Bowman lecture on the role of inflammation in degenerative disease of the eye

JV Forrester^{1,2}

Abstract

Inflammation, in the pathogenesis of many diseases previously thought to be strictly genetic, degenerative, metabolic, or endocrinologic in aetiology, has gradually entered the framework of a general mechanism of disease. This is exemplified by conditions such as Parkinson's disease, Alzheimer's disease, atherosclerosis, diabetes, and the more recently described Metabolic Syndrome. Chronic inflammatory processes have a significant, if not primary role, in ophthalmic diseases, particularly in retinal degenerative diseases. However, inflammation itself is not easy to define, and some aspects of inflammation may be beneficial, in a process described as 'para-inflammation' by Medhiztov. In contrast, the damaging effects of inflammation, mediated by pro-inflammatory macrophages through activation of the intracellular protein-signalling complexes, termed inflammasomes, are well recognised and are important therapeutic targets. In this review, the range of inflammatory processes in the eye is evaluated in the context of how these processes impact upon retinal degenerative disease, particularly diabetic retinopathy and age-related macular degeneration.

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Introduction

It is the finest honour for any ophthalmologist to be awarded the Bowman Medal by the Royal

College of Ophthalmologists and I am genuinely humbled, especially when I reflect, in embarrassed inadequacy, how I have reached this point in my career. Bowman was a world-class Clinician Scientist, to use a modern term, not only for his work in Ophthalmology but also in the Basic Sciences especially Anatomy, and his microscopy drawings of histological material are still unequalled today. It defies imagination how he could achieve the detail he did, given the technological limitations of the optical instruments he had at his disposal. Bowman was also a renowned lecturer and President of the Royal Society of Medicine, Section of Ophthalmology. He moved in rarified circles as when he part organised the Great Exhibition in London (1869) at which his 'networking' skills initiated a collaboration between Charles Darwin and that other eminent Clinician Scientist of Ophthalmology, FC Donders.

The subject of my presentation, inflammation is not an area in which Bowman was especially active, but he was acutely aware of the scourge of sympathetic ophthalmia (SO), as evidenced in his introductory comments to the delegates at the Royal Society of Medicine meeting, on Professor Snellen's lecture which he would deliver during the conference on the topic.¹ Bowman's incisive insight regarding the complexity and mysterious nature of SO was recorded in his comment '*.....and it is to experiments in lower animals that we may look with most probability for a clue to the enigma.*'

For this reason, I think the topic of my presentation can be justified. SO can be regarded as the first autoimmune disease, known for centuries as a consequence of war injures and first reported in the English language in a clinico-pathological description by Mackenzie² even before Immunology as a discipline was born. It was not for another century or more, however, with the use of

¹Section of Immunology and Infection, Institute of Medical Sciences, University of Aberdeen, Aberdeen, Scotland, UK

²Department of Immunology, Lions, Eye Institute, University of Western Australia, Nedlands, Perth, Western Australia

Correspondence: JV Forrester, Section of Immunology and Infection, Division of Applied Medicine, Institute of Medical Sciences, University of Aberdeen, Room 4.24, Foresterhill, Aberdeen, AB25 2ZD Scotland, UK. Tel: +44 1224 437505, Fax: +44 1224 437506. E-mail: j.forrester@abdn.ac.uk

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Bowman's 'lower' animal models, that the nature of ocular autoantigen(s) was discovered^{3,4} and the pathogenic mechanisms of intraocular inflammation (IOI), or more commonly uveitis, began to be unravelled (reviewed in Caspi⁵).

I have chosen to use this information on the mechanisms of how inflammatory processes develop in uveitis to address the role of inflammation in degenerative eye disease more generally, particularly the two common retinal diseases: age-related macular degeneration (AMD)⁶ and diabetic retinopathy (DR) and to see whether insights gained from uveitis can shed light on similar processes in AMD and DR.

Uveitis/IOI

Sight-threatening IOI until recently has been a somewhat neglected disease for a number of reasons. First, the condition comprises an increasingly large range of infectious and non-infectious causes and syndromes, which can also implicate systemic diseases (reviewed in Dick *et al*⁷ and Forrester *et al*⁸). Accordingly, IOI collectively ranks as the fourth commonest cause of blindness, almost equal in morbidity to DR.⁹ This complexity in definition causes some degree of confusion and diagnostic disagreement, even among experts. As a result, the recent initiative to standardise uveitis nomenclature is greatly welcomed¹⁰ and should allow progress in multicentre clinical trials for common and rare causes of IOI. However, even more difficult is the current management of both non-infectious and infectious sight-threatening IOI which in severe cases demands high level expertise in both medical and surgical care.

