Immunopathology of *Toxocara canis* and *Ascaris suum* infections of the eye: the role of the eosinophil

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Intravitreal injection of guinea pigs with second-stage larvae of *Toxocara canis* and second- and fourth-stage larvae of *Ascaris suum* induced intraocular IgE antibodies and a dense eosinophil infiltration in the anterior chamber and throughout the uveal tract. The eosinophil infiltrate began within 1 day after infection and persisted for as long as 51 days. By day 12 after intravitreal infection, the injected ascarid larvae were surrounded by granulomas which consisted almost entirely of eosinophils. Firm adherence of eosinophils to the parasite cuticle, morphologic alterations and degranulation of eosinophils with the deposition of free eosinophil granules on the parasite surfaces, and ingestion of eosinophils and eosinophil granules by the parasite larvae also were observed. Intravitreal injection of a soluble antigen derived from third-stage *A. suum* molting to the fourth-stage in defined media in vitro also induced intraocular IgE antibody and a diffuse ocular eosinophil infiltrate. Dense eosinophil infiltration of the choroid, not immediately adjacent to a parasite larva, was accompanied by destruction of the overlying outer retina, with cystic changes in the retina and between the retina and the choroid. Few eosinophils were observed within the retina, and the retinal destruction may be the result of direct toxic action of constituents of the choroidal eosinophils. Evidence which indicates that the eosinophil is the principal effector cell in immunity to helminth infections and is cytotoxic for the parasites, possible mechanisms for the induction of the ocular eosinophil infiltrates, and evidence for autotoxicity by eosinophils are briefly reviewed, and the potential roles of the eosinophil in the ocular response to helminth parasites are discussed.

Key words: eosinophils, *Toxocara canis*, *Ascaris suum*, ocular immunopathology, eosinophil granuloma, choroiditis, retinal degeneration

Infection of the human eye with larvae of *Toxocara canis* is a potentially blinding disease whose immunopathologic mechanisms are incompletely understood. Infection of the guinea pig eye with *T. canis* or the closely related ascarid *Ascaris suum* provides a useful model of this process. A rapidly growing body of evidence implicates the eosinophil as the primary killer cell for parasitic helminths and some protozoa and as a crucial element of host defense against helminths. Eosinophils have been shown to damage or kill helminths and protozoa in vitro, depletion of eosinophils has
been shown to abrogate immunity to certain helminths, and analysis of eosinophil granule contents has revealed many potentially cytotoxic enzymes.

In the present studies, the experimental intravitreal infection of guinea pig eyes with one or two doses of second-stage larvae (L2) of *T. canis*, L2 or fourth-stage larvae (L4) of *A. suum*, or a soluble *A. suum* antigen induced intraocular IgE antibodies and a dense eosinophil infiltration of the anterior chamber and uveal tract. Individual larvae were surrounded by granulomas or abscesses composed almost entirely of eosinophils. Histopathologic studies of these granulomas showed an intimate interaction between the eosinophils and the parasite larvae, ingestion of eosinophils and eosinophilic granules by parasites, and degranulation of adherent eosinophils. Eosinophils may fulfill an important role in the killing of intraocular parasites. Eosinophil infiltrations of the choroid also may directly result in toxic effects upon the adjacent retina.

Materials and methods

Infective eggs of *T. canis* were obtained from gravid uterus of adult female worms recovered at necropsy from infected dogs. *A. suum* eggs were obtained from adult female worms collected from the intestines of swine slaughtered at a local abattoir. *T. canis* and *A. suum* L2 were prepared in vitro according to the method of Jaskoski and Colucci as modified by Stromberg and Soulsby. Infective eggs of *A. suum* or *T. canis* were suspended in sterile 0.85% saline at 37°C and gassed with CO2 for 30 sec. A few (20 to 30) small glass beads were added, and the preparation was rotated at 37°C for 30 min. Live L2 were separated from eggshells and other debris by the Baermann technique, where the suspension was layered over a 6 mm thick cotton pad and the active larvae migrating through the pad were collected underneath. Larvae were concentrated by sedimentation at 1 X g, washed repeatedly in sterile 0.85% saline, incubated for 3 hr in saline containing penicillin (10° U/ml), streptomycin (1.3 mg/ml), and nystatin (250 U/ml), and washed twice more in saline before culture. The culture medium consisted of Medium 199 (GIBCO, Grand Island, N.Y.) supplemented with 0.02 mg/ml glucose, a synthetic tripeptide (Gly-His-Lys), penicillin (15 U/ml), and streptomycin (0.015 mg/ml), gassed with a mixture of N2/CO2/O2 (90/5/5). Cultures were maintained at 37°C in 200 ml French square bottles on a roller drum for 7 days. At this time more than 95% of the third-stage larvae had undergone ecdysis and emerged as L4. Larvae were collected by sedimenting at 1 x g, washed, and resuspended at a concentration of 500/ml in sterile saline for intravitreal injections.

