Polymorphonuclear Leukocyte Infiltration Into the Subretinal Choroid and Optic Nerve in Response to Leukotrienes

Achim H.-P. Krauss and David F. Woodward

Purpose. To describe the pattern of leukocyte infiltration in ocular anterior and posterior segment tissues in response to local administration of LTB₄ and LTD₄.

Methods. Leukocyte infiltration after intravitreal administration of LTB₄ or LTD₄ was assessed in ocular sagittal cross-sections and compared with vehicle-treated control eyes.

Results. A dose-dependent eosinophil infiltration was observed in the subretinal choroid and the ora serrata region of the ciliary body in response to both LTB₄ and LTD₄, but only LTB₄ behaved as a chemoattractant for neutrophils. Subretinal eosinophils achieved Bruch’s membrane in response to LTB₄ but, though gathered in several foci, this important barrier was not breached and leukocytes did not reach the neural retina. Eosinophils and some neutrophils also achieved the optic disc in response to LTB₄. Tissue damage to the optic nerve head coincided with the presence of degranulating eosinophils, indicating that visual impairment may result from damage to the optic nerve head, with the retina left intact. Apart from the ora serrata and pars plana, no leukocyte infiltration in other anterior segment tissues—such as the pars plicata, ciliary process, or iris proper—was apparent.

Conclusions. It appears that the ingress of leukocytes into intraocular tissues of the eye in response to leukotrienes is discretely regulated, probably at the level of the vasculature.

Intraocular inflammations can be characterized histopathologically as purulent or nonpurulent, granulomatous or nongranulomatous, depending on the stage of the disease and the pattern of infiltrating white blood cells. An inflammatory response is a protective mechanism of the host to defend against infections and to promote tissue repair. However, an acute or prolonged inflammatory process can lead to deleterious tissue alterations.

Leukotrienes (LTs) are widely considered as potentially important inflammatory mediators. Leukotriene B₄ (LTB₄) is a potent chemoattractant for neutrophil and eosinophil leukocytes, whereas peptidoleukotrienes have been shown to cause eosinophil infiltration in the conjunctiva and increased microvascular permeability in several other tissues. The effects of LTs on the conjunctiva are pronounced. In addition to the marked eosinophil infiltration that occurs in response to LTB₄, LTD₄, and LTE₄, the peptidoleukotrienes are potent microvascular permeability factors in the conjunctiva. Studies with leukotriene antagonists also indicate that peptidoleukotrienes play a significant role in experimental allergic conjunctivitis. However, the microvascular permeability increases evoked by LTs in the conjunctiva do not readily occur in ocular anterior segment tissues. Moreover, LTB₄ and the peptidoleukotrienes do not seem to disrupt substantially the ocular blood-aqueous barrier. LTB₄ causes marked leukocyte infiltration into the anterior chamber but, apart from peptidoleukotriene-induced miosis in the cat, this appears to be the most consistent LT effect in the ocular anterior segment.

Many previous investigations on LT-induced ocular segment inflammation have involved sampling of aqueous humor for protein and leukocytes and measurement of intraocular pressure. Such studies do not, however, provide information regarding the pa-
thology of the actual tissues that comprise the ocular anterior segment. Therefore, the intention of the studies described herein was to examine directly the pathologic consequences of locally administered LTs on ocular anterior segment tissues. Posterior tissues, including the retina, were similarly examined to assess the potential pathologic consequences of local LT release.

