Vitreous Levels of CD4, CD8 and CD28 T-Lymphocytes in Patients with Proliferative Diabetic Retinopathy and Proliferative Vitreoretinopathy

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Abstract: This is a prospective comparative non-randomized study aimed to detect T-lymphocyte subsets expression in the vitreous fluid of patients with proliferative diabetic retinopathy (PDR) and proliferative vitreoretinopathy (PVR) and to evaluate their role in the pathogenesis of these diseases. This study included 3 groups of patients undergoing vitrectomy for PDR (Group I), PVR (Group II) and for other pathologies serving as control (Group III). Vitreous and blood samples were collected from all patients. Flow cytometry was used to detect CD4, CD8 and CD28 expression in the vitreous as compared to peripheral blood. Results revealed that ten (43.4%) patients had PDR. Six patients (26%) had PVR. The control group consisted of 7 patients (30.4%). T-lymphocytes were detected in vitreous fluid of all PDR patients and all PVR patients but in none of the 7 control patients. In conclusion, flow cytometry analysis of vitreous fluid promotes better understanding of the intraocular immune response implicated in the pathogenesis of PDR and PVR.

Key words: Flow Cytometry Analysis • T-lymphocyte subsets • Intraocular Immune Response • Cytokines

INTRODUCTION

Both PDR and PVR follow a common downstream cascade towards development of tractional retinal detachment due to fibrovascular or cellularly induced membranes. Such cascade parallels the normal wound healing process that implicates inflammatory, proliferative and remodeling components that set in through synergism between cytokines and growth factors [1].

In the course of this process, human retinal pigment epithelial (RPE) cells, macrophages, neutrophils, fibroblasts and glial cells play critical roles in the inflammatory stages of proliferative disorders, either through exposure as target cells or by producing cytokines [2].

While a normal vitreous owes its immune-privileged status to the blood-retina-barrier (BRB) that enables it to escape recognition by the immune response, breakdown of the BRB in PDR/PVR patients renders the vitreous accessible to the immune system with free inflow of inflammatory cells and cytokines not normally present and that eventually instate chronic inflammation with subsequent fibrosis and scarring[1].

Many studies have so far addressed many of these growth factors and cytokines in PDR/PVR. [1-7]. The present study aims at exploring the role T-lymphocytes subsets (CD4-CD8 and CD28) might have in the pathogenesis of proliferative disorders in PDR and PVR patients.

This is a Prospective comparative non-randomized study aimed to detect CD4, CD8 and CD28 T lymphocytes expression in the vitreous fluid of patients with proliferative diabetic retinopathy (PDR) and proliferative vitreoretinopathy (PVR) and to evaluate their role in the pathogenesis of these diseases.

MATERIALS AND METHODS

Subjects: In this study recruited patients were divided into 3 groups. Group I (PDR), patients enrolled in this
group had vitrectomy for PDR with or without associated vitreous and/or pre-retinal hemorrhage. Group II (PVR), in this group, patients had vitrectomy for PVR complicating rhegmatogenous retinal detachment. Group III (control group), included patients who underwent vitrectomy for pathologies other than PVR/PDR, including dropped nucleus, dislocated IOL, asteroid hyalosis, idiopathic macular hole and cellophane maculopathy.

Pre-operative examination included fundus examination using indirect ophthalmoscopy with scleral indentation for evaluation of the periphery and bio-microscopy using fundus contact lens. The modified Early Treatment Diabetic Retinopathy Study retinopathy severity scale was used for grading the severity of PDR. Whereas the Silicone Study Group modified classification of proliferative vitreoretinopathy and the expanded classification of proliferative vitreoretinopathy by the Retina Society were used for grading the severity of PVR.

Fundus findings were confirmed preoperatively by standardized fundus color photography and fluorescein angiography (Topcon TRC-501A fundus camera, an image-net system (Tokyo Optical Co Ltd. Tokyo, Japan) when indicated. In cases where media opacity hindered fundus photography, both A-scan and B-scan ultrasonography were performed.

Sample Collection: For all patients vitreous samples were obtained at the start of vitrectomy. Vitreous samples were collected undiluted by manual suction into a syringe through an air-flushed aspiration line of the vitrectome before opening the infusion line.

