Natural adaptive immune responses in humans against Toxoplasma gondii and Herpes simplex virus type I
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Chapter 4

Herpes Simplex Virus Infection of the Human Eye Induces a Compartmentalized Virus-Specific B cell Response

Submitted

Ron Peek, Georges M.G.M. Verjans and Bob Meek

Abstract

Intra-ocular infection with herpes simplex virus (HSV) can cause uveitis, a potentially sight-threatening disease. The disease is characterized by an ocular infiltration of inflammatory cells like macrophages, B and T cells. The characteristics of the local humoral and cellular immune responses elicited upon intra-ocular HSV infection are poorly understood. The local herpes virus-specific antibody production, routinely used for confirmation of a clinical diagnosis of herpetic uveitis, has never been analyzed in detail. In the present study we have analyzed the humoral immune response against HSV type 1 (HSV-1) in paired samples of intra-ocular fluid and serum of patients with intra-ocular herpes virus infection. In addition, the B cell epitope distribution on a single HSV-1 type-specific antigen, glycoprotein G, was compared for these paired samples. The results presented in this study indicate that inflamed eyes of patients with HSV-induced uveitis display a compartmentalized B cell response directed to the triggering virus.
**Introduction**

Infection with herpes simplex virus type 1 (HSV-1) is widespread in the human population and a significant health concern. The clinical expression of HSV-1 related disease varies and ranges from self-limiting illness to fatal disease. Ocular complications include infection of the eyelid, conjunctiva, cornea and retina. HSV infection of the eye is the most common infective cause of blindness in developed countries, mainly due to its recurrent nature [73,74]. Although HSV-2 infection has also been implicated in ocular disease, especially in younger patients [75-77] most cases of orofacial herpes infection are attributable to infection with HSV-1 [73,78,79].

Inflammation of the human eye elicited upon herpetic infections is characterized by the influx of mononuclear inflammatory cells. The cell infiltrate is mainly composed of T cells, whereas limited numbers of macrophages and plasma cells are predominantly found in the affected ocular tissues [80-83]. However, the local microenvironment strongly influences the expression of immunity to local antigens, a phenomenon known as "immune privilege". Various anatomical, cell bound and soluble factors contribute to the immune privilege of the eye resulting in impaired T cell responses (reviewed in [84] and [85]). The humoral immune response remains intact and HSV-specific antibodies can be detected in intra-ocular fluids [86]. In addition to detection of viral DNA by PCR, the relative titers of these locally produced virus-specific antibodies are routinely used for confirmation of a suspected clinical diagnosis of ocular HSV infection. However, the specificity and functional properties of these virus-specific intra-ocular antibodies remain enigmatic [87]. Recently, we have analyzed the specificity of intra-ocular antibodies of uveitis patients with an ocular infection caused by the protozoan parasite *Toxoplasma gondii*. Marked differences between the intra-ocular and systemic *T. gondii*-specific IgG response were detected. Some antigens were preferentially recognized by serum or ocular-derived antibodies, suggesting a non-random distribution of IgG producing plasma cells between theocular and systemic compartment [88,89]. Besides differences in clinical presentation between HSV- and *T. gondii*-induced uveitis the life cycles of these two pathogens differs considerably. While *T. gondii* has a complex life cycle involving several stages that uniquely express numerous antigens, HSV displays only a very limited variability in antigens. Therefore, egress and presentation of their antigens intra-ocularly and the subsequent induction of a local immune response may differ between both uveitis entities.

In the present study, the HSV-specific intra-ocular humoral immune response in eyes of patients with HSV-induced uveitis was characterized. The IgG antibody repertoires from paired serum and ocular fluid (IOF) samples were analyzed by immunoblotting of a total HSV-1 extract. Additionally, the B cell response to the HSV-1 type-specific antigen glycoprotein G (gG1) and a series of C-terminally truncated mutants of gG1 was studied by IgG epitope mapping. The data presented show that affected eyes of patients with HSV-induced uveitis contain antibodies with different specificities than the serum. This difference was not only observed for different HSV-1 antigens, but also for the distribution of B cell epitopes on gG1. To our knowledge this is the first report on a compartmentalized B cell response in humans during viral infection.

