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Perspectives: Age Related Macular Degeneration and the Immune Response - Implications for Therapy

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Abstract

Purpose—Review of the available information concerning the immune mediation of age related macular degeneration (AMD); speculate on proposed mechanisms and immuno-therapy.

Design—Interpretative essay

Methods—Literature review and interpretation

Results—An ever growing body of evidence is gathering concerning the role of the immune system in AMD. Evidence to date suggests that the underlying mechanism leading to AMD is the decline of the ocular downregulatory immune environment (DIE). The subsequent activation of the immune system would lead to T cell sensitization. When combined with local anti-angiogenic therapy, several existing immuno-therapies could be used to downregulate the immune response and potentially leading to a more efficient inhibition of choroidal neovascularization.

Age related macular degeneration is currently the leading cause of blindness in the United States and its prevalence will continue to increase in the coming decades unless new prevention strategies can be developed.¹ Currently over 1.75 million US citizens are affected with advanced AMD, and this number is likely to increase to nearly 3 million by the year 2020 due to the increased number of aging US citizens. Population studies have helped to determine risk factors for this disorder.² Some of the non-modifiable factors include age, family history/genetic factors, light colored iris, and hyperopia. Some of the modifiable risk factors include cigarette smoking, diet, high blood pressure, and possibly elevated serum cholesterol or increased light exposure. The association of these risk factors with the risk of advanced AMD can provide clues as to what causes the disease to develop and how one might prevent the disease. For example, cigarette smoking may increase the risk of AMD through enhancement of the immune cascade.³ The progression of AMD may be similar to the mechanisms of progression for atherosclerosis, which is now considered to be an inflammatory disease.⁴ This provides a new approach to understanding the development and potentially the treatment of this disease. The atheromatous lesion is believed to be inflammatory and it is conjectured that the autoantigen LDL is a driving factor.^{5, 6} The immune response and cytokine status appear to have profound effects on the disease, with IL-10 loss associated with an increase in the numbers of atheromatous lesions, while deficiencies in IFN-gamma and IL-18 are associated

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with a decrease in the numbers of atheromatous lesions.^{7, 8} Similar findings are being reported in Alzheimer's disease.⁹

The mechanisms leading to the development of both the early and late lesions of AMD remain largely unknown. Recent information supports the notion that immune mechanisms play an important and perhaps central role in AMD. The underlying mechanism that leads to AMD and choroidal neovascularization (CNV) seems to mimic mechanisms of other degenerative disorders in older people, but the specific disease presentation in the eye may be reflective of the special immune characteristics of the intraocular environment.

What is the Evidence that Age Related Macular Degeneration is an Immune Mediated Disease?

Histopathology in Humans

Histopathological evaluation of AMD lesions has illustrated the presence of inflammatory cells, including macrophages, mast cells and lymphocytes.¹⁰ Grossniklaus and colleagues reported pathological features in 199 surgically excised subfoveal CNV membranes from AMD patients.¹¹ Based on electron microscopy, the cellular components most often seen were RPE, macrophages, erythrocytes, fibroblasts and vascular endothelium. Dastgheib and Green observed multinucleated giant cells in intimate association with the breaks of Bruch's membrane in an eye with neovascular AMD.¹² These observations cannot determine whether immune dysfunction is primary or in response to an initiating event.

Anderson and colleagues evaluated the composition of drusen in over 400 eyes with AMD.¹³ They noted that RPE debris collected between RPE cells that had changed morphologically. Further, they reported a subgroup of RPE cells that appeared to be undergoing cell death, some with an accumulation of C-reactive protein and the 5th component of complement (C5) in their cytoplasm. Drusen were shown to contain both C3 and C3 activation fragments and complement reactivity was identified in sub-RPE deposits associated with the inner and outer collagen layers of Bruch's membrane. These findings led Anderson and colleagues to propose that immune factors, perhaps the activation of the complement cascade and resultant release of inflammatory mediators, mediated the changes they identified.