Pathology of non-infectious IOI and correlation with experimental models

Clinical data on pathology of active IOI are limited for obvious reasons, and most of the pathology comes from eyes with long-standing disease or other complications such as trauma. Probably, the most well-reported pathology is from cases of SO but pathology from other cases of severe IOI has also been variously documented, such as retinal vasculitis and rarer conditions including birdshot retino-choroidopathy and subretinal fibrosis syndrome.^{11–13} The clinico-pathology of human IOI bears close resemblance to the model of experimental autoimmune uveoretinitis (EAU), an organ-specific disease induced in experimental animals by intradermal inoculation of retinal auto-antigens emulsified in an oily 'adjuvant' containing extract of heat-killed attenuated *Mycobacterium tuberculosis* (*Mtb*) organisms (termed complete Freund's adjuvant, CFA). The disease can be

induced in several animal species and by a number of antigens, and the antigenic epitopes have been well described and are different for different species and strains.^{14,15} However, the variety of pathological lesions is relatively restricted (granulomas, cellular infiltration, oedema, and vasculitis) and remarkably experimental uveitis induced by a single antigen can reproduce much of the spectrum of disease similar to that found in human IOI, suggesting a common variably severe pathogenic mechanism.¹⁶ EAU as a model has been described in great detail in several excellent reviews previously^{5,17} and only brief summary details of the acute disease are given here.

The disease is a CD4 Th1 T cell-mediated disease, which has an induction phase, a peak of severity and a resolution phase in many species such as the B10.R111 mouse and disease evolution can be followed *in vivo* by clinical fundoscopy, scanning laser ophthalmoscopy (SLO), and retinal flat mount analysis. The kinetics of disease are such that around 7 days after antigen administration, there is a reduction in shear stress detectable by SLO, and an upregulation of adhesion molecule expression in the retinal vessels that sharply increases over the following 24 h (Figure 1). A simultaneous breakdown of the blood retinal barrier reaches its peak somewhat later (48 h) coinciding with a massive influx of inflammatory cells into the retina. This sequence of events predicts that invasion of the retina with tissue-destroying inflammatory cells in autoimmune disease requires a period of extraocular pre-sensitisation of T cells (minimally 7 days in this model). Since antigen delivery and presentation to T cells in the eye draining lymph node (DLN) takes place within minutes of inoculation (reviewed in Bajenoff¹⁸), with T cell activation and egress from the DLN occurring within 48–72 h, activated T cells with the potential to access the target site (retina in this case) are present in the circulation for several days before they enter the retinal tissue.¹⁹ Furthermore, before they can do so changes in the retinal vessels (reduced shear stress, increase in adhesion molecule expression, initial barrier breakdown) have to occur. Once all these prior events have occurred, only then can uveoretinitis develop and be detected clinically. It is likely that the changes which are induced in the vessels to allow egress of cells into the tissues have occurred as a result of repeated interactions with the circulating activated T cells (many hundreds to thousands given that the circulation time in the mouse is measured in seconds), which probably increase as more activated T cells are released into the circulation. Eventually a threshold is crossed, allowing entry of large number of antigen-specific cells into the retina (Figure 1). As indicated above, Th1 cells appear to be selectively recruited into the retina in this process.²⁰

Figure 1 Kinetics of inflammatory cell infiltration into the retina. The development of EAU in the mouse was evaluated *in vivo* using SLO by adoptive transfer of labelled T cells into mice who had been immunised with IRPB peptides 161–180 and flat mount preparations of the retinal tissue. Seven days after immunisation, the first change observed was a decline in shear stress and an increase in adhesion molecule expression on the endothelium of the retinal vessels. This was followed by breakdown of the blood retinal barrier and infiltration of T cells into the retinal tissue, which maximally occurred 9 days after immunisation. Courtesy Dr Heping Xu.

