Uveitis Induced by Lymphocytes Sensitized against a Transgenically Expressed Lens Protein

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PURPOSE. Previously established experimental models for lens-associated uveitis (LAU) are all mediated by antibodies. The present study analyzed the features of a novel experimental intraocular inflammatory eye disease that is mediated by lymphocytes targeted at a lens antigen.

METHODS. Conventional technologies were used to generate three lines of transgenic (Tg) mice, expressing hen egg lysozyme (HEL) under the control of the αA-crystallin promoter. To induce intraocular inflammation, these Tg mice were injected with lymphocytes from syngeneic wild-type donors sensitized against HEL. Before their injection, the cells were stimulated in culture with HEL. To release lenticular material, some eyes were capsulotomized. Ocular histopathologic changes were examined by routine methods. Levels of HEL antibody were measured by enzyme-linked immunosorbent assay, whereas cellular immunity was determined by the lymphocyte proliferation assay.

RESULTS. Intraocular inflammation developed in HEL–Tg mice injected with syngeneic lymphocytes sensitized against HEL. The severity of inflammation was directly related to the number of injected cells, as well as to the accessibility of HEL. The most intense inflammation was seen in Tg mice in which the lens was disintegrated due to high production of HEL. In mice with no apparent lenticular changes the inflammation was enhanced by capsulotomy. The inflammation affected all segments of the eye and persisted for at least 39 days after adoptive transfer of cells. Four days after cell injection, the inflammation consisted of subacute infiltration, with both mononuclear and polymorphonuclear leukocytes, whereas more chronic infiltration was seen at later times. Vigorous cellular immunity but no antibody to HEL was found in recipient mice, thus demonstrating the exclusive participation of cellular immunity in the pathogenesis of this experimental disease.

CONCLUSIONS. Transgenic mice expressing HEL in their lenses develop intraocular inflammation after injection of syngeneic lymphocytes sensitized against HEL. This experimental disease is a novel cell-mediated model for LAU. (*Invest Ophthalmol Vis Sci.* 1999;40:2735–2739)

Lens-associated uveitis (LAU) is a severe inflammatory disorder that has been attributed to an autoimmune response against released lens proteins.[1,2] An experimental disease resembling the human condition can be induced in animals by immunization with lens crystallins followed by puncture of the lens capsule. The experimental disease, which has been thoroughly studied by Marak and his coworkers, is a type III, Arthus-like, hypersensitivity reaction mediated by antigen-antibody complexes and the complement cascade.[1,2] The preferred involvement of antibodies against lens antigens in the pathogenesis of established experimental models of LAU can be attributed to the selective immunotolerance of the T-cell compartment toward crystallins. Early studies have demonstrated that mice fail to mount a T-cell response against autologous crystallins but do produce antibodies to these proteins.[3] More recent experiments have indicated that selective T-cell tolerance to self-crystallins is due to the presence of these proteins in the thymus of the animals.[4]

The potential involvement of cellular immunity in the pathogenesis of LAU in humans is unclear, but lymphocyte responses against crystallins were found in patients with cataracts, in particular after extracapsular lens extraction.[5] To investigate whether LAU can be mediated by a cellular immune response, we developed a novel experimental system in which transgenic (Tg) mice expressing a foreign antigen, hen egg lysozyme (HEL), in their lens are injected with lymphocytes specifically sensitized against HEL. The lymphocytes are obtained from wild-type (WT) syngeneic mice that are immunized against this foreign antigen. Preliminary observations on this system have been briefly described elsewhere[6]; the present communication reports new data of an extended study in which the following parameters of the experimental system have been examined: detailed analysis of the histologic changes in different lines of Tg mice, after adoptive transfer of various numbers of activated lymphocytes, kinetics of these changes, the effect of capsulotomy on the inflammatory reaction, and the cellular and humoral immune responses in the recipient mice.

