Letters

Using Interleukin 10 to Interleukin 6 Ratio to Distinguish Primary Intraocular Lymphoma and Uveitis

We were interested to read the article by Ongkosuwito et al.,1 whose work expands the knowledge on cytokine expression in intraocular fluids. They used enzyme linked immunosorbent assay to examine the cytokine profile in the vitreous or aqueous humor of 44 eyes with infectious uveitis. The results were compared to 51 control samples. Increased interleukin (IL)-6 levels were found in 44 control eyes and 45 eyes with infectious uveitis. IL-10 was detected in 10 eyes with acute retinal necrosis (ARN) and 13 eyes and 43 eyes with infectious uveitis. IL-10 level in the patients with ARN. We calculated the ratio of IL-10 to IL-6 in all patients with PIOL was greater than 1.0.

In a subsequent article2 we reported that PIOL is strongly associated with an increased IL-10 to IL-6 ratio (greater than 1.0). Four of 13 uveitis patients had both elevated vitreal IL-6 levels and increased IL-10. In these four patients the IL-10:IL-6 ratio was less than 1.0 (0.13, 0.26, 0.67, 0.90), whereas, the vitreal IL-10:IL-6 ratio in all patients with PIOL was greater than 1.0.

To date we have performed cytokine analysis with ELISA on 52 vitrectomy specimens from 50 patients with infectious and noninfectious uveitis. We have found elevated IL-6 levels in 31 samples (59%) but elevated IL-10 in only 6 (12%). In those 6 with increased IL-10 levels, the IL-6 was higher, with a calculated IL-10:IL-6 ratio less than 1.0 in all. Of 5 patients with ARN, only 2 had elevated IL-10 levels, whereas 4 had increased IL-6. Although IL-6 levels were increased in both toxoplasmosis cases, only one had detectable IL-10. We are unable to establish a correlation between the duration of disease at time of the vitrectomy and the IL-10 level in the patients with ARN. We calculated the vitreal IL-10:IL-6 ratio of the patients with infectious uveitis reported in Ongkosuwito’s study. All, except 2 (1.04, ARN; 1.91, inactive toxoplasmosis), had a ratio less than 1.0.

Recently, we have a case of PIOL in which the vitreal IL-10:IL-6 ratio was less than 1.0. We speculated that this represented an early stage in the tumor course. The presence of IL-10 in the eyes of uveitis patients is not diagnostic of malignancy; however, in those cases in which the vitreal IL-10 level is higher than the IL-6 the diagnosis of a PIOL should be strongly considered.

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References


The Authors Respond

We appreciate the calculations reported by Drs. Buggage, Nussenblatt, Chan, and Whitcup based on the data of our recent article,1 in which we described the detection of interleukin (IL)-2, IL-4, IL-6, IL-10, and interferon (IFN)-γ in patients with acute retinal necrosis (ARN) and toxoplasmosis and in control subjects. In their letter Buggage et al. speculate that the fact that the IL-10:IL-6 ratio was greater than 1.0 in two of our patients could possibly indicate an early stage in the tumor course. We believe that this assumption is unlikely; since the samples were taken in 1995, we have been able to evaluate the follow-up of both patients. In one patient, who had acute retinal necrosis, the sample was taken only 2 weeks after the start of symptoms. This sample also was examined pathologically and revealed normal lymphocytes, monocytes, plasma cells, and a few erythrocytes. Further evaluation revealed that after 2 years, the bulbus was enucleated because of ptosis bulbi, and pathologic examination did not reveal signs of an intraocular lymphoma. Furthermore, the sample we used for the detection of cytokines also revealed a positive polymerase chain reaction for varicella-zoster virus, and intraocular production of IgG antibodies against this virus also could be established, confirming the diagnosis of ARN caused by varicella-zoster virus.

The other patient with an IL-10:IL-6 ratio exceeding 1.0 had ocular toxoplasmosis, and further follow-up did not show any signs of an intraocular lymphoma. After our manuscript was submitted, we extended our cytokine studies and also have had the opportunity to test two samples from patients with a suspected intraocular lymphoma (in collaboration with Professor Marc de Smet). The IL-10:IL-6 ratio was 3.29 and 6,
and pathologic examination of the ocular fluid samples confirmed the diagnosis of intraocular lymphoma in both cases. Routine tests for infectious causes of uveitis were negative. Thus, we agree that an IL-10:IL-6 ratio may become an important test in the diagnosis of intraocular lymphoma but would like to point out that the exact cutoff ratio indicating a positive test result still has to be established and may vary between laboratories. Furthermore, we would like to stress that tests excluding infectious causes should be performed at the same time.

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Is Lipofuscin Eliminated from Cells?

Katz et al. 1 recently concluded that the age-related increase of lipofuscin within the retinal pigment epithelium (RPE) “results from an imbalance in the rates of lipofuscin formation and its disposal rather than from a complete absence of a disposal mechanism.” This leads the authors to discuss the possibility of reversing lipofuscin accumulation by enhancing the disposal processes.

Katz et al. base their concept on the observation that lipofuscin-like bodies, formed within RPE cells after a single intravitreal injection of the protease inhibitor leupeptin, gradually disappear over time. These bodies, and those observed by Ivy et al. 2–5 (who developed the protease inhibition model of lipofuscin/ ceroid formation), do resemble lipofuscin in ultrastructural and autofluorescence properties. However, there is no proof that these inclusions (representing large auto/heterophagocytic vacuoles) arising from short-term leupeptin treatment and lipofuscin (age pigment) are identical.

