation [34]. However, using a combination of parabiosis and myeloablation techniques, it was shown most recently that infiltrating myeloid cells during inflammation within the central nervous system contribute to both the initiation and progression of disease (in this case experimental autoimmune encephalomyelitis), and moreover, infiltrating cells do not contribute to the resident pool of microglia during disease [35]. If we look at all concepts and data together, one paradigm that emerges is that microglia remain the sentinel cell population that govern and regulate immune health within the tissue, whilst in extremes, where systemic inflammatory responses drive tissue-specific inflammation, the activated infiltrating cells orchestrate the initiation and perpetuation of inflammatory responses. Whilst this is defensible to some extent for autoimmune or auto-inflammatory disorders (uveitis), we still need to explain under what circumstances, if at all, microglia-mediated tissue control is dysregulated to permit ongoing para-inflammatory-induced tissue damage, which is seen most commonly during degeneration or angiogenesis.

Control of myeloid cells is mediated by a close interaction between both cognate as well as cytokine signals, the understanding of which has generated a categorization of macrophage phenotype-function [36–38]. Macrophages

are highly adaptable cells which are conditioned and respond to signals in the environment they encounter. We have shown in experimental auto-immune uveoretinitis (EAU) [39, 40] that during the course of disease, retinal infiltrating monocyte/macrophage populations display significant phenotype and functional differences. That is, during the peak of disease and tissue destruction, macrophages are activated (classical activation) by TNF-dependent IFN- γ stimulation that generates a pro-inflammatory NOS2+ macrophage response (so-called M1 macrophages), contributing to tissue destruction [41], whereas during resolution of inflammation, as observed in the rat model, macrophages demonstrate alternative activation (*Arg-1*+, M2 macrophages) with a corresponding phenotype and function, which promotes healing [41]. Understanding the pathways which generate or inhibit each form of macrophage will enable us to better design therapeutic approaches for the future.

Why are microglia able to regulate and marshal immune responses? CD200 is a ligand that is highly expressed on neurons and widespread in the retina [42]. Its cognate receptor, CD200 receptor (CD200R) is predominantly expressed by myeloid cells, including microglia [43]. The receptor signals to down-regulate macrophage function [43–46]. Therefore, there remains in-built control against unwanted pro-inflammatory activation of myeloid cells, which we corroborated in human microglia, showing that CD200R ligation maintains retinal microglial ability to migrate and regulate [33]. If, as is currently understood, we wish to restore homeostasis, and given the notion that the most harm is generated via infiltrating cells [35], then we may be able to address this by augmenting natural homeostatic mechanisms. The evidence suggests this is the case, as when the CD200R agonist monoclonal antibody is administered either systemically or locally (intravitreal route), EAU is attenuated [47]. When a similar approach is taken by reconstituting the principal regulatory cytokine released from microglia, IL-10, via gene therapy, again EAU is suppressed [48].

A mechanistic overlap at tissue sites between para-inflammation and (auto)-inflammation in the retina and choroid is revealed through complement activation [49–51]. In the ageing retina there is increased complement activation with negative regulation of complement components by oxidative stress and pro-inflammatory cytokines [52, 53], principally at the level of the retinal pigment epithelium. Further, no matter what the initial stimulus, macrophages may be activated via their C5a receptor following cleavage of C5 during complement activation. This can be exploited therapeutically by inhibit-

ing C5 cleavage with a monoclonal antibody (BB5.1), which when administered either locally (intravitreal route) or systemically suppresses both EAU [54] and laser-induced choroidal neovascularization (CNV) [Liu et al., pers. commun.].

