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Evidence for a Compartmentalized B Cell Response as Characterized by IgG Epitope Specificity in Human Ocular Toxoplasmosis

Vincent N. A. Klaren1 and Ron Peek

Infectious agents in the eye induce both a local and a systemic humoral immune response. Previously, differences in Ag recognition were observed between systemic and ocular derived IgG of patients with ocular toxoplasmosis. This finding implied a nonrandom distribution of IgG-producing B cells in the inflamed eye. In the present study, we compared the intraocular and systemic B cell responses of patients with ocular toxoplasmosis to a single Toxoplasma gondii Ag. Two series of C-terminally deleted recombinant T. gondii GRA-2 proteins were constructed to delineate IgG B cell epitopes of paired ocular and serum samples. Differences in epitope region recognition between the ocular and systemic compartment were detected in 9 of 13 patients. The difference in distribution of GRA-2 epitopes between paired samples is indicative of a local GRA-2 specific B cell population functionally different from the systemic GRA-2-specific B cell population. Our results suggest a selective activation of a subset of B cells locally in nonlymphoid tissue. The Journal of Immunology, 2001, 167: 6263–6269.

The eye is an immunologically privileged site in which the local microenvironment influences both the induction and the expression of immunity to local Ags (1, 2). A number of factors contribute to the immune privilege of the eye including anatomical barriers, lack of lymphatic drainage, and cell-bound and soluble factors (3–6). Together these factors influence the cellular immune responses in ocular, resulting in an impaired cell-mediated cytotoxicity. This is believed to be a dynamic state of immune regulation to prevent damage to ocular tissue by inflammatory responses and to preserve vision (7). The humoral branch of the ocular immune response remains intact, and specific Ab is produced systemically and locally in the eye during ocular infection (8). A mouse model of anterior chamber-associated immune deviation, introducing Ag in the eyes of mice, revealed the activation of B cells that secrete non-complement-binding Abs (9). To date, this has not been confirmed by studies in humans. Although intraocular Ab production to pathogens is used for confirmation of clinical diagnosis by ophthalmologists (10), little is known about the specificity or the underlying mechanisms of this localized B cell response.

In previous work, our group has studied the intraocular B cell response by analyzing the intraocular Abs of patients with ocular toxoplasmosis, a recurrent inflammation of the retina caused by the intracellular parasite Toxoplasma gondii. With immunoblotting, it was found that the intraocular Ab response (of 11 of 13 patients) differed markedly from the systemic Ab response (11). Some T. gondii Ags were preferentially recognized by serum-derived Abs, whereas others were recognized by ocular-derived Abs. This finding implied a nonrandom distribution of IgG-producing B cells in the inflamed eye.

Evidence for accumulation of clonally related B cells was recently reported for the CSF of patients with multiple sclerosis (12), the synovial tissues of patients with rheumatoid arthritis (13), and the salivary glands of patients with Sjögren’s syndrome (14, 15). Knopf et al. (16) concluded in a study in which T cell-dependent Ags were injected into rat brains that B cells have the ability to detect (seek) the locally applied Ags and to differentiate into plasma cells within the brain. These studies suggested that oligoclonal B cell populations in immune privileged sites could be involved in autoimmune diseases. To date, no studies have been undertaken to investigate local B cell responses of infections in immune privileged sites.

To implicate similar mechanisms in the local, deviant response to Ag in eyes of patients with intraocular inflammation, the present study aimed to functionally characterize the clonality of intraocular B cell responses in eyes of patients with ocular toxoplasmosis. We determined the epitope specificity of IgG from paired serum and ocular samples by using a recombinant T. gondii Ag, GRA-2. This is one of the T. gondii Ags preferentially recognized by IgG from ocular fluid (OF)2 samples of patients with ocular toxoplasmosis (11). GRA-2 is a 28-kDa glycoprotein secreted by the parasite and plays an important role during infection. GRA-2 has been shown to induce both a cellular and humoral response during chronic infection (17–20).

A series of experiments was performed to determine the epitope specificity of serum- and ocular-derived IgG specific for GRA-2. Two series of C-terminally deleted rGRA-2-GST fusion proteins were designed to identify sequences involved in B cell epitope formation. Paired serum and OF samples of 13 patients with active ocular toxoplasmosis were selected for these experiments. Most samples revealed the intraocular epitope specificity of anti-GRA-2 IgG to be different from that of paired serum-derived IgG. These results indicate the presence of a different set of B cells in the eyes...
of patients with ocular toxoplasmosis as compared with the systemic anti-GRA-2 specific B cell population.