Whole blood samples were obtained by venous puncture directly before vitrectomy. Vitreous and blood samples were immediately transferred to a vacuupainer EDTA tube (Clinilab) to avoid sample coagulation and/or cells aggregation. No dilutions were required. Harvested samples were preserved on ice until reaching the laboratory then prepared on the same day. Analysis was performed on the same day or the following one. For samples analyzed on the following day, stained cells were preserved in 1% paraformaldehyde (75% PBS and 25% paraformaldehyde).

Vitreous Fluid: Vitreous specimens were centrifuged at 2500 rpm for 15 minutes at 4°C and the pellet obtained was re-suspended in staining buffer (phosphate-buffered saline plus 1% fetal calf serum plus 0.1% sodium azide). Surface markers were stained by incubation with saturating amounts of the different mAbs. Stained cells were then washed with staining buffer, re-suspended and then analyzed with a single argon blue laser flow cytometer. All samples were protected from light during incubation throughout the procedure. Negative control isotype IgG-matched mAbs were used for each case. Cells gating and analysis were performed on the lymphocytic rich region with an acquisition and analysis program (Partec FloMax, Dakocytomation). In case of samples with vitreous hemorrhage, red cells were lysed as described earlier. Results were expressed as percentages of positive cells.

Analysis of Surface Markers: Reagents: Monoclonal antibodies (mAbs) against CD28, CD8 and CD4 conjugated to fluorescein isothiocyanate (FITC), propedium iodide (PI), propedium iodide cya-5 (PI-Cya-5) respectively. FL1 indicates FITC; FL2 indicates propedium iodide, FL3 indicates propedium iodide Cya-5, were obtained (Dako Denmark A/S, Produktionsvej 42, DK-2600 Glostrup, Denmark).

Whole Blood: Cells were analyzed by means of multi-parameter flow cytometry according to standard protocols described by the manufacturer. Briefly, tricolor staining of surface markers (CD4⁺, CD8⁺ and CD28⁺) was performed by incubation of the whole blood with saturating amounts of the different mAbs mentioned previously. Stained cells were then washed with staining buffer (phosphate-buffered saline plus 1% fetal calf serum plus 0.1% sodium azide) and red blood cells were lysed with erythrocyte lysis (ammonium chloride, EDTA and Sodium bicarbonate) (Partec PAS III; Dakocytomation flow cytometer). After washing, cells were re-suspended and then analyzed with a single argon blue laser flow cytometer (Partec PAS III; Dakocytomation flow cytometer).

Vitreous Fluid: Vitreous specimens were centrifuged at 2500 rpm for 15 minutes at 4°C and the pellet obtained was re-suspended in staining buffer (phosphate-buffered saline plus 1% fetal calf serum plus 0.1% sodium azide). Surface markers were stained by incubation with saturating amounts of the different mAbs. Stained cells were then washed with staining buffer, re-suspended and then analyzed with the flow cytometer. All samples were protected from light during incubation throughout the procedure. Negative control isotype IgG-matched mAbs were used for each case. Cells gating and analysis were performed on the lymphocytic rich region with an acquisition and analysis program (Partec FloMax, Dakocytomation). In case of samples with vitreous hemorrhage, red cells were lysed as described earlier. Results were expressed as percentages of positive cells.

All cases were operated at the Research Institute of Ophthalmology, Egypt, by two surgeons (AS) and (ML), whereas sample analysis was performed in the Microbiology and Immunology department of the Research Institute of Ophthalmology (R.I.O.) - Ministry of Scientific Research, Egypt and the Clinical Pathology department of the National Cancer Institute (N.C.I.) - Cairo University, Egypt. This study was performed in accordance with the tenets of the Declaration of Helsinki of 1975 (the 1983 revision). The Research Committee of
both The Research Institute of Ophthalmology and The National Cancer Institute approved the protocol of collection of vitreous fluid and blood samples. All patients provided an informed consent prior to the procedure.

