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$CD4^+$ T Cell/$CD8^+$ T Cell Ratio in the Anterior Chamber of the Eye After Penetrating Injury and Its Comparison With Normal Aqueous Samples

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Abstract: We have recently reported that significantly more $CD8^+$ T-cell activity is present in the aqueous humor compared to peripheral blood. The aim of the current study is to investigate the effect of the ocular trauma on the number of the T lymphocyte subsets in the aqueous humor. $CD4^+/CD8^+$ T-cell ratios in the aqueous and blood samples of 12 patients who suffered from traumatic iridocyclitis because of a corneal perforation were compared to those of patients with senile cataracts. We found a relatively higher $CD4^+/CD8^+$ cell ratio in the aqueous samples of traumatized patients than cataractous patients. Meanwhile, no difference was present between the aqueous and blood samples of the traumatized patients with respect to the above-mentioned ratio. We suggest that one of the most important factors in maintaining a lower $CD4^+/CD8^+$ cell ratio in normal aqueous humor compared to peripheral blood is an intact blood-aqueous barrier. Blood-aqueous barrier may play a participating role in the pathogenesis of immunosuppressive properties of the anterior chamber of the eye by establishing higher $CD8^+$ T-cell activity in the aqueous humor relative to the peripheral blood. **Jpn J Ophthalmol 1998;42:204–207**

Key Words: Anterior chamber-associated immune deviation, aqueous humor, $CD4^+$ T cell, $CD8^+$ T cell, human, immunology, traumatic iridocyclitis.

Introduction

The anterior chamber (AC) of the eye is an immunologically privileged site. Ocular immune "privilege" is the result of the interaction of various factors such as blood-aqueous barrier, the absence of lymphatic vessels, the sequestration of retinal antigens, the presence of immune modulators in the aqueous humor, and anterior chamber-associated immune deviation (ACAID).[1] The ACAID is characterized by a selective systemic immune deficiency in which both antigen-specific delayed hypersensitivity and complement-fixing antibody production are impaired by populations of $CD8^+$ T lymphocytes, although other immune effector modalities are preserved.[2] Although the ACAID is important, it is not the only factor responsible for the presence of the immunosuppressive microenvironment in the AC of the eye. Numerous studies have documented that this unique privileged site is mediated by several processes.[3] For example, we have recently reported that $CD4^+$ T lymphocyte/$CD8^+$ T lymphocyte ratios were significantly lower in the aqueous humor than in the peripheral blood.[4] A high concentration of $CD4^+$ and a low concentration of $CD8^+$ lymphocytes in the aqueous humor may be one of the contributing factors in the pathogenesis of immunosuppressive behavior of AC. One of the most reasonable explanations for finding a lower $CD4^+/CD8^+$ cell ratio in the aqueous than in the blood is the effect of the blood-aqueous barrier. The current study was designed to investigate the effect of breakdown of blood-aqueous barrier function on the number of T lymphocyte subsets in the aqueous humor. We determined $CD4^+/CD8^+$ cell ratios in the aqueous and in the blood samples of patients with traumatic iri-

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docyclitis because a corneal perforation among patients who suffered from uncomplicated or senile cataracts. The results are discussed later.

Materials and Methods

Twelve patients (seven men and five women between 45 and 72 years of age) with traumatic iridocyclitis because of a corneal perforation constituted the first group (group A). Preoperatively, slit-lamp examination showed a trace of 2^+ cells and one to two aqueous flares in the AC using the criteria described by Hogan et al.[5] All patients were examined within 24 hours following injury, and there was no presence of any cell or flare in the aqueous humor of fellow eyes. The second group (group B) contained 15 patients (eight men and seven women between 44 and 69 years of age) who were operated on because of their senile cataracts.

