Inflammation and, in some cases, Soluble Fas ligand (sFasL) is an important cytokine. Histopathological studies have suggested that loss of targets, but can be involved in inflammation, functioning through binding to a death receptor, Fas, and activating apoptosis. Blood sFasL levels are elevated in many pathological conditions. We have recently shown that there is an increase of plasma sFasL in association with age and with AMD. This article will discuss the prospect of sFasL as a promising biomarker for the assessment of risk for AMD and the possible role of Fas-mediated apoptosis of retinal pigment epithelium (RPE) in AMD.

**Age-related Macular Degeneration**

AMD is a progressive degenerative disease of the RPE, Bruch’s membrane, and choriocapillaris. It is classified into two types: non-exudative or ‘dry’ AMD (90%), characterized by drusen, pigmentary changes, and geographic atrophy, and exudative or ‘wet’ AMD (10%), characterized by choroidal neovascularization and, eventually, disciform scarring. The introduction of anti-angiogenic agents, such as bevacizumab and ranibizumab, has revolutionized the treatment of neovascular exudative AMD. However, there is no effective treatment for the more prevalent non-exudative AMD other than antioxidant supplements (vitamin C, vitamin E, beta-carotene, and zinc), which are effective in a 25% reduction of the risk of progression for patients with intermediate and advanced AMD. Non-exudative AMD is triggered by abnormalities in the RPE, a monolayer of cells that lies beneath the photoreceptors and normally provides critical metabolic support to these light-sensitive cells. Histopathological studies have suggested that loss of RPE is an early and pivotal event in the development of AMD. The dysfunction and loss of RPE result in photoreceptor degeneration, leading to the irreversible loss of vision. The molecular mechanism for RPE dysfunction likely involves multiple factors including genetic predisposition, oxidative stress, formation of drusen, accumulation of lipofuscin, and local inflammation. Death of RPE in AMD demonstrates characteristic features of apoptosis, a highly regulated cell death program. In cultured RPE cells, apoptosis can be induced by different death stimuli including light exposure, oxidants, and activated cytotoxic T lymphocytes.

**Soluble Fas Ligand**

FasL is a 40kDa type II transmembrane protein of the tumor necrosis factor (TNF) family. Its receptor, Fas, is a 45kDa type I transmembrane protein of the same family. Binding of the FasL to Fas induces apoptosis. Fas is ubiquitously expressed, whereas FasL is principally expressed on activated T cells, natural killer cells, and tumor cells and in ‘immune-privileged sites’ such as the testis, placenta, and eye. The FasL–Fas interaction plays a critical role in the regulation of immune responses, killing of tumor cells, and maintenance of immune-privileged sites. Similar to other members of the TNF family, FasL also comes in two forms, a membrane-bound Fasl (mFasl) and a soluble variant of 26–29kDa. mFasL is produced by proteolytical cleavage of mFasl by metalloproteinase. For both membrane and soluble sFasL, a trimer needs to be formed to trigger the death signal via the Fas receptor. The cleavage site for metalloproteinase is located within the extracellular domain of mFasl and outside the region that is required for trimerization and self-assembly. Thus, although sFasL lacks the intracellular and transmembrane parts of mFasl, sFasL can form trimers. The exact function of sFasL as a death inducer, a death inhibitor, or even as a chemoattractant depends on the given cellular microenvironment. In some conditions, sFasL not only fails to induce apoptosis of Fas+ targets, but can actually inhibit the cytotoxicity mediated by the cell-bound mFasL. In other conditions, sFasL is able to kill certain target populations that express a high level of Fas. SfasL can also act in synergy with anti-Fas monoclonal antibody (mAb) in activating the Fas-mediated signal transduction pathways. Therefore, excessive production of sFasL may have deleterious effects in humans by causing systemic tissue damage. Indeed, as indicated above, blood sFasL levels are elevated in many pathological conditions, including cancer, infection, and autoimmune diseases.

**Fas Ligand/Fas in the Retinal Pigment Epithelium**

In the normal eye, FasL is weakly expressed in the RPE monolayer. However, increased FasL expression has been found in the residual RPE monolayer of the
choroidal neovascular membranes from AMD patients. FasL-expressing RPE cells are found to surround new Fas+ vessels in the center of the neovascular membranes. In a murine model, Fas-deficient (lpr) and FasL-defective (gld) mice have a significantly increased incidence of neovascularization compared with normal mice. Overexpression of Fasl in RPE cells also reduces laser-induced neovascularization in mice. These studies lead to a speculation that first, Fasl expressed on RPE may control the growth and development of new subretinal vessels, and second, neovascularization in AMD may result from the decreased inhibitory effects of Fasl positive RPE on angiogenesis. However, a study by Lambooij et al. has shown that Fasl expression in RPE is not decreased with age or early AMD. In another early study using the excised neovascular membrane from AMD patients, an association between Fas expression, but not Fasl expression, and the extent of apoptosis of RPE is found. Little attention has been given to the fact that RPE cells also express Fas. Our study using cultured human RPE has shown that the level of cell-surface Fas is much higher than that of Fasl in RPE. Under normal culture conditions, these expression levels are insufficient to activate apoptosis on single or neighboring RPE cells. However, when oligomerization of Fas is facilitated with a high concentration of recombinant sFasL and agonist anti-Fas antibody, apoptosis can be induced. Oxidative stress upregulates both Fas and FasL expression in human RPE (hRPE) cells. Antioxidants such as glutathione and N-acetylcysteine inhibit oxidant-induced apoptosis by downregulating Fas and Fasl. In vitro, interferon-γ, TNF-α, and lipopolysaccharide also increase Fas expression in RPE and facilitate Fas-mediated apoptosis. In vivo, an increased expression of Fasl or Fas in RPE cells has been detected in eyes infected with herpes simplex virus and cytomegalovirus. Thus, in conditions where Fas or Fasl expression is upregulated, such as oxidative stress or inflammation, RPE becomes sensitive to Fas-mediated apoptosis. This may act as an important mechanism of RPE loss that is the earliest finding in both non-exudative and exudative AMD.