A proteomic analysis of drusen provides more information. Crabb and coworkers¹⁴, using liquid chromatography tandem MS analyses of drusen preparations from 18 "normal" and 5 AMD donors, identified 129 different proteins within the drusen of patients with early through advanced AMD. Tissue metalloproteinase inhibitor 3, clusterin, vitronectin, and albumin were commonly seen in drusen from normals, while crystallines were most frequently detected in the drusen of the AMD patients. These authors suggest an oxidative stress mechanism, which could involve TIMP3, clusterin, and vitronectin.

Complement

As noted above, several reports have noted the presence of components of the complement cascade in drusen and in the surrounding spaces. Complement is part of the innate immune system, and this is one of oldest immune systems organisms possess. There are three routes that can lead to complement activation. Antigen/antibody complexes activate the classic pathway, lectins activate the lectin pathway, and foreign organisms will activate the alternative pathway. Bora and co-workers¹⁵ have reported that the complement activation via the alternative pathway plays a central role in the development of a laser induced CNV lesion. Complement activation inside the eye is carefully controlled by regulatory proteins that are found inside the eye.¹⁶ In particular, the alternative pathway needs to be constantly regulated. The major factor that downregulates this constantly active system is Factor H. If this dampening

system is dysfunctional then complement will be constantly activated, leading to inflammation. Several reports have now associated a common variant (single nucleotide polymorphism [SNP]) to susceptibility and protection from AMD. Several groups have reported SNPs in the Complement H genome that are associated with AMD. A single copy of the risk associated haplotype increases the risk of AMD by 2–4 fold while those with a dual copy may increase their lifetime risk by 5–7 fold.^{17–21} Other variations in regulatory proteins of the same pathway may confer protection.²²

The confluence of work from separate groups supports the notion that complement plays a role in this disease process. Gehrs and colleagues²³ suggest that debris in the sub-RPE space acts as a nidus for activation of the innate immune system, inducing significant bystander damage. The finding of potential immunologic antigens in drusen suggests an extension of this concept, wherein the RPE releases antigens. These antigens may then become the nidus for activation of the innate immune system with complement augmenting this response by affecting macrophage function.

Other Genes

Significantly, several other genes controlling the immune response have been reported to be associated with either protection or added risk to developing AMD. These associations include a report by Jakobsdottir et al of the PLEKHA 1 gene, yielding an odds ratio of 5, similar to those reported in the studies of complement factor H.²⁴ The PLEKH1 gene is believed to regulate TAPP1 proteins, which are activators of lymphocytes. Recently another immune regulator in the eye, transforming growth factor beta (TGF-beta), has been associated with AMD. TGF-beta is both a product of immune cells and RPE cells.²⁵ It is a molecule that appears to play a central role in the normal downregulatory intraocular environment and it is one of the major mediators of one of the best characterized of these intraocular immune altering phenomena, the Anterior Chamber Associated Immune Deviation (see below), which alters the immune response, preventing several T-helper 1 (TH1) responses.²⁶ Recent observations have shown that the HTRA1 gene plays an important role as an antagonist of the TGF-beta family proteins, particularly when TGF-beta plays an important regulatory role.²⁷ The HTRA1 gene is a member of the High Temperature Requirement A (HTRA) serine protease family.²⁷ Dewan and coworkers²⁸ reported an association between a SNP in the promoter region of HTRA1 and AMD in patients of Asian descent. Individuals with this genotype were estimated to have a 10 times greater likelihood of developing AMD than those with the wild type genotype. Of interest is that the wet-type of AMD, albeit more frequently seen without drusen or as a polypoidal variant, is more prevalent in Asians than in Caucasians²⁹ and the complement factor H Y402H variant is less frequently seen in people with Japanese and Chinese ancestry (less than 5% compared with approximately 35% in Caucasians).^{30–32} An article by the same group identified this SNP in the promoter region of Caucasian AMD patients as well³³, with a calculated attributable risk of 49.3%. Further, in a preliminary evaluation of analyses of lymphocytes and the RPE of 3 AMD patients, those with the high risk allele had elevated expressions of the HTRA1 mRNA and the protein.