**Statistical Analysis:** Independent-Samples T-Test was used for statistical analysis. The Independent-Samples T-Test procedure compares means for two groups of cases. Ideally, for this test, the subjects should be randomly assigned to two groups, so that any difference in response is due to the treatment and not to other factors. For each variable: sample size, mean, standard deviation and standard error of the mean. For the difference in means: mean, standard error (SE). The standard deviation (SD) was given by:

\[
\Sigma X^2 - \left(\frac{\Sigma X}{2}\right)^2 \frac{1}{N}
\]

**RESULTS**

The study included 28 eyes of 28 patients. Sixteen men and twelve women. Mean age 53.6 years; range 12-65. All patients had type II diabetes mellitus. Five patients were excluded at the time of analysis due to presence of nonviable cells in the vitreous fluid. Therefore, data from 23 patients were considered suitable for analysis. Ten (43.4%) of the 23 patients had PDR, 7 (30.4%) of those had associated vitreous/pre-retinal hemorrhage whereas, the remaining 3 (13%) had no vitreous/pre-retinal hemorrhage. Six patients (26%) had PVR. The control group consisted of 7 patients (30.4%). None of them had vitreous hemorrhage or PVR. T-lymphocytes were detected in vitreous fluid of all diabetic patients with and without hemorrhage and in all PVR patients but in none of the 7 control patients.

**PDR With Hemorrhage Patients:** Polychromatic flow cytometry assay used to analyze paired vitreous fluid and peripheral blood lymphocyte samples from a representative patient with PDR with hemorrhage are displayed in Figure 1.

The percentages of T cells obtained as well as their phenotypical analysis in vitreous fluid and in peripheral blood are presented in Table 1.

The percentages of T cells subsets in the vitreous and in the peripheral blood were comparable in PDR with hemorrhage; instead the percentages of CD4+ and CD28+, were significantly higher in the vitreous fluid than in the peripheral blood (39.7 versus 26.7). The percentages of CD4+CD28+ were lower in vitreous fluid than in peripheral blood.

**PDR Without Hemorrhage Patients:** Flow cytometry assay of both vitreous fluid and blood from a representative patient with PDR without vitreous hemorrhage are displayed in Figure 2.

The percentages of CD4+ and CD8+ T cells obtained, as well as their phenotypical analysis in peripheral blood and in the vitreous fluid are presented in Table 2.

There is a slight reduction in the percentage of CD4+ and a significant increase in the percentage of CD8+ in the vitreous fluid in comparison with the peripheral blood (47.7% versus 58.1%) and (52.3% versus 41.8%) respectively. However, the percentage of CD4+ CD28+ T cells in the vitreous fluid was significantly higher than in peripheral blood (29.4% versus 6.4%), subsequently the percentage of CD4+ and CD28+ was lower in the vitreous fluid than in the peripheral blood (15.8% versus 37.2%). As regard CD8+ CD28+ there was significant increase in the vitreous fluid than in the peripheral blood.

<table>
<thead>
<tr>
<th>Table 1: PDR with hemorrhage</th>
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<tr>
<td>Mean</td>
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<tr>
<td>CD4</td>
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<td>CD8</td>
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<td>CD4/CD28 Co-expression</td>
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<td>CD4 + CD28+</td>
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<tr>
<td>CD8/CD28 Co-expression</td>
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<td>CD8+ CD28+</td>
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Where P-value is the probability (reflect of null hypothesis)
P-value > 0.05 non-significant (NS)
P-value < 0.05 significant (*)
P-value < 0.01 highly significant (**)
Table 2: PDR without hemorrhage

<table>
<thead>
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<th>Peripheral Blood</th>
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<th>P-value</th>
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<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
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</tr>
<tr>
<td>CD4</td>
<td>47.70</td>
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<tr>
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<td>CD4⁺ CD28⁻</td>
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<td>CD8/CD28 Co-expression</td>
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<td>4.81</td>
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<tr>
<td>CD8⁺ CD28⁻</td>
<td>9.63</td>
<td>1.59</td>
<td>11.93</td>
<td>2.22</td>
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Where P-value is the probability (reflect of null hypothesis)
P-value > 0.05 non-significant (NS)
P-value < 0.05 significant (*)
P-value < 0.01 highly significant (**)

Table 3: PVR

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<td></td>
<td>Mean</td>
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<tr>
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<td>4.55</td>
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<td>CD8⁺ CD28⁻</td>
<td>4.38</td>
<td>0.72</td>
<td>5.06</td>
<td>0.50</td>
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</table>

Where P-value is the probability (reflect of null hypothesis)
P-value > 0.05 non-significant (NS)
P-value < 0.05 significant (*)
P-value < 0.01 highly significant (**)