*A. suum* culture fluid (ACF) antigen was prepared from supernatants of cultures in which third-stage *A. suum* larvae had molted to the fourth-stage, by ultrafiltration on an Amicon YM-10 membrane (final protein concentration 310 μg/ml). This process retains components of 10,000 molecular weight or greater.

Female Hartley strain guinea pigs weighing 500 to 650 gm (Skippack Farms) were used for intravitreal infections and 6-day passive cutaneous anaphylactic testing (P-K).

Intravitreal injections of *T. canis* or *A. suum* L2 were made with a 30-gauge needle on a 1 ml tuberculin-type syringe. One-tenth milliliter of larval suspension containing 3000 or 5000 *A. suum* L2 or 500 *T. canis* L2 was placed in the syringe, and the syringe was inverted, allowing the larvae to settle into the needle. One half to two thirds of the inoculum, containing all the larvae, then was injected. Residual inoculum was examined microscopically to ensure that no larvae remained. Intravitreal injections of 50 *A. suum* L4 were administered in a similar fashion, with a 26-gauge needle because the L4 are too large (2 to 4 mm long) to pass through a smaller needle. A relatively small number of *T. canis* L2 was used for in-
Intravitreal injection because of the greater tendency of these larvae to migrate into the central nervous system, causing death of the experimental animal. Injection of 500 T. canis L2 caused 5% to 10% mortality within 2 weeks, and use of greater numbers would have caused a higher, unacceptable mortality rate. A. suum L4 are more than 100 times larger than L2, so that 50 L4 would provide an antigenic mass similar to that of 3000 to 5000 L2. Intravitreal injections of 10 μl of ACF antigen (3.1 μg of protein) were administered with a Hamilton microsyringe.

Animals were systemically immunized to A. suum by three subcutaneous injections of 10,000 embryonated A. suum eggs each at intervals of 10 days, followed 10 days later by an oral dose of 10,000 A. suum eggs.

Eyes were enucleated for histologic study 11 and 28 days following a single intravitreal injection of 500 T. canis L2, 42, 47, and 49 days following two intravitreal injections of 500 T. canis L2 each on days 0 and 21; 1 to 21 days following a single injection of 3000 live A. suum L2; 21 and 42 days following a single injection of 3000 heat-killed A. suum L2, and 30 and 51 days following two injections of 3000 A. suum L2 each on days 0 and 14.

Animals previously systemically immunized to A. suum were given a challenge intravitreal injection of 3000 live or heat-killed A. suum L2 each on days 0 and 14, and the eyes were enucleated on day 21. The eyes of animals injected intravitreally with a single dose of 50 A. suum L4 on day 0 or with two doses given on days 0 and 14 were enucleated 14 and 21 days, respectively, after the initial infections. Animals injected with ACF antigen were given two intravitreal doses of 3.1 μg each on days 0 and 14, and eyes were enucleated on days 21 and 42.

Eyes for routine histopathologic study were fixed in 5% formalin–0.5% glutaraldehyde for 24 hr and processed as described by Menocal et al. Sections 7 or 8 μm in thickness were cut, dried overnight at 56° C, and stained with hematoxylin-eosin, periodic acid–Schiff, Gomori’s trichrome, Masson’s trichrome, Luna’s stain for eosinophil granules, and Unna’s mast cell stain. Eyes for thin (1 μm) sections were fixed in 2% P-formaldehyde–2.5% glutaraldehyde–0.025% CaCl₂ in 0.1M sodium cacodylate buffer, pH 7.5, overnight at 4° C. Fixed specimens were divided into eight pieces by horizontal, vertical, and transverse cuts, postfixed in 1.6% osmium tetroxide in 0.05M cacodylate buffer for 2 hr at 25° C, rinsed, and dehydrated. Specimens were infiltrated overnight at 4° C in the plastic embedding mixture of Epox and Araldite described by Dvorak et al. diluted 1:1 with propylene oxide, in undiluted medium for 4 hr at 4° C, and embedded in fresh medium in rubber molds. Blocks were polymerized at 25° C for 4 hr under a vacuum of –25 mm Hg, for 20 hr at 37° and for 40 hr at 60° C. Sections 1 μm in thickness were cut on a Sorvall Porter-Blum automatic microtome and dried at 60° C for 3 to 5 min. Sections were stained with Giemsa stain (Harleco) buffered with either 2% sodium borate or 0.05M acetic acid buffer, pH 5.0, for 45 min at 60° C, for visualization of basophil and eosinophil granules, respectively.