We suggest that the underlying mechanisms may lie in the differences of Ag uptake and presentation in the eye compared with the spleen, whereby factors like Ag retention within the eye, competition for Ag binding by B cell receptor (BCR), and local T cell help may be key factors to the local differentiation of specific Ab-secreting B cells.

Materials and Methods

Patients

OF and serum samples were sent to the Netherlands Ophthalmic Research Institute with ophthalmologists associated with the Dutch Uveitis Study Group (21) for confirmation of a clinical diagnosis of ocular toxoplasmosis. Samples were routinely tested for intraocular Ab production against *T. gondii*, HSV, varicella-zoster virus (VZV), and CMV. The ocular samples used in this study were either aqueous humor from the anterior chamber, or vitreous fluid from the posterior chamber of the eye.

Intraocular Ab production was determined as described earlier using an immunofluorescence assay (10). Briefly, *Anti-Toxoplasma* IgG titters were measured with a commercially available immunofluorescence test kit (Belhveringe, Marburg, Germany). Total IgG concentrations in the samples were determined by radial immunodiffusion (22). Intraocular Ab production was calculated and expressed as a Goldmann-Witmer coefficient (GW), which is the quotient of the relative amounts of specific Abs in the eye and serum (specific Ab titer GW/specific Ab titer serum)-(total IgG level total IgG level serum) (23). The GW was used for diagnostic and clinical purpose to determine whether specific Ab present within the eye during uveitis is produced locally. Intraocular production of specific Abs gives an indirect indication of the pathogen responsible for the ocular inflammation and thus confirms a suspected clinical diagnosis (10, 24, 25). A coefficient exceeding 3 was considered as evidence for *T. gondii*-specific Ab production within the eye and hence confirmed the clinical diagnosis of ocular toxoplasmosis (10). Paired samples from 13 patients with a GW of 3 or higher for anti-*T. gondii* IgG were selected for this study. Average age of patients included in the study was 37 years (median, 39 years). Although paired samples from patients 4 and 10 contained anti-HSV Abs and both samples from patient 11 contained anti-HSV and anti-VZV Abs, none of these patients showed intraocular Ab production for HSV, VZV, or CMV. OF samples from the patients included in the present study showed a low intraocular IgG concentration, on average 81-fold lower (median, 22-fold) than total serum IgG, indicating that the blood-retina barrier had not been compromised by the inflammation. Control patient V1 had a net anti-CMV intraocular Ab production (GW = 3.2), and control patient V2 had a net anti-VZV intraocular Ab production (GW = 9). A pool of 10 sera from individuals with no detectable anti-*T. gondii* IgG was used as a negative control.

*T. gondii* Ag extract

A crude *T. gondii* extract was prepared according to the method described earlier (11). Briefly, tachyzoites from the RH strain were obtained from the peritoneal cavity of mice 72 h after i.p. inoculation. After prolonged sonication (5 × 15 s at 30-kHz microprobe; Soniprep 150; Making Science Effective, Loughborough, U.K.), the lysate was centrifuged at 15,600 × g for 10 min with wash buffer, the strips were incubated with HPO-conjugated goat anti-human IgG Ab (DAKO, Glostrup, Denmark) at a dilution of 1/1000 (in wash buffer) for 5 min with wash buffer, the strips were incubated with HPO-conjugated goat anti-human-IgG Abs (DAKO) at a dilution of 1/1000 for 1 h. After four washings, the strips were soaked in a solution of 1 mg/ml diaminobenzidine (Sigma, Zwijndrecht, The Netherlands), 0.1% H2O2 in PBS. The color development was stopped by rinsing with PBS.

Construction of GRA-2 recombinant fusion protein

Total RNA was isolated from *T. gondii* tachyzoites by extraction with RNAzol (Campro Scientific, Elst, The Netherlands). The RNA was used for the amplification of the GRA-2 by RT-PCR using primers based on the sequence obtained from the National Center for Biotechnology Information GenBank. These primers had a flanking restriction site BamHI at the 5’ coding border and a KpnI site in the 3’ noncoding sequence. PCR primers used for amplification of the GRA-2 open reading frame were 5’-GAGAGGAGTCCCCCATGTGCAGCTGTTAACAACTG-3’ and 5’-GGAGGATCTTTAAGGAAATCTTATAGGGTTC-3’. The PCR product was digested with BamHI and KpnI and ligated using T4 DNA ligase with BamHI and KpnI and ligated using T4 DNA ligase (Boehringer Mannheim, Mannheim, Germany) in a derivate of pgEX-3X plasmid (Pharmacia, Uppsala, Sweden). This resulted in a recombinant protein with GRA-2 fused to the C-terminal end of GST.