Fig. 1: Whole blood (top) and vitreous fluid (bottom) CD4⁺ and CD8⁺ T lymphocytes from a representative patient with PDR with vitreous hemorrhage. Samples were stained immediately after collection with CD28-fluorescein isothiocyanate (FITC), CD4-propedium iodide Cya-5 (PI-Cya-5), CD8-propedium iodide (PI) and CD3-peridium chlorophyll protein (PerCP). First, a gate was drawn around the lymphocyte population as shown. From the gated lymphocytes, we selected CD3⁺ (T cells) and analyzed the distribution of CD4⁺ and CD8⁺ cells. FL1 indicates FITC; FL2 indicates propedium iodide, FL3 indicates propedium iodide Cya-5.
Fig. 2: Whole blood (top) and vitreous fluid (bottom) CD4+ and CD8+ T lymphocytes from a representative patient with PDR without vitreous hemorrhage. Samples were stained immediately after collection with CD28-fluorescein isothiocyanate (FITC), CD4-propidium iodide Cya-5 (PI-Cya-5), CD8-propidium iodide (PI) and CD3-peridium chlorophyll protein (PerCP). First, a gate was drawn around the lymphocyte population as shown. From the gated lymphocytes, we selected CD3+ (T cells) and analyzed the distribution of CD4+ and CD8+ cells. FL1 indicates FITC; FL2 indicates propidium iodide, FL3 indicates propidium iodide Cya-5.

Fig. 3: Whole blood (top) and vitreous fluid (bottom) CD4+ and CD8+ T lymphocytes from a representative patient with PVR. Samples were stained immediately after collection with CD28-fluorescein isothiocyanate (FITC), CD4-propidium iodide Cya-5 (PI-Cya-5), CD8-propidium iodide (PI) and CD3-peridium chlorophyll protein (PerCP). First, a gate was drawn around the lymphocyte population as shown. From the gated lymphocytes, we selected CD3+ (T cells) and analyzed the distribution of CD4+ and CD8+ cells. FL1 indicates FITC; FL2 indicates propidium iodide, FL3 indicates propidium iodide Cya-5.
PVR Patients: Flow cytometry assay of both vitreous fluid and in the peripheral blood from a representative patient with PVR are displayed in Figure 3. The percentage of CD4\(^+\) and CD8\(^+\) T lymphocytes and expression of CD28 are represented in Table 3. There is a slight decrease in the percentage of CD4\(^+\) T cells and a slight increase in the percentage of CD8\(^+\) T cells in the vitreous fluid in comparison with the peripheral blood; however, these differences are non-statistically significant (60.2% versus 66.3%) and (39.8% versus 33.6%) respectively. However, the percentages of CD4\(^+\) CD28\(^+\) and CD8\(^+\) CD28\(^+\) T cells in the vitreous fluid were significantly higher than in the peripheral blood (40.1% versus 15.2%) and (24.5% versus 4.5%) respectively.

**DISCUSSION**

While the immunopathogenesis of vitreoretinal disorders remains obscure, it is thought that local inflammatory cells and a variety of cytokines may be involved in mediating the immune response including interleukins IL-1, IL-6, IL-8, interferon-gamma and tumor necrosis factor (TNF). Human RPE cells, monocytes, macrophages, T-lymphocytes, endothelial cells and fibroblasts have all been suggested as sources of these cytokines in the vitreous humor [2].

Several studies extensively investigated the role of many of these factors in the pathogenesis of PVR/PDR [1-7]. In the current study we employed flow cytometry to identify T-lymphocyte subsets namely CD4, CD8 and CD28 in the vitreous and their concentration as compared to peripheral blood in PDR and PVR patients, as well as in control group.

CD4\(^+\) cells (Helper T cells) help the activity of other immune cells by releasing T cell cytokines. Whereas, CD8\(^+\) (Cytotoxic T cells) help the immune system through their antigen recognition and binding capacity and also through production of T cell cytokines. Finally, CD28\(^+\) help production of T cell cytokines through providing crucial co-stimulatory signal [7].

In the current study, flow cytometry analysis of vitreous samples detected T-lymphocytes in vitreous fluid of all PDR patients with and without hemorrhage and in all PVR patients but in none of the 7 control patients. This finding is in accordance with the notion that the vitreous is an immune-privileged site being unrecognized by the immune system owing to the presence of intact BRB. Disruption of the BRB is therefore a pre-requisite for immune system recognition of the vitreous and subsequent free inflow of T-lymphocytes among other factors [1]. Our results seem to be consistent with the work of Kase et al. [8] in which lymphocyte infiltration was observed in all PDR membranes. These authors suggested that high-level infiltration of T lymphocytes correlated well with poor visual prognosis.

