

Differential Modulation of Allergic Eye Disease by Chronic and Acute *Ascaris* Infection

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PURPOSE. To assess alterations in allergic ocular responses to nonparasite antigens in an experimental system in which mice were skewed toward a Th2 cytokine profile by helminth infection.

METHODS. Mice were inoculated with *Ascaris suum* (*A. suum*) eggs concurrent with ragweed (RW) sensitization (RW/acute) or by repeated inoculation before RW sensitization (RW/chronic). Control subjects were divided into RW, *A. suum*, and sham-sensitized groups. Animals were RW-challenged in the eye and examined for changes in ocular responses, inflammatory cell infiltrates, and in vitro assessment of cytokines after antigen restimulation. In subsequent experiments, CD4⁺/CD25⁺ T regulatory and CD4⁺/CD25⁻ control T cells were adoptively transferred into mice before ocular challenge.

RESULTS. RW sensitization and challenge increased ocular symptoms and eosinophil infiltration into the conjunctiva over PBS control eyes. Acute *A. suum* infection significantly increased RW-induced clinical symptoms and eosinophil infiltrates in the conjunctiva ($P = 0.0001$) and resulted in the development of anterior uveitis. In contrast, RW/chronic infection provided protection from allergic responses to RW with significantly fewer eosinophils in the eye and reduced eotaxin levels. Transfer of CD4⁺/CD25⁺ T cells from RW/chronic mice into RW/acute animals also decreased disease intensity, suggesting that T regulatory cells may contribute to protection from allergic eye disease.

CONCLUSIONS. The current studies suggest acute parasitic infections exacerbate allergic symptoms, whereas chronic infections offer protection and provide possible explanations for the role of parasitic infection in susceptibility and resistance to nonparasite allergens. (*Invest Ophthalmol Vis Sci.* 2005;46:2772-2780) DOI:10.1167/iovs.04-0899

Consequences of allergic disease range from the comparatively irritating to the life-threatening responses associated with asthma and anaphylaxis.¹ Several mucosal organs are associated with allergic disease, including the eyes, nose,

lungs, and skin; however, ocular disease represents the most prevalent site of early symptoms and affects 30% of people in westernized nations.² Patients with allergic conjunctivitis experience itching and burning sensations in the eye, and chemosis, tearing, redness, and blepharitis (lid edema) develop in response to allergens that do not affect normal individuals.³ Although this study focused on allergic conjunctivitis as the primary measurement of allergic response, similar underlying mechanisms are observed in other mucosal sites. Therefore, information gained from these studies will have direct relevance to asthma and other allergic diseases.

There has been a significant increase in the prevalence of allergic diseases worldwide, with a rapidly increasing incidence in industrialized nations and urban areas of developing countries.⁴ The swift development of this epidemic within a single generation cannot be entirely explained by a genetic shift. Therefore, environmental factors must contribute to the problem. Studies in rural areas of industrialized countries where the incidence of allergic disease is low have suggested that chronic childhood infections with bacterial and/or viral pathogens may abrogate the development of Th2-based allergic disease through early systemic polarization toward a Th1 immune response (i.e., the "hygiene hypothesis").⁵ Conversely, improved hygiene, vaccination, and the use of antibiotics may decrease polarization toward an infection-induced Th1 immune response and result in the expression of allergy-inducing Th2 cytokines (e.g., IL-4, -5, and -6).⁶ However, studies examining populations with endemic parasite infections, in particular helminth infections, suggest other mechanisms may play roles in allergic disease.⁴ Despite the increased concentrations of IgE and Th2 cytokines normally associated with development of allergic disease, these helminth-infected populations exhibit decreased atopy.^{4,5} Indeed, patients undergoing long-term antihelminthic treatment have been found to have increased mite skin test reactivity after treatment, indicating that helminth infection itself may lead to direct suppression of allergic reactions.⁷ Thus, although a similar Th2 pattern occurs in helminth infections and allergic diseases, the final outcome is clearly dissimilar.

The mechanism by which helminth infection mediates protection from allergic disease is unknown but may involve an increased concentration of both nonspecific and helminth-specific polyclonal IgE,⁸ which could theoretically block the binding and cross-linking of FcεR1, the high affinity IgE receptor, with allergen-specific IgE and prevent degranulation by FcεR1-bearing cells, such as basophils and mast cells. An alternate hypothesis is that protection from allergic disease is attributable to an increase in anti-inflammatory cytokines produced by regulatory T cells in response to chronic helminth infection.⁹

To examine the role of helminth infection in allergic disease, a model of combined *A. suum* infection and allergic eye disease was developed. Ocular responses were used to gauge allergic inflammation. *A. suum* larvae migrate to mucosal sites in the intestines and lungs but do not normally develop in the eye.^{10,11} These studies demonstrate that *A. suum* infection can either be protective or can exacerbate allergic disease in re-