These observations have clear significance for uveitis pathogenesis in humans. Assuming a similar process in autoimmune/immune-mediated IOI, extraocularly activated T cells likely circulate in the system for a variable period of time (weeks) before they breach the retinal barrier, implying that uveitis is a systemic disease initiated outside the eye. What activates these T cells and why they target the retina is not known but infectious agents of many types have been implicated and a variety of mechanisms suggested include cross-reactive antigens, bystander activation, and molecular mimicry (reviewed in Forrester *et al*²¹). It is relevant that EAU in the mouse cannot be induced without the use of adjuvants, especially *MTb* extract, since there is an increasing awareness of the role of *MTb* in a range of uveitis syndromes.²²

Resolution of IOI and EAU

The resolution phase of EAU varies with the species and strain. In the guinea pig there is progressive, continuing disease until much of the retina is destroyed²³ when inflammation declines. In the Lewis rat, the disease has a

more rapid kinetic but once more there is extensive, if not complete, retinal destruction before resolution of inflammation.^{24–26} In these models, much of the damage is mediated by pro-inflammatory macrophages as revealed by macrophage depletion studies and by inhibitors of macrophage mediators, such as $\text{TNF}\alpha$, complement, CD44, and nitric oxide.^{27–32,33} In the B10.RIII mouse, disease progression³³ is similarly aggressive³¹ but in the C57/BL6 mouse, EAU onset and development is less aggressive and inflammation settles considerably after a variable period of 3–4 weeks.^{31,34} Immunopathological studies reveal that there is a contest between different types of inflammatory cells: T effector cells compete with T regulatory cells which both are induced by the same antigen but with different kinetics³⁴ while inflammatory macrophages, such as those expressing sialoadhesin³⁵ compete with suppressive macrophages (myeloid suppressor cells, MSCs).³⁶ If the suppressor mechanisms win, then the disease resolves. However, the situation remains unstable for some time and recurrence can occur. This less aggressive model of EAU, with its potential for recurrence, has closer similarity to many human forms of posterior uveitis⁵ and has therefore gained in popularity for preclinical studies (reviewed in Forrester *et al*²¹).

Chronic IOI and EAU

However, further study of the EAU model has shown that, while there was considerable resolution of inflammation after 4–6 weeks, inflammatory cells persisted within the retina, and clinical disease did not completely resolve. In the Lewis rat, some suggestion of chronic disease with retinal/subretinal neovascularisation had occasionally been observed³⁷. In the C57 BL6 mouse, the majority of cells associated with chronic disease were macrophages and recent understanding of the physiology and phenotype of macrophages has led to an investigation of how such cells might allow disease to persist.³⁸ Not only was there continued chronic inflammation, but as in some rat models, retinal angiogenic complexes could be identified.³⁹ Many of these vascular sprouts were associated with proliferation of retinal pigment epithelial (RPE) cells²⁵ making them readily identifiable and quantifiable as pigmented foci on retinal flat mounts. It was thus possible, using gene deleted mice, to determine some of the factors that promoted or prevented retinal angiogenesis in chronic EAU. Local production of thrombospondin, for instance by the RPE, seemed to promote resolution of disease while the pro-inflammatory chemokine ligand-receptor pair, CCL2-CCR2, seemed to promote greater levels of disease. Interestingly, the alternatively activated, arginase-producing macrophage appears to play a

significant role in the focal angiogenic sprouts, since they appeared to be selectively associated with these complexes. However, CCL2 + macrophages were also involved in the complexes once more demonstrating how local competitive cellular behaviour determines outcomes.

This model has considerable relevance for certain forms of IOI, which appear to have a minimal inflammatory component but are characterised by subretinal neovascular membranes (SRNVM), such as punctate inner choroidopathy (PIC) and serpiginous retinochoroidopathy. In addition, this 'local competitive behaviour' among subsets of macrophages may offer insight into how to resolve some of the controversy concerning the role of macrophages in AMD (see below).

Diabetic retinopathy

Current concept of diabetes pathogenesis, and more broadly the Metabolic Syndrome, highlights the role of inflammation in pathogenesis of disease and particularly its complications (reviewed in Johnson *et al*⁴⁰). For a number of years now, the role of inflammation specifically in DR has been recognised,⁴¹ particularly recent studies on the role of bone marrow-derived monocyte/macrophages.^{42,43}

Clinical features of DR and correlation with experimental models

The characteristics of DR are well known: in the early stages, there may be minimal signs for periods up to 5 years after diagnosis; this is followed by the hallmark sign of DR, the microaneurysm, which later progresses to a more severe phenotype with retinal haemorrhages, exudates, oedema (central visual loss if the macula is affected), increasingly large patches of retinal ischaemia and eventually neovascularisation. This leads to the secondary complications of vitreous haemorrhage, pre-retinal gliosis, and traction retinal detachment with eventual global visual loss.