**Material & Methods**

*Patient materials:* The patients' samples analyzed in this study were sent to the Netherlands Ophthalmic Research institute by ophthalmologists for confirmation of a clinical diagnosis of herpes-virus-induced uveitis. All patients were sampled during an acute episode of uveitis and did not receive pre-operative anti-viral or immunosuppressive treatment. Serum and IOF samples, either aqueous humor from the anterior chamber or vitreous fluid from the vitreous body, were obtained from patients during therapeutic pars plana vitrectomy or paracentesis for diagnostic purposes. The IOF and serum were routinely tested for IgG antibody against *T. gondii*. Herpes simplex
**Table 1**: Patient data. The HSV Goldmann-Wittmer coefficient (HSV GWc) is a measure for specific anti-HSV intraocular antibody and is expressed as the quotient of the relative amounts of HSV-specific antibody, corrected for total IgG, in the IOF sample and serum (see materials and methods). Anti-HSV IgG antibody titers were determined by HSV-specific ELISA, or by an immunofluorescence assay (indicated by °). Note that the IOF sample of patient 18 was PCR positive for HSV-2.

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**Intraocular B cell response induced by HSV infection**

**SDS-PAGE and Western blot analysis:** A commercially available purified HSV-1 extract (MacIntyre strain; ABI, Columbia, Maryland) was used to prepare immunoblots containing total HSV-1 proteins. Proteins were size fractionated by electrophoresis on 10% SDS polyacrylamide slab gels and transferred to PVDF membranes.
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(Immobilon-P, 0.45 μm pore size; Millipore, Bedford, MA). Transferred proteins and markers were visualized using ponceau red dye staining. Protein binding capacity of the blots was saturated by incubation with Tris-buffered saline (TBS: 50 mM Tris-HCl and 150 mM NaCl [pH 10]) containing 0.5% Tween-20 and 2% nonfat powdered milk (blocking buffer; Biorad, Hercules, CA). Blot strips were incubated with patients’ samples diluted 1:500 (except when stated otherwise) in TBS containing 0.5% Tween-20 and 0.03% nonfat powdered milk (TBS-T). Paired IOD and serum samples were always tested on adjacent blot strips from the same gel allowing precise alignment afterwards. After washing in TBS-T strips were incubated with horseradish peroxidase (HRPO)-conjugated goat-anti-human-IgG (DAKO, Glostrup, Denmark) for one hour. After washing in TBS-T strips were soaked in detection reagents (ECL, Pharmacia, UK) and covered with a polyethylene sheet. Exposures for various time intervals to X-ray film were made. Following analysis of the patients’ samples, blots were stripped from antibody by incubation with 62.5 mM Tris pH 6.8, 2% SDS and 100 mM β-mercaptoethanol for 30 min at 70°C. Blots were again blocked and re-probed with horseradish peroxidase-conjugated anti-GST mAb (Biotech. Buckinghamshire, UK) to visualize the quantity and integrity of the recombinant glutathione S-transferase (GST)-gG1 fusion proteins.

Construction of the HSV-1 glycoprotein G expression plasmid and deletion mutants: The open reading frame (ORF) of HSV-1 gG was amplified from a purified HSV-1 extract (ABI, Columbia, Maryland) using *Pfu* DNA polymerase (Stratagene, La Jolla, California) with the addition of 5% DMSO and equimolar amounts of dGTP and 7-deaza-2'-dGTP to circumvent the formation of secondary structures due to the high G+C content of the target sequence [91]. The sequences of the primers for amplifying the complete ORF (amino acid (aa) 1-238) were: 5'GAGACCATGCGGCGGGCGCCAT 3' (primer 1) and 5'GAGAATTCTACCCGCGTTCGGACGGC 3'. This cloning procedure created an *NcoI* site at the 5' end covering the initiation codon and introduced an amino acid substitution at position 2 (Ser→Ala). For constructing the C-terminal deletion clones primer 1 was used in combination with