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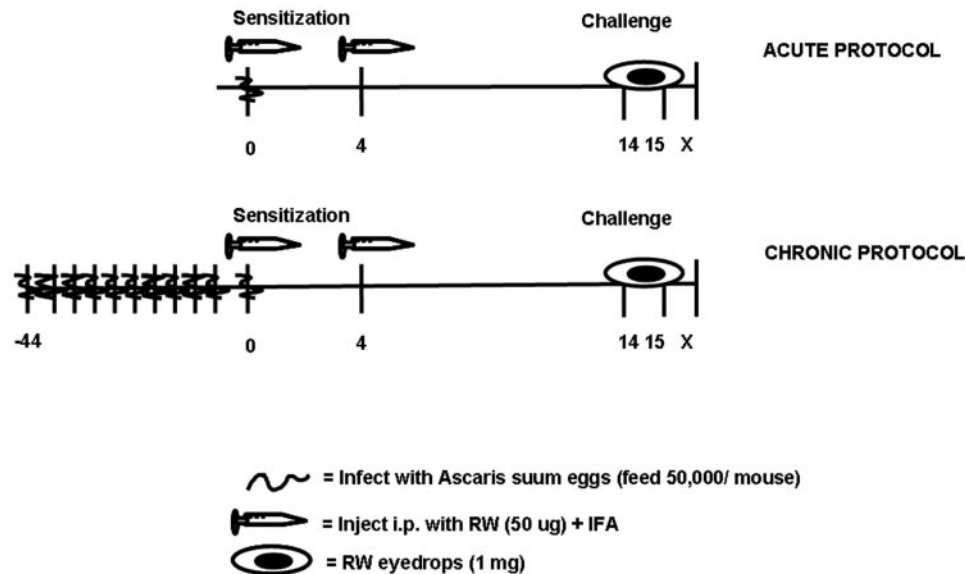


FIGURE 1. *A. suum* RW/acute and RW/chronic infection with RW sensitization and challenge protocols. For the RW/acute protocol, mice were sensitized on day 0 by oral inoculation with ~50,000 *A. suum* eggs concurrent with an IP injection of RW and then sensitized again on day 4 with a second IP injection of RW. Days 14 and 15 after sensitization, the mice were challenged in the eye with 5 μ L/eye of a 200 mg/mL RW (in PBS). The mice were killed on day 16. For the RW/chronic protocol, mice were sensitized every third day from days 44 to 0 by inoculating ~50,000 *A. suum* eggs concurrent with a single IP injection of RW on day 0 and then sensitized again on day 4 by IP injection on RW. Days 14 and 15 after sensitization, the mice were challenged in the eye with 5 μ L/eye of a 200 mg/mL RW and PBS solution. Control mice were sensitized with PBS or RW without *A. suum* infection (RW) or received *A. suum* infection without RW (*A. suum*).

sponse to a nonparasite allergen, depending on the time and duration of the infection. If animals are infected with a single *A. suum* inoculation concurrently with ragweed sensitization, they become increasingly susceptible to allergic conjunctivitis. In contrast, if chronic *A. suum* infection occurs before allergen sensitization, the severity of the immunologic response to allergen exposure decreases significantly.

The protective effect from chronic infection correlated with decreased eotaxin levels and subsequent abrogation of eosinophil infiltrates into the tissue.^{12,13} This protection may be due to an increase in T regulatory cells, as adoptive transfer of CD4⁺/CD25⁺ T regulatory cells removed from RW/chronic mice into RW-sensitized mice resulted in significant reductions in eosinophil infiltration into the eye in response to RW challenge, suggesting that these T regulatory cells provide protection in this model. Investigating the influence of helminth infections on the development of allergic responses to non-parasite antigens such as allergens may reveal other factors that control expression of allergic diseases and ultimately aid in the development of novel therapies for the treatment of allergic eye disease and other allergic disorders.⁶

METHODS

Animals

Six-week-old A/J and BALB/c mice were obtained from the National Cancer Institute (Frederick, MD). BALB.B/Ai[KO]JL6N9 mice (IL-6 KO line 30) were obtained from Taconic, Inc. (Germantown, NY). These studies conformed to the principles for laboratory animal research outlined by the Animal Welfare Act (NIH/DHHS) and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the National Institutes of Health Animal Care and Use Committee. Each group contained 8 to 10 animals.

Antigen Challenge Protocol

RW sensitization and challenge were used to elicit an ocular allergic response. Mice were sensitized on days 0 and 4 with a 200- μ L intra-peritoneal (IP) injection containing 50 μ g RW extract (Greer Laboratories, Lenoir, NC) emulsified in an equal-volume mixture with incomplete Freund's adjuvant (IFA; Sigma-Aldrich, St. Louis, MO). The mice were subsequently challenged on days 14 and 15 with 200 mg/mL RW extract in PBS drop-wise in the eye (5 μ L/eye). To control for a nonspecific response to the deposition of protein on the eye surface, control mice were sensitized IP with PBS/IFA and challenged with RW in the eye. Animals were killed 24 hours after the challenge, to evaluate conjunctival eosinophil infiltration (Fig. 1). In all cases, the mice were deeply anesthetized with ketamine (100 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (10 mg/kg; Phoenix Pharmaceuticals, St. Joseph, MO) and exsanguinated by cardiac puncture.

A. suum Infection

Mice are semipermissive hosts to *A. suum* infection with normal migration from the cecum to the liver, lung, and small intestine, but not farther.¹⁰ *A. suum* eggs were obtained from adult worms isolated from infected pigs and embryonated to the infective stage.¹⁴ In the acute protocol (RW/acute), mice were infected on day 0 by feeding them 5×10^4 *A. suum* eggs concurrent with RW sensitization. To mimic chronic exposure that occurs in humans in countries where *A. suum* is endemic, a chronic protocol (RW/chronic) was established. RW/chronic mice were infected two to three times per week from day 44 until day 0 (RW sensitization). Control animals were either sham sensitized with PBS emulsified with IFA (PBS group), sham sensitized and infected with *A. suum* (*A. suum* group), or given RW sensitization without *A. suum* infection (RW group; Fig. 1).