**Group I (PDR Patients):** Subgroup analysis revealed that 7 out of 10 patients with PDR had associated vitreous/pre-retinal hemorrhage. In those patients flow cytometry demonstrated similarity in percentage and pattern of CD4\(^+\) and CD8\(^+\) cells between vitreous and blood which is a further evidence of breakdown of the BRB. These findings are consistent with those of Canton et al. [7] who confirmed the presence of T lymphocytes infiltrating the vitreous fluid of all patients with PDR using flow cytometry. The authors presumed that intravitreal cellularity reflects peripheral blood entering the intraocular cavity because of disruption of the BRB in PDR. However, they concluded that T lymphocytes infiltrating the vitreous were related to improved prognosis in patients with PDR.

In contrast to Canton et al. [7], flow cytometry in this study demonstrated that in this subset of patients the percentages of CD4\(^+\)/CD28\(^+\) T cells as well as CD8\(^+\)/CD28\(^+\) T cells were higher in the vitreous fluid than in the peripheral blood. The association of CD4\(^+\)/CD28\(^+\) T cells with the disease status could indicate direct contribution of these cells to disease manifestations.

CD4\(^+\)/CD28\(^+\) T cells differ from conventional CD4\(^+\)/CD28\(^-\) helper T lymphocytes in both phenotype and function. Activation of naive T cells requires not only the recognition of antigen presented by dendritic cells (DCs) but also the interaction of B7, present on DCs with the CD28 receptor. The only co-stimulatory receptor constitutively expressed on naive T cells is CD28. Therefore CD28 is pivotal for the induction and maintenance of T cell-mediated immune responses [9].

Despite the loss of the CD28 molecule, these CD4\(^+\) T cells are functionally active and have the ability to release cytokines in the absence of a co-stimulatory pathway showing that co-stimulatory molecules other than CD28 are involved in complete activation of CD4\(^+\) CD28\(^+\) T cells. CD4\(^+\)/CD28\(^-\) T cells are terminally differentiated and have pro-inflammatory functions characterized by the production of high levels of interferon-g (IFN-g), tumor necrosis factor-a (TNF-a) and IL-2 [10].

In addition, CD4\(^+\)/CD28\(^-\) T cells are cytotoxic and effectively kill endothelial cells *in vitro* [11] This is mediated by cytolytic enzymes, such as perforin, granzyme A and granzyme B expressed by CD4\(^+\)/CD28\(^-\) T cells [12].
These cytolytic enzymes are usually present in cytotoxic CD8+ T lymphocytes and natural killer (NK) cells, whereas classical CD4+ T cells lack them. Thus, the presence of significant numbers of CD4+ CD28+ T cells could shift immune response from B-cell activation and production of immunoglobulins toward activation of type-1 T helper cells and involvement of macrophages releasing matrix-degrading proteases. Recent studies demonstrated that de novo expressed NK receptors on CD28+ T cells in an additive pattern compensate for suboptimal T cell receptor stimulation [13].

In addition, decreased susceptibility to immunoregulation by T regulatory cells can potentially further strengthens the pathogenic role of CD28+ T cells [14]. Several authors have suggested that in chronic inflammatory autoimmune diseases such as diabetes, multiple sclerosis and rheumatoid arthritis, Th1 cells are pathogenic and Th2 cells are protective. Therefore, a successful deviation from a Th1-dominated to a Th2-dominatet response could have clinical benefits for individuals suffering from these diseases [15].

In some individuals, CD28+ T cells make up more than half of the CD4 T cell population, in this way diminishing immunological space and survival factors that are otherwise available for functional T cells, including Treg cell subsets. This change may cause a breakdown in tolerance and contribute to autoaggressive immune manifestations [14,16].

CD4+ CD28+ T cells are infrequent in healthy individuals (comprising 0.1-2.5% of T cells) [17], whereas higher levels seen in patients with unstable angina, multiple sclerosis, Wegener's granulomatosis and rheumatoid arthritis with extra-articular manifestations support their involvement in autoimmune pathogenesis [18,19]. Therefore, high levels of the stated T cells will be found in PDR patients.

