Soluble gp130, an Antagonist of IL-6 Transsignaling, Is Elevated in Uveitis Aqueous Humor

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PURPOSE. To determine the concentrations of soluble gp130, a natural antagonist of IL-6 transsignaling, in the serum and aqueous humor (AqH) of patients with uveitis.

METHODS. Serum was obtained from the peripheral blood of patients with active uveitis and healthy control subjects. AqH samples were collected from patients with active uveitis and those without uveitis who were undergoing routine cataract surgery. Samples were centrifuged and the cell-free supernatant frozen at −80°C. Concentrations of sgp130, sIL-6R, and IL-6 were determined by a sandwich ELISA or multiplex bead immunoassay, using standard curves of known concentrations of recombinant cytokines.

RESULTS. Serum concentrations of sgp130 were not significantly different between control individuals and patients with active anterior uveitis, regardless of the degree of intraocular inflammation cells. By contrast, the concentration of sgp130 in AqH was very low in patients with no or little inflammation, but increased significantly with disease severity. The greatest elevations of AqH sgp130 were found in patients with the highest cellular activity. Simultaneous measurement of IL-6, sIL-6R, and sgp130 revealed a high degree of correlation between the levels of these molecules, especially for sIL-6R and sgp130.

CONCLUSIONS. Soluble gp130 is increased in the AqH of patients with active uveitis. It is likely that sgp130 partially inhibits the process of IL-6 transsignaling during inflammation. However, the concentration found is still far below that in serum, suggesting that increasing the level of sgp130 further may assist in reducing the inflammatory changes induced by IL-6 transsignaling. (Invest Ophthalmol Vis Sci. 2008;49:3988–3991) DOI:10.1167/iovs.08-1953

The pleiotropic proinflammatory cytokine IL-6 acts on a wide variety of cells, including leukocytes, endothelial cells, and fibroblasts, and induces acute-phase protein synthesis in the liver.1 The bioactivity of IL-6 is controlled by the availability of the complex of gp130 and IL-6R, which mediate signal transduction and IL-6 high-affinity binding, respectively. Unlike most of the cytokines for which a soluble cytokine-binding receptor is an antagonist, soluble IL-6R (sIL-6R) can bind with IL-6 to surface gp130 and mediate signal transduction in a process termed IL-6 transsignaling. This pathway allows cells that do not express surface IL-6R to respond to the presence of IL-6. To ensure that IL-6/sIL-6R transsignaling is tightly regulated, there is counterregulation by a soluble form of gp130 (sgp130),1,2 present at high concentrations (100–300 ng/mL) in serum. This natural inhibitor forms a complex with IL-6/sIL-6R, preventing the binding of IL-6/sIL-6R to membrane-bound gp130.

The importance of IL-6 transsignaling has been highlighted in several inflammatory situations. During an inflammatory response there is a switch from the initial recruitment of neutrophils during acute inflammation, to lymphocytes in the chronic phase. This process does not occur in the absence of IL-6 and can be inhibited by the presence of sgp130, indicating that IL-6 transsignaling is critical.3 IL-6 transsignaling has also been implicated in inflammatory bowel disease,4 and the addition of sgp130 also ameliorates collagen-induced experimental arthritis.5,6

IL-6 cannot be detected in the aqueous humor (AqH) from the noninfamed eye, but is elevated to very high levels (up to 1 μg/mL) in AqH from patients with active uveitis.7 In animal models of intraocular inflammation, there is also an elevation of IL-6, and this has been shown to antagonize the tolerogenic effects of AqH TGF-β2.8 Although IL-6 could act directly on some cells during episodes of uveitis, sIL-6R can also be found in uveitis AqH,9 leading to IL-6 transsignaling and the inhibition of T cell apoptosis.10 It is therefore likely that IL-6 transsignaling contributes to the persistence of inflammation during episodes of uveitis. Nevertheless, it is unclear whether the naturally occurring antagonist of IL-6 transsignaling, sgp130, is present in AqH. In this study, we sought to measure the quantity of sgp130 in the serum and AqH of patients with uveitis.

MATERIALS AND METHODS

Patients, Diagnoses, Serum, and Aqueous Humor Samples

Matched serum and AqH samples (~100 μL) were collected from 65 patients with anterior uveitis, according to the IUSG (International Uveitis Study Group) classification scheme. The AqH was collected as published elsewhere.11 Anterior chamber activity was scored at the time of sampling. The cohort of patients studied included only those with idiopathic or HLA-B27-associated anterior uveitis, treated with topical glucocorticoid or not treated. The uveitis was classified as idiopathic if investigations failed to reveal an associated or underlying cause. A further 45 serum samples (without AqH) and 20 AqH samples (without serum) were collected from patients with uveitis who fulfilled the same criteria. Most patients were receiving no treatment at the time of sampling (serum 83/110; AqH 67/85) with the remainder using topical glucocorticoids. No patients were taking oral corticoсте-
roid or immunosuppressive agents. The age range of the patients with anterior uveitis was 45.2 ± 15.2 (median ± SD). Noninflammatory control group AqH specimens (n = 11; median age, 71.9 ± 9.9 years (SD)) were collected from individuals undergoing routine cataract surgery and peripheral blood from normal healthy volunteers (n = 12; median age, 38.5 ± 8.9 years). All sample collection complied with tenets of the Declaration of Helsinki and was approved by the Dudley Local Research Ethics Committee. Written informed consent was obtained from each individual. AqH was centrifuged at 300g for 5 minutes, the cell-free supernatant (subsequently referred to as AqH) was removed and frozen in aliquots at −70°C.