Soluble Fasl and Age-related Macular Degeneration

Altered cytokine production, along with lymphopenia and abnormal immune response, is frequently observed in aging and age-related diseases. Oxidative stress, infection, and inflammation can upregulate Fasl expression and stimulate sFasL release from T cells, monocytes, macrophages, endothelial cells, and other cell types. Our laboratory has previously shown that redox states of human plasma pools become oxidized in association with increased age. In an ancillary study to the Age-Related Eye Disease Study (AREDS), we found that the AREDS antioxidant supplements prevent oxidation of cysteine/cysteine redox in AMD patients. Therefore, we hypothesized that oxidative stress, in addition to its local effect in the eye—i.e., upregulating Fasl and Fas expression in RPE and triggering autocrine and paracrine death in RPE cells—may also exhibit its systemic effect by elevating Fasl expression in circulating blood cells such as T cells and macrophages or endothelial cells in blood vessels and stimulating release of sFasL from these cells into the blood circulation. When blood with high plasma sFasL circulates to the retina, sFasL may reach the subretinal space and bind to the Fas receptor on RPE and cause cell death. To test this hypothesis, we measured plasma sFasL concentrations in 230 subjects with ages ranging from 45 to 85 years, including 69 patients with AMD. In non-AMD subjects, the levels of plasma sFasL ranged from 0 to 1.63 ng/ml. In AMD patients, sFasL ranged from 0 to 2.43 ng/ml. When plasma sFasL levels were examined as a function of age, a significant correlation was identified between the sFasL and age in the non-AMD subjects (see Figure 1). In addition, gender appeared as an important factor. The sFasL levels in non-AMD females were lower than those in non-AMD males. However, the linear regression lines had very similar slopes (p=0.94), indicating that the effect of age on sFasL is similar for both sexes. The AMD subjects tended to have significantly higher sFasL levels than the non-AMD group (see Figure 2).

The increased sFasL with aging may be a consequence of increased oxidative stress, infection, or inflammation, and thereby may contribute to other age-related disease events. The observed difference of sFasl between females and males may be a result of the sexual dimorphism of the neuroendocrine and immune systems. In a chronic disease such as AMD, metalloproteinase activation or a decline in clearance mechanisms of generated sFasl could result in elevated circulating sFasL concentrations. As a result of elevated sFasL, critical cells, such as those of the RPE would be vulnerable to elimination by apoptosis. On the other hand, changes in plasma sFasL can be one of the consequences of gene–environment interactions that are associated with various chronic diseases. AMD is likely to be a multifactorial disease. In recent years, it has been well established that AMD is tightly
associated with genetic variations of the complement system and certain pro-inflammatory cytokines. The polymorphisms of the complement factor genes, including complement factor H (CFH), factor B (BF), and component 2 (C2), are associated with wet AMD. Analysis of C-reactive protein (CRP), an alternative complement pathway activator, in participants in the AREDS and the Rotterdam Study demonstrates a link between elevated serum levels of CRP and increased risk for developing AMD. Some evidence for the role of inflammation in AMD also arises from links between past infections and AMD. It has been shown that serum antibodies for Chlamydia pneumoniae proteins are associated with increased risk for AMD development and progression. At lower concentrations or in the absence of accessory molecules, sFasL exerts its pro-inflammatory effects. A correlation between serum sFasL and CRP is observed in inflammatory diseases.

Summary
The combination of the findings, i.e. that sFasL is increased in plasma with age, that increased plasma concentration of sFasL is associated with AMD, that RPE cells are preferentially lost during AMD, and that RPE cells are sensitive to sFasL, suggest that Fas-mediated apoptosis could be an important mechanism contributing to the development and progression of AMD (see Figure 3). Aging, oxidative stress, inflammation, and infection may directly act on RPE and upregulate FasL and Fas expression and trigger autocrine and paracrine cell death. These stimuli may also elevate FasL expression in cells such as T cells, macrophages, or endothelial cells and stimulate release of sFasL. A high serum sFasL could facilitate Fas ligation in RPE and cause cell death. When cells expressing increased Fas infiltrate to the subretinal space, as occurs in inflamed eyes, they may activate Fas-mediated apoptosis in RPE. In exudative AMD, a choroidal neovascular membrane grown under or through the RPE may attract more FasL-expressing blood cells and allow access to FasL-bearing endothelial cells or exposure to circulating sFasL, and thereby cause death of RPE. Thus, if Fas-mediated apoptosis in RPE contributes to the pathogenesis of AMD, it may be involved in both non-exudative and exudative AMD and both initiation and progression of the disease.
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