The reason for the enhancement of CD4+ CD28+ observed in the vitreous fluid of PDR patients in the present study is as yet unknown. One possibility is that co-stimulation independent auto-reactive CD4+ cells undergo activation in the periphery by the mechanism of molecular mimicry or bystander activation. Subsequently, activated CD4+ CD28+ cells may preferentially tend to leave the bloodstream, migrate through the impaired BRB and initiate an inflammatory response within the eye. In this regard, co-stimulation-independent activation is particularly important for antigen recognition within the posterior chamber of the eye, where competent antigen presenting cells are sparse. Alternatively, the resistance to the apoptosis reported for CD4+ with lack of CD28 surface could be a reliable explanation for its increased percentage within the vitreous fluid of diabetic patients [7].

Furthermore the increased level might be explained on the basis of environmental and genetic mechanisms which underline the expansion of these unusual subsets of T-cells. Therefore, the development of CD4+ CD28+ T cells might be influenced by persistent poor glycemic control and might be related to the presence of high levels advanced glycosylated end-products, known to be implicated in the onset of diabetic vascular complications [8].

The remaining 3 patients in the PDR group did not have associated vitreous/pre-retinal hemorrhage. In these patients flow cytometry demonstrated CD8+ CD28+ T cell subsets to be increased in the vitreous fluid than in the peripheral blood. This finding is in accordance with another study conducted by Urbančič et al., [20] confirming the hypothesis that local intravitreal inflammation plays an important role in the development of PDR.

High intravitreal levels of several cytokines and inflammatory mediators detected in diabetic patients with PDR causing cytotoxic T lymphocyte activation and proliferation might explain their increased levels in the vitreous fluid more than in the peripheral blood [21].

Group II (PVR Patients): In this group flow cytometry demonstrated lower percentage of CD4+ T cells and higher percentage of CD8+ T cells in the vitreous fluid in comparison with the peripheral blood; however, these differences were not statistically significant. The percentages of CD4+ CD28+ and CD8+ CD28+ T cells in the vitreous fluid were significantly higher than in the peripheral blood indicating an ongoing process in the vitreous, (40.1 versus 15.2) and (24.5 versus 4.5) respectively. By contrast, no statistically significant difference was observed in terms of the percentage of CD4+ CD28+ and CD8+ CD28+ T cells between the vitreous fluid and the peripheral blood.

Our results agree with the study done by Charteris et al. [22] that concluded that T lymphocytes are present in PVR subretinal membranes and have the potential to interact with other cell types in the pathogenesis of this condition. Our results also seem to be consistent with the study done by Limb et al. [23] that revealed that T lymphocytes may play an important role in the pathogenesis of PVR. Our results contradict the study done by Zhang et al. [24] which suggested that T- and B-cell immunity is not essential for the induction of PVR.
PVR patients display signs of active immune processes in their epiretinal or subretinal membranes, vitreous cavity, subretinal fluid and serum samples [25,26].

This means activation of type-2 T helper cells. Thus, deposits of IgG, IgA and IgE antibodies and complement fragments C1q, C3c and C3d can be detected in the epiretinal or subretinal membranes of PVR lesions, along with infiltrating CD4 and CD8 T cells, B cells and macrophages. Moreover, increased expression of CD95-ligand and/or HLA-DR molecules on these immune cells suggests that they are in an activated state. Similar signs of immune activity have been reported for the cells present in the vitreous cavity or subretinal fluid of PVR patients [2,27,28].

The PVR process can be considered as a modified wound healing process, which is initiated by injury (retinal break) that causes a retinal detachment. The most important factor is probably the excessive inflammatory reaction that occurs in some clinical situations that predispose to PVR. The exact pathogenic mechanisms involved in the formation of PVR are not completely understood [29].

CONCLUSION

Flow cytometry analysis of vitreous fluid promotes better understanding of the intraocular immune response implicated in the pathogenesis of PDR and PVR and helps searching for more effective therapies. This technology offers simultaneous measurements of multiple analytes in a single run/cycle of the assay, in addition to being applicable in vivo. Further research is required to identify the various subsets of T lymphocytes expressed in the vitreous fluid in PDR and PVR patients and the various mechanisms of innate immunity that act inside the eye in these cases.

REFERENCES