Histopathologic lesions were scored on the basis of the distribution of five types of cells: eosinophils, neutrophils, basophils/mast cells, mononuclear cells, and plasma cells. Eosinophil counts were performed on sections stained by Unna’s method for eosinophil granules. Basophil/mast cell counts were performed on sections stained by Unna’s method for mast cells or on Epon sections stained with alkaline Giemsa. Hematoxylin-eosin–stained sections were evaluated for neutrophils, mononuclear cells, and plasma cells. Infiltrates were scored as negative if fewer than 3 cells of a given type were present in one microscopic field (40 x Zeiss objective), as 1+ if 3 to 49 cells were present, as 2+ if 50 to 149 cells were present, or as 3+ if 150 or more cells of a given type were present per field. Counts were performed on anterior chamber, iris, ciliary body, pars plana–choroid, and representative regions of retina, choroid, and sclera within 90 degrees of the posterior pole. Fields immediately adjacent to parasites were excluded in order to measure the extent of the generalized cellular infiltrate. A more detailed analysis was performed on the eosinophil infiltrate following a single intravitreal injection of A. suum L2.

Results

T. canis L2. Following a single intravitreal injection of 500 T. canis L2, a dense (2+) eosinophil infiltrate of the ciliary body and pars plana–choroid and a diffuse (0 to 1+) infiltrate of the anterior chamber and posterior choroid were observed on day 11. By day 28 this was reduced to a diffuse eosinophil infiltrate of the ciliary body and the pars plana and posterior choroid. Relatively few (0 to 1+) mononuclear cells were observed, diffusely scattered throughout the ciliary body, pars plana, and posterior choroid on days 11 and 28. A diffuse infiltrate of basophils (1 to 4
Fig. 1. Eosinophil response in different ocular structures to a single intravitreal injection of 3000 *A. suum* L2 on day 0. Twelve eyes were removed each at 1, 3, 6, 7, 9, 12, 15, 19, and 21 days after infection. Five Luna-stained sections from each eye were examined. Sections were coded, mixed, and scored separately by two investigators before decoding. The eosinophils in 10 microscopic fields (Zeiss Universal Microscope, 100× oil-immersion objective, widefield system) were counted per eye for each tissue. •, Anterior chamber; ○, iris; +, ciliary body; ◦, pars plana–choroid; ▲, posterior choroid; ■, retina; △, sclera. Fields including or immediately adjacent to an ascarid larva were avoided in order to measure the generalized eosinophil infiltration. Randomly selected fields were counted in each case except for anterior chamber fluid, where because of eosinophil settling during fixation, 10 fields were counted in one section beginning with the most dense and progressing to less dense eosinophil collections. The average number of eosinophils per field is plotted against days after infection. Eosinophil counts at day 0 are the averages from six normal (noninfected) eyes. The changes in slopes of the eosinophil density curves vs. time occurring at 6 days indicates that one or more new eosinophilotactic stimuli, possibly a parasite metabolic product or an immunologic factor, has appeared in the infected eye at this time. Eosinophil infiltration in the iris shows a noticeable gradient, with low relative eosinophil densities in the central iris progressing to higher densities at the iris base. Note the markedly greater eosinophilia of the choroid in comparison with that of the retina.