MATERIALS AND METHODS

Mice

Transgenic mice expressing HEL under control of the αA-crystallin promoter were generated as described in detail elsewhere.[4] Transgenic mouse lines used here included those expressing low or high copy numbers of the membrane-bound HEL (M-HEL) and those expressing the soluble form of the protein (S-HEL).[4] Normal female FVB/N mice, 6 to 8 weeks old,

provided by the National Cancer Institute breeding facility (Frederick, MD), were used as the syngeneic WT donors of splenocytes. All procedures with mice were carried out in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Adoptive Transfer of HEL-Sensitized Splenocytes

Donor WT mice were immunized with 50 μg HEL (Sigma, St. Louis, MO), emulsified with CFA containing *Mycobacterium tuberculosis* at 2.5 mg/ml (Difco, Detroit, MI). The emulsion, in a volume of 0.2 ml, was injected subcutaneously into the base of the tail and the two hind thighs. Spleen cells of these donors were collected 12 to 14 days postimmunization and stimulated in culture with HEL as follows: aliquots of 4×10^6 cells were cultured in 12-well cluster plates in 2 ml RPMI-1640 medium supplemented with 5% fetal calf serum, 50 μM 2-mercaptoethanol, antibiotics, and HEL at 10 μg/ml. After incubation for 3 days, the cells were collected, washed, and injected intraperitoneally into Tg mice, as indicated. Unless otherwise stated, the recipient mice were killed 4 days after cell injection. Experimental groups consisted of 2 to 8 animals. The inflammatory changes and the immune responses were remarkably similar among mice of each group.

Capsulotomy

After deeply anesthetizing the mouse, the eye to be operated on was first treated topically with a drop of AK-DILATE (Akorn, Abita Springs, LA). Subsequently, this eye received topically a drop of Ophthetic 0.5% (Allergan; Hormigueros, Puerto Rico) for additional anesthesia. After the eye was sufficiently dilated, a 30-gauge needle was inserted into the anterior chamber through the peripheral third of the cornea. The lens capsule was then incised in a linear fashion using the tip of the needle (iris was not punctured). Triple antibiotic ointment (Phoenix Pharmaceutical, St. Joseph, MO) was applied topically at the completion of the procedure.

Histologic Examination

Eyes were fixed in 4% glutaraldehyde for 30 minutes before being transferred to 4% formaldehyde. Sections were prepared and stained with hematoxylin–eosin by conventional methods.

Immune Responses of Donor and Recipient Mice

Blood samples and spleens were collected immediately after the mice were killed and tested for humoral and cellular immunity to HEL, using the enzyme-linked immunosorbent assay (ELISA) and the lymphocyte proliferation assay, respectively. The two assays are described elsewhere.[4]

RESULTS

Ocular Inflammation in Recipients of HEL-Sensitized Lymphocytes

In accordance with our previous communication,[4] severe noninflammatory morphologic changes were seen in eyes of high copy number M-HEL–Tg mice (Fig. 1A). These changes include disruption of the lens fibers and distortion of the lens capsule, as well as microphthalmia. When injected with splenocytes sensitized against HEL, these mice developed intense intraocular inflammation characterized by severe keratitis, iridocycli-

tis, vitritis, and retinitis (Figs. 1B, 1C, and 1D). The cellular infiltration was of subacute type, consisting of both mononuclear (MN) and polymorphonuclear (PMN) leukocytes (Fig. 1E). The severity of inflammatory changes was directly related to the number of injected cells; the changes shown in Figures 1B through 1E were induced by injection of 10^8 cells, whereas markedly milder changes developed after injection of 10^7 cells (Figs. 1F and 1G). The pathogenic capacity of the sensitized splenocytes also depended on their stimulation in culture with HEL; no pathogenic changes were detected in recipients injected with 10^8 HEL-sensitized but unstimulated splenocytes (data not shown).