Cytokine Measurement

Serum and AqH samples were diluted 1:200 and 1:5, respectively. Diluted samples (50 μL) were analyzed by ELISA for soluble gp130 (R&D Systems Europe Ltd., Abingdon, UK), according to the manufacturer’s instructions. Briefly, microplates were coated overnight at 20°C with capture antibody diluted in PBS. After the reaction was blocked with PBS-1% BSA for 1 hour, 50 μL of sample or standards were added for 2 hours, followed by the detection antibody for 1 hour, streptavidin-HRP for 20 minutes, and substrate for 20 minutes. After measurement of the optical density, the concentration of sgp130 for each sample was determined from standard curves of known concentrations of recombinant human sgp130. The sensitivity of the assay was <10 pg/mL, and the value for buffer alone (PBS, 1% BSA) was subtracted for each sample.

The simultaneous measurement of IL-6, sIL-6R, and sgp130 in AqH used multiplex bead analysis assays (Millipore UK Ltd., Watford, UK) as described previously.7 AqH samples were diluted 1:5 with PBS, 1% BSA, and 0.05% Tween 20 and incubated with monoclonal antibody-coated beads for 2 hours at 20°C. Washed beads were further incubated with biotin-labeled anti-human cytokine antibody for 2 hours followed by streptavidin-phycerothyrin for 30 minutes. Samples were analyzed with a bioassay analysis system (model 100; Lumirex, Austin, TX) and commercial software (Starstation 2.0; Applied Cytometry Systems, Sheffield, UK). Standard curves of known concentrations of recombinant human cytokines were used to convert fluorescence units to cytokine concentration. The sensitivities of each assay were <1, <2, and <10 pg/mL for IL-6, sIL-6R, and sgp130, respectively. The value for buffer alone (PBS, 1% BSA) was subtracted for each sample.

Statistical Analysis

The Kruskal-Wallis test (subsequently referred to as Kruskal-Wallis) followed by the Dunn multiple-comparison test, when significant differences were found (subsequently referred to as Kruskal-Wallis and Dunn post tests), were used to compare the levels of each cytokine measured between multiple groups. Mann-Whitney (two-tailed) tests were used when just two groups were compared (subsequently referred to as Mann-Whitney). Spearman (two-tailed, nonparametric) correlations were used to assess the significance of correlations between each cytokine (subsequently referred to as Spearman). All statistical analyses were performed with commercial software (Prism 4.03; GraphPad, San Diego, CA). The level of confidence at which the results were judged significant was P < 0.05.

RESULTS

All serum samples showed high levels of sgp130, but they did not differ significantly between control individuals (median 186.1 ng/mL) and patients with active anterior uveitis (P = 0.11; Kruskal-Wallis), regardless of the degree of intraocular inflammation cells (median, 248.4, 221.3, 162.6, and 233.0 ng/mL for 1+, 2+, 3+, and 4+ AC cells, respectively; Fig. 1A). By contrast, the level of sgp130 in AqH increased significantly as the number of anterior chamber (AC) cells increased (P < 0.0001; Kruskal-Wallis; Fig. 1B). In noninflammatory AqH sgp130 was detectable (median, 5.2 ng/mL), but significant elevations were found in uveitis AqH that increased with increasing numbers of AC cells (median, 11.35, 18.2, and 21.5 ng/mL; P < 0.001, P < 0.001, and P < 0.01 with the Dunn post test for 1+, 2+, and 3+ AC cells, respectively). For most of the uveitis samples, matched serum and AqH was available, but there was no significant correlation between the levels of sgp130 in AqH and serum (r = −0.06, P = 0.63, Spearman; Fig. 1C). It should be noted that the levels in serum were at least 10 times greater than those found in the AqH. The median age of the control AqH group was significantly greater than in the anterior uveitis group (P = 0.0005; Mann-Whitney). However, there was no significant correlation between patient age and the level of AqH sgp130 in the uveitis group (r = −0.05, P = 0.60, Spearman; data not shown).