Construction of GRA-2 deletion mutants

C-terminally truncated mutants were obtained by digestion of the GRA-2-GST DNA construct with restriction enzymes to remove fragments of DNA between various points in the GRA-2 sequence and the polylinker of the vector. Mutant ΔC1 was obtained after digestion with SmaI and SnaI followed by treatment with S1 nuclease to remove the 3’ overhang. Mutant ΔC2 was obtained using SnaI and Smal. Mutant ΔC3 was constructed by digestion with SfaI. The double-digested vectors were agarose gel purified and closed by ligation with T4 DNA ligase.

To create mutants of which the C termini mapped between aa 99 and aa 36, a series of deletion clones was constructed by digesting the GRA-2-GST DNA construct with KpnI/SalI followed by unidirectional digestion with exonuclease III (Boehringer)/S1 nuclease (Life Technologies, Gaithersburg, MD) followed by ligation. After transformation of bacteria, clones were selected on basis of the insert length using PCR analysis. The inserts of selected clones were sequenced to determine their precise length. Depending on the reading frame, 3-, 12-, or 15-vector-encoded amino acids were added to the C terminus of mutant proteins.

Expression and purification of recombinant proteins

Overnight cultures of Escherichia coli BL21(DE3) transformed with the rGRA-2-GST DNA constructs were diluted 1/20 in 500 ml fresh media and grown at 37°C to an OD600 of 1.0. Protein expression was induced by the addition of isopropyl-β-D-galactopyranoside to a final concentration of 0.5 mM. After 2 h at 37°C, the cells were pelleted and resuspended in 5 ml PBS, 0.1% Triton X-100, supplemented with a mixture of protease inhibitors (Complete; Boehringer Mannheim). The cells were lysed by three cycles of freezing and thawing followed by DNase I treatment (50 μg/ml) for 20 min at 37°C. After the addition of 0.1 mg lysozyme and EDTA to a final concentration of 1 mM, the suspension was incubated on ice for 15 min. Nonsoluble material was removed by centrifugation for 10 min at 14,000 × g. The supernatant was filtered through a 0.45-μm pore size filter and incubated with 0.25 ml glutathione agarose beads (Pharmacia) for 1 h at room temperature. After extensive washing with PBS, 0.1% Triton X-100, the glutathione agarose was resuspended in 500 μl sample buffer (2% SDS, 63 mM Tris-HCl (pH 6.8), 10% glycerol, 0.01% bromophenol blue, 5% 2-mercaptoethanol). SDS-PAGE and immunoblotting

Gel electrophoresis was performed with 12.5% SDS-polyacrylamide slab gels. Similar amounts of mutant proteins were used as determined by Coomassie staining of the gels and anti-GST staining of the blots. Proteins were transferred to polyvinylidene difluoride membranes (Immobilon-P, 0.45-μm pore size; Millipore, Bedford, MA) following treatment with 50 mM NaCl, 0.5% Tween 20 containing 2% nonfat dry milk powder (blocking buffer; Bio-Rad, Hercules, CA). The membranes were incubated for 1 h with serum and OF 1/40 in 50 mM Tris 150 mM NaCl, 0.5% Tween 20 containing 0.3% nonfat milk powder (wash buffer). After addition of 450 μl wash buffer, the preabsorbed samples were used for immunoblot analysis of size-fractionation of *T. gondii* extract and compared with untreated serum and vitreous fluid samples diluted 1/1000. One Western blot strip was stained with GRA-2 Ab (kindly provided by T. W. C. C. Schuurman, Erasmus MC, Rotterdam, The Netherlands) and an anti-GST Ab (kindly provided by Dr. H. Vlieghe, Erasmus MC, Rotterdam, The Netherlands) for detection of GST and GRA-2, respectively. A Kodak X-OMAT AR film (Eastman Kodak, Rochester, NY) was exposed to the strips at −80°C. Films were developed and scanned. The bands were quantified using IPLab software (Scanalytics Inc., Fairfax, VA) and normalized to the signal of GRA-2.
identified. Reactivity of paired samples with total GRA-2-GST was quantified by measuring trace density (OD × millimeters) of signals from Western blot using ImageMaster software from Pharmacia (Uppsala, Sweden). A GRA-2-GST-specific GW coefficient was calculated to determine the relative amount of intraocular GRA-2-specific IgG.