Unlike in high copy number M-HEL–Tg mice, no abnormal morphology was detected in eyes of low copy number M-HEL–Tg mice or S-HEL–Tg mice.[4] When injected with HEL-sensitized splenocytes, mice of these two lines did develop ocular inflammation, but of considerably lower intensity than that seen in the high copy number M-HEL–Tg recipient mice. Figures 2A and 2B show typical changes in eyes of low copy M-HEL–Tg and S-HEL–Tg recipients, respectively. The ocular changes in eyes of these two lines of mice were remarkably similar and consisted mainly of proteinaceous exudate, as well as mixed cellular infiltration in the anterior segment. Mild vitritis and retinal vasculitis were also often observed in these eyes (Fig. 2C). The coparticipation of MN and PMN leukocytes in the inflammatory process is clearly depicted in Figure 2D, which shows an eye section of a S-HEL–Tg mouse injected with 10^7 HEL-sensitized cells. The limbus is packed with mostly lymphocytes, whereas the infiltration of the ciliary body consists mainly of PMN leukocytes.

Enhancement of Ocular Inflammation by Capsulotomy

The lens capsule of both S-HEL–Tg and low copy number M-HEL–Tg mice seems to be intact,[4] thus restricting the exposure of the lens antigens to the adoptively transferred HEL-sensitized splenocytes. To investigate the effect of increased exposure of lens HEL, we performed capsulotomy on eyes of recipient mice of these two lines. Figure 2E shows a section of a capsulotomized eye from a S-HEL–Tg mouse, 4 days after injection of 10^8 HEL-sensitized splencytes. The site from which the lenticular material was released is heavily infiltrated with a mixture of inflammatory cells. The cellular infiltration in the posterior segment of these eyes (Fig. 2F) was also often more intense than in untreated eyes. Minimal or no inflammation was seen in the eyes of control mice examined 4 days after capsulotomy without adoptive cell transfer (not shown).

Persistence of Ocular Changes in Recipient Mice

Eyes of recipient mice were routinely examined 4 days after cell injection. Eyes examined after longer periods revealed that the inflammation persisted for at least 39 days after adoptive transfer of the cells. An eye section with typical changes at 39 days after cell injection is shown in Figures 2G and 2H. The changes include a granuloma-like structure with fibroblasts and neovascularization extending toward the retina, as well as retinal vasculitis, with mainly MN leukocytes.

Immune Responsiveness to HEL in Recipient Mice

To further elucidate the immunologic capacity of the adoptively transferred splenocytes in the recipient mice, we