5'GAGAATTCTCTTTGAGGTAGTCGGGGT 3' (∆C1; aa1-165), 5'GAGAATTCTCTTGAGGTAGTCGGGGT 3' (∆C2; aa1-145), 5'GAGAATTCTCTTGAGGTAGTCGGGGT 3' (∆C3; aa1-125), 5'GAGAATTCTCTTGAGGTAGTCGGGGT 3' (∆C4; aa1-105), 5'GAGAATTCTCTTGAGGTAGTCGGGGT 3' (∆C5; aa1-85), 5'GAGAATTCTCTTGAGGTAGTCGGGGT 3' (∆C6; aa1-65),

5'GAGAATTCTCTTGAGGTAGTCGGGGT 3' (∆C7; aa1-45) and

5'GAGAATTCTCTTGAGGTAGTCGGGGT 3' (∆C8; aa1-25). PCR fragments were gel purified, digested with *Neol/EcoRI* and ligated into a derivative of pGEX-3X plasmid (Pharmacia, Uppsala, Sweden) to produce GST-gG1 fusion proteins.

Expression and purification of recombinant proteins: Cultures of *Escherichia coli* strain DH5α transformed with GST-HSV-1 expression constructs were grown at 37°C to an OD₆₀₀ of 1. Expression of fusion protein was induced by the addition of isopropyl-α-D-galactopyranoside to a concentration of 1 mM. After 2h at 30°C bacteria were pelleted, resuspended in PBS containing protease inhibitors (Complete, Boehringer Mannheim, Germany) and lysed by repeated freezing and thawing. After sonication the extract was treated with DNase I (50 μg/ml) for 20 min at 37°C. Insoluble material was removed by centrifugation and the supernatant containing the recombinant protein was filtered through a 0.45 μm filter. To purify the recombinant protein, the cleared extract was incubated with glutathione agarose beads (Pharmacia) for 1h at room temperature. After extensive washing with PBS the glutathione agarose beads were resuspended in SDS-sample buffer. The concentration of the purified recombinant protein was determined by analysis of a serial dilution on protein gels stained by Coomassie, using BSA as a standard,
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The signal obtained with the anti-GST mAb.

Absorption of gG1-specific antibody: Patient sera were diluted 1:2000 in TBS-T and incubated with blot strips containing 25 µg of recombinant GST-gG1 or control blot strips containing E. coli protein. To saturate all protein binding sites, the strips were soaked in blocking buffer for 1 h. Blot strips were incubated for 1 h with diluted serum in 500 µl wash buffer. Part of this pre-absorbed serum was used for a second and third round of depletion. After each round of absorption part of the diluted serum was incubated with blot strips containing recombinant gG1 protein and blot strips containing size fractionated total HSV-1 protein. To determine the exact position of the various glycosylated forms of native gG1 on the blots strips containing total HSV-1 protein, an anti-gG1 mAb was used (clone LP10; kindly provided by A.C. Minson). Bound antibodies were visualized by incubation with HRPO-conjugated secondary antibodies followed by chemiluminescence.