It would seem that this observation has importance as a mechanism of wet AMD. It would suggest that each factor, i.e. CFH and HTRA1, can play a role in a larger mechanism but neither is the central mechanisms leading to disease. Careful genetic associations of the typical neovascularization associated with AMD versus the polypoidal neovascularization more common in Asian populations have not yet been investigated.

In some cases the data being collected does not fit into easy answers. One example is that of Toll-like receptors, which are related to Interleukin-1 receptors and have been conserved between insects and humans. This family of receptors may play an important role in AMD because they are a bridge between innate and acquired immunity.³⁴ Toll-like receptors bind

to various pathogens, inducing the production of interferons and Interleukin-12 and -18, cytokines which will cause differentiation of T helper cells. Interestingly, Zarepari et al reported an association of Toll-like receptor 4 and AMD³⁵, in contradistinction to atherosclerosis, where this seems to induce protection. However, in an evaluation of a cohort of AMD patients in India, no association was found with Toll-receptor 4, while one was found with complement factor H.³⁶

The Retinal Pigment Epithelium and the Immune Response

The retinal pigment epithelium plays a central role in the visual cycle.³⁷ It is also centrally involved in immune responses involving the posterior segment, and in the pathologic changes associated with AMD. Are there characteristics of this cell that may give insight into its role in the development of AMD? The RPE appears to be an accessory cell that could be important in the local immune response. It has characteristics generally associated with immune cells. It has obvious phagocytic abilities, similar to that of a macrophage. The RPE can modulate the immune response in the eye.³⁸ Zamiri and colleagues³⁹ have shown that soluble factors produced by the RPE, such as PEDF, will inhibit IL-12 production (pro-inflammatory but anti-angiogenic) and increase IL-10 production (anti-inflammatory). Thus the RPE's usual role is to participate in the ocular downregulatory environment. However, under certain conditions the RPE is able to activate T cells.⁴⁰ The RPE produces other pro-inflammatory cytokines and receptors as well. Complement activation can induce the release of the pro-inflammatory cytokines, IL-1, IL-6, and TNF-alpha, which are harbingers of angiogenic activity and all are produced by the RPE when these cells are stimulated.^{41, 42} The glucocorticoid-induced TNF related receptor ligand (GITRL) is a receptor that has been associated with the abrogation of immunoregulation associated with the CD25 subpopulation of CD4 T cells.⁴³ It has been further shown to be constitutively expressed at low levels in the human eye, including the RPE.⁴³ Enhancing GITRL expression on human RPE cells has been shown to have a marked effect on the immune response. A downregulation of CD3+ T cells is usually noted when RPE cells are co-cultured with these cells, and this downregulation is associated with a large production of TGF-beta. The enhanced expression of GITRL on RPE cells could theoretically reverse the inhibitory effect of the RPE on neovascularization. In addition to this potential mechanism, there is an increase in the production of several pro-inflammatory chemokines/lymphokines by the T cells, including interferon-gamma, TNF-alpha, and IL-2.⁴⁴ Thus, activated RPE cells may augment a pro-inflammatory response and may, under these circumstances, aid in abrogating ocular immune privilege. Penfold et al's¹⁰ observations would support this notion. While probably unusual, the examination of at least one case with AMD has shown a granulomatous inflammatory response at the level of the RPE and Bruch's membrane¹² accompanied by a T cell infiltration in the choroid, further supporting the notion that the immune environment can become proinflammatory at least in some AMD eyes.

To further support this concept, Li and associates⁴⁵ demonstrated that damaging the RPE resulted in the production of chemoattractants by that cell, thus enhancing the migration of bone marrow derived stem cells. Higgins and coworkers⁴⁶ have shown that the ingestion of oxidized photoreceptor outer segments by cultured RPE cells induces the expression of angiogenic cytokines (IL-8 and CCL2/MCP-1) in these cells.