FIGURE 1. Histopathologic changes in eyes of recipient HEL-Tg mice injected with HEL-sensitized splenocytes. (**A**) Eye of an uninjected control high copy number M-HEL–Tg mouse. The eye is dystrophic, and the characteristic morphology of the lens is completely disrupted. Yet, this eye is free of inflammation (magnification, ×25). (**B**) Eye of high copy number M-HEL–Tg mouse injected with 10^8 HEL-sensitized splenocytes. Intense inflammation is seen in all tissues of this eye (magnification, ×25). (**C**) Anterior segment of the eye shown in (**B**). Heavy cellular infiltration in the edematous cornea, ciliary body, and iris. Proteinaceous and cellular exudate in the anterior chamber. Leaking lenticular material is seen in the vitreous (arrow; magnification, ×100). (**D**) Posterior segment of the eye shown in (**B**). Intense inflammation in the extensively edematous retina. The vitreous is filled with proteinaceous exudate. The lens capsule is disintegrated (magnification, ×100). L, lens. (**E**) A sample of the cellular exudate in the anterior segment (**C**), showing a mixture of PMN and MN leukocytes (magnification, ×500). (**F**) Eye of a high copy number M-HEL–Tg mouse injected with 10^7 HEL-sensitized splenocytes. Inflammation in the anterior segment affecting the limbus, cornea, and ciliary body (magnification, ×100). (**G**) The posterior segment of the eye shown in (**F**). Perivascular infiltration in the retina and light exudate in the vitreous. The lens is disintegrated (magnification, ×100).
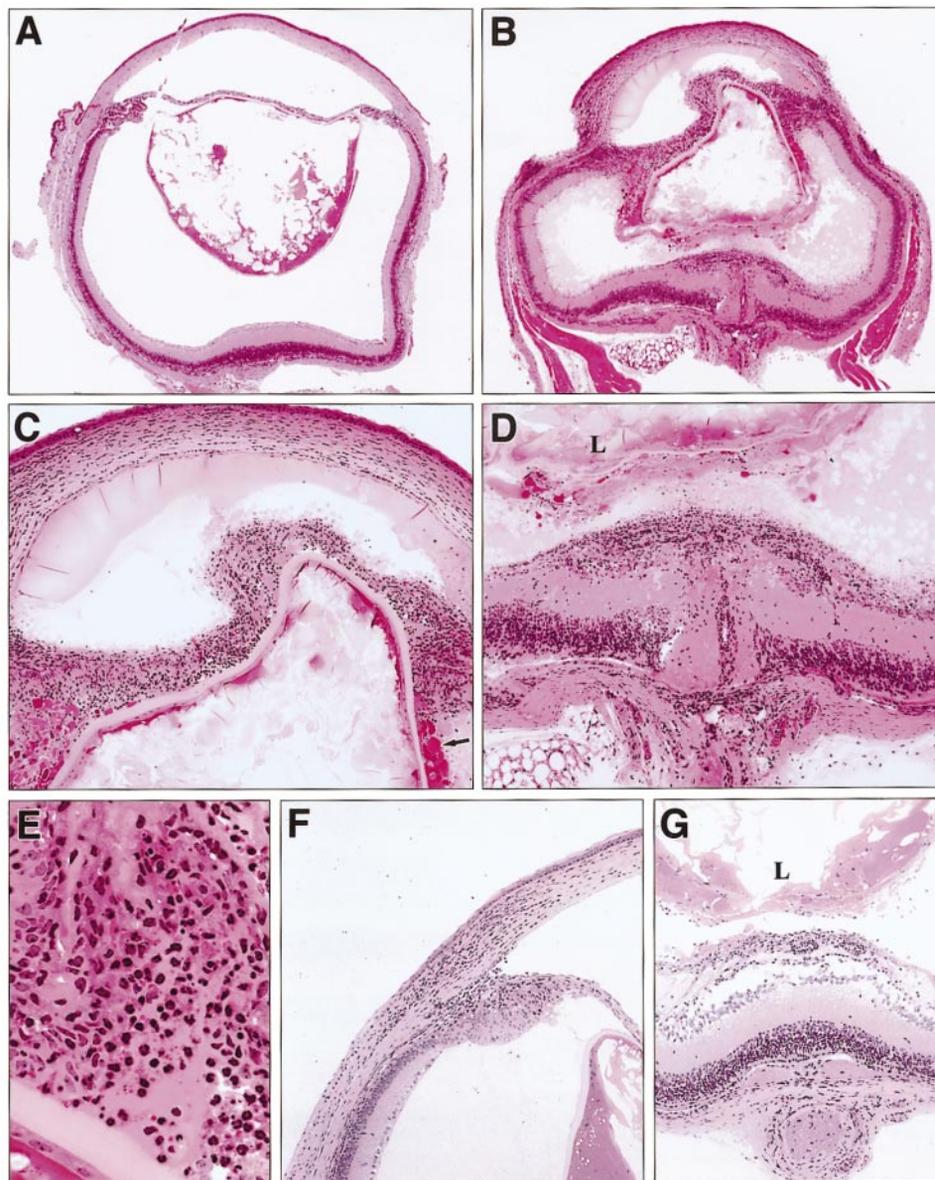
tested these mice for HEL antibodies in their serum and for proliferative responsiveness against HEL by their spleen lymphocytes. Figures 3 and 4, respectively, summarize data of representative recipient mice. For comparison, these figures also record the immune responses of typical WT mice actively immunized with HEL. High levels of HEL antibodies were measured in sera of the immunized WT mice, but no antibody could be detected in sera of recipient mice at any tested time after transfer of HEL-sensitized splenocytes (Fig. 3). In contrast, spleen cells of recipient mice responded vigorously to HEL, with stimulation indices similar or even higher than those monitored in cultures of spleen cells from the actively immunized mice (Fig. 4). The high level of cellular immune response to HEL was retained in recipient mice for at least 39 days after cell injection (Fig. 4).