To check the quantity and integrity of the recombinant proteins, the blots were stripped from patient Abs by incubation with 62.5 mM Tris (pH 6.8), 2% SDS, 100 mM 2-ME for 30 min at 70°C.

Results

Evaluation of anti-GRA-2 reactivity of samples

To validate the use of the rGRA-2 fusion protein for the analysis of the major B cell epitopes, paired serum and OF samples of two patients were preincubated with solid phase bound rGRA-2-GST. The reduction of the anti-GRA-2 response in these samples was evidently decreased after preabsorption of the samples with solid phase bound rGRA-2-GST (Fig. 1, lanes 2 and 4). This clearly demonstrated that most anti-GRA-2 specific IgG of the samples could be bound by rGRA-2-GST, indicating that rGRA-2-GST can be used to identify regions involved in B cell epitope formation. Similar results were obtained with paired samples from the second patient (not shown).

To further assess the anti-GRA-2 reactivity of patients’ sera and OF samples selected for intraocular anti- T. gondii Ab production, immunoblotting was performed with purified GRA-2-GST fusion protein (Fig. 2). Anti-GRA-2 IgG was detected in the samples of all patients with ocular toxoplasmosis with the exception of the OF from patients 4 and 6 and the serum from patient 10. These patients were excluded from further analysis. The control patients with viral retinitis had no indication of ocular T. gondii infection, but both serum and OF contained anti-T. gondii IgG. No anti-GRA-2 IgG was detected in the OF from these patients however. A monoclonal anti-GST Ab was used to stain the rGRA-2-GST and its degradation products. As a negative control, a pool of 10 sera from individuals with no detectable anti-T. gondii IgG was used. This control showed no staining of the rGRA-2-GST. Relative amounts of intraocular anti-GRA-2 IgG were determined by calculation of a GW coefficient for GRA-2 (see Materials and Methods). All patients showing a GRA-2-specific staining of both serum and OF, with the exception of patient 7, had a GW coefficient of >3, indicating local anti-GRA-2-specific Ab production (Table I). Lack of signal in either the OF or serum sample from patients 4, 6, and 10 and the controls precluded the calculation of a GRA-2 GW coefficient.

Analysis of B cell epitope regions of GRA-2

To analyze which part of the GRA-2 protein contained the immunodominant epitopes, we used internal restriction sites in the GRA-2 cDNA to construct three C-terminally truncated rGRA-2-GST molecules (see Materials and Methods). The rGRA-2-GST mutants C1, C2, and C3 with respective C-terminal deletions of 72, 86, and 149 aa of the total 185-aa GRA-2 open reading frame are depicted in Fig. 3. Paired serum and OF samples containing detectable anti-GRA-2 IgG levels were used to analyze differences in epitope region recognition. Samples were incubated with strips cut from the blots containing only the full length mutant GRA-2 protein (Fig. 2). Anti-GRA-2 IgG was detected in the samples of all patients with ocular toxoplasmosis with the exception of the OF samples selected for intraocular anti-GRA-2 IgG were determined by calculation of a GW coefficient for GRA-2 (see Materials and Methods). All patients showing a GRA-2-specific staining of both serum and OF, with the exception of patient 7, had a GW coefficient of >3, indicating local anti-GRA-2-specific Ab production (Table I). Lack of signal in either the OF or serum sample from patients 4, 6, and 10 and the controls precluded the calculation of a GRA-2 GW coefficient.

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length rGRA-2-GST. This shows that the OF lacked IgG recognizing the region between aa 36 and 113. Serum from patient 9 recognized ΔC1 only, whereas paired OF recognized all three truncated proteins.

Paired samples from patients 1, 3, 7, and 11 strongly recognized ΔC1 and ΔC2, but truncation up to aa 36 completely abolished recognition of rGRA-2-GST by these samples. This implies that the major B cell epitopes of the paired samples from these patients involved sequences between aa 36 and aa 99.