Histology

To assess the cellular infiltrate in the conjunctiva and surrounding tissue, eyes and lids were removed intact, immediately fixed in 10%

phosphate buffered formalin (EMD Chemicals, Gibbstown, NJ), and embedded in resin (Histo-resin; Leica Instruments GmbH, Wetzlar, Germany). Serial sections were cut from each eye and lid and were stained with Giemsa (Volu-Sol, Inc., Salt Lake City, UT), hematoxylin and eosin (H&E; Biochemical Sciences, Swedesboro, NJ), or Congo red (Sigma-Aldrich). Slides were masked and counted by two independent investigators trained in assessment of ocular pathology, as described by Magone et al.¹⁴ Five sections were examined from each mouse per treatment group, and cells were counted in four 400 $\times$ nonoverlapping fields (which included the fornical conjunctiva) per animal.

Eosinophil Determination

Blood (25 μ L) obtained on exsanguination was transferred to a test for eosinophil determination (Unopette; BD Biosciences, Franklin Lakes, NJ). The number of eosinophils per cubic millimeter in the blood was determined according to the manufacturer's instructions.

Quantitation of Cytokine Production in Ag-Specific In Vitro Assays

Draining (cervical) lymph nodes and spleens were removed, cells were made into single-cell suspensions, and red blood cells were lysed with buffer (ACK; Biosource, Camarillo, CA), according to the manufacturer's instructions. The remaining cells were then cultured in one of the following: medium alone (AIM-V; Invitrogen, Carlsbad, CA), medium and 2.5 μ g/mL concanavalin A (ConA; Sigma-Aldrich) as a positive control, medium and 50 μ g/mL of RW extract, or medium and 10 μ g/mL extract of *A. suum* in the third and fourth larval stages. Data shown do not include the positive responses in all cultures tested in response to ConA or the baseline responses to medium alone. Cells were harvested after 48 hours in culture. EIA kits (Opt; BD Biosciences) containing the following antibody pairs were used for ELISA: IL-5 (TRFK4 and TRFK5), IL-6 (MP5-20F3 and MP5-32C11), and IL-10 (JES5-2A5 and SXC-1).

Sera ELISAs

Eotaxin measurements were made from the freshly isolated sera according to the manufacturer's instructions, with a mouse eotaxin ELISA construction kit (Antigenix America; Huntington Station, NY). Total IgE measurements were performed according to the manufacturer's instructions, with the EIA IgE ELISA kit (Opt; BD Biosciences). The plates were developed with 50 μ L/well of avidin peroxidase (ABTS; Kirkegaard and Perry, Gaithersburg, MD). They were then read at 450 nm (Spectra Max; Molecular Devices, Sunnyvale, CA) and quantitated (Soft Max Pro software; Molecular Devices).

T Cell Adoptive Transfer

CD4⁺/CD25⁺ T cells (T regulatory cells) or CD4⁺/CD25⁻ (control T cells) were purified from draining (cervical) lymph nodes and spleens of RW/chronic mice by cell sorting, as described previously.¹⁵ The T-cell subsets were >98% pure, as analyzed by flow cytometry. CD4⁺/CD25⁺ or CD4⁺/CD25⁻ T cells ($1-2 \times 10^5$) were transferred intravenously to RW/acute mice 24 hours before RW challenge in the eye. Sham-challenged RW/acute mice receiving no cells, as well as animals sensitized with PBS, RW, or *A. suum*-infected alone were the control subjects.

Data Analysis

Data are reported as the mean $\pm$ SE. Significant differences between groups were determined by analysis of variance using the Fisher least significant difference test for multiple comparisons (StatView; SAS Institute Inc., Cary, NC). Statistical significance was set at $P < 0.05$.