DISCUSSION

The present study provides new data concerning a novel experimental system in which intraocular inflammation is in-

duced in mice by adoptive transfer of lymphocytes sensitized against a foreign antigen transgenically expressed in the recipient's lens. Unlike all previously developed models of LAU, which are antibody mediated,[1,2] this new experimental model is cell mediated. The lack of humoral immunity in the pathogenesis of the current model was established by the absence of antibody against HEL, the neo–self-lens antigen, in recipient mice (Fig. 3). In contrast, vigorous cellular responses to HEL were recorded in cultures of splenocytes from the recipient mice (Fig. 4). Interestingly, the splenocytes exert their pathogenic activity only after specific stimulation in culture with the antigen; unstimulated splenocytes had no pathogenic effect. A similar requirement for stimulation in culture was also found in other systems in which sensitized lymphocytes adoptively transfer inflammatory diseases such as experimental autoimmune uveoretinitis,[7] or immune-mediated blepharoconjuctivitis.[8] The process of stimulation in culture modifies the expression of certain surface molecules[9] and, consequently, enhances the lymphocyte capacity to cross tissue-blood barriers.
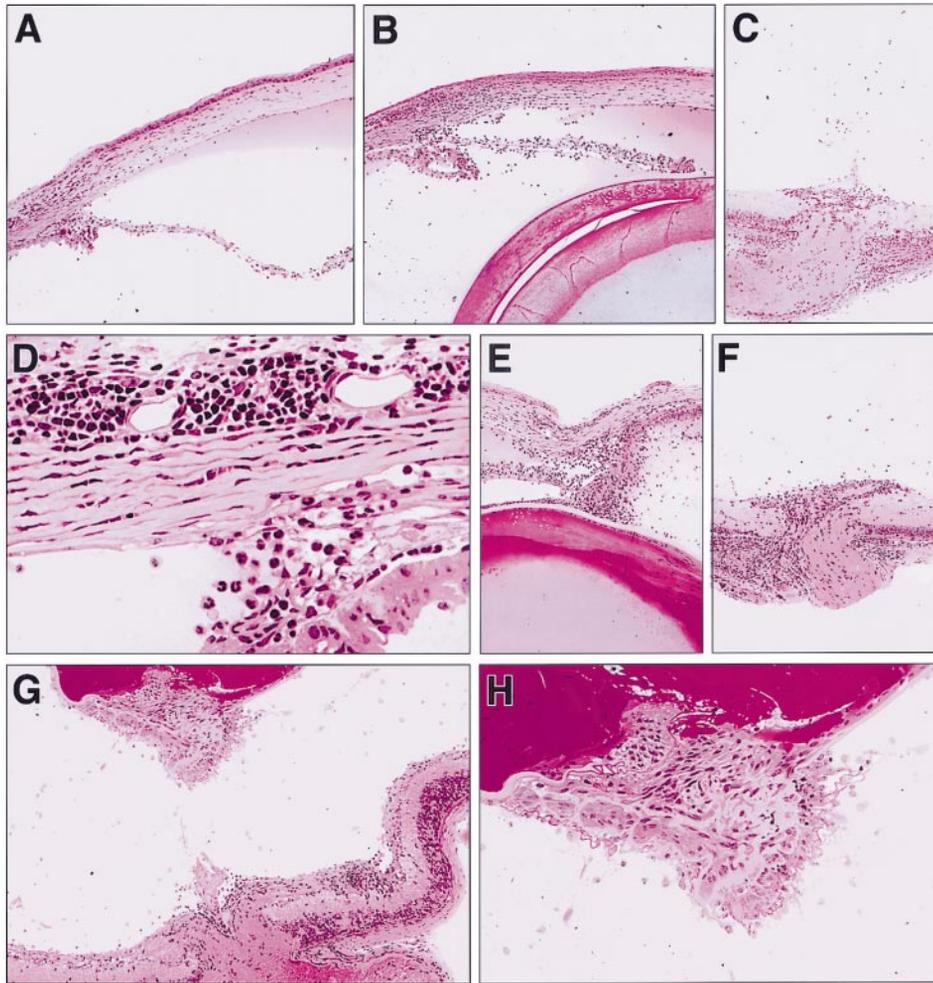
FIGURE 2. Changes in eyes of HEL-Tg mice injected with HEL-sensitized splenocytes. (**A**) Eye of a low copy number M-HEL–Tg mouse injected with 10^8 HEL-sensitized splenocytes. The limbus and adjacent cornea, as well as the ciliary body, are infiltrated with inflammatory cells. Proteinaceous exudate with a few cells in the anterior chamber (magnification, ×100). (**B**) Eye of a S-HEL-Tg mouse injected with 10^8 HEL-sensitized splenocytes. Cellular infiltration in the limbus, cornea, ciliary body, and iris, as well as proteinaceous exudate, with cells, in the anterior and posterior chambers (magnification, ×100). (**C**) Posterior segment of the eye shown in (**B**). Cellular infiltration at the optic nerve head, as well as in the vitreous (magnification, ×100). (**D**) Eye of a S-HEL–Tg mouse injected with 10^7 HEL-sensitized splenocytes. Dense accumulation of MN leukocytes in the limbus, but mainly PMN leukocytes infiltrating the ciliary body (magnification, ×500). (**E**) Eye of a S-HEL–Tg mouse capsulotomized and injected with 10^8 HEL-sensitized splenocytes. Intense inflammation along the needle track, as well as cellular and proteinaceous exudate in the anterior chamber (magnification, ×100). (**F**) Posterior segment of the eye shown in (**E**). Perivascular infiltration in the retina, mainly at the optic nerve head. Inflammatory cells in the vitreous (magnification, ×100). (**G**) Eye of a high copy number M-HEL–Tg mouse injected with 10^8 HEL-sensitized splenocytes 39 days before enucleation. A granuloma-like structure attached to the posterior section of the lens with extension toward the infiltrated retina at the optic nerve head (magnification, ×100). (**H**) A high-power magnification of the granuloma-like structure shown in (**G**). The structure consists of fibroblasts, inflammatory cells, and neovascular tissue (magnification, ×500).