per high-power field) was present throughout the uvea on day 11 and was confined to the choroid on day 28. Plasma cells were observed in the ciliary body and pars plana–choroid on days 11 and 28. IgE antibodies were detected in serum beginning on day 7 and in aqueous beginning on day 14. Administration of two intravitreal injections of 500 *T. canis* L2 each at 0 and 21 days induced a reaction of longer duration. A dense (1+ to 2+) eosinophil infiltrate, similar to that seen on day 11 of the primary reaction, was observed on days 42 to 49 in the anterior chamber, ciliary body, and pars plana and posterior choroid. A mononuclear cell infiltrate (0 to 1+) of the iris, ciliary body, and pars plana–choroid was observed on days 42 to 49, similar to the early primary response. Plasma cells were observed in the ciliary body and pars plana–choroid on days 42 to 49. IgE antibody was found in aqueous fluid and serum on days 21, 28, 35, 42, and 49. Administration of two intravitreal injections of a sin-
A single dose of 3000 A. suum L2 induced a histopathologic reaction more severe than that induced by 500 T. canis L2. Diffuse eosinophilia of the choroid and ciliary body was observed by day 3. By 6 days after infection, eosinophils could be found diffusely in the anterior chamber and iris and densely in the base of the ciliary processes and in pars plana–choroid. Increased numbers of eosinophils were present on day 9 in the anterior chamber and in the entire uveal tract, particularly in the ciliary body and pars plana–choroid where they constituted over 90% of the infiltrating cells. The time course (1 to 21 days) of eosinophil infiltration in the guinea pig eye following a single intravitreal injection of live A. suum larvae is detailed in Fig. 1. Formation of eosinophil granulomas or abscesses around parasites were noted in the anterior chamber, iris, ciliary body, and choroid by day 12. Mononuclear cells (principally large lymphocytes and macrophages) were observed throughout the uveal tract in fairly constant numbers (1+ to 2+), constituting a diminishing fraction of the total infiltrate as eosinophil numbers increased on days 3 through 9. On day 12 an increase (2+) in mononuclear infiltrate of the ciliary body and pars plana–choroid was noted, but the mononuclear cell infiltrate returned to the previous levels by day 21. A small but perceptible basophil infiltrate was observed on day 12, consisting of 1 to 5 cells per ciliary body section and 1 or 2 per high-power field in the choroid. Plasma cells were first observed in the ciliary body on day 6 and in greater numbers (1+) in the iris, ciliary body, and pars plana–choroid on day 12. IgE antibody was detected in aqueous humor from infected eyes by day 14 and in serum by day 19. Neutrophils (1+) were found in the choroid and ciliary body on day 1 but were minimal in number throughout the remainder of the reaction.

In contrast, eyes injected with 3000 heat-killed (56°C, 20 min) A. suum L2 showed a minimal inflammatory reaction with few eosinophils and a diffuse (1+) mononuclear cell infiltrate. As late as 42 days after injection, decomposing parasites were found to be surrounded by small numbers of macrophages and no eosinophils. Plasma cells were not numerous in these eyes, and IgE antibody was not detected in the aqueous humor.

Administration of two intravitreal injections of 3000 A. suum L2 each on days 0 and 14 induced 1+ to 2+ eosinophilia throughout the uvea and particularly in the choroid by day 30, which diminished to a mild (0 to 1+) infiltrate involving only the choroid by day 51. Mononuclear cells were found in the anterior chamber and densely (2+) throughout the entire uvea on day 30 and were still present, although in much reduced numbers, on day 51. Plasma cells (1+) were observed in the iris, ciliary body and pars plana–choroid on days 30 and 51. Intracocular areas of bone metaplasia were observed by day 51. IgE antibody was present in aqueous humor and serum on days 19 and 29.

Intravitreal challenge of guinea pigs previously systemically sensitized to A. suum was effected with 3000 A. suum L2 10 days after the final systemic immunization. Twenty-one days after challenge, a dense (2+) eosinophil infiltrate of the iris, ciliary body, and pars plana–choroid and a diffuse (1+) eosinophil and neutrophil infiltrate of the anterior chamber, choroid, and sclera were observed. Mononuclear cells (principally large lymphocytes) were observed in the anterior chamber, iris, and ciliary body and densely (2+) in the pars plana and posterior choroid. A small number of basophils were observed in the choroid, and a few (0 to 1+) plasma cells were seen in the pars plana on day 21. IgE antibody was found in aqueous humor of 90% of eyes tested 7 and 14 days after intravitreal challenge. In contrast, leakage of IgE antibody from serum into the aqueous of the normal eye prior to challenge was not observed even in the presence of serum 6-day P-K titers as high as 1:1,000.

A. suum L4. Intravitreal injection of 50 A. suum L4 induced a dense (2+) eosinophil infiltrate of the ciliary body and pars plana–choroid and a milder (1+) eosinophil infiltrate in the anterior chamber and iris by day 14. Mononuclear cells (1+) were observed in the anterior chamber, iris, ciliary
body, and choroid, whereas plasma cells (1+) were observed in the ciliary body and pars plana–choroid 14 days after infection. Challenge infections of 50 L4 were given on day 14. By day 21 more intense inflammation was observed throughout the eye. Eosinophilia of the ciliary body and pars plana–choroid (2+) and of the anterior chamber and iris (1+) was present, and in addition, prominent accumulations of mononuclear cells in the pars plana–choroid (3+) and ciliary body (2+) were observed. Plasma cells and small lymphocytes were also prominent (1+) in the ciliary body and pars plana–choroid. Only very small numbers of basophils were observed on day 14. However, by day 21 an infiltrate of from 2 to 7 basophils per high-power field was present in the choroid and ciliary body. IgE antibodies were detected in serum and aqueous humor beginning on day 14.