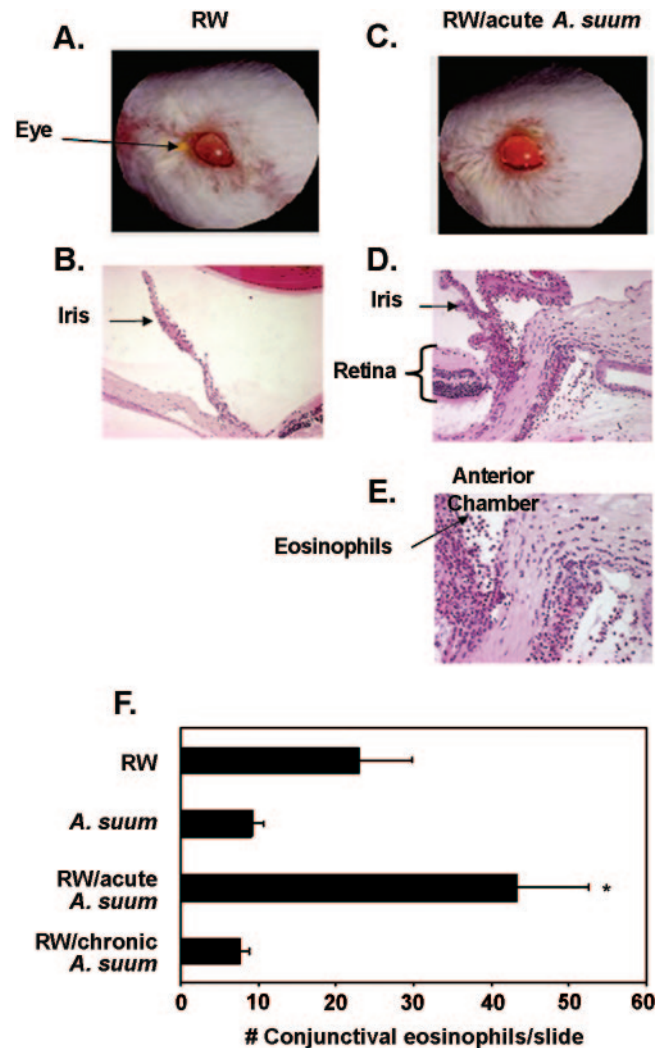


FIGURE 2. Eosinophil infiltration as a marker of allergic inflammation. (A) Gross appearance of a mouse eye that was sensitized and challenged with RW. (B) There was no cellular infiltration of the anterior chamber of the eye. (C) Gross appearance of a mouse eye in the RW/acute protocol and corresponding histologic images that display an increased infiltration of inflammatory cells (D) and (E), stained with H&E. These animals had a significant enhancement in the number of eosinophils migrating into the conjunctival matrix, the iris (iridocyclitis), and anterior chamber. (F) Animals were sensitized and challenged with RW or received concurrent acute or chronic *A. suum* infection. Control mice were sensitized with PBS or RW without *A. suum* infection (RW) or received *A. suum* infection without RW (*A. suum*). Five sections per mouse per treatment group were quantitated to determine the number of eosinophils. Results are expressed as the number of eosinophils that infiltrated the conjunctival matrix per slide. Magnification: (B, D) $\times 200$; (E) $\times 400$.

RESULTS

Effect of Acute Infection with *A. suum* versus Chronic Infection

RW/acute infection significantly enhanced clinical allergic ocular symptoms over animals given RW sensitization alone (Figs. 2C, 2A, respectively). Over 90% of the animals in the RW/acute group experienced blepharitis (eyelid swelling and inflammation), iridocyclitis, and the development of a novel eosinophil-prominent anterior uveitis in addition to conjunctivitis (Figs. 2D, 2E), suggesting a synergistic effect of *A. suum* and

RW in these animals. The chronic infection unexpectedly appeared to protect animals from allergic eye symptoms, as they were not distinguishable clinically from PBS-sensitized control animals ($P = 0.808$). In support of this finding, the number of eosinophils infiltrating the conjunctiva was significantly lower in RW/chronic animals than in RW/acute animals (Fig. 2F; $P = 0.0001$). Animals sensitized with RW had a significant increase in conjunctival eosinophils after RW challenge compared with *A. suum* alone ($P = 0.0038$). No cases of either anterior uveitis or iridocyclitis were observed in any other mouse group.

IL-6 Production in Response to Antigen Stimulation

The anterior uveitis in the RW/acute mice was characterized by an eosinophil influx into the iris, ciliary body (iridocyclitis), and anterior chamber of the eye. The development of uveitis in an allergic eye model was a surprising finding and exemplified the overwhelming eosinophil influx into the ocular tissues. Although eosinophil migration into the anterior chamber is exceedingly rare, case reports have been published describing such an influx in patients with systemic ascariid infections.¹⁶ In most of these cases, *Ascaris* larvae were found to have aberrantly migrated into ocular tissues, including the nasolacrimal gland^{17–20} and anterior chamber, resulting in the development of parasitic uveitis with secondary eosinophilic granuloma formation.^{16,21} Animal models of ocular *A. suum* infection^{22,23} and clinical investigations examining ocular infection with a closely related ascariid, *Toxocara canis*, have allowed for further characterization of eosinophilic uveitis^{17,24–26} and suggest that the eosinophil influx observed is elicited by a strong immune response to the presence of either the parasite or parasite products in the ocular tissue. Although we did not observe any direct histologic evidence of larval presence in the ocular tissue of either RW/acute group or any other mice in this study, we cannot rule out the transient migration of the larvae.²³ The observed eosinophilic anterior uveitis may also result from an enhanced response to systemically released *A. suum* antigen present in the RW/acute animals, similar to that observed in animal models of ocular ascariid infection using parasite antigen instead of whole larvae.²⁶ We do not believe endotoxin contamination of either the (ragweed) RW or the *A. suum* eggs was contributing to the eosinophil influx into the anterior chamber of the eyes in the RW/acute mice, as animals given either RW or *A. suum* infection alone did not show development of anterior uveitis nor did animals that had been chronically infected with *A. suum* before RW sensitization. In addition, the cellular infiltrate into the anterior chamber was predominantly eosinophils instead of neutrophils, which are the cells generally associated with models of endotoxin-induced anterior uveitis.²⁷

The development of anterior uveitis in patients and in endotoxin-induced models has been found to correlate positively with IL-6.²⁷ Because IL-6 had been associated with other models of anterior uveitis, we assessed the in vitro capacity of lymphocytes obtained from the cervical lymph nodes to produce IL-6 in response to RW or *A. suum* antigen (Fig. 3A). Lymphocytes from RW/acute animals, showed significant increases in IL-6 production during allergen-specific recall assays compared with all other groups ($P < 0.05$).

Eosinophil Infiltration into the Conjunctiva of IL-6 KO Mice

We used IL-6 deficient mice to determine whether the increased IL-6 observed in the RW/acute group contributes to development of the eosinophil-prominent anterior uveitis. The RW/acute protocol was observed in BALB/c mice genetically

deficient in IL-6 and wild-type (WT) control mice (Fig. 3B). WT BALB/c mice displayed a similar increase in eosinophil entry into the conjunctiva, as was observed in the previous experiments in A/J animals (Fig. 2F). Genetic depletion of IL-6 did not significantly alter the number of eosinophils migrating into the conjunctiva after RW challenge in any of the mouse groups ($P = 0.0657$) compared with their respective WT controls.