The ocular inflammatory process was particularly severe in high copy number M-HEL-Tg recipient mice, in which the lens capsule was disintegrated and allowed exposure of lens proteins (Fig. 1). However, inflammation was also observed in eyes of S-HEL-Tg or low copy number M-HEL-Tg mice (Figs. 2A-C), in which no apparent morphologic changes were seen in the lens.[4] The latter finding differs from observations in the two experimental models of antibody-mediated LAU, i.e., "experimental lens-induced granulomatous endophthalmitis" and "Arthus-type panophthalmitis"; in both models inflammation developed only after puncture of the lens capsule.[1,2] Moreover, we have recently found that lymphocytes from αB-crystallin knockout mice sensitized against murine αB-crystallin induce intense ocular inflammation when injected into naive WT recipient mice, but only after capsulotomy; no inflammation was detected in uncapsulotomized recipient eyes (M. Gelderman et al., unpublished data). It is proposed, therefore, that despite their normal appearance, lenses of the S-HEL-Tg and low copy number M-HEL-Tg mice are altered by the transgenic production of HEL in a manner that makes HEL more

accessible to the immune system than are lens crystallins in a normal eye.

The relevance of the experimental system described here to clinical LAU is not clear since no direct evidence is available to show involvement of cell-mediated immunity in the pathogenesis of the human disease. Had such involvement been established, the animal disease described here could become a model for the human entity.