METHODS

The studies were performed using albino guinea pigs of the Hartley strain. Treatment of animals was in compliance with the ARVO Resolution on the Use of Animals in Research. Animals of both sexes weighing 300 to 500 g were employed. Cellular infiltrates were investigated 6 hours after intravitreal administration of LTs. For that purpose, the guinea pigs were anesthetized with a mixture of 37.5 mg/kg ketamine and 1.5 mg/kg xylazine. Each animal received a solution containing LTB₄, LTD₄, or a combination of equal doses of these LTs into the test eye, and saline into the contralateral control eye. Solutions were administered in a 20-μl volume using a 50-μl Hamilton syringe. After intravitreal injection, each eye was immediately washed with 1 ml of a sterile saline solution to remove any LT solution that may have leaked onto the surface of the globe during removal of the needle. The animals were returned to their familiar environment, and the anesthesia wore off after approximately 45 minutes. The test animals and, for control purposes, two untreated animals were sacrificed 6 hours after intravitreal injection with 1 ml of T-61. The eyes were carefully removed and fixed overnight in Karnovsky buffer containing 1% glutaraldehyde and 4% formaldehyde in a neutral buffer. Fixation was continued for an additional 24 hours in neutral buffered formalin. The fixed globes were washed in water for approximately 6 to 8 hours and then transferred for at least 24 hours into 60% reagent alcohol. The hardened globes were sagittally hemisected. After gentle dehydration with increasing concentrations of reagent alcohol and finally chloroform, the hemisected globes were embedded in paraffin. Two 6-μm sections of each eye, at least 500 μm apart, were stained with Luna’s stain for eosinophils.

Eosinophils and neutrophils were counted at 400X magnification using an ocular lattice grid. To avoid biased results, a number of high-power fields (1 HPF = 0.025 mm²) per tissue type and section were examined according to predetermined rules. For each tissue type and eye, cell counts were expressed as the average of all HPFs investigated. The number of HPFs was dependent on the tissue size and was as follows (per eye): iris proper, 12; ciliary process, 8; pars plicata, 8; pars plana, 8; ora serrata, 4; choroid, 12; retina, 12. For each animal, cell counts were expressed as the contralateral difference of test eye minus control eye.

Statistical analysis was performed using the Student’s t-test or the Wilcoxon signed rank test for paired observations (test versus contralateral tissues).

RESULTS

Cell counts in different ocular tissues after challenge with LTB₄, LTD₄, and the combination of both LTs are depicted in Figures 1, 2, and 3, respectively. LTB₄ elicited a dose-dependent eosinophil and neutrophil infiltration into the subretinal choroid and the pars plana region of the ciliary body, with the largest response in the ora serrata. Although no neutrophils were observed in LTD₄-treated eyes, LTD₄ was a potent chemoattractant for eosinophils that dose-dependently accumulated in the choroid and pars plana region of the ciliary body, including the ora serrata. An infiltration pattern similar to that of LTB₄ alone was obtained with LTB₄/LTD₄ combinations in virtually all tissues. Despite the large cell numbers in the posterior uvea, anterior ocular tissues, such as iris, pars plicata, and ciliary processes of the ciliary body, remained virtually unaffected. With respect to the anterior segment, these findings are in close accordance with the inability of intracameral administered LTs to cause an ingress of inflammatory cells into the anterior chamber. These results appear to eliminate the iris and ciliary processes as a possible site for inflammatory cells to enter the vitreous in favor of the pars plana region of the ciliary body.

The patterns of leukocyte infiltration in response to locally administered LTB₄ and LTD₄ in anterior and posterior regions of the globe are shown photomicrographically in Figures 4 and 5, respectively. Interestingly, influx of leukocytes into the vitreous was observed only when LTB₄ was present (Fig. 4). For LTB₄, eosinophils in the choroid and pars plana were clearly directed toward the vitreal chemoattractant, as depicted in Figures 4 and 5. In the subretinal choroid, eosinophils achieved Bruch’s membrane in response to LTB₄ gathering in several foci (Fig. 5a). Despite the apparent attack, Bruch’s membrane—the important barrier separating the retina from the highly vascularized choroid—remained intact, preventing infiltration into the neural layers of the retina. LTD₄ appeared to cause a diffuse eosinophil infiltrate that did achieve Bruch’s membrane (Fig. 5b). Although neutrophils emigrated into the subretinal choroid in response to LTD₄, these did not exhibit clear directional motility toward the neural retina (Fig. 5a).