The presence of alpha B crystallin in drusen is very intriguing. While it could certainly be an immunogen, most evidence would suggest it to be a stress-induced anti-apoptotic protein which can be generated by oxidative stress. Alge and colleagues have shown that the retinal pigment epithelium is protected against programmed cell death by this molecule.⁴⁷ Further, intracellular reactive oxygen species play a critical role in tumor necrosis alpha signaling, particularly the activation of nuclear factor-kB, an important step in adaptive immunity activation.⁴⁸ Therefore a link exists between oxidative stress and acquired immunity.

Antigen Presenting Cells and Other Parts of the Immune System

Antigen presenting cells (APCs), such as macrophages and microglia, have been implicated in AMD for some time, in various and sometimes diversely different ways.⁴⁹ APCs appear central to any immune response, including both acquired and innate immunity, and often provide the bridge between the two. The diversity of the macrophage family is striking. They can appear in such forms as Langerhans cells, dendritic cells, resident peritoneal macrophages, bone marrow macrophages, Kupffer cells, and alveolar macrophages.⁵⁰ Each has different functions and lives in a different environment. As a whole, they are involved in immunosurveillance, immune activation, and immune mediated killing.

The putative role of macrophages in the pathogenesis of AMD has been studied in animal models and in humans. Some of the data could be construed as contradictory with results supporting upregulation of macrophage activity with other results suggesting a downregulation.

Ambati and co-workers⁵¹ reported retinal changes similar to those seen in human AMD in mice deficient in either the macrophage chemoattractant Ccl-2 or its cognate Ccr-2. They hypothesize that impaired macrophage recruitment allows for the accumulation of immunoactive molecules such as complement and immunoglobulins, which then stimulate RPE induced VEGF. Apte and colleagues⁵² used the laser induced CNV mouse model in IL-10^{-/-} mice. They reported that, in this model, there was reduced CNV associated with larger macrophage infiltrates, while the prevention of macrophage infiltrates promoted CNV. Using the laser model in C57BL/6 mice, Espinosa-Heidmann et al⁵³ reported that macrophage depletion diminished the lesion size and severity of the CNV induced. They therefore concluded that macrophages contributed to the severity of CNV lesions.

Tuo and colleagues⁵⁴ evaluated the possible association of a sequence variation of a receptor for fractalkine, a leukocyte chemoattractant protein, CX3CR1 and its expression in the retina of AMD. Two single mutation polymorphisms (SNPs) were found to be associated with AMD, more so with the histologically documented cases than those diagnosed clinically, accounting for about 10% of the study risk in the study group reported. CX3CR1 is expressed on many immune cells, including monocytes, microglia, and T cells.⁵⁵ Fractalkine has been reported to be present in the iris and retina. Therefore, decreased binding affinity in this system could result in fewer inflammatory cells, particularly macrophages, entering into an inflammatory process. Thus, diminished recruitment of macrophages could lead to a diminished inflammatory response, a build up of debris in the retina and an associated degenerative process. These SNPs are also associated with an apparent protective effect in patients with atherosclerosis but we do not know if these SNPs confer decreased functionality of the fractalkine system.

On the other hand, there are data to suggest that macrophages are active during AMD in humans. Cousins and coworkers⁵⁶ reported that patients who had the wet type of AMD were more likely to have circulating, “activated”, macrophages, which produce high amounts of the pro-inflammatory cytokine TNF-alpha than controls without AMD.

APCs are known to differentiate into different cell types depending on the environment they find themselves.⁵⁰ One such cell is the microglia. These cells are abundantly found in the eye⁵⁷ and are conjectured to play a role in anterior chamber associated immune deviation ACAID (see below for definition). One of the most interesting and potentially important observation has been the recognition of macrophage polarization, meaning that just as Th1, Th2 T and Th17 cells have been identified, we now recognize at least two subgroups of macrophages, M1 and M2 macrophages.^{58–60} The M1 macrophage is induced by IFN gamma and participates in proinflammatory inducer and effector Th1 responses. The M2 macrophages produce IL-4, IL13 and TGF-beta, participates in scavenging, tissue repair and remodeling, and immunoregulation.