Results

Differential HSV-1 antigen recognition by IgG in paired serum and IOF samples: The 18 patients included in this study had a clinical picture of herpetic uveitis. The patients 1-10 and 12-17 had an intra-ocular antibody synthesis against HSV, but not to VZV, CMV or T.gondii,(Table 1). Control patients 11 and 18 had a VZV- or HSV-2-induced uveitis, respectively. On the basis of clinical and laboratory data the ocular lesions were typed as HSV-induced uveitis. The seroprevalence to HSV-1 and -2 was determined using gG-based HSV type-specific commercial assays. Whereas all patients were seropositive for HSV-1, the sera of patients 2 and 6-8 also contained antibodies against HSV-2 (Table 1). Paired serum and IOF samples of 10 of these patients were analyzed for the presence of anti-HSV-1 IgG by Western blotting, using a purified extract of total HSV-1 proteins. A diverse banding pattern was observed on the immunoblots (Fig. 1). In addition to major antigens of mainly high molecular weight, which were efficiently recognized by most of the samples, minor antigenic viral proteins of various molecular weights were observed. Large differences in antigen recognition were detected between sera of individual patients. More interestingly, the paired serum and IOF samples of all patients with a positive GWc for HSV displayed clear differences in antigen recognition (Fig. 1). For example, several high molecular weight antigens are intensely stained by IgG from the ocular compartment of patient 2, but hardly visible in the corresponding serum lane. In some patients particular antigens appeared to be recognized in one compartment only, as for example the antigen running at ~100 kDa in the ocular compartment of patient 10, and the ~80 and ~50 kDa antigens in the corresponding serum lane. There were no particular antigens consistently recognized by the patient samples. Also patient 18, with a confirmed diagnosis of intra-ocular HSV-2 infection and no detectable anti-HSV-1 IgG in either serum or IOF (Table 1),

Figure 1: Immunoblot analysis of a size fractionated total HSV-1 protein extract with paired serum (s) and ocular fluid (o) samples of herpetic uveitis patients.

Patients 1-10 and 18 suffered from a HSV-1 or -2-induced uveitis, respectively. Patient 11 was diagnosed with an intra-ocular VZV infection. Numbers on the left represent molecular weight markers (in kDa).
showed a differential antigen recognition profile between the serum and the IOF (Fig. 1). As disease controls several patients with a negative GWc (<3) for HSV-specific IgG and suffering from intra-ocular infection due to T. gondii or VZV were tested. While some of these patients did have HSV-1-specific IgG in either serum or both compartments (Fig. 1, patient 11 and data not shown) no differences in antigen recognition were observed. These results demonstrate that the HSV-1 antibody response is compartmentalized, but does not provide any information on the epitopes of a single HSV antigen recognized by antibodies in these compartments.

**Recognition of a single HSV antigen by IgG in serum and IOF samples:** To further characterize the HSV-1-specific intra-ocular humoral immune response as compared to the serum, the IgG epitopes of a single defined HSV-1 antigen were studied in detail. For this purpose gG1 was selected. The humoral immune response to this virus envelope protein is devoid of serologic cross-reactivity between the HSV serotypes or other human herpes viruses [92-94]. Furthermore, human IgG serum epitopes of gG1 have been identified [95]. The complete gG1 ORF was amplified by PCR and cloned in a bacterial expression vector allowing production of a recombinant glutathione (GST)-gG1 fusion protein. Based on the amino acid sequence, the calculated molecular mass of gG1 is about 27 kDa. In contrast to bacterial expressed gG1, the native protein is differentially glycosylated in HSV-1-infected cells, leading to several products with apparent molecular masses between 38 and 50 kDa [93,95]. This indicates that a major part of native HSV-1 gG is composed of carbohydrate residues that, in addition or in combination with the primary amino acid sequence, might also be targeted by the IgG antibody response. To validate the use of the (non-glycosylated) GST-gG1 recombinant fusion protein for the analysis of IgG epitopes, a patient serum with a high anti-gG1 IgG titer (patient 9) and a patient serum with a low titer (patient 4; results not shown) were incubated with solid-phase bound GST-gG1. The efficiency of absorption was monitored by incubating the non-absorbed and absorbed fractions of the patient sera on immunoblot strips containing either recombinant GST-gG1 or an extract of total HSV-1 proteins. As shown in Fig. 2, a single pre-incubation of diluted serum of patient 9 with solid phase bound recombinant fusion protein reduced the reactivity with recombinant GST-gG1 considerably (compare lanes 1 and 2), while additional absorptions completely abolished recognition of the recombinant protein (lanes 3 and 4). These same pre-absorbed fractions were tested in parallel with an anti-gG1 mAb (lane 5) on blot strips containing total HSV-1 protein extract (lanes 5-9). These analyses demonstrated that the antibodies against the various forms of native gG1 are efficiently and specifically absorbed from the diluted serum (compare lane 6 with 7-9). Control pre-incubations of diluted serum with solid phase bound E. coli proteins did not show a specific absorption (lanes 10-17). Similar results were obtained with serum of patient 4 (not shown). These data suggest that the IgG immune response to gG1 is mainly directed against the primary amino acid sequence and not against the extensive post-translational
Intraocular B cell response induced by HSV infection