ACF antigen. Injection of 10 μl of ACF antigen (3.1 μg of protein) intravitreally on days 0 and 14 induced a milder cellular reaction. A diffuse (0 to 1+) eosinophilia of the anterior chamber, ciliary body, and pars plana was observed on day 21. Mononuclear cells were observed diffusely (0 to 1+) throughout the anterior chamber, uveal tract, and retina in equal or greater numbers than eosinophils. Plasma cells were observed in the ciliary body and pars plana–choroid on day 21. IgE antibodies were detected in the serum by day 14 and in aqueous humor by day 21.

Eosinophil-parasite interactions. An intimate interaction between eosinophils and the parasite larvae was a striking feature of the histopathology of the ascarid-infected guinea pig eyes (Fig. 2). Shortly after intravitreal injection (day 6 or before), ascarid larvae were observed in the posterior chamber, on the iris, and within the retina, but the eyes were largely free of the eosinophil granulomatous reaction noted later. By day 12 parasite larvae in the anterior chamber were enclosed within eosinophil granulomas with dense central cell collections composed of 80% to 100% eosinophils immediately surrounding the ascarid larvae and an outer layer of mononuclear cells, histiocytes, epithelioid cells, and, in some instances, giant cells. Eosinophil abscesses, where the dense central nodule of essentially pure eosinophils showed early signs of cell necrosis, also were seen. Eosinophils were found to have invaded the alimentary tract of some larvae, whereas eosinophilic granules were observed within the alimentary tracts of other larvae (Fig. 2, A). The eosinophils immediately adjacent to the ascarid larvae frequently showed degranulation, and free eosinophilic granules were observed upon the parasite surfaces (Fig. 2, B). The inner layer of eosinophils was firmly adherent to the parasite surfaces or to a refractile membrane which was almost universally observed to have detached from the parasite in some region due to an artefactitious shrinkage caused by fixation and/or processing (Fig. 2, C). The membrane may be of parasite or eosinophil origin. Dense eosinophil granulomas about ascarid larvae also were found in the posterior choroid beginning 9 to 12 days after intravitreal infection (Fig. 2, D). In contrast to this reaction, as late as 12 days after infection, some ascarid larvae could be found in the posterior choroid with a minimal eosinophil reaction, having scattered diffuse eosinophilia in their vicinity but none or only an occasional eosinophil in direct contact with the parasite (Fig. 2, E). The latter type of choroidal reaction may isolate and protect the parasite from the killer function of the host eosinophils and permit the ascarid larvae to survive for an extended period.

As late as 9 days after intravitreal infection, ascarid L2 were found which had penetrated into or beneath the retina but not through Bruch’s membrane. These lesions were remarkable in that no eosinophils or other inflammatory cells were present in the adjacent retina or in contact with the parasite even though there was extensive eosinophilia of the underlying choroid as a part of a general uveal reaction. The adjacent retina showed loss of rod outer segments (vide infra), but otherwise the retinal architecture immediately adjacent to the ascarid larvae was relatively normal.
Fig. 2. For legend, see facing page.
Acellular retinal destruction. Alterations of the retina were observed as early as 6 to 9 days after intravitreal infection with ascarid larvae. The retina overlying choroidal parasites usually was replaced with scar tissue by day 12. In areas where parasites were not present, acellular destruction of the retina was observed when the adjacent choroid was densely infiltrated with eosinophils (Fig. 2, F). The earliest retinal change associated with a dense eosinophil infiltration of the choroid was a loss of the photoreceptor outer segments. Subsequently, serous edema, cystic changes, and loss of the normal retinal architecture and cellularity were observed, especially affecting the outer layers of the overlying retina. The pigment epithelial cells showed hydropic degeneration, with coalescences of vacuoles in some regions to form large cystic spaces between the heavily infiltrated choroid and the residual retina (Fig. 2, F). Few eosinophils or other inflammatory cells were present in the degenerating retina in marked contrast to the dense eosinophil infiltrate in the adjacent choroid (Fig. 2, F).