Quantity of Blood Eosinophils and the Severity of Allergic Disease in RW/Chronic-Infected Animals

As in humans, naïve mice have very low levels of circulating eosinophils (~2%).²⁸ Animals exposed to RW sensitization and challenge have a significant increase in the number of eosinophils found in the blood over PBS control animals ($P = 0.0035$). RW/acute animals, which had the highest concentration of eosinophils in the conjunctiva, had significant increases in circulating eosinophils compared with the RW control ($P = 0.0001$; Fig. 4A) as did mice inoculated with *A. suum* alone. Conversely, RW/chronic mice had the highest concentration of circulating eosinophils, but significantly fewer eosinophils in the eye than either the RW/acute ($P = 0.0309$) or RW-alone ($P = 0.001$) groups.

IL-5 has been shown to correlate with the number of eosinophils in the circulation and is also essential for the growth and differentiation of these cells.²⁹ To determine the capacity of the cervical lymph node cells to produce antigen-specific IL-5, lymphocytes were restimulated with RW or *A. suum* antigen (Fig. 4B). RW/chronic animals restimulated with *A. suum* antigen produced significantly greater levels of IL-5 than all other groups ($P = 0.002$), which probably contributed to the enhanced circulating eosinophilia observed in these animals (Fig. 4A).

Effect of Chronic *A. suum* Infection on Eotaxin Levels in Sera

Levels of the C-C chemokine eotaxin (CCL11) have been shown to be strongly associated with eosinophil migration into sites of allergic inflammation.^{30–32} Examination of sera showed a significant decrease in eotaxin levels in RW/chronic mice compared with all other groups ($P = 0.0173$; Fig. 5). This decrease in eotaxin was reflected by decreased eosinophil migration into the tissue. In contrast, RW/acute animals displayed the highest levels of eotaxin in the sera and had the highest number of eosinophils found in the conjunctiva and anterior chamber.

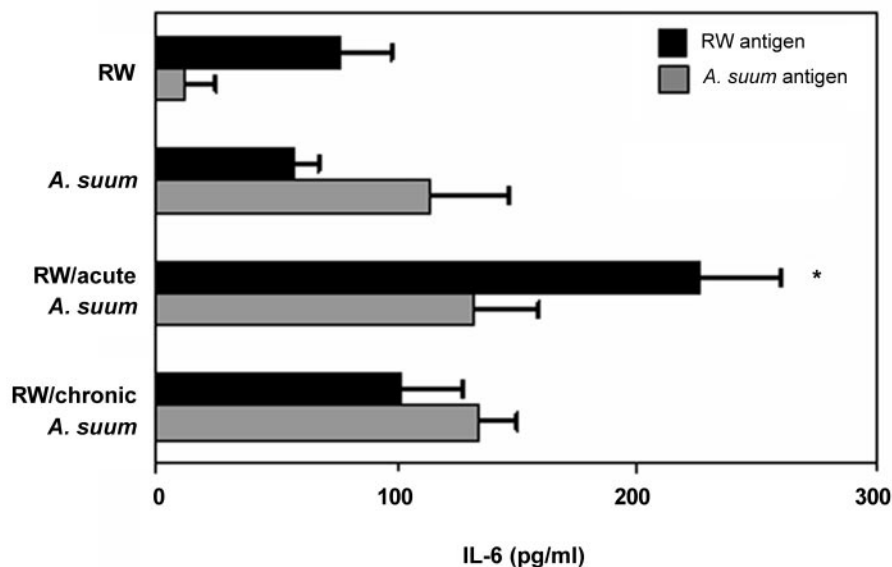
Chronic *A. suum* Infection and Polyclonal IgE and Mast Cell Degranulation

Chronic infection by *A. suum*, with or without RW sensitization, resulted in a significant increase in polyclonal IgE compared with either the RW/acute or RW alone groups (Fig. 6A, $P < 0.05$). RW/chronic mice also demonstrated a decrease in the number of intact mast cells in the conjunctiva (Fig. 6B) similar to that observed in the RW-alone and RW/acute groups; therefore, chronic infection did not block mast cell responses to RW.

Effect of Adoptive Transfer of CD4⁺/CD25⁺ T Regulatory Cells on Allergen-Specific Eosinophil Infiltration

In vitro-produced T regulatory cells have been found to decrease significantly the eotaxin and eosinophil influx into the tissue, in other models of allergic diseases.³³ Adoptive transfer of 1×10^5 CD4⁺/CD25⁺ T regulatory cells 24 hours before ocular challenge significantly decreased eosinophil infiltration into the conjunctiva compared with sham-transferred RW/

A.



B.