Figure 2: Depletion of serum-derived HSV-1 glycoprotein G (gG1)-specific antibodies by solid phase bound recombinant GST-gG1.

Diluted serum of patient 9 was sequentially incubated with blot strips containing 25 μg of GST-HSV-1 gG fusion protein. After incubation part of the diluted serum was tested on blot strips containing recombinant protein (lanes 2-4) or total HSV-1 protein (lanes 7-9). In lane 5 the gG1-specific mAb was used and in lanes 1 and 6 reaction of the non-absorbed serum is shown. The control experiment is shown at the right (lanes 10-17), where the serum was sequentially incubated with blot strips containing solid phase bound E.coli proteins. Arrowheads point to the various glycosylated forms of gG1. The molecular weight markers (in kDa) are indicated. Note that the recombinant fusion protein is highly susceptible to proteolytic degradation.

modifications. Furthermore, this demonstrated that the GST-gG1 fusion protein is suitable to identify regions of gG1 involved in IgG epitope formation.

To exclude the possibility that HSV-2-specific IgG cross-reacts with gG1, the sera of 4 healthy HSV-2 seropositive controls and the paired serum and IOF of a patient with HSV-2 induced uveitis (patient 18; Fig. 1 and Table 1), were tested for reactivity to GST-gG1. In concordance with other studies [92-94], the HSV-2 specific samples did not show any cross-reactivity with gG1 (data not shown).

To determine the gG1-specific IgG response in serum and IOF, paired samples of 17 patients were tested by immunoblotting with purified GST-gG1 (Fig. 3). All patients with ocular HSV infection had gG1-specific antibodies in their serum, although in some patients the signal was relatively weak (patients 4-6). However, in the IOF sample of 6 patients (3, 4, 7, 12, 14 and 15) no IgG against GST-gG1 could be detected, not even after overexposure (data not shown).

Mapping of B cell epitope regions of gG1 in paired serum and IOF samples: In order to define the gG1 epitopes recognized by antibodies in the patients’ samples, 8 C-terminally truncated gG1 deletion mutants were generated (designated as ΔC1-ΔC8) and expressed as GST fusion proteins (Fig. 4). The mutant GST-gG1 fusion proteins and GST alone were purified and used for immunoblots containing similar amounts of each protein. From the panel of paired samples shown in Fig. 3, seven patients were selected that had gG1-specific IgG in both compartments (patients 1, 2, 8-10, 13 and 16). To minimize the volume of ocular fluid required, the 8 C-terminal GST-gG1 mutants and GST protein were blotted onto small immunoblot strips. Protein sequences involved in B cell epitope formation were identified by a discrete change in the intensity of the chemiluminescent signal of two subsequent mutant proteins (Fig. 5). As expected, the gG1-specific mAb LP10 displayed only a single epitope region between gG1 aa 65 (ΔC6) and aa 45 (ΔC7) (Fig. 5, panel B). The low molecular weight protein consistently

Figure 3: Immunoblot analysis of purified recombinant GST-gG1 fusion protein with paired serum (s) and ocular (o) patient samples.