Figure 6 shows infiltrates predominantly comprised of eosinophils, with some neutrophils in the optic disk and the optic nerve in eyes challenged with LTB₄. It appears that the optic nerve head suffered
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FIGURE 1. Eosinophil (left panel) and neutrophil (right panel) infiltration into intraocular tissues after intravitreal injection of LTB₄. Cell counts are given as mean ± SEM of the contralateral difference between test and control eyes and were obtained as described in the Methods section. Symbols for the different tissues are explained in the insert in Figure 2. n = 6 per dose. * P < 0.05, + P < 0.01.

some structural damage in the center of the disk. The tissue structures that distinctly outline the optic disk in control eyes (Fig. 6b) lost their normal integrity in LTB₄-treated eyes (Fig. 6a). These findings were coincident with the presence of fragmented eosinophils and eosinophil-derived granular deposits, indicating that these changes were due to the action of inflammatory cells.

The retina did not appear to be the target of inflammatory cells from the vitreal side despite the large numbers of cells in the vitreous at high LTB₄ doses. Vitreal infiltrates were comprised of eosinophils, neutrophils, and mononuclear cells, none of which could be observed in any retinal layer regardless of the chemotactant used.

DISCUSSION

In this study, we assessed the pattern of leukocyte infiltration into ocular tissues of the anterior and poste-

FIGURE 2. Eosinophil (left panel) and neutrophil (right panel) infiltration into intraocular tissues after intravitreal injection of LTD₄. Cell counts are given as mean ± SEM of the contralateral difference between test and control eyes and were obtained as described in the Methods section. Symbols for the different tissues are explained in the insert. n = 6 per dose. * P < 0.05, + P < 0.01.
prior segments in response to locally administered LTB4 and LTD4. The guinea pig was selected as a model because it has been used by many investigators to study allergic and inflammatory responses in the eye and skin. Like humans, the guinea pig is particularly responsive to exogenously administered peptidoleukotrienes.5-10 Immediate hypersensitivity reactions in the eye also appear to be similar in humans and guinea pigs.8,16,17 Furthermore, synthesis of lipoxygenase products has been demonstrated in ocular tissues of many species, including guinea pigs.18

Our investigations confirm that leukotrienes are potent inflammatory mediators with pronounced effects on leukocyte recruitment at nanogram doses. Yet, these effects are tissue specific. The most striking observation was the vast number of polymorphonu-
clear leukocytes appearing in the choroid and the pars plana region of the ciliary body, which are highly vascularized uveal tissues. The iris, pars plicata of the ciliary body, and ciliary processes showed no or little ingress of inflammatory cells. Although it may be argued that only minute amounts of leukotrienes reach these sites after intravitreal administration, \(^{19}\) LTs also lack chemoattractant activity in the anterior segment tissues after intracameral administration, \(^{11}\) where they achieve intimate contact with the iris, ciliary body, and ciliary processes. The differential cellular response of anterior and posterior segment tissues to LTs suggests that leukocyte emigration may be regulated at the level of the ocular vasculature.

As has previously been demonstrated in the conjunctiva, \(^{3,8}\) eosinophil infiltration into the posterior segment tissues occurred in response to both LTB\(_4\) and the peptidoleukotriene LTD\(_4\). Similarly, neutrophil infiltration occurred in response to LTB\(_4\) but not to LTD\(_4\). Stimulation with LTD\(_4\) resulted in a uniform distribution of eosinophils in the choroid and pars plana region of the ciliary body, with the densest infiltration occurring at the ora serrata. After challenge with LTB\(_4\), on the other hand, neutrophils and eosinophils were present in the entire choroid and pars plana of the ciliary body was left as the only possible vitreal entry site. The integrity of Bruch's membrane is of particular importance given the deleterious activities of such eosinophil- and neutrophil-derived products as reactive oxygen metabolites and lysosomal enzymes.