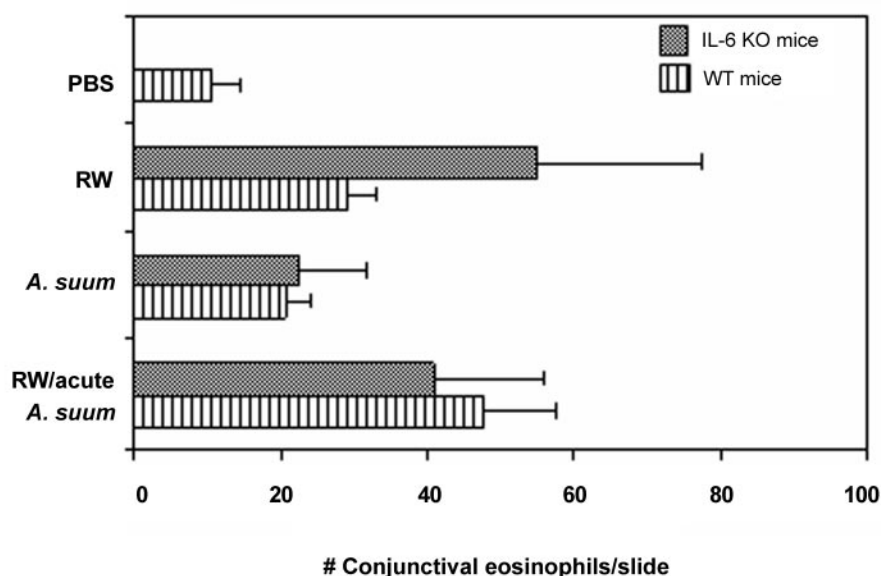


FIGURE 3. IL-6 production in response to antigen stimulation. (A) Concentration of IL-6 in draining lymph node supernatants from mice that were sensitized and challenged according to the labeled groups. The IL-6 was made in response to antigen-specific recall assays of pooled draining lymph nodes. Cells were incubated for 48 hours with medium, ConA, RW antigen (50 μ g/mL), or *A. suum* antigen (20 μ g/mL). Supernatants were collected, and IL-6 levels were detected by ELISA. Results are expressed as picograms per milliliter of IL-6 in the supernatant. (B) Number of eosinophils infiltrating the conjunctiva of IL-6 KO mice and BALB/c WT mice. Animals were subjected to the RW/acute protocol and control mice were sensitized with PBS or RW without *A. suum* infection (RW) or received *A. suum* infection without RW (*A. suum*).

acute mice ($P \leq 0.0001$; Fig. 7). Transfer of a similar number of $CD4^+/CD25^-$ control T cells did not confer the same protection, as these animals did not have any significant reduction in eosinophil infiltration compared with sham-transferred RW/acute mice ($P = 0.067$). This observation is in agreement with our finding of decreased systemic levels of eotaxin in the same RW/chronic mice.

DISCUSSION

Recent clinical studies in patients with light helminth infections found that clinical allergic symptoms were alleviated after treatment with anti-helminthics. That symptoms were lessened in individuals with heavy worm burdens corroborated our findings that heavy helminth infections protect against allergy.^{34,35} This suggests that acutely infected mice may reflect human disease with occasional light hel-

minth exposure, whereas chronic mice resemble individuals with heavy worm burdens and continuous exposure. Thus, RW/acute and RW/chronic models provide a method of investigating the underlying immune protective mechanisms that occur during the spectrum of clinical infections, complete with alterations of eosinophil influx into the ocular tissues.

Since IL-6 has been associated with Th1 polarized uveitis and was upregulated in RW/acute animals, IL-6 KO mice were used to determine whether IL-6 was responsible for the increase in ocular eosinophils. Genetic depletion of IL-6 did not alter eosinophil infiltration after RW/acute treatment compared with WT control animals. This observation is in agreement with Rosenbaum et al.,³⁶ who found that IL-6 does not play an initiating role in endotoxin-induced uveitis and suggests that other mechanisms may be responsible for the increase in anterior uveitis and conjunctivitis in RW/acute animals.

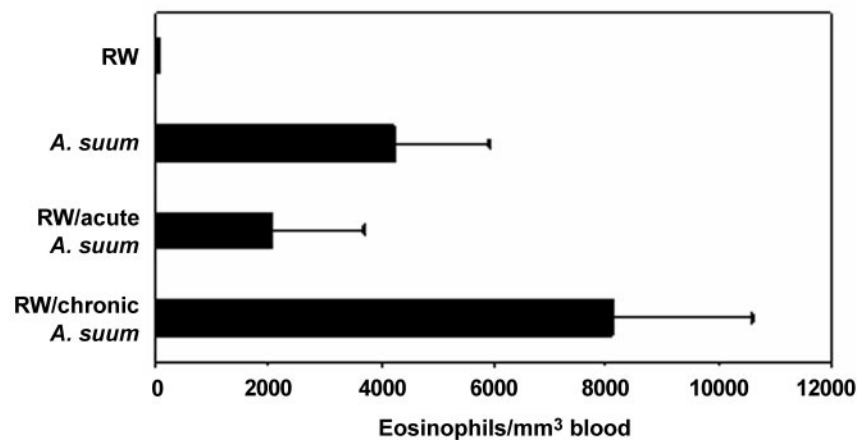
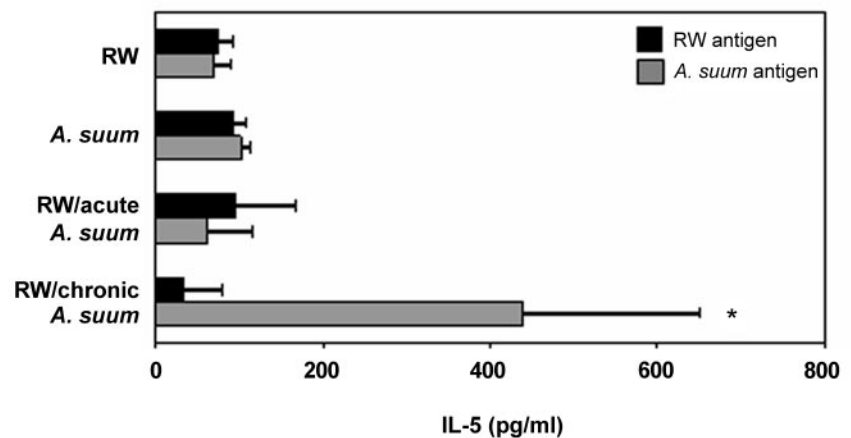
A.

FIGURE 4. RW/chronic infection significantly increased the number of circulating eosinophils and IL-5 concentration in draining lymph nodes. **(A)** The number of eosinophils per cubic millimeter of blood. Mice were sensitized and challenged with RW or received concurrent acute or chronic *A. suum* infection. Control mice were sensitized with PBS or RW without *A. suum* infection (RW) or received *A. suum* infection without RW (*A. suum*). **(B)** In each treatment group, draining lymph nodes were removed and pooled. Cells were incubated for 48 hours with either RW antigen (50 μ g/mL) or *A. suum* antigen (20 μ g/mL). Supernatants were harvested, and IL-5 levels were determined by ELISA. Results are expressed as picograms per milliliter IL-5 in the supernatant.