The position of the full-length fusion protein is indicated at the left. Part of the blot was stained with an anti-GST monoclonal antibody (GST). The position of the molecular weight markers (in kDa) is shown at the left.
recognized in mutants GST-gG1 ΔC1-ΔC6 is most likely a gG1-derived degradation product. Interestingly, major differences in reactivity to the truncated gG1 proteins were observed within and between paired serum and IOF samples of 4 out of 7 patients analyzed. The serum of patient 1 (panel C) was still fully reactive with ΔC4 but showed less intense staining of ΔC5, indicating that the region between ΔC4 and ΔC5 is involved in epitope formation. Further truncation to gG1 aa 65 (ΔC6) completely abolished recognition by the serum of this patient. In contrast, the paired IOF (panel D) displayed only a single epitope region between gG1 aa 125 and 105 (ΔC3-ΔC4). The serum of patient 2 revealed the recognition of two epitope regions (between ΔC2-ΔC3 and ΔC3-ΔC4), while the IOF of this

Figure 4: Schematic representation of the native gG1 (top) and recombinant GST-gG1 fusion proteins.

The positions of the signal sequence (signal), the immuno-dominant region (IDR) and the putative transmembrane region (TMR) are indicated. Mutants ΔC1 – ΔC8 were generated by PCR amplification. The numbers refer to the position of the amino acids of gG1.
Figure 5: Mapping of B cell epitopes on HSV-1 glycoprotein G.

Blot strips with similar amounts of different mutant GST-gG1 proteins (ΔC1–ΔC8) or GST were incubated with patient samples (panels C–P). Panel B was incubated with a gG1-specific mAb and panel A with an anti-GST mAb. Arrowheads indicate discrete changes in signal intensity between two subsequent deletion clones. Note that of each blot several exposures for various time intervals were made but a single, most informative exposure is shown.

Patient (panel F) did not have a major epitope in these regions but displayed only one epitope region between gG1 aa 105-85 (ΔC4–ΔC5). The serum of patient 10 (panel K) recognized three epitopes in the region between gG1 aa 165-85. The paired ocular fluid had no major epitopes in this region but depended on sequences between aa 85-45 for full recognition of gG1. The IOF sample of this patient showed a similar reactivity pattern as the gG1-specific mAb LP10 (panel B), recognizing an epitope located between gG1 aa 65-45 (panel L). Both samples of patient 16 (panels O and P) depended on sequences between gG1 aa 85 and 65 (ΔC5–ΔC6) for epitope formation, while the serum displayed an additional epitope region between gG1 aa 105 and 85. For the paired samples of patients 8, 9 and 13 no differences in epitope recognition were observed (panels G–J, M and N).

Discussion

In the present study we have analyzed the HSV-specific IgG repertoire in the ocular compartment of patients with herpetic uveitis and compared this with the systemic compartment. In the patients tested for reactivity to total HSV-1 protein extract, several antigens were predominantly or exclusively recognized in one compartment. Ocular inflammation can disrupt the blood-eye barrier, leading to influx of serum proteins like immunoglobulins. This phenomenon may account for the recognition of HSV-1 proteins by IOF samples of patients with non-HSV-induced uveitis (e.g. patient 11 suffering from a VZV-induced uveitis). However, these patients will have a GWc less than 3 for HSV, indicating that the observed anti-HSV antibodies in the IOF are not produced locally but probably are extravasated from the systemic compartment. The differential recognition of various HSV-1 antigens by patients with a GWc exceeding 3, strongly suggests a compartmentalized HSV-1-specific B cell response. Within this cohort, sera of 4 patients contained IgG against both HSV serotypes, suggesting that some differences in intensity of antigen recognition observed in either compartment might be the result of cross-reactive antibodies. Antibodies against HSV-2 are known to cross-react with HSV-1 proteins [96] and the paired serum and IOF sample of a patient with HSV-2-induced uveitis (patient 18) tested by immunoblot indeed confirmed this. However, also these anti-HSV-2 antibodies clearly demonstrated compartmentalization of the antibody response. To further exclude the possibility of HSV cross-reactivity and to study the compartmentalized B cell response in more detail, the analyses were extended by mapping the IgG epitopes of a single HSV-1 antigen: gG1. In concordance with previous studies [92-94], our data show that gG1 is not recognized by sera of HSV-2 seropositive donors or an HSV-2 positive ocular sample, illustrating that gG1 enables the analysis of HSV-1 type-specific B cell responses. Furthermore, our results suggest that the gG1-specific IgG response is mainly directed towards the primary amino acid sequence and not to the extensive post translational modifications of gG1. Therefore, the HSV-1 gG fusion protein is suitable to map the major linear epitopes of this antigen.