Experimental models of diabetes are numerous. Spontaneous, genetically modified, virus-induced, transgenic antigen-specific and chemically induced models have been used for many years (reviewed in Minhas *et al*⁴⁴ and Robinson *et al*⁴⁵). Extensive studies of the retinal pathology have also been performed. In humans, the classical pathology in the early stages includes microaneurysms, pericyte loss, basement membrane thickening, and later ghost vessel formation. Neovascular outgrowths and vitreo-retinal traction also occur in the later stages. In experimental models, similar changes are also seen except for neovascularisation which has never been observed. (Recently it has been

reported (Han *et al*⁴⁶) that the Akita mouse model of hyperglycaemia develops intraretinal neovascularisation, similar to the intraretinal microvascular abnormalities characteristic of severe diabetic retinopathy in humans.)

Inflammation and DR

Most of the pathological studies in human tissues and in experimental models are on fixed specimens, which do not permit an analysis of the dynamics of evolution of the disease. Using *in vivo* SLO imaging techniques in experimental models combined with flat mount retinal pathology, it is possible to develop a concept of the early stages of disease. Retinal ischaemia due to capillary occlusion is understood to be the trigger for the sight-threatening damage including the capillary leakage and neovascularisation, proposed many years ago by Michaelson *et al*⁴⁷ and promulgated by Ashton.⁴⁸ Previously, the capillary occlusion in DR was attributed to the hypercoagulability of diabetes (also a criteria of the Metabolic Syndrome⁴⁰) and low fibrinolytic activity of diabetic plasma. In 1990, Schroder *et al*⁴⁹ demonstrated that activated leucocytes were the major contributors to capillary occlusion in alloxan diabetic rats, and this has been confirmed in many models (see review in Zhang *et al*⁴¹). Diabetic patients also have increased adhesiveness of leucocytes, including T cells.⁵⁰ Most recently, the bone marrow-derived CCR5 + monocyte has been identified as the culprit leucocyte involved in this process,⁵¹ confirming Michaelson's concept of the pathological role of the macrophage/monocyte in DR pathogenesis.⁵² In addition, pathogenic changes due to diabetes in the autonomic regulation of bone marrow-derived cells have been implicated in the many complications of diabetes (reviewed in Yellowlees Douglas *et al*⁴³).

We have therefore proposed that DR retinopathy is a low-grade, progressive chronic inflammatory disease, mediated by activated bone marrow-derived monocytes, which leads to repeated leucocyte trapping in small capillaries. We suggest that activation of bone marrow-derived circulating leucocytes coincides with the onset of diabetes, and specifically correlates with the hyperglycaemia, and persists throughout the disease. In the early stages of disease, the tissues including retinal tissues can probably deal with this low level chronic leucocyte activation (para-inflammation, see below) by recanalising vessels or opening new capillaries, but eventually the reparative compensatory mechanisms fail and DR develops (dysregulated para-inflammation, see below). This might explain the delay in the appearance of clinical signs of DR for some time after the onset of diabetes, best observed in Type I diabetes where the onset can usually be clearly determined.

A hypothesis for DR-associated angiogenesis

The role of inflammatory cells is not restricted to the early stages of DR. The devastating complication of neovascularisation in DR is also likely due to monocyte/macrophage activity. New vessels develop in an area of retinal ischaemia which will also be oedematous and filled with inflammatory cells, and which is due to capillary and post-capillary venular occlusion. Occlusion of vessels by blood clot and inflammatory cells leads to activation of the endothelial cells which proliferate and invade the clot, eventually

fusing within the vessel to form endothelial bridges forming smaller tubes (Figure 2a and b). Subsequent remodelling of the tissues by the associated macrophages eventually leads to the formation of a set of much smaller vessels, forming an outgrowth or fan from the occluded venule. This concept fits with the clinical manifestations, and will likely involve contributions from both inflammatory macrophages and pro-angiogenic alternatively activated macrophages. Furthermore, it is supported by Michaelson's early pathological studies of occluded retinal vessels in humans.⁵³ This macrophage behaviour

Figure 2 (a) Cartoon demonstrating a possible mechanism for neovascularisation arising from an occluded post-capillary venule; (b) intravascular neovascularisation (1) showing a small neovessel filled with red blood cells in an occluded vessel in a case of diabetic retinopathy. Note the intravascular nuclei from migrating endothelial cells in the area of previous occlusion (2). Haematoxylin and eosin stain courtesy of Professor WR Lee.

is also mirrored in dysregulated adipose tissue by infiltrating monocytes/macrophages in the Metabolic Syndrome, once more highlighting the common pathology of these conditions.⁴⁰