Various microorganisms, viruses, autoimmune reactions, and foreign bodies can cause intraocular inflammation with participation of white blood cells. \(^{20-26}\) Both neutrophils and eosinophils are equipped with an arsenal of enzymes necessary for the host-defense against invading microorganisms. \(^{27-35}\) Reactive oxygen metabolites and lysosomal enzymes formed by these cells within the eye could impair or threaten vision, giving particular importance to these polymorphonuclear leukocytes in intraocular inflammatory disorders. Some ocular autoimmune diseases can be mimicked in experimental allergic uveitis (EAU), where the pattern and severity of leukocyte infiltration depends on the dose and type of antigen used. \(^{22,36,37}\) In guinea pigs, eosinophils were only observed after sensitization with high doses of S-antigen, which caused a severe panuveitis. The histopathologic features of EAU, however, do not correlate well with our findings. EAU-induced cellular infiltrates in guinea pigs are predominantly comprised of mononuclear cells. It has been suggested that EAU is a delayed type, immune complex-mediated response. Thus, it remains unlikely that LTs play a role in the development of EAU, at least in mild to moderate forms where eosinophils are absent.

In ocular inflammation, many investigators concentrate their attention on the retina. However, nerve fibers originating in the light-sensitive neurons of the retina eventually merge in the optic nerve. Our studies revealed damage to the optic nerve head inflicted by a cellular infiltrate consisting largely of eosinophils (Fig. 6). Interestingly, the retina was spared though it is potentially in the pathway of polymorphonuclear leukocytes from the choroid and the vitreous. Thus, with the retina left intact, vision could be impaired by gradual destruction of nerve fibers in the optic nerve head. These findings are of particular interest with respect to parasitic ocular lesions that had been, at least in part, ascribed to eosinophilic leukocytes in an animal model and humans. \(^{38,39}\) As a result of a Schistosoma infection in humans, funduscopic changes included swelling of the optic disk and numerous lesions at the level of the RPE and chorioid, leading to the deterioration of visual acuity. \(^{38}\) Examination of a skin biopsy specimen and blood sample revealed an eosinophilic infiltrate around Schistosoma mansoni ova and eosinophilia, respectively. Thus, it is conceivable that the focal ocular lesions described were eosinophil granulomas around the choriocapillaris causing secondary inflammation of the RPE and optic disk. These reports underline the possible deleterious activities of eosinophils on intraocular tissues. When considered with our results, it appears that vision-threatening lesions can develop secondarily to an eosinophil infiltration. However, it remains to be elucidated if and to what extent leukotrienes play a role in inflammatory reactions of parasitic origin, particularly because other inflammatory mediators (e.g., PAF, histamine, ECF-A, and cytokines) are chemotactic for eosinophils as well.

Vitreal infiltration was not achieved by migration of inflammatory cells through the retina. Because the ciliary processes and the pars plicata of the ciliary body were free of PMNs, the pars plana region of the ciliary body was left as the only possible vitreal entry site. The presence of PMNs and mononuclear cells in the epithelium, as demonstrated in Figure 4, is evidence that cells in the normally acellular vitreous must have emigrated from the vascularized uvea along the pars plana region.

Our studies confirm the potent chemoattractant properties of leukotrienes in the eye. Both LTB\(_4\) and
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LTD₄ caused an eosinophilic infiltrate, whereas only LTB₄ provoked an additional ingress of neutrophils. Infiltration was predominantly confined to the highly vascularized choroid and pars plana of the ciliary body, as well as to the avascular vitreous. Although the retina was spared as a target, it is conceivable that the damage inflicted by PMNs in the optic nerve head could result in visual impairment. The role of leukotrienes in the pathophysiology of uveo-retinal inflammatory diseases, however, remains speculative because the therapeutic activity of LT-antagonists or 5-lipoxygenase inhibitors, and other mediators of immune responses, remains to be extensively investigated.

Key Words
PMN, leukotrienes, choroid, optic nerve, leukocyte infiltration

References