B.

The observed increase in ocular eosinophil infiltrates in RW/acute mice may be due to enhanced production of IL-5 and circulating eosinophils. However, RW/chronic mice demonstrated not only an inverse relationship between IL-5 levels and systemic eotaxin, but also a decrease in eosinophil infiltration into the ocular tissue. This suggests that in RW/chronic mice a compartmentalization occurs wherein eosinophils in the peripheral blood do not enter the conjunctiva, perhaps due to decreased levels of eotaxin.

The IgE-blocking hypothesis is a paradigm that suggests the high concentration of helminth-induced polyclonal IgE blocks the binding to and subsequent cross-linking of Fc ϵ R on the surface of mast cells with allergen-specific IgE.^{7,37} Consistent with this hypothesis, we observed significantly higher levels of total IgE production in the RW/chronic mice; however, the ratio of polyclonal to specific IgE that is needed to block cross-linking in vivo is currently unknown. The required level may be nonphysiologic and a ratio greater than 1000:1 may be

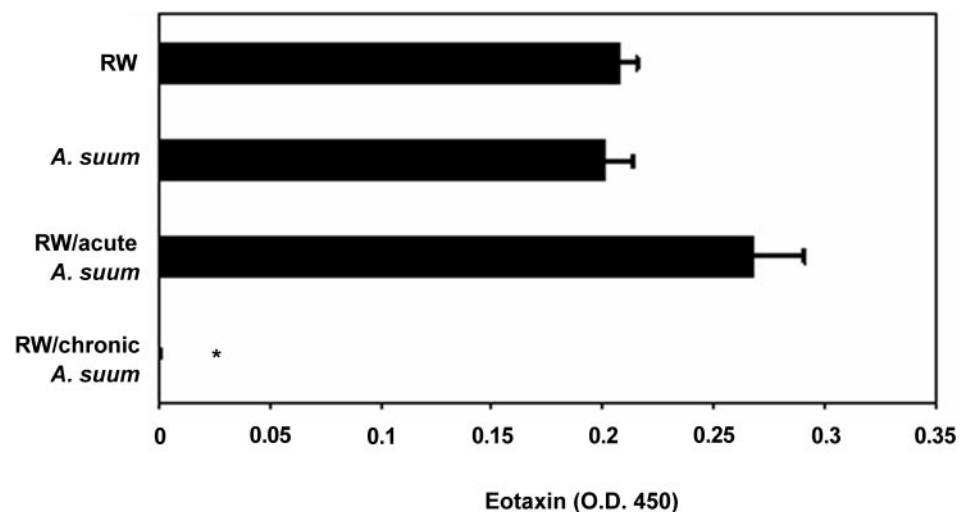
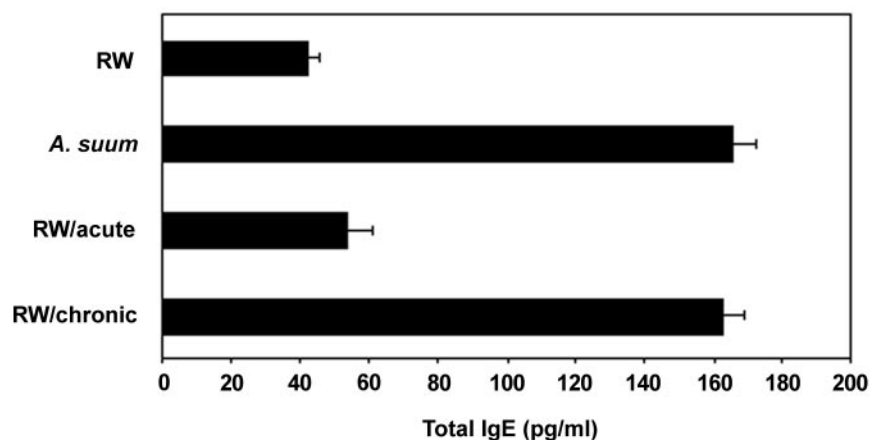


FIGURE 5. Serum eotaxin levels. Eotaxin levels obtained from sera after exsanguination were quantitated by ELISA. Results are expressed as ocular density (O.D. 450).

A.



B.

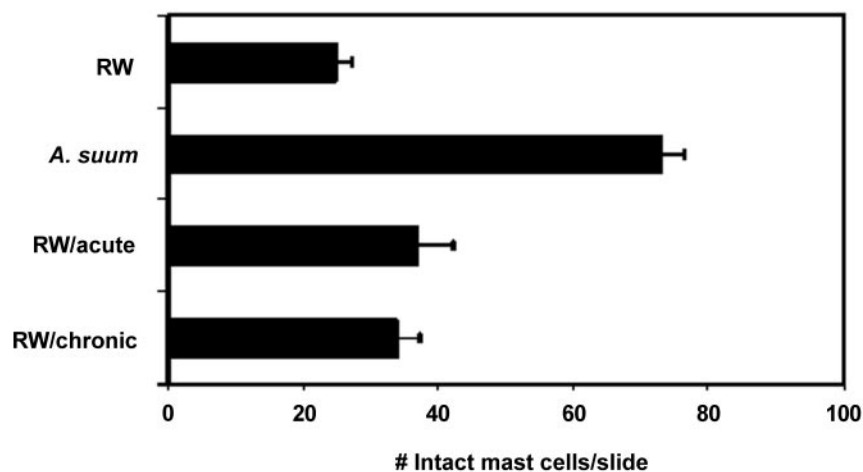


FIGURE 6. Serum IgE levels and mast cell degranulation. **(A)** Total (polyclonal) IgE levels in sera of mice sensitized and challenged as noted and quantitated by ELISA. Animals were sensitized and challenged with RW or received concurrent acute or chronic *A. suum* infection. Control mice were sensitized with PBS or RW without *A. suum* infection (RW) or received *A. suum* infection without RW (*A. suum*) **(B)** The number of intact mast cells in the conjunctiva of mice sensitized and challenged as noted on the y-axis.