The histopathologic changes in eyes of the recipient mice were subacute when examined 4 days after cell injection (Figs. 1C-E) and became more chronic and granulomatous in eyes collected at later time points (Figs. 2G and 2H). The inflammatory changes induced by sensitized lymphocytes in mice in the present study resemble in certain aspects those seen in rats with experimental LAU, in which the pathogenic processes are antibody-mediated.[1,2] Of particular interest is the finding that the cellular infiltration in recipient mice in the present study consisted to a large extent of PMN leukocytes that typically are the hallmark for Arthus-like, antibody-mediated inflammatory reactions. This observation resembles our previous finding,[7] that PMN leukocytes are a major component of the cellular infiltration in eyes of rats in which experimental autoimmune uveoretinitis is adoptively transferred by lymphocytes sensitized against retinal antigens. It is assumed that in both systems the initial immunopathogenic process is triggered when small numbers of specifically sensitized lymphocytes recognize their target ocular antigen, become activated, and release a battery of inflammation-promoting cytokines, including chemokines. The PMN leukocyte involvement in lymphocyte-mediated ocular inflammation is attributed to the activity of certain chemokines that selectively attract PMN leukocytes.[10] These chemokines are assumed to be produced by the inflammatory cells, as well as by resident ocular cells.[10] The finding that similar PMN infiltrations
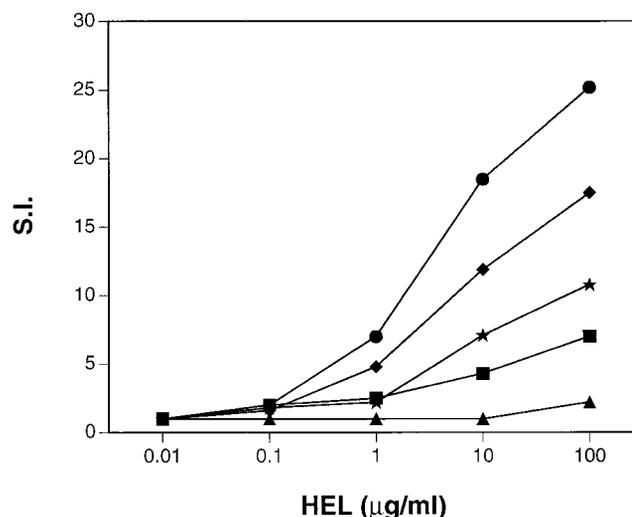
FIGURE 4. Proliferative response to HEL of spleen cells from representative HEL-immunized WT control mice (★ and ■) and HEL-Tg recipients of 10^8 HEL-sensitized splenocytes, injected 4 days (●) or 39 days (◆) before being tested. The response curve of a naive WT control mouse is depicted by the ▲ symbol. The responses are presented as stimulation indices (S.I.).

characterize ocular inflammation of various origins suggests that chemokines produced by resident ocular cells play a major role in these disease processes. These notions are currently under investigation.

Acknowledgments

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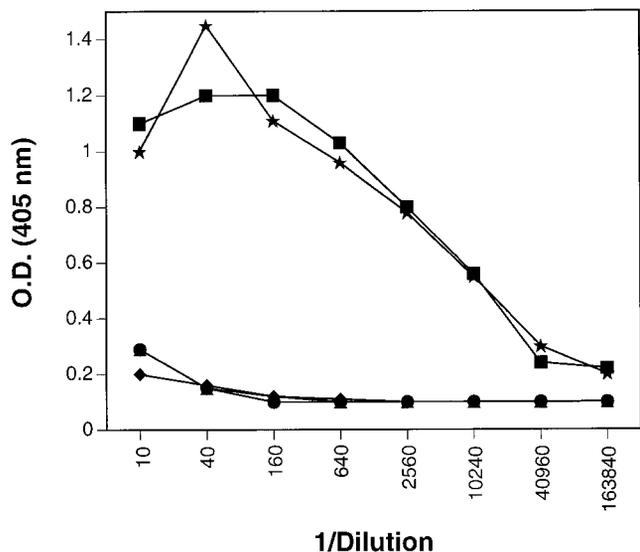
FIGURE 3. Levels of HEL antibody in sera of representative HEL-immunized WT control mice (★ and ■) and HEL-Tg mice injected with 10^8 HEL-sensitized splenocytes 4 days (●) or 39 days (◆) before serum collection. Also shown is the response curve of a naive WT control mouse (▲). Antibody levels were measured by ELISA, and the data are presented as optical density (O.D.) units.