Age-related macular degeneration

Considerable interest in recent years has been generated in the possible role of inflammation in AMD. This is part of a more general enquiry into the role of inflammation in degenerative diseases including Parkinsonism and Alzheimer's disease, in which inflammation is considered to be central to the pathogenesis of disease. The inclusion of AMD in this set of conditions has arisen because of the suggestion that complement may have a role in AMD, based on the altered susceptibility risk to carriers of mutations in certain genes such as complement factors H and B.^{54,55} Several other genetic links to AMD have since been identified although not with such strong associations.⁵⁶ In the initial rush to provide a mechanism, it was proposed that defects in complement activation were related to the ingress of pathogenic inflammatory cells, which had been identified as part of the pathology of AMD by Penfold *et al*^{57,58} in the early 1980s. However, since then it has been difficult to prove cause and effect and trials of various anti-inflammatory agents⁵⁹ have not been shown to be effective. In particular, the role of the innate immune system, in the form of macrophages, appears to be somewhat ambivalent, not to mention the possible pathogenic role of the adaptive immune system.^{60,61} As an aside, it has recently been shown that the main dietary supplement for which there is evidence of AMD preventive properties, lutein, also has anti-inflammatory properties.⁶²

Innate immunity and the danger hypothesis

Recent advances in Immunology have returned to Metchnikov's discovery of innate immune cells (reviewed by Tauber⁶³) and revealed once more the importance of this bone marrow-derived 'myeloid cell' system (previously known as the reticulo-endothelial system and the mononuclear phagocyte system) not only in inducing the adaptive immune system with its exquisitely specific T and B cell responses, but also in highlighting its primary role in defence against attack. As indicated above in the section on uveitis, experimental models of uveitis and indeed of autoimmunity generally, require the use of adjuvants such as CFA. Janeway described this at the 'immunologist's dirty little secret' since CFA activates the innate immune system, specifically cells of the myeloid system. In seminal work, he and Medzhitov⁶⁴ identified receptors on myeloid cells

that responded to patterns of generic molecules on pathogens (pathogen-associated molecular patterns, PAMPs), which were related to similar molecules in the fruit fly *Drosophila*, termed Toll receptors (Toll-like receptors, TLRs). TLRs were known for sometime as regulators of dorso-ventral patterning in *Drosophila*, and had in addition anti-fungal activity. Janeway's identification of TLRs as pattern recognition receptors (PRRs) in mammalian myeloid cells, changed and expanded the direction of the discipline of Immunology.⁶⁵ Since then several TLRs have been identified in mouse and man, as well as three other classes of PRRs.^{66,67} Different TLRs respond more or less generically to different classes of pathogens, for example, fungi, virus, and bacteria although there is considerable overlap among different PRR's. However, the central tenet that there are generic PRRs which respond to pathogens in this way, appeared to hold and has generated much fruitful research.

In immunological terms, however, this notion partly undermines the concept of self/non-self recognition which was built upon the high specificity of the adaptive immune response and the non-specificity of the innate immune response, that is, inflammation. In 1994, Matzinger⁶⁸ proposed an alternative hypothesis to the self/non-self paradigm which stated that the organism did not differentiate between self and foreign antigens but simply responded to 'Danger', and thus certain materials such as urate crystals and hyaluronic acid oligosaccharides,⁶⁹ which could be generated endogenously by sterile injury, were sufficient to activate the innate immune system and initiate damage, even autoimmune damage. Gradually, both concepts are merging and an alternative category of disease pathogenesis is now recognised, namely auto-inflammatory disease, in which the tissue damage is mediated *via* innate immune cells and components of the inflammasome (see below).

This has led to questions concerning the precise role of inflammation in homeostasis generally. In this scenario, disruption of health or homeostasis by exogenous or endogenous attack⁷⁰ leads to activated and responding, but still healthy, cells and tissues, with production of among other things, inflammatory mediators. Medzhitov⁷¹ has described this situation as 'para-inflammation' that may settle spontaneously after removal of the Danger signal, or progress to inflammation proper with some damage but eventual resolution of the disease. In contrast, para-inflammation itself may become dysregulated, for example, if the defenses are impaired as with mutations in Complement Factor H in AMD or with defects in the NOD-like receptors in Blau syndrome,⁷² and the condition can persist with progression either to degenerative disease

(ill-health) or overt but low level (chronic) inflammation which fails to resolve. As such, chronic inflammation represents a state in which the disease process is inadequately controlled, and the organism attempts to respond with alternative strategies if it cannot restore structural cellular integrity. However, in this situation after the initial 'danger' has passed, tissue damage may continue due to an uncontrolled host/healing response. In the eye, a 'healing' response such as this involves mesenchymal tissue cells (fibroblasts) and new blood vessels, equivalent to typical responses such as the SRNVM of AMD and low-grade chronic uveitis, which actually causes more damage.