necessary to be effective (Nutman T, Mitre E, personal communications, 2004). As an indirect measure of IgE binding to FcεRI, conjunctiva was examined for evidence of mast cell

degranulation at the time of death (24-hours after allergen challenge). Animals sensitized with RW, RW/acute, or RW/chronic all showed increased mast cell degranulation in re-

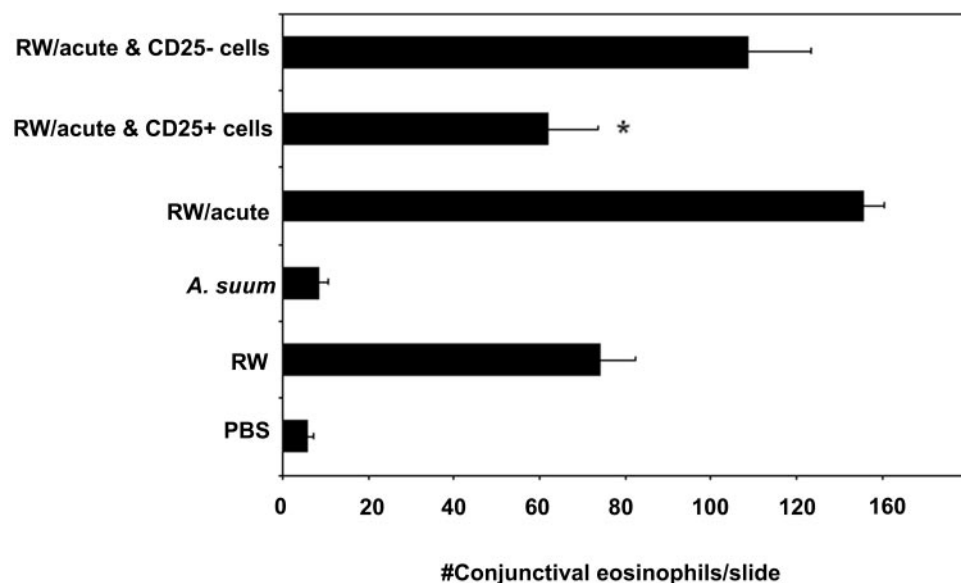


FIGURE 7. Number of eosinophils in the conjunctiva after adoptive transfer of T regulatory cells. Animals were sensitized with RW or received concurrent acute *A. suum* infection before transfer of CD4⁺/CD25⁺ regulatory T cells or CD4⁺/CD25⁻ control T cells isolated from chronic *A. suum*-infected mice. Control mice were sensitized with PBS or RW without *A. suum* infection (RW) or received *A. suum* infection without RW (*A. suum*). Five sections per mouse per treatment group were quantitated to determine the number of eosinophils. Results are expressed as the number of eosinophils that had infiltrated the conjunctival matrix per slide.

sponse to RW stimulation compared with *A. suum* alone, which suggests that RW-specific antibodies elicited mast cell degranulation, regardless of the presence of polyclonal or *A. suum*-specific IgE.

Several recent epidemiologic studies have suggested that T regulatory cells induced by chronic helminth infections may be responsible for protection from allergic eye disease, in part through the induction of immunosuppressive cytokines such as IL-10,³⁸ as patients who are protected from allergic disease have a higher number of CD4⁺/CD25⁺ T regulatory cells and produce a higher amount of IL-10. Consistent with this hypothesis, RW/chronic mice displayed increased IL-10 production (data not shown), concurrent with decreased eotaxin and eosinophil egress into the ocular tissue. RW/acute and RW/chronic protocols in either IL-10-deficient mice or WT mice treated with anti-IL-10Rab were unsuccessful, as the *A. suum* infection was lethal in these animals (data not shown), because of the development of an overwhelming Th1 cytokine response (IFN- γ and TNF- α).³⁹

T regulatory cells have been found to inhibit several eosinophilic chemokines, including eotaxin,³³ and to increase anti-inflammatory mediators such as IL-1 receptor antagonists,⁴¹ and have been shown to inhibit Th2 cytokines, leading to an overall reduction in responses to allergic inflammation.^{41,42} In addition, in recent studies Hesse et al.⁴² have found that the pathogenesis of another helminth, *Schistosoma mansoni*, is controlled by both IL-10 producing innate effector and regulatory T cells. In addition, in vitro-induced T regulatory cells have been shown to alter allergic responses.^{33,43} Preliminary studies in our laboratory have shown that a T regulatory cell component may contribute to the protection demonstrated in the RW/chronic model. In these studies, transfer of 1×10^5 CD4⁺/CD25⁺ regulatory T cells, but not CD4⁺/CD25⁻ control T cells significantly reduced eosinophil infiltration into the conjunctiva of RW/acute animals. However, transfer of these T regulatory cells did not ablate eosinophil influx into the conjunctiva completely, suggesting that either additional T regulatory cells or other mechanisms are needed to confer complete protection from allergic ocular disease. Additional studies will be conducted to clarify the precise role of IL-10 and regulatory T cells in the development of ocular allergic disease.

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