Discussion
A single intravitreal injection of 3000 A. suum L2 provoked a rapid eosinophil infiltrate and intraocular IgE antibody formation. Primary intravitreal injection of heat-killed A. suum L2 provoked only a limited inflammatory reaction and no intraocular IgE antibody. Administration of a second intravitreal injection of live A. suum L2 potentiated the destructive inflammatory reaction and IgE antibody response. Intravitreal challenge with live or heat-killed A. suum L2 of animals sensitized to A. suum by systemic infection provoked greatly potentiated IgE antibody and inflammatory responses of essentially equal intensity. Thus, although live L2 were much more effective inducers of primary intraocular inflammatory and IgE
Table I. Eosinophil chemotactic factors

<table>
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<th>Factor *</th>
<th>Source</th>
<th>Reference</th>
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<tbody>
<tr>
<td>ECF-A</td>
<td>Basophil granules, neutrophils</td>
<td>17</td>
</tr>
<tr>
<td>Intermediate mol. wt. ECF</td>
<td>Basophil granules</td>
<td>18</td>
</tr>
<tr>
<td>Histamine</td>
<td>Basophil/mast cell granules</td>
<td>19</td>
</tr>
<tr>
<td>High mol. wt. ECF</td>
<td>ACA lesion</td>
<td>20</td>
</tr>
<tr>
<td>ESP</td>
<td>T cells</td>
<td>21</td>
</tr>
<tr>
<td>ECF-P</td>
<td>T cells</td>
<td>22</td>
</tr>
<tr>
<td>C5a</td>
<td>Complement</td>
<td>26</td>
</tr>
<tr>
<td>C5b</td>
<td>Complement</td>
<td>26</td>
</tr>
<tr>
<td>C5b7</td>
<td>Complement (alternative pathway)</td>
<td>25</td>
</tr>
<tr>
<td>Kallikrein</td>
<td>Plasma protein</td>
<td>17</td>
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<tr>
<td>Flasminogen activator</td>
<td>Plasma protein</td>
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</tr>
<tr>
<td>HHT</td>
<td>Platelet cyclooxygenase</td>
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<td>HETE</td>
<td>Platelet lipoxigenase</td>
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</tr>
<tr>
<td>Parasite-derived factors</td>
<td>Ascaris lumbricoides</td>
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<td>Trichinella spiralis</td>
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<tr>
<td></td>
<td>Anisakis spp.</td>
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</table>

* ECF-A = eosinophil chemotactic factor of anaphylaxis; ESP = eosinophil stimulation promoter; ECF-P = eosinophil chemotactic factor precursor; HHT = 12-l-hydroxy-5,8,10-heptadecatrienoic acid; HETE = 12-l-hydroxy-5,8,10,14-eicosatetraenoic acid; ACA = active cutaneous anaphylaxis.

antibody responses, both live and heat-killed larvae proved equally effective inducers of secondary intraocular reactions.

Intravitreally injected ascarid L2 can migrate out of the eye. Systemic sensitization of the animal by the migrating larvae could contribute to the histopathologic and IgE antibody responses observed. The responses to the A. suum L4 were studied because L4 are much larger than L2 (2 mm vs. 250 μm) and are less able to penetrate tissues. Despite their greatly reduced migratory capacity, intravitreally injected A. suum L4 were able to induce both primary and secondary intraocular IgE antibody and histopathologic responses.

Even though A. suum L4 do not leave the eye, antigen produced by them may do so. The ACF antigen is a soluble allergen and immunogen produced by third-stage A. suum molting to the fourth stage in defined media in vitro. Intravitreal injection of this antigen did not provoke a primary intraocular IgE antibody response. Administration of a challenge injection did elicit IgE antibody and diffuse eosinophilia in the anterior uvea. As with A. suum L2, the capacity to induce histopathologic and IgE antibody responses following primary intravitreal injection may reside in the living organism, although soluble antigens may provoke a secondary response.

Intravitreal administration of T. canis L2 provoked primary and secondary intraocular IgE antibody and histopathologic responses similar to those observed with A. suum L2. The greater tendency of T. canis to enter the brain and kill the paratenic host necessitated the administration of a smaller intravitreal dose than that used with A. suum. Nevertheless, the frequency of intraocular IgE antibodies was greater in animals infected with T. canis than in those given A. suum. The histopathologic reaction to T. canis included eosinophilia and subsequent plasma cell infiltrations, as observed with A. suum, but the infiltrates were confined largely to the ciliary body with less choroidal inflammation than observed in A. suum-infected eyes. These apparent quantitative differences may result from the smaller intravitreal dose of T. canis.