Medzhitov introduced the idea therefore that inflammation might have a physiological role and that a little inflammation might be beneficial: he views a spectrum of inflammation, enshrined in his concept of para-inflammation, which he regards as a property of resident tissue myeloid cells. One might ask if, in fact, this is a new concept, or simply restatement of an existing idea in a novel guise? For instance, the scavenger role of splenic macrophages for aged red cells is known to every medical student, while the recent tolerising role of macrophages such as the F4/80 + macrophages in the mouse has been revealed.⁷³ The new and interesting information on heterogeneity in macrophages, some with inflammatory functions, others with anti-inflammatory roles, is highly relevant to disease in the eye, such as AMD, diabetes, and uveitis and probably many other conditions associated with the degenerative diseases of ageing. The balance of outcome seems to depend on whether the effects of low level chronic inflammation are more or less deleterious to the host compared with the potentially destructive effects of the healing response, which are more than amply played out in the retina. The following sections develop this theme.

Pathology of macular degeneration

AMD as a disease has been recognised since the introduction of the ophthalmoscope and drusen as an entity was first described by Donders.⁷⁴ The landmark systematic investigation of the pathology of AMD was reported by Sarks,⁷⁵ in which different degrees of severity of the disease were described from the minimal deposits in the subRPE/Bruch's membrane layer of the retina through to stages of basal linear deposit, (BLD), drusen, geographic atrophy, and eventually SRNVM with haemorrhage. The inference was that these represented stages in the natural progression of AMD, but it has also been generally agreed that the geographic atrophic form of the disease (dry AMD) and the neovascular disease (wet AMD) may represent divergent pathologies in the later stages of disease progression.⁷⁶

Indeed it is not clear that BLD and drusen presage development of dry or wet AMD, or indeed of either form: only 3% of individuals with minimal disease (small hard drusen) progress to AMD over a period of 15 years while around 50% of patients with soft, diffuse drusen progress to wet AMD during the same period (reviewed in Williams *et al*⁷⁷). In one study, a large area of hard drusen was suggested to be a risk factor for AMD development but this has been challenged by one of the participants in that study when undertaking a follow-up investigation.⁷⁸

Pathologically, drusen contain many components, including inflammatory proteins such as complement (C) components, fibrin and fibrin degradation products, lipids and lipoproteins, glycosaminoglycans and their fragments, and proteins associated with degenerative diseases such as amyloid.⁷⁹ In particular, the lipid component is considered by some to represent a significant factor in the pathology of AMD.⁸⁰ Different types of drusen contain different materials.⁸¹ Formation of drusen has been attributed to RPE cell waste deposits and/or to accumulating material derived from the choroid in reverse flow.⁷⁹ Drusen are associated with myeloid cells including macrophages and dendritic cells (reviewed in Williams *et al*⁷⁷), but it is unclear whether such cell are attracted to drusen or are part of the normal resident retino-choroidal myeloid cell population.⁸² The retina contains resident microglia, which become activated and migrate into the subretinal space with age as large autofluorescent structures filled with lipofuscin that can mimic drusen on clinical imaging.⁸³ Interestingly, microglial activation is a constant response of the retina to stress including that associated with diabetes, and may be a sign of para-inflammation (see below). The ageing retina can also endogenously generate C components (ie, they do not have to be derived from the blood) as well as express a restricted set of chemokines and chemokine receptors which are associated with inflammation.⁸⁴ As a result, even in the absence of drusen, the ageing retina sets the scene for a pro-inflammatory environment and as Anderson *et al*⁸⁵ suggest drusen may be an epiphenomenon.

An increasing number of genes involved as risk or protective factors for AMD are increasingly being recognised (reviewed in Liu *et al*⁸⁶ and Lotery and Trump⁸⁷). Interestingly, in the majority of patients with drusen who do progress to more severe forms of AMD, selective genetic risks appear to be at play with HDL genes being an important factor in the early progression from stage 1 to 2 while the C factor genes appeared to be more relevant once drusen had started to accumulate.⁷⁶ It has been suggested that this may relate to the activation of inflammation by C factors and is more likely in the later stages of the disease.

There are thus many questions regarding the pathophysiological development and role of drusen in the context of AMD and visual loss. Indeed, the relationship between drusen, dry AMD, and wet AMD is unclear, and as stated already, a direct progression may not be assumed.