The operations were performed within 12–36 hours following injury, and none of the patients had used either topical or systemic antiinflammatory drugs during the preoperative period. Immediately before the operation, venous blood samples were obtained from the antecubital vein. During the operation, 200 to 300 μL (235 μL ± 11.4 μL) of aqueous samples are withdrawn into a plastic syringe, using a 27-gauge Rycroft needle (Visitec Corp., Sarasota, FL, USA). Separate syringes were used for each person. We analyzed the specimens 15 minutes after collection. Blood and aqueous specimens were dispensed equally into two pieces, as each one contained 100 μL of sample. We fixed the aqueous specimens with a specific solution that contained sodium azide 1% and paraformaldehyde 1%. Erythrocytes in the blood samples were lysed with Multi Q prep machine and fixed in paraformaldehyde 1%. Some 20 μL of isotopic antibody conjugated with fluoroisothiocyanate (FITC) (Coulter Corp., Hialeah, FL, USA; cat no. 6603796) were added to the control tubes. These tubes were used as internal controls for each specimen. Some 20 μL of CD4 RD1 (conjugated with rhodamine)/CD8 FITC (cat no. 669379; shows T-cell subsets) were added to the study tubes. All pieces were incubated at room temperature for 10 minutes.

Analysis was performed with an Epics-Elite EST machine. We used PMT-log2 and PMT-log3 parameters as well as forward-side scatters. After studying cells that were analyzed with forward-side scatters restricted to a fixed area, we studied at least 500 of the aqueous cells and 5000 blood-sample cells. Results were presented in a quadrant graphic and outlined as Th/Ts ratios. Differences between groups A and B were analyzed by Mann-Whitney *U* Test, whereas the Wilcoxon matched pair signed rank test was used for demonstrating differences between the values obtained from the aqueous and those from the blood samples of the same patient.

Some 100 μL of aqueous humor was also withdrawn at the time of the operation and cultured in blood and thioglycolate agar. No microorganism growth was observed.

Results

The results of flow cytometric analyses are presented in Tables 1 and 2. In the aqueous of group B patients, $CD4^+/CD8^+$ cell ratios did not show any significant difference from their blood samples ($P > 0.05$), but $CD4^+/CD8^+$ T-cell ratios in the aqueous of group B patients were higher than those of group A patients ($P < 0.05$). $CD4^+/CD8^+$ lymphocyte ratios in the aqueous samples of group A patients were significantly lower than in their blood samples ($P < 0.05$). There was no difference between the blood samples of both groups ($P > 0.05$) (Table 3).

Discussion

Allografts placed into the AC of the eye enjoy prolonged and sometimes permanent survival, whereas similar grafts are promptly rejected if transplanted to nonprivileged sites.[6] Some researchers believe that the immunologic privilege of the AC results, in large part, from the systemic suppression of delayed-type

Table 1. $CD4^+/CD8^+$ Cell Ratios in Patients with Traumatic Iridocyclitis (Group A)

Patient Number	$CD4^+/CD8^+$ Cell Ratio in the Aqueous Humor	$CD4^+/CD8^+$ Cell Ratio in the Blood
1(+)	1.844	1.561
2(++)	1.483	1.685
3(+)	1.577	1.623
4(trace)	1.15	1.638
5(+)	1.563	1.384
6(++)	1.567	1.263
7(++)	1.683	0.972
8(trace)	1.416	1.547
9(+)	2.429	2.266
10(+)	1.383	1.957
11(++)	1.606	1.315
12(++)	1.475	1.325
	1.468154 ± 0.533118[a]	1.425846 ± 0.537099[a]

Plus signs indicate the density of cells in the aqueous humor.
[a]Mean ± standard deviation.

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Table 2. $CD4^+/CD8^+$ Cell Ratios in Patients with Senile Cataract (Group B)

Patient Number	$CD4^+/CD8^+$ Cell Ratio in the Aqueous Humor	$CD4^+/CD8^+$ Cell Ratio in the Blood
1	0.914	2.011
2	0.523	1.708
3	1.109	1.741
4	0.498	1.598
5	1.106	1.789
6	0.741	2.024
7	0.826	1.508
8	0.432	1.396
9	1.211	1.768
10	0.647	2.097
11	0.887	1.614
12	0.512	1.524
13	0.701	1.485
14	0.489	1.476
15	0.812	1.276
	0.774438 ± 0.357958[a]	1.563438 ± 0.477454[a]

[a]Mean ± standard deviation.