Although the increase in AqH sgp130 levels appeared to be due to the increased disease activity, other factors could be contributing to these changes, including the use of topical glucocorticoid treatment. We analyzed the levels of sgp130 in serum and AqH, dividing the patients into those who had yet to receive any treatment (untreated) and those taking topical glucocorticoids. There was no significant difference in the serum level of sgp130 between these two groups (P = 0.91, Mann-Whitney; Fig. 2A). However, the treated uveitis AqH group showed a significant increase in sgp130 (Fig. 2B; median, 14.2 and 28.1 ng/mL for untreated and treated, respectively; P = 0.0009, Mann-Whitney). As most patients receiving treatment at the time of sampling were those with disease that had not resolved or had disease that had significantly progressed, many more of the treated group would also have had an increased number of AC cells. To dissociate between these two factors, we analyzed both the untreated and treated group,
each divided into those with low AC cells (1+ and 2+) and those with high AC cells (3+ and 4+). Before accounting for treatment there was a significant difference between the sgp130 AqH levels in the 1+/2+ and 3+/4+ AC cells. sgp130 was measured by ELISA. Groups were compared by using the Mann-Whitney test. NS, not significant (P > 0.05); *P < 0.05; **P < 0.001.

DISCUSSION

IL-6 transsignaling allows IL-6 to signal in the absence of the high-affinity surface-bound IL-6R, and has been implicated in the pathology of several inflammatory conditions. The natural antagonist, sgp130, is found at high levels in the serum and has the ability to ameliorate disease in animal models of inflammation. The levels of sgp130 have not been examined in uveitis serum or AqH, and in this study, we have shown that in anterior uveitis patients there is no change in the peripheral blood levels of sgp130. However, in the AqH the levels of this antagonist increase with disease activity.

In both animal models of uveitis and human disease, IL-6 is found at very high levels in the AqH, but is not significantly elevated in the serum. Although this IL-6 could act directly on cells expressing surface IL-6R, the restricted expression of surface IL-6R prevents much of this activity. Instead, IL-6 transsignaling, through binding of a sIL-6R/IL-6 complex to surface-bound sgp130 can occur, and in uveitis and colitis, this has been shown to inhibit T-cell apoptosis. In this study we have demonstrated an elevation in the natural antagonist of IL-6 transsignaling, sgp130. This increase suggests that the capacity for IL-6 transsignaling in the inflamed ocular microenvironment is restricted by sgp130 and that, in its absence, there may be even more severe inflammation. These data are consistent with studies in rheumatoid arthritis, in which sgp130 is found in the synovial fluid. In these studies, the researchers identified a unique isoform of sgp130. We have not determined whether this isoform is present in uveitis AqH. As well as IL-6, several other molecules, including IL-11, IL-27, leukemia inhibitory factor, and oncostatin M, use the surface-bound gp130. Transsignaling may also occur for other gp130 family members, and, although sgp130 does not appear to inhibit IL-27 signaling, it remains possible that sgp130 also has an antagonistic effect on other of these cytokines. In addition to the inhibition of IL-6 transsignaling by sgp130, there may be similar changes in other cytokine pathways. In particular the IL-1β pathway can be inhibited by increases of IL-1 receptor antagonist (IL-1RA), and IL-1RA has been detected in uveitis AqH. We could not directly determine the source of the sgp130 in AqH. It is possible that sgp130 is derived from the infiltrating leukocyte population, either produced as a secreted molecule or cleaved from the surface of gp130-expressing cells. However, we favor the hypothesis that it originates from the serum. During episodes of intraocular inflammation, there is breakdown of the blood–ocular barrier, leading to the influx of serum proteins into the aqueous, resulting in detectable flare. Although the number of AC cells does not always directly correlate with the degree of flare, there is no doubt that AqH...
with higher numbers will contain increased levels of serum proteins. Another indication that sgp130 may derive from serum is the close correlation we observed between sgp130 and sIL-6R; both molecules were found at high levels in the serum, but at lower levels in the AqH. By contrast, the levels of IL-6 in uveitis AqH were much greater than those in the serum, in which IL-6 was frequently undetectable. This result indicates that IL-6 is probably synthesized locally, by resident macrophages, fibroblasts, and infiltrating leukocytes. From animal models of uveitis it is clear that IL-6 is only transiently expressed. It is possible that in some of our patients with low cell numbers, there had been previous elevations in sgp130 that were, at least partially, responsible for decreasing disease activity to the lower level observed at the time of AqH sampling.

Our results show that there is an increased level of sgp130 in AqH from patients with anterior uveitis with higher AC activity. Although this difference was present in both the untreated and treated groups, it is possible that topical glucocorticoid therapy may in itself elevate AqH sgp130 levels. To test this hypothesis it would be necessary to carry out serial sampling of AqH from individual patients. A study of this nature would be very valuable in the analysis of the IL-6 transsignaling pathway and several other key cytokines involved in the regulation of ocular immunity, but it would require careful ethical consideration.

Transgenic expression of a sgp130-Fc fusion protein or administration of sgp130 has been shown to reduce disease severity in animal models of inflammation. It is likely that there is already some sgp130 present in these systems as well, indicating that increasing the levels of sgp130 above the physiological regulatory response may be of benefit. The use of sgp130 as a therapeutic agent for the treatment of uveitis would necessitate further investigation—notably, testing efficacy in animal models of intraocular inflammation.

References