Paired serum and IOF samples of 16 patients with HSV-induced uveitis were analyzed for reactivity to recombinant GST-gG1. Whereas all patients had gG1-specific antibodies in their serum, 7 IOF samples did not contain detectable amounts of gG1-specific IgG. Because these IOF samples did contain antibodies against several other HSV-1 antigens when tested on blots containing total native HSV-1 protein, this again demonstrated a compartmentalized B
cell response in these patients. Among the 7 patients with anti-gG1 reactivity in both compartments, 4 showed marked differences in IgG epitope distribution on gG1 between the IOF and the serum sample. Although the other 3 patients appeared to recognize the same regions of gG1 in both compartments, this does not exclude the possibility that also these patients display differences in IgG epitopes between both compartments. The 20 aa deletions between sequential GST-gG1 deletion mutants may contain more than one B cell epitope. Furthermore, the technique employed to identify epitopes was based on discrete changes in intensity of the chemiluminescent signal and will probably not detect minor epitope regions. The immunodominant region of gG1 identified in this study maps in the central part of the protein between aa 65–125 and overlaps with the region delimited by aa 112–127 identified using gG1-specific synthetic peptides [95].

In autoimmune diseases local oligoclonal IgG production has been reported for the synovial tissues of patients with rheumatoid arthritis [97], salivary glands of patients with Sjögren’s syndrome [98,99] and cerebrospinal fluid of patients with multiple sclerosis [100]. In contrast to the current study however, the antigens recognized by these locally produced antibodies in autoimmune disease were not defined.

A possible cause that may be responsible for compartmentalized antibody responses could be that several pathogens have various stages during their life cycle that display marked differences in antigenic composition. These differences may be responsible for a compartmentalized humoral immune response against complex eukaryotic pathogens like T. gondii [88,89], but are unlikely to account for the observed compartmentalized antibody response against pathogens like HSV, that have a limited set of invariable antigenic components.

The uveitis patients analyzed in our study are likely to have acquired HSV-1 prior to development of ocular disease [101]. This implies that mainly memory B cells are triggered in the draining LN of ocular fluid and subsequently infiltrate the eye. It has been demonstrated that memory B cells do not depend on secondary LN or spleen to develop into plasma cells, but do require complementary and activated T helper cells. The ocular compartment contains several Th2 cell associated cytokines that support B cell responses and differentiation [102-105]. Ocular inflammation in patients with HSV-induced uveitis is commonly associated with the ocular infiltration of T cells directed to the triggering agent, hence the entire process of activation and differentiation into plasma cells could take place in the eye, depending on availability of specific antigen. It is not known if B cells are able to cross the blood-retina barrier without prior instruction in LN. However, in vitro studies with retinal pigment epithelial cells have shown that these cells respond to infection by secreting cytokines and expression of adhesion molecules [106], which might support chemotaxis and entry of lymphocytes into the ocular compartment.

In this paper we present evidence for a compartmentalized B cell response in eyes of patients suffering from an HSV-induced uveitis. This phenomenon is not restricted to viral infections, since analogous results have recently been observed in patients infected with the protozoan parasite T. gondii [89]. The type of immune cells activated, the combined activity of local immunoregulatory molecules and the antigenic load are likely to be the key factors to determine the intra-ocular differentiation of B cells into plasma cells upon infection with either HSV or T. gondii. HSV-specific IgG synthesized within the affected eye may have a beneficial role in clearing the virus from the eye (e.g. virus neutralizing activity) or exert a pathogenic effect on ocular cells by the induction of antibody dependent cell-mediated cytotoxicity. Future studies are mandatory to unravel the role of these antibodies in HSV-induced uveitis.