Whether macrophages are from different cell lineages or switch function depending on the milieu they find themselves remains to be seen. Further, there is a shift from an M2 to a M1 macrophage type infiltrate in an induced obesity and insulin resistant model in mice.⁶¹

Retinal microglia are believed to be of hemangioblastic mesodermal origin. All are CD45+ with a subpopulation bearing macrophage markers. Because these cells have been shown to have antigen presenting properties in the brain, Gregerson and colleagues⁶² evaluated the antigen presenting activity of resident adult microglia in the retina. These resident CD45+ cells downregulated T cell responses when these two cell types were co-cultured. However, others have conjectured that microglia are actively recruited from the circulation.⁶³ It has been suggested that they are required for proper retinal blood vessel formation.⁶⁴ While, still others have shown that these bone marrow derived myeloid progenitors can be recruited to the eye by the release of myeloid specific hypoxia inducible factor 1alpha, differentiate into microglia, and facilitate vascular repair with the aid of endogenous microglia.⁶⁵ Others have shown that retinal pigment epithelium damage enhances the expression of chemoattractants for bone marrow derived stem cells.⁶⁶ Sheridan et al⁶⁷ further supported this notion by demonstrating the presence of AC133 positive cells, endothelial progenitor cells, in the retina. These observations all strongly suggest the participation of bone marrow derived stem cells in angiogenesis, both de novo vasculogenesis and the formation of new blood vessels from pre-existing vasculature.

Autoimmunity in Age Related Macular Degeneration

Autoantibodies have been reported to be present in a spontaneous simian model of AMD.⁶⁸ Several authors have reported the presence of circulating autoantibodies in patients with AMD.^{69, 70, 71} It is not clear whether these antibodies play a role in the pathogenesis of the disease. Even if they were simply the result of the clearing of the debris, it would indicate that macrophages or other antigen presenting cells are transmitting information to T cells, resulting in an acquired immunologic response, since these B cell (autoantibody) responses would be T cell mediated.

Other Concepts

Downregulatory Intraocular Environment (DIE)

It would seem teleologically reasonable that nature would wish to carefully control immune reactions in the eye. A small nidus of inflammation in the eye has far greater repercussions than a similar one in the kidney. The posterior segment appears to have several such mechanisms. One of them is the downregulatory effect of Muller cells in the presence of lymphocytes, which appeared to require cell to cell contact⁷²; retinal pigment epithelial cells play this role as well, unless overwhelmed by an inflammatory response.⁴⁰ The anterior chamber associated immune deviation (ACAID) is perhaps one of the best known and evaluated of these mechanisms. ACAID is a phenomenon that has been extensively evaluated over the last few decades. Antigen placed into the eye will elicit an altered immune response that selectively decreases T cell mediated delayed hypersensitivity responses (Th1 cells), and suppresses the B cells that secrete complement fixing antibodies. It also causes an expansion of cytotoxic T cell precursor cells and B cells that secrete noncomplement fixing antibody (IgG1). In addition to ACAID, the immunosuppressive intra-ocular environment entails several mechanisms that function in parallel or are intertwined, and may also include microglia cells, the RPE, and Muller cells. Many factors have been reported to contribute to this intraocular environment. It could be argued that the DIE mechanism(s) plays a critical role in maintaining a normally tight control of the immune environment, allowing certain immune functions, such as APC clearing of debris, but not allowing further recruitment or activation

of other immune cells. A disruption in this system may be one of the key elements to the development of AMD.

Is Age Related Macular Degeneration a Local or Systemic Immunologic Disease?

This basic question can be extended to many ocular disorders and has important implications for therapy. The data accrued to date would suggest that AMD including the wet type of AMD is a systemic immunologic disease with local expression. Systemic factors include the recruitment of bone marrow derived microglia (and vascular endothelial) precursors, activated macrophages in the blood, and evidence of autoantibodies in AMD patients, as mentioned in the text. Systemic immunologic involvement suggests the possible need for systemic therapy, perhaps in combination with local therapy.

How Can One Put These Concepts Together? An Hypothesis

Many reports suggest that various parts of the immune system participate in the pathogenesis of AMD or its sequelae. Is it possible to bring these observations together into a single hypothesis?