### Fine mapping between aa 36 and aa 99

Whereas several paired serum and OF samples showed major differences in sequences required for epitope formation using only three truncated mutants (ΔC1, ΔC2, ΔC3), four patients (1, 3, 7, 11) had their major epitope(s) in the region between aa 36 and aa 99 for both serum and ocular sample. To reveal possible differences in recognition between these paired serum and OF, a series of C-terminally deleted mutants was constructed by unidirectional digestion (see Materials and Methods) which allowed the production of truncated proteins with C termini in this 63-aa region (see Fig. 3). Results obtained with these truncated proteins, which in some cases differ only by a few amino acids in length, are shown in Fig. 5. Samples of patients 1, 2, 3, and 7 were analyzed as described above. Patient 11 could not be tested further because of insufficient amount of OF.

### Incubation of the paired samples with strips containing ΔC2, ΔC3, and nine exonuclease-generated mutants with C termini mapping between ΔC2 and ΔC3, revealed the presence of at least two major B cell epitopes in this region. Surprisingly, the serum samples of the four patients tested gave similar results and required a stretch of 6 aa between aa 94 and 88 for their main epitope, whereas an additional region between aa 48 and 42 was involved in the formation of a minor epitope. The OF from these patients showed a more heterogeneous distribution of epitopes within this region of the GRA-2 protein. The OF from patient 1 also depended on the sequence between aa 94 and aa 88 for full reactivity but unlike the paired serum did not recognize the region between aa 48 and aa 42. OFs of patients 3 and 7 did not depend on sequences between aa 94 and aa 88 for formation of their main epitope as did their paired serum samples but instead required the sequence between aa 68 and aa 63 (patient 7) and between aa 48 and aa 42 (patients 3 and 7). These results are summarized in Table II and indicate that of the 10 patients tested at least 9 showed a marked difference in the distribution of the major B cell IgG epitopes on the GRA-2 Ag between the systemic and the ocular compartment.

### B cell epitopes are generally located in stretches of hydrophilic amino acids exposed at the protein surface accessible to Ab.

To investigate whether the three short peptides (aa 42–48, aa 63–68, and aa 88–94) were indeed such hydrophilic stretches, a hydrophobicity plot was calculated according to the work of Kyte and...
Doolittle (26). As shown in Fig. 6, all three peptides were located within hydrophilic stretches of the GRA-2 protein.

**Discussion**

By using a panel of deletion mutants of a single *T. gondii* Ag, we were able to analyze the differences in epitope specificity of the systemic and ocular IgG of patients with ocular toxoplasmosis. Bearing in mind that each Ab with its unique specificity reflects the presence of an Ab-producing B cell, we were able to identify B cell population differences between the ocular and the systemic compartment.

Paired serum and OF from 10 of 13 patients with ocular toxoplasmosis contained detectable anti-GRA-2 IgG as tested with full length rGRA-2-GST. By using a set of three C-terminally truncated proteins, epitope recognition differences between the serum and OF could be detected in samples of six patients. A panel of nine truncated proteins revealed recognition differences between paired samples of three more patients. In all, these findings indicate that from a set of B cell epitopes across the Ag, serum and OF IgG recognition of these epitopes is different.

The in vivo activation of B cells takes place in regional lymph nodes (tissue-derived Ag) or spleen (blood-borne Ag) after Ag is transported there, either alone or associated with APC (9, 27). Affinity maturation, class switching, and formation of plasma cells and memory B cells take place in germinal centers. Ab-secreting

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**Table II.** GRA-2-IgG epitope region differences between paired serum and OF samples

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<thead>
<tr>
<th>Patient</th>
<th>Serum</th>
<th>OF</th>
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<tr>
<td>Full length GRA-2</td>
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</tr>
<tr>
<td>2</td>
<td>36–99</td>
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<td>5</td>
<td>36–99</td>
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<td>36–99</td>
<td>113–185</td>
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<td>Region 36–99</td>
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plasma cells rarely circulate in the blood and are thought to reside mainly in the peripheral lymph nodes, tonsils, spleen, and mucosa. This concept is inconsistent with the local Ab production and presence of B cells in the eyes of patients with uveitis (28–30). Because the eye is not equipped with lymphatic drainage, immunity is generated in the spleen (31). Selective influx of B cells into the eye during ocular infection seems an unlikely explanation of eye-specific recognition of certain epitopes as found in this study. The results reported here suggest a regional B cell response within the eye during inflammation. This is a concept of humoral immunity, which has been reported by others in several autoimmune inflammatory responses but not in infectious diseases. Evidence for the compartmentalized clonal expansion of B cells based on PCR analysis was reported for the brains of multiple sclerosis patients (12) and the salivary glands of patient with Sjögren’s syndrome (14, 15). Secondary gene rearrangements, involving VH replacements were found in clonally related B cells that expanded locally in the synovial tissues of patients with rheumatoid arthritis (32). High Ag load may be the driving force for localized B cell expansion. Although these studies report local clonal expansion of B cells, they lack information on Ab specificity.