Experimental Models Highlighting Mechanisms and Pathways to Restore Immune Health

The debate thus far has been centred on the ability to harness mechanisms to restore homeostasis and suppress inflammation during auto-immune inflammation. As discussed, many of the conditions we treat are potentially the result of para-inflammation, including uveitis, which at the time we need to institute treatment is in its intractable, persistent phase (where clinical inflammation is less apparent, but tissue-damaging effects of cell loss and vascular damage are apparent). This is supported by recent data which show that in the persistent phases of EAU, angiogenic responses are pronounced in the absence of obvious inflammation [55]. Nevertheless, we know, despite clinical features of resolution, that the retina remains infiltrated with leucocytes [39, 40, 56]. During the evolution of retinal angiogenesis following acute phases of uveitis in EAU, there remains significant infiltration of arginase-1+ VEGF+ macrophages up to 120 days after immunization. Whether such findings are secondary to para-inflammation or maintained low grade inflammation from infiltrating cells has yet to be resolved. Nevertheless, we do know that removing a pathway of local control of angiogenesis via deletion of a matricellular protein, thrombospondin-1, accentuates the neovascular response, whereas in the absence of a pivotal chemokine for macrophage recruitment, CCR2, diminished angiogenic responses are observed. Such data point toward an inability to restore tissue homeostasis and suggest that damage is persistent as a result of continually recruited cells.

The role macrophages play with respect to the control of vascular barrier and angiogenic responses has been actively investigated [57, 58]. The quandary is the fundamental understanding of mechanisms that govern macrophage responses in this regard. One notion is that with age, upregulation of IL-10 and downregulation of FasL contribute to regulation of abnormal vascular growth in laser-induced CNV. However, despite the finding that loss of IL-10 reduces the extent of laser-induced CNV, does this make IL-10 an attractive target for therapy? Angiogenic or anti-angiogenic function of macrophages is

dependent upon how macrophages are activated. Whilst IFN- γ and LPS may generate NOS2+VEGF+ M1 macrophages, PGE₂ remains a more potent stimulus generating Arg-1+ VEGF+ M2+ macrophages [59]. Whilst IL-10 inhibits the M1 VEGF production, IL-10 fails to inhibit the M2 VEGF production and, moreover, fails to suppress VEGF production in hypoxic conditions [59]. Consequently, therapies (directly or indirectly) to suppress macrophage activation that result in IL-10 generation may result in support of CNV. However, when macrophages are stimulated with IL-4 or IL-13 to generate Arg-1⁺ M2 macrophages, VEGF is reduced and large quantities of sFlt-1 are generated [Wu et al., pers. commun.] (fig. 2). In all, the contribution of ‘inflammation’ in terms of macrophage infiltration is dependent upon the signals the cell will receive and the extent of tissue hypoxia. Dissecting such mechanisms will illuminate more specific pathways to prevent neovascular-associated tissue damage.

Prevent Entry of Cells

If we continue a tenet that most damage incurred is secondary to the maintenance of infiltrating cells, which in turn may prevent homeostatic redress (sustain para-inflammation), then one aspect of therapy is to prevent infiltration of cells to the target site. To this end we noted that when we used FTY720 (Novartis) to prevent principally T cell, but also myeloid cell, migration to inflammatory sites by down-regulating the expression of sphingosine-1 phosphatase receptor required to permit egress of cells from secondary lymphoid tissue, the inflamed retina became rapidly devoid of infiltrating cells [60]. Taking this further, we noted in EAU that the reduction in infiltrating retinal cells occurred at clinically relevant therapeutic doses of FTY720 over a 24-hour period without direct alteration to the vascular barrier, demonstrating the potential to use this as a potent *rescue* therapy [Copland et al., in press]. Moreover, this effect was not segregated to the T cell compartment as, either directly or indirectly, myeloid cell and activation within the tissue was also suppressed.

Conclusions

Immune responses are either the direct cause or underpin the pathology we observe in many of the chorio-retinal disorders we currently attempt to treat. Under-

Fig. 2. Schematic represents the effect of macrophage conditioning stimuli on phenotype and function in relation to angiogenesis.

standing the pathology and mechanisms will contribute to development of therapies to either counter directly the inflammatory response, such as in uveitis, or assist in prevention of recurrence by redressing homeostasis, orchestrated by maintaining tissue immune health. Together with the increasing ability and potential to use genome-wide technologies to individualize diagnosis and treatment we are entering an era of not just stratifying patients groups but predicting individual responses and instituting appropriate and targeted therapy (personalized medicine). Together with understanding immune health through, for example understanding microglial behaviour, the role of complement and trafficking and activation of immune cells, novel therapeutics will be translated appropriately and to greater effect over the next decade.

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