The common denominator of all patients with AMD begins with the gradual deposition of debris by the RPE, which is eventually excreted into the environment particularly at the level of Bruch's membrane. This may be associated with the eventual loss of a robust DIE. This debris includes immunogenic antigens, such as oxidatively altered self-proteins. APCs such as M2 type macrophages and perhaps microglia may attempt to clean up the debris. These antigens, though ingested by the APC do not initiate an immune reaction because of the downregulatory nature of these APCs and the multiple mechanisms associated with DIE which are in play, including the release of downregulatory cytokines (chemokines) by the RPE as well as microglia. Thus M2 macrophages, which scavenge and immunosuppress are the dominant subtype initially. In particular, part of this multi-layered system of intraocular immunotolerance would include the production of TGF-beta and complement factor H. Thus, the deposition and accumulation of debris by the RPE cell is a life long process. But this antigen stimulation and the potential resultant response does not occur if DIE is robust. If there is a defect in macrophage recruitment, as might occur if the fractalkine system is not working efficiently, the debris will collect around the RPE in larger amounts and the wrong types of macrophages may be called in. As we age, a number of alterations in our immunosurveillance mechanisms occur.⁷³ These alterations can lead to an increase in autoimmunity and tumors that are seen in the aging population. One such change is that DIE, our intraocular immunotolerance system, becomes less robust, resulting in an increased inflammatory process.

Therefore, with aging, DIE becomes a less effective immuno-downregulatory mechanism. The increasing number of drusen, which may initially be related to both the increased debris from the RPE and the initially decreased macrophage scavenging (M2) functionality, will ultimately lead to macrophage (M1)/microglia recruitment (therefore this polarization causes a shift from an innate immune response to an acquired immune response takes place) in order to clear the accumulating debris, carrying out the normal function of the defective APCs. This recruitment of a small number of pro-inflammatory APCs leads to antigen presentation to T cells. This inflammatory reaction is low grade and chronic and may involve Th17 T cells which are produced in an environment containing IL-6 and TGF-beta.. These recently described cells are believed to mediate chronic inflammatory activity⁷⁴, while Th1 cells mediate the acute episodes. With this activation, there would be proinflammatory cytokines and autoantibodies produced. These proinflammatory cytokines could stimulate the RPE to produce its own pro-inflammatory and vasculogenic products, stimulating the migration of angiogenic cells from

the bone marrow. Antibodies produced could block important downregulatory functions or help further pro-inflammatory activity. DIE is further disrupted. If long term pro-inflammatory cytokine and autoantibody production are toxic to the RPE, then apoptosis of these cells will occur as will pro-inflammatory cytokines. This would result in the eventual development of an atrophic lesion.

Any genetic propensity towards a decrease in downregulatory functionality will have an important contributory role to the development of AMD. Older patients with a functionally inefficient CFH or HTRA1 would be at even higher risk of AMD, since DIE's controlling mechanisms would be lost more quickly over time. Most of these changes would not be seen histologically but result in the development of the large drusen. The presence of large drusen could be seen as an attempt to wall off the active components of the immune system such as complement factors and immunogenic products with chaperone molecules in order to prevent an even heightened immune response. These large drusen probably should be given another name, since they are excellent predictors of AMD. The large drusen are thus a sign of a chronic inflammatory response. The chronic inflammatory response would now involve acquired immunity and would lead to further loss of RPE. More M1 macrophages would be recruited. There would be a greater number of vascular endothelial cell precursors coming from the bone marrow and the result of this is the development of choroidal neovascularization (CNV). Thus there are factors within the eye that stimulate and perpetuate the development of CNV, but systemic recruitment of cells would seem to be equally important as would the polarization of macrophages to an M1 type and the involvement of acquired immunity, as is seen in insulin intolerance.⁶¹

What are the Implications for Therapy?