The pronounced eosinophil infiltrate in the eye caused by the intraocular ascarid (T. canis and A. suum) infections may reflect the effects of a number of eosinophilotactic agents (Table I). The ocular eosinophilia was accompanied by an intraocular IgE antibody response. Degranulation of mast cells or basophils by the interaction of antigen with cell-bound homocytotropic (IgE) antibody releases at least three classes of eosinophil cytotoxins: eosinophil chemotactic factor of anaphylaxis (ECF-A); intermediate-molecular-weight factors; and histamine. ECF-A is a mixture of two tetrapeptides of amino acid sequences Val-Gly-Ser-Glu and Ala-Gly-Ser-Glu, which are selectively chemotactic
for eosinophils in vitro. Intermediate-molecular-weight (1500 to 2500) eosinophilotactic activity of mast cells is physically associated with the basophil granule and consists of three distinct species of differing charge and hydrophobicity. histamine interacts with eosinophils in a complex fashion, showing variable in vivo eosinophilotactic activity.

An additional protein of 70,000 molecular weight with eosinophilotactic activity in vitro, which produces delayed, prolonged eosinophilia when injected into guinea pig skin, has been isolated from active cutaneous anaphylactic lesions produced in guinea pigs immunized with a single injection of dinitrophenyl-Ascaris extract.

Thymus-derived (T) lymphocytes also produce eosinophil chemotactic principles when activated by antigen or lectins. Eosinophil stimulation promoter (ESP), a protein of 26,000 molecular weight isolated from cultures of immune thymocytes exposed in vitro to parasite antigens, is specifically chemotactic for eosinophils and also enhances eosinophil random motility and stimulates eosinophil cytotoxicity for parasites. An additional T cell eosinophilotactic factor (ECF-P), with in vivo and in vitro activity dependent on the interaction with IgG antibody complexed with homologous antigen, has been isolated from cultures of antigen-stimulated guinea pig lymph node cells. The importance of T lymphocytes in the production of peripheral eosinophilia in response to parasite infection has been demonstrated repeatedly in vivo, and eosinophil reactions associated with delayed-type hypersensitivity have been adoptively transferred by immune lymphocytes in syngeneic guinea pigs. Delayed eosinophil-rich reactions in the uveal tract of the guinea pig have been observed following intravitreal challenge of animals primed with antigen in complete or incomplete Freund's adjuvant.

Activation of complement produces C3a, C5a, and C567 and C3B complexes, all of which are chemotactic for eosinophils. Kallikrein and plasminogen activator also are eosinophilotactic in vitro. Kallikrein may be produced by cleavage of prekallikrein by an arginine esterase released from mast cells and basophils during degranulation. Platelet activation also may lead to eosinophil infiltration mediated by 12-1-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE), and 12-1-hydroxy-5,8,10-heptadecatrienoic acid (HHT). HETE also may be released from mast cells.

Helminth parasites may produce substances which are directly eosinophilotactic or which may degranulate mast cells, causing release of their chemotactic agents (Table I). Extracts from perienteric fluid of adult Ascaris lumbricoides have selective chemotactic activity for eosinophils in vitro. A factor of approximately 10,000 molecular weight which is capable of degranulating mast cells in vitro has been isolated from extracts of adult A. suum and T. canis. The appearance of eosinophils within 1 day of primary intraocular infection with ascarid L2 suggests that the parasites themselves produce an eosinophil chemotactic factor responsible for this effect. The formation of eosinophil granulomas by 12 days following primary infection and the more intense and prolonged eosinophilia observed after secondary challenge may be mediated largely by antibody or T cell-dependent processes.

The apparent synchronization of appearance of plasma cells in the uvea and IgE antibody in the aqueous humor furnishes further evidence that this antibody may be locally produced.

The functions of the eosinophil and its role in host responses to helminth infections are only now beginning to be understood. The characteristic granule of the eosinophil leukocyte contains zinc, a peroxidase, and a major basic protein (MBP). A second, smaller, cytoplasmic organelle contains acid phosphatase and a sulfatase. Eosinophils also contain histaminase, plasminogen, plasminogen activator, reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, prostaglandin synthetase, kininase, β-glycerophosphatase, β-glucuronidase, phospholipases B and D, collagenase, and other hydrolytic enzymes and have surface recep-
tors for C4, C3b, C3d, and for IgG, Fc. Binding of aggregated IgE and IgE immune complexes to eosinophils also has been reported.