Table 3. The Results of the Statistical Analyses

Aqueous group A	$P = 0.00271$
Aqueous group B[a]	$P < 0.05$
Blood group A	$P = 0.126796$
Blood group B	$P > 0.05$
Aqueous group A	$P = 0.705315$
Blood group A[b]	$P > 0.05$
Aqueous group B	$P = 0.0000208$
Blood group B[b]	$P < 0.05$

[a]Statistical analysis was performed by using Mann-Whitney *U* Test.

[b]Statistical analysis was performed by using Wilcoxon matched pair signed rank test.

hypersensitivity that is induced by AC presentation of allo-antigens and is considered to be an ACAID.[7,8] However, other reports have indicated that both the aqueous humor and cells in the iris and ciliary body have immune inhibitory properties in vitro, suggesting that these components of the anterior segment might contribute to the unique properties of this microenvironment.[9,10] Moreover, inhibitory activity of iris and ciliary body cells was reported to be produced by parenchyma rather than by hematogones.[10] Iris and ciliary body cells show their immunosuppressive effects by secreting a soluble factor locally and into the aqueous humor, which endows resident, mature macrophages with ACAID-inducing capabilities.[11–14] These reports suggest that some intraocular factors also may play an important role in the development of immunosuppressive properties in the AC of the eye in addition to ACAID. Among these intraocular factors, $CD8^+$ lymphocyte activity in the AC is of interest, because expression of delayed-type hypersensitivity response in the AC after injection of foreign antigens is curtailed by $CD8^+$ lymphocytes.[15] It was even reported that $CD8^+$ cells are important to and necessary for the expression of ACAID and immunosuppressive properties of AC.[16] We have recently reported that AC of the eye has a significantly higher $CD^+/CD8^+$ cell ratio than blood.[4]

However, the factor(s) responsible for enhanced activity of $CD8^+$ lymphocytes in the AC is not clear. A possible explanation is that the AC of the eye lacks internal lymphatics, so an antigen placed in the AC will be presented to the immune system through the Schlemm's canal and the bloodstream, not through the lymphatics.[16] Indeed, the induction of $CD8^+$ lymphocytes is enhanced when antigen processing bypasses the lymphatic system.[17] On the other hand, the blood-aqueous barrier may be responsible, at least partly, for enhanced activity of $CD8^+$ lymphocytes in the AC compared with the blood. The blood-aqueous barrier limits the interaction between the systemic immunity and the eye.[18] Our current study shows that the blood-aqueous barrier breakdown by eye trauma had an enhancing effect on the $CD4^+/CD8^+$ lymphocyte ratio in the AC. The blood-aqueous barrier breakdown is a well-known consequence of ocular trauma.[19] It is probable that the blood-aqueous barrier has an important function in maintaining a lower $CD4^+/CD8^+$ lymphocyte ratio relative to the blood. Enhanced $CD8^+$ lymphocyte activity in the AC may be one of the contributing factors in the generation of the immune privilege of the AC. The role of the blood-aqueous barrier in the induction of the immune privilege of the AC has been suggested by some researchers.[1] First of all, the blood-aqueous barrier sequesters the intraocular antigen from systemic immunity. On the other hand, the blood-aqueous barrier maintains the presence of local immunomodulators in the aqueous humor and prevents the unabated entering of the systemic immune cells into the AC from the blood.[1,18] The lower $CD4^+/CD8^+$ cell ratio in the normal AC of the eye may have an important role in the AC immunology, because $CD8^+$ lymphocytes are necessary for the induction of the immunosuppressive microenvironment of the AC.[14,15] In the current study, it was shown that traumatic breakdown of the blood-aqueous barrier increased the $CD4^+/CD8^+$ lymphocyte ratio to the

blood levels. Based on these data, it may be suggested that lower $CD4^+/CD8^+$ lymphocyte ratio in the AC of the normal eye compared to the blood samples is maintained by the blood-aqueous barrier.

In conclusion, we postulate that the blood-aqueous barrier contributes to the induction of the immunosuppressive microenvironment of the AC by regulating the $CD4^+/CD8^+$ cell ratio in the aqueous humor. Additional observations, maybe by using fluorophotometry, will yield more data.

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