Interestingly, while much attention has been paid to drusen development, ageing changes in the choroid are also observed, which are more difficult to assess. In the mouse, changes include an increase in thickness, rounding of melanocytes, rounding and detachment of RPE cells, signs of endothelial cell activation such as high endothelial venule (HEV)-like changes, a marked increase in the number of myeloid cells overall, and the development of fibrotic changes in the outer choroid. Similar changes have been observed in human ageing choroid, but detailed studies are limited (Figure 3). Of interest is the marked evidence of melanocyte rounding: this is well recognised as sign of melanocyte activation and stress that occurs in such conditions as vitiligo.⁸⁸ Most recently, the phenotype of the macrophages associated with drusen in human choroid associated with geographic atrophy has been reported as CD68 + macrophages, while those associated with wet AMD also express iNOS, that is, appear to express a more pro-inflammatory phenotype.⁸⁹

The pathogenic process of AMD: wet vs dry disease

While drusen are considered to be pathognomic of AMD, the major and somewhat neglected aspect of the disease is that the primary pathology is RPE cell death. The RPE is classically described as a monolayer of terminally

Figure 3 Pathology of dry AMD showing geographic atrophy. Note junction (arrow) of a patch of geographic atrophic retina (absence of photoreceptors and damaged/loss of RPE cells) with area of retina choroid where RPE and photoreceptor cells are still present. Mallory Trichrome stain. Section courtesy of Professor WR Lee.

differentiated neuro-ectodermal cells with minimal/*nil* turnover *in vivo*, in the absence of disease. However, when isolated and cultured *in vitro* the RPE has strong proliferative potential, while recent studies have shown that, even *in vivo*, adult peripheral RPE cells can proliferate.⁹⁰ In some conditions, RPE cell proliferation can be observed usually in association with significant degrees of inflammation such as uveitis, or prolonged retinal detachment. With age however, slowly progressive RPE cell death is the more likely outcome, most probably in the form of apoptosis (reviewed in Williams *et al*⁷⁷).

Accordingly, it is possible to envisage a sequence of events in AMD in which the RPE cell attempts to repair the defect following single cell senescence and death in which, as in other epithelial cell layers, contiguous cells enlarge and slide to fill the defect. Meanwhile, the dying cell undergoes apoptosis with deposition of cellular material in the subRPE space, which acts as a focus for binding of extracellular blood-derived proteins seeping through Bruch's membrane from the leaky choroidal vessels. Thus, many and varied molecular components will be present in this concentrated 'BLD', which might become sufficiently large to form a 'drusen'.

The normal response of tissues to deposits of waste is to recruit the locally resident myeloid cells to clear the debris and to restore tissue homeostasis. As stated above, microglia are the tissue resident myeloid cells of the retina⁸² which, being located in the ganglion cell layer, are some distance from the site of action of drusen deposition, but interestingly with age they become activated and migrate towards the subretinal space.⁸³ However, if the RPE cell monolayer has achieved full homeostasis with full adhesion junction formation between migrated and newly adjacent cells and restoration of barrier integrity, then microglial cells will have limited access to the subRPE layer. In contrast, choroidal myeloid cells of which there are large resident networks have long dendritic processes which penetrate through Bruch's membrane^{82,91} (as they do in other tissues lined with epithelia such as the gut, trachea, and cornea; reviewed in Forrester *et al*⁸²) and thus can endocytose and remove BLD and even drusen. Scavenging of such material requires cell surface receptors on the myeloid cells such as specific scavenger receptors including the AGE and RAGE receptors, the mannose receptor, F4/80 in mice, and C3 receptors to facilitate complement mediated clearance of debris (reviewed in Gordon and Martinez³⁸ and Gordon *et al*.⁹² However, this process occurs with minimal inflammation (para-inflammation) generated by resident myeloid cells, which behave like 'silent performers'.

It is clinically well recognised that drusen come and go, and also as stated above, that <50% of patients with

drusen go on to develop AMD (reviewed in Williams *et al*⁷⁷). Thus, assuming drusen represent cellular waste (which remains an open question), it can be taken that a majority of individuals with drusen have the normal scavenging homeostatic machinery in place to cope with this type of cellular physiology in an ageing environment. However, the system is not failsafe, either experimentally or clinico-pathologically in human eyes, and breaks down when defects occur as in the CCR2/CCL2/CX3CR1 and the NRF2 gene-deficient mouse models of AMD in which macrophage function is impaired,⁵⁶ and in humans in which complement Factor H mutations do not allow normal regulatory control of complement (see above). In this situation, dysregulated para-inflammation ensues (Figure 3).