Eosinophils appear to be capable of modulating hypersensitivity reactions induced by homocytotropic (e.g., IgE) antibodies by limiting the effects of pharmacologic mediators of anaphylaxis. Eosinophil arylsulfatase (type II-B) cleaves and inactivates slow-reacting substance of anaphylaxis (SRS-A); phospholipase D inactivates platelet-activating factor, and histaminase oxidatively deaminates histamine. The MBP of eosinophilic granules precipitates and neutralizes heparin, inactivates SRS-A, and because it has two free sulfhydryl groups, is capable of activating proteases such as pepsin. Eosinophils also may modulate anaphylactic reactions by regulating the rate at which intracellular stores of histamine are replenished. An eosinophil-derived inhibitor of mediator release, a mixture of prostaglandins E1 and E2, has been reported to be synthesized and released following interaction of eosinophils with anti-IgE or ragweed allergen E. The role of prostaglandins in ocular parasite infections, however, may be more complex, since PGE1 and PGE2 directly cause inflammation and vascular leakage in ciliary processes.

The eosinophil may play a dominant role in protective immunity and in destroying intraocular parasites. Recent evidence indicates that eosinophils function directly as primary parasite killer cells. This cytotoxic effect is mediated by antiparasite IgG antibody and is independent of complement. An intimate interaction between eosinophils and the ascarid larvae was seen on light microscopic examination of the parasitized guinea pig eyes. Electron microscopic studies of eosinophils adherent to parasite larvae in vitro have demonstrated close association of eosinophil plasma membranes and parasite surfaces, with degranulation of the adherent cells onto the parasite. The cytotoxic effect of the eosinophil may result from these surface interactions.

Eosinophils, in addition, may directly produce serious damage to the host. A reduction in the number of mature eosinophils with antieosinophil sera decreases the tissue damage around schistosome egg granulomas, supporting a role for the eosinophil in this tissue damage. Humans with hypersensitivity syndromes may acquire a thickening and fibrosis of the myocardial wall (Loeffler's cardiomyopathy) associated with vacuolation of peripheral blood eosinophils and reversal of eosinophil granule electron densities (indicating that degranulation may be taking place) and increased C3b rosette formation and eosinophil phagocytic activity. Eosinophil autotoxicity was described in an early diagnostic test for Hodgkin's disease. Patients' lymph node cell suspensions, injected intracerebrally into guinea pigs, produced an ataxia accompanied by destruction of cerebellar Purkinje cells. Subsequent studies demonstrated that the effect was due to the presence of eosinophils and that intracerebral injection of homologous or heterologous eosinophils or purified eosinophil granules would produce the same effect. The acellular degeneration and destruction of the outer retina overlying regions of choroid heavily infiltrated with eosinophils but not directly adjacent to a parasite larva which we have observed frequently in guinea pig eyes infected with T. canis and A. suum, may, at least in part, be a direct result of such a toxic reaction to constituents of the eosinophils. The dense eosinophil infiltrate also may produce a vascular congestion in the choroid leading to retinal ischemia.

The degeneration of the outer retinal layers also could reflect an autoimmune reaction to retinal constituents (e.g., rod outer segments, rhodopsin) (ref. 44 and A.H.S. Rahi, personal communication). The early onset of the degeneration (6 to 9 days after infection) argues against this mechanism. The possibility of autoimmune reactions to retinal antigens in the parasite-infected eye is presently under investigation in our laboratories.

Experimental intravitreal injection of guinea pig eyes with larval stages of T. canis and A. suum produced an inflammatory response.
characterized by intraocular IgE antibody and a dense infiltrate of eosinophils. The eosinophil reaction may have been induced directly by parasite-derived chemotactic factors, particularly during early phases of the immune response. Immunologic mechanisms may be of greater importance later in the reaction. Firm adherence of eosinophils may represent a cytotoxic mechanism for specific destruction of the intraocular parasites. Widespread intraocular release of eosinophil constituents also may be responsible for the retinal destruction associated with the choroidal inflammatory responses. The roles of the eosinophil in the destruction of intraocular parasites and in the mediation of autologous ocular damage merit continued study.

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REFERENCES


23. Parish, W.E., Luckhurst, E., and Cowan, S.I.: Eosinophila. V. Delayed hypersensitivity, blood and bone marrow eosinophilia, induced in nontoxic guinea pigs by adoptive transfer of lymphocytes...