Ocular toxoplasmosis is a recurrent disease whereby the parasite periodically reactivates from intracellularly hidden tissue cysts that are invisible to the (humoral) immune system. GRA-2 is expressed by the encysted and tachyzoite stages of the parasite and has been shown to induce both a humoral and cellular immune response during chronic infection (17–20). Activation of GRA-2-specific B cells should include all Ag-activated B cells carrying the BCR specific for GRA-2 epitopes. Given the possibility of regional expansion of B cells, it seems possible that Ag-activated B cells expand locally in the eye after reactivation of the T. gondii cysts. The results from this study suggest that the microenvironment of the eye supports a selective differentiation of B cells into Ab-secreting plasma cells. Because the eye contains cellular infiltrates of both B and T lymphocytes during inflammation (28, 29), there is reason to believe that Ag-specific T cell help occurs for these B cells. It has recently been shown that also memory B cells can be restimulated to differentiate to plasma cells outside of germinal centers and lymphoid tissue (33). From the combined observations of several studies concerning Ab synthesis in the CNS in response to Ags injected into rat brains reviewed by Harling-Berg et al. (34), it was hypothesized that at some point during B cell development two populations of Ag-activated lymphocytes emerge. Whereas one population remains in the lymph nodes expressing effector functions (Ab secretion by plasma cells), a second population of Ag-activated B and Th2 lymphoblasts exits from the nodes while paused in their development. The local Ab synthesis is the result of these Ag-activated lymphoblasts resuming their lymphocyte development after they locate their cognate Ag in other organs (including nonlymphoid). Wilbanks and Streilein (35) have demonstrated that Ags persist in the eye for long periods of time after inoculation. A possible explanation for the results of this study therefore would be that differentiation of intraocular B cells into plasma cells may originate in the competition for Ag binding. B cells with their specific BCR are efficient APC; therefore, B cells with a BCR that binds most efficiently would expect to get more T cell help for differentiation and expansion as compared with B cells with BCR with lower affinity for the same Ag. This mechanism of selection would not occur in the same manner in the spleen because activation of B cells in this compartment requires Ag, or APC loaded with Ag to travel to the spleen followed by the activation of specific B cells. Although selection of B cells carrying specific BCR in the spleen also takes place by competition for Ag on the surface of follicular dendritic cells, it may be to a lesser extent and/or different from that in the eye. Therefore, whereas the selection of specific B cells in the lymphoid organs requires processed Ag expressed by professional APC, the selection within the inflamed eye may depend on Ag capture by Ag-activated B cells paused in their development and available T cell help. It may reflect a mechanism to steer the humoral response locally toward higher efficiency. Consecutive recurrences of the inflammation would be expected to show a shift from the initial peripheral humoral response toward the epitopes that are most efficiently recognized by B cells.

The proposed mechanism of regional humoral immune deviation should be studied in more detail to confirm its validity. Immune privilege may not be a prerequisite for this phenomenon, but the compartmentalized inflammation, recurrence of inflammation (infectious or autoimmune), and Ag load may be the provisional factors.

If our hypothesis is valid, the analysis of paired serum and OF samples could help in the diagnosis of ocular toxoplasmosis. Differences in Ag specificity within paired samples would be indicative of a recurrence of infection whereas equal Ag reactivity should indicate a primary ocular infection with T. gondii. This notion is corroborated by a previous observation of similar T. gondii Ag recognition patterns between paired serum and OF samples of only 2 of 13 patients with ocular toxoplasmosis. These two patients showed evidence of a recently acquired T. gondii infection (11).

To our knowledge, this is the first study to report functional differences in epitope recognition of locally produced IgG compared with systemically produced IgG. We suggest that the underlying mechanisms are the differences of Ag uptake and presentation in the eye compared with the spleen whereby competition for Ag binding by BCR and local T cell help may be key factors to the local differentiation of specific B cells into Ab-secreting plasma cells. Whatever the underlying mechanisms of the deviant ocular Ab response may be, our results suggest that selection and/or activation does occur locally outside of lymphoid tissue, leading to a nonrandom distribution of B cells. This may contribute to our understanding of the regulation of B cell responses during infection.

**Acknowledgments**

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