Given the biologic plausibility of the importance of the immune response in the pathophysiology of AMD, devising therapeutic strategies addressing this response, both systemic and local approaches seem appropriate. At the initial stage of a disease, purported to be immune driven in large part, it may be that local factors are the major drivers, inducing a toxic effect on RPE cells and changing the milieu from one of immuno-downregulation to one of immuno-upregulation. Therapies to intervene at this level still need to be developed. However, as the disease progresses, and assuming that the immune component to the disease process is significant, one needs to also consider other strategies. The evidence to date suggests that systemic recruitment, whether immunologic or vasculogenic, may be important in the underlying disease. Thus addressing the disease with local therapy alone may result in a transient benefit, but would not be sustained over time. For example, anti-VEGF therapy alone results in a reduction of fluid leakage and improvement in visual acuity but the neovascular complexes often remain, requiring retreatment for extended periods. Combining an anti-VEGF therapy with systemic therapy directed against the immune system seems logical. Initially, one might choose anti-T cell therapies, since these approaches have been used for human disease and for ocular inflammatory disorders and these cells contain the immune memory for ongoing chronic inflammation. Remicade, directed against the TNF axis, is widely used for ocular disease and one report suggests it is useful in the treatment of CNV secondary to AMD.⁷⁵ Another therapy to consider would be rapamycin. This medication would combine both anti-angiogenic properties and anti-immune properties that have been shown to abrogate the expression of experimental autoimmune uveitis⁷⁶ and of posterior segment neovascularization.⁷⁷ Indeed, this medication has been shown to prevent the recurrence of CNV in a case of punctuate inner choroidopathy.⁷⁸ Yet another medication that could be considered would be daclizumab, an antibody directed against the IL-2 receptor with a secondary effect on macrophage activation⁷⁹. This drug has been used in the treatment of intraocular inflammatory disease⁸⁰ and was shown to increase a subpopulation of NK cells in the circulation of treated uveitis patients.⁸¹ These medications are presently being tested in combination with local anti-

VEGF therapy at the National Eye Institute in early phase 1–2 clinical trials. Finally, newer agents that may be most applicable to AMD are appearing.

The population with advanced AMD has a median age in the late 70s in most studies, and systemic side effects are a major concern. Although this is an important concern, these medications are being used in older populations for other indications.⁷⁵ However, close surveillance within clinical trials is absolutely necessary, particularly without current evidence of a long term benefit.

Development of a long term strategy that could affect the peripheral immune response but with less potential secondary effects would be particularly attractive. One such strategy is vaccination against antigen driven immune responses.⁸² Another approach would be the use of oral and nasal tolerance using antigens which stimulate the adoptive immune response, which has been shown to have an effect on uveitis and other disorders^{83, 84} and recently has been shown to have an effect on atherosclerosis.⁸⁵ Therapy could begin once large drusen and pigmentary changes are noted, since this is the hallmark of increased risk of advanced AMD.^{86, 87}

The available evidence points to the importance of the immune system in the pathogenesis of AMD, just as it appears to be important for atherosclerotic disease⁴ and Alzheimer's disease.⁹ Whether this is so central to these diseases that therapy directed against it will have a positive clinical effect remains to be tested. The potential for newer therapies is great, with important new approaches possible as we learn about the intricate interaction between the eye and the body.

In summary, we believe there is strong evidence for an immunologic mechanism resulting in the development of advanced AMD. The hypothesis is as follows. Debris gradually accumulates in the RPE for decades. Eventually, when this build-up reaches a critical point, this debris is extruded and accumulates as small drusen. These small drusen serve as a potential antigenic stimulus, especially in persons with relative immune dysfunction. Initially, normal macrophage (M2) scavenging occurs with no inflammatory response. However, if the DIE is less robust because of aging and/or genetic polymorphisms, a macrophage polarization to an M1 type and a subsequent shift from innate to acquired immunity occurs. A sign of this immune response is the development of large drusen and apoptosis of the retinal pigment epithelium, which in concert are the hallmark of an increased risk for the development of advanced AMD. Short circuiting the process at this point with anti-inflammatory treatment has the potential to prevent AMD. For those who already have neovascular AMD, downregulating the immune response has the potential to decrease the angiogenic stimulus and hopefully result in vascular closure. The potential for new therapies is great, as we learn about the intricate interaction between the eye and the body.

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