Two outcomes are thus possible: progressive RPE cell death with failure to clear cell debris but with minimal additional inflammation (geographic atrophy, dry AMD); or an enhanced attempt to clear the debris by recruitment of pro-inflammatory bone marrow-derived monocytes and macrophages which also have angiogenic potential to add to that of the RPE cell itself: this leads to subretinal neovascularisation (SRNV membrane formation, wet AMD). Clinical variants occur as in the 'RPE rip' which is due to the extreme attenuation of the RPE as further cell death occurs in a monolayer trying to maintain barrier (cell adhesive) integrity.

How might RPE cell death and the associated pathology recruit pro-inflammatory macrophages? The hallmark of pro-inflammatory macrophages is secretion of the cytokines IL-1 and IL-18 through activation of a protein complex termed the inflammasome *via* the PAMP-PRR 'Danger' system, described above.⁹³ Cell surface PAMPs such as TLRs, and even the IL-1 receptor itself, are activatable by many exogenous and endogenous agents but the physical nature of these agents plays a significant part in the process. It is well known that PAMPs presented to the cell in particulate rather than soluble form are much more likely to activate the inflammasome.^{94,95} Interestingly, a recent study has shown that isolated drusen harvested from AMD eyes have the ability to activate the inflammasome in bone marrow-derived macrophages with the release of both IL-18 and IL-1.⁹⁶ IL-18 has further been shown to promote RPE cell damage and atrophy (akin to dry AMD)⁹⁷ while there is also evidence that the IL-18 may be anti-angiogenic⁹⁶ and thus protective for wet AMD. It is intriguing to consider the possibility that the local secretion and tissue concentration of a single cytokine may determine the outcome and type of AMD and thus bear directly on visual prognosis (dry *vs* wet *vs* no AMD).

The RPE itself is not standing on the sidelines during this process. For instance, it is noteworthy that the RPE cell constitutively produces IL18³⁰ and this infers some

kind of critical role for the cytokine. More generally, the RPE cell deals with a massive amount of photoreceptor material during its lifetime and has processes in place to manage this, including phagocytosis/endocytosis, phagosome/lysosome fusion, and enzymatic degradation of the material in a process termed autophagy. Entrainment of autophagy in cells is identified by formation of an intracellular organelle termed the phagophore before the solubilisation of autophagosomal material. However, when the material cannot be fully solubilised, insoluble material accumulates in the form of lipofuscin. Importantly, induction of autophagy limits inflammasome activation suggesting that autophagy acts a para-inflammatory regulatory brake on the process, thereby limiting excessive inflammation.⁹⁸

Many tissue cells express TLRs including RPE cells, which express TLR4. Ligation of TLR4 on RPE cells by exogenous or endogenous PAMPs either during inflammation or by infectious agents upregulates autophagy as does ageing itself and autophagy genes such as LCR3 are increased in AMD and in drusen. Indeed, it has been proposed that exosomes, small particle released as micro- or nano-particles from many cell types are the origin of drusen, that is, that drusen accumulate due to altered activity of 'para-inflamed' stressed but live RPE cells, rather than represent dead cell waste.^{99,100} A protective role for drusen may thus be possible.

Concluding remarks

It is self-evident that inflammation and survival are intimately linked in the precarious balance between health, disease, and death. However, while overt sight-threatening inflammation is, in the eye at least, often very recognisable for instance in uveitis, some forms of chronic inflammation are less obvious and in many diseases a possible role for inflammation has not yet been considered. However, this perception is changing as we learn more from gene linkage studies, which have thrown up many unexpected associations between inflammation and a range of eye diseases. However, Cell Biology and Immunology have also provided us with many new concepts concerning the specific functions of different cells during the evolution of the inflammatory process, and indeed not all inflammation is deleterious. Janeway's discovery of receptor specificity for innate immune cells for classes of pathogens, Matzinger's development of the Danger hypothesis and its inevitable reassessment of the self/non-self paradigm, and Medzhitov's notion of physiological inflammatory processes all have direct relevance to our concepts of how inflammatory processes might be involved in eye

disease. In addition, it is important to consider that, while inflammatory processes may be fuelling diseases associated with ageing and the metabolic syndrome generally, it remains a viable proposition that these processes are driven by active, previous, or latent infection by agents which may lurk within us, with a special affinity for immune privileged sites.

Conflict of interest

The author declares no conflict of interest.

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