We recently have investigated the consequences of short- and long-term leupeptin treatment of neonatal rat cardiac myocytes and density-inhibited human fibroblasts. 4,5 A short-term (24 hour) administration of leupeptin does induce formation of electron-dense (osmiophilic) inclusions showing some yellowish autofluorescence when excited with blue light, which superficially resemble the authentic lipofuscin inclusions that form slowly under natural conditions. However, the vacuoles induced by short-term leupeptin treatment disappear relatively rapidly (within 4 days) from the cells when the drug is withdrawn, as also observed by Dr. Katz and colleagues. 1 In contrast, when the leupeptin exposure is continued for 2 weeks, a portion of these leupeptin-induced vacuoles acquire new properties, including enhanced autofluorescence and, ultrastructurally, a predominance of crystalloid and myelin-like structures. Moreover, this material does not disappear when the cells are returned to standard culture conditions for another 2 weeks.

Therefore, when auto/heterophagocytosed material stays for a long time within lysosomes, it undergoes transformation into mature and nondegradable lipofuscin/ceroid. This may occur through the intermediacy of slow processes such as oxidative modification of macromolecules within lysosomes, eventually leading to lipofuscin/ceroid formation. Prolonged (but not short-term) inhibition of intralysosomal degradation by leupeptin appears to provide sufficient time for such modification. 4,6 This interpretation also is consistent with the fact that the formation of nondegradable material (authentic lipofuscin/ceroid) is much accelerated when the leupeptin-treatment is combined with oxidative stress. 5 Reactive oxygen species form continuously during normal oxygen metabolism and—despite the activity of cellular antioxidant systems—mild oxidative stress seems to be an inevitable side effect of aerobic life. However, under normal conditions, the formation of lipofuscin is relatively slow in postmitotic cells, since lysosomal degradation occurs much faster than that of leupeptin-treated ones.

In a number of studies, lipofuscin/ ceroid has been shown to be undegradable and not exocytosed. 4,5,7 Of course, one might dispute the validity of in vitro experiments or possibly unique characteristics of the lipofuscin-ceroid that accumulates in different cell types. Nevertheless, the catabolism and/or disposal of authentic pigment has not been demonstrated convincingly. The reduction of lipofuscin/ceroid formation by pharmacologically decreasing oxidative stress may represent a more promising approach to the problem.

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References


The Author Responds

Drs. Terman and Brunk make several points in their letter to which I would like to respond.

1. These individuals state in their letter that “no sufficient grounds exist to consider that leupeptin-induced bodies...and lipofuscin (age pigment) are identical.” We certainly did not make such a claim in our article, but rather characterized the leupeptin-induced bodies as “lipofuscin-like.” There are a number of strong similarities between retinal pigment epithelium (RPE) lipofuscin and the leupeptin-induced inclusions, including (a) both are derived in large part from the phagocytosed photoreceptor outer segments1–3; (b) both have similar fluorescence spectral properties4,5; and (c) the development of autofluorescence in both lipofuscin and the leupeptin-induced inclusions is dependent on vitamin A.6,7

On the basis of these similarities, we believe that the leupeptin-induced accumulation of lysosomal storage bodies is a good model for studying factors involved in regulating the RPE content of lipofuscin.

2. In their letter, Drs. Terman and Brunk describe some of their own experiments on leupeptin-treated cells in culture. They indicate that in cultured cells, short-term treatment with leupeptin resulted in an accumulation of autofluorescent inclusions that was reversible, but when the leupeptin treatment was maintained for 2 weeks, the autofluorescent inclusions did not disappear, at least during the first 2 weeks after termination of the leupeptin treatment. On the basis of their experiments, they conclude that long-term but not short-term leupeptin treatment is a good model for studying lipofuscin turnover because, they propose, the material within the phagosomes undergoes a time-dependent chemical modification that prevents its subsequent elimination from cells. Their conclusion is inconsistent with two observations: (a) in our experiments, we found that by one measure the RPE content of leupeptin-induced inclusions was still elevated 4 weeks after the leupeptin treatment8—this is longer than the 2 weeks that they propose from their experiments is required for the inclusions to make them “non-degradable”; and (b) in experiments by Ivy,9 there was at least partial reversal of accumulation of leupeptin-induced inclusions in brain neurons of rats that had been given continuous infusions of leupeptin for more than 2 weeks. Thus, the finding of irreversible accumulation of leupeptin-induced inclusions reported by Terman and Brunk may be an artifact of culturing cells. Indeed, one cell type they used in their culture experiments was the fibroblast, which we have not observed to accumulate lipofuscin in vivo.

3. Terman and Brunk state that “The undegradability, and the absence of exocytosis of lipofuscin/ceorid has been shown in a number of studies.” However, all the studies that they cited were done using cultured cells. In contrast, there is precedent for the demonstration of the reversibility of lipofuscin accumulation in vivo. In a study using squirrel monkeys, Manocha and Sharma10 showed that lipofuscin accumulation in cortical neurons induced by protein malnutrition was reversible by returning the animals to a nutritionally adequate diet. Thus, with respect to regulation of cellular lipofuscin content, findings obtained with cultured cells are unlikely to be applicable to the in vivo situation.

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References