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Interleukin-10 and Interleukin-12 Levels in the Vitreous of Patients with Large Cell Lymphoma and in Patients with Uveitis

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There is considerable experimental evidence for the concept that T lymphocytes and cytokines play a major role in the pathogenesis of uveitis. T lymphocytes have been demonstrated to be the most abundant cell type in the uveal tissue, retina, aqueous humor and vitreous of patients with intraocular inflammation. Studies have implicated that the pathogenic cell in uveitis may be CD4+Th1-like. IL-12 is a potent immunoregulatory molecule that is critically involved in a wide range of diseases. It is mainly produced by monocytes, macrophages, B cells and connective tissue type mast cells.

IL-12 is a heterodimeric cytokine comprising p35 and p4O chains. Its presence drives CD4 cells towards type-1 (Th1) cells that mediate delayed-type hypersensitivity, activate macrophages and switch antibody production from IgM to IgG2. Other potential direct effects of IL-12 include regulation of the homing of T cells and other inflammatory cells to a particular organ via modulation of adhesion molecules. Further, it acts as a T-cell growth factor and it suppresses induction of counter-regulatory cytokines such as IL-4 or TGF-b. It is known that the main antitheses of IL-12 are IL-4 and IL-10. IL-4 drives CD4 cells towards type 2 (Th2) cells that mainly provide help for B cells, by promoting antibody class switching from IgM to IgG1 and IgE.

Interleukin-10 has primarily been described as a cytokine- synthesis inhibitor factor. It acts as a negative regulator for IL-12 induced inflammation. Monocytes, B Lymphocytes and Th2 lymphocytes are the main sources for IL-10. Its secretion has been shown to be enhanced by high levels of IL-12.

We measured the levels of IL-12 and IL-10 in the aqueous humor and vitreous in 22 patients with uveitis to determine whether or not the levels of these cytokines are significantly different from those of normal aqueous and vitreous.

Undiluted vitreous samples were collected during pars plana vitrectomy, before the infusion line was opened. Aqueous humor and vitreous samples were centrifuged immediately after collection at 600 G for 10 minutes and were immediately frozen to -70$_{o}$ C until assayed. The samples were diluted 1:1 with diluant.

Interleukin-12 and IL-10 levels were measured using commercially available enzyme linked immunosorbent assay (ELISA) kit (ENDOGEN, Mass.). These kits do not cross react with human IL-1a , IL-1b, IL-3, IL-4, IL-6, IL-7, IL-8, TNF-a, TNF-Ñ or IFN-g. The sensitivity of the ELISA was determined to be less than 3 pg/ml and 5 pg/ml for IL-10 and IL-12, respectively.

The ELISA was performed following the manufacturer's protocol. The results were analyzed with the use of Kaleidograph data analysis/graphical application software, (Abelbek Software, Synergy Software PSC Inc. Reading, PA).

Statistical analysis was performed using PC-SAS (version 6.08, SAS Institute, Cay, NC) Interleukin levels were compared using the Wilcoxon rank-sum test for non-parametric data. Correlation was expressed in terms of the Pearson correlation coefficient. No adjustment was made for multiple comparison. The levels of IL-12 in the aqueous humor of our control group, consisting of normal individuals undergoing cataract surgery, ranged from 9 to 14 pg/ml (median 10.5 pg/ml).

The IL-12 level in the aqueous humor and/or vitreous of patients with active inflammation on the day of surgery ranged from 72 to 293 pg/ml (median 95pg/ml). Patients with moderately active uveitis or uveitis in remission presented with IL-12 levels ranging from 15-94 pg/ml (median 32 pg/ml). A close correlation of the IL-12 levels and the activity of the intraocular inflammation was found. Il-12 levels were higher in the active patients compared to patients with moderate or no active uveitis ($p<0.001$), and Il-12 levels in uveitis patients were higher than in the control group ($p< 0.01$).

A difference of IL-12 levels in the various uveitis entities could not be detected. In patients in whom we could compare the IL-12 levels of the aqueous humor to IL-12 levels of the vitreous, the IL-12 levels in the vitreous were higher than the IL-12 levels in the aqueous humor ($r=0.90$, $p<0.05$) . The finding of higher levels of IL-12 in the vitreous was independent of the primary location of the intraocular inflammation (anterior versus posterior).

Low levels of IL-10 were detected in aqueous humor or in the vitreous from 3 patients with long-standing active uveitis (8-23 pg/ml). Very elevated levels of IL-10 were found in the vitreous body of two patients with large cell lymphoma; these patients had low levels of IL-12.

We have shown that the levels of IL-12 in the aqueous humor and in the vitreous in patients with active intraocular inflammation are elevated. The levels of IL-12 in the aqueous humor and in the vitreous of uveitis patients with low grade inflammation, or in patients in whom the uveitis is in clinical remission for up to 2 years were also significantly higher than in normal subjects.

The presence of high levels of IL-12 in patients showing no clinically obvious inflammation might help explain why patients with a history of uveitis so often respond to surgery (or even non ocular infections) with exuberant recurrence of inflammation, and why immunomodulatory therapy begun before, and continued after surgery provides some protection to such patients from a post surgical flare-up of their uveitis.

Chi Chou Chan from the National Eye Institute described three patients with intraocular lymphoma with high levels of IL-10 in the vitreous. Seven patients with uveitis did not have high levels of IL-10 in the vitreous. We believe that our findings of high levels of IL-12 in the vitreous bodies in patients with uveitis, and our confirmation of low levels of IL-10 in such patients but the presence of higher levels of IL-10 in the vitreous bodies of patients of large cell lymphoma sets the stage for an important role for measurements of these cytokines in the future for the diagnosis of primary intraocular lymphoma, and particularly the definitive discrimination between masquerade secondary to lymphoma and primary uveitis.