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Inducible immune regulation following autoimmune disease in the immune-privileged eye

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Abstract

The immune-privileged eye has the potential to induce regulatory immunity along with local mechanisms of immunosuppression. Rodent models of human autoimmune uveoretinitis [experimental autoimmune uveoretinitis (EAU)] recover without spontaneous recurrence of uveitis, which differs from uveitis in some humans. This raises the possibility that the mechanism of immune privilege in the rodent eye can reimpose itself during autoimmune uveoretinitis and re-establish tolerance to autoantigen. To investigate this possibility, we examined the spleens of EAU-recovered mice for regulatory immunity. We detected regulatory immunity when we adoptively transferred post-EAU spleen cells into other mice immunized for EAU. We could not detect this regulatory immunity in enucleated mice nor in naive mice. Moreover, unlike the mechanisms of anterior chamber-associated immune deviation, the suppression was only mediated by post-EAU $CD4^+$ T cells, which required activation with autoantigen presented by post-EAU spleen antigen-presenting cells (APC). Our results demonstrate that when the immune-privileged ocular microenvironment recovers from an autoimmune response, it has influenced systemic immunity to retinal autoantigen by affecting APC and mediating induction of potential regulatory $CD4^+$ T cells laying in wait in the post-EAU spleen for restimulation.

Keywords

regulatory T cells; ocular immunity

INTRODUCTION

The eye is an immunosuppressive microenvironment that uses multiple mechanisms to suppress the activation of T cells that mediate inflammation and cytotoxicity [1]. Some of these mechanisms involve soluble factors found in aqueous humor, which suppress interferon-γ production by effector T cells and the activation of delayed-type hypersensitivity (DTH) by T helper cell type 1 (Th1) cells [2]. The aqueous humor factors also prevent antigen-presenting cells (APC) from presenting antigen in a manner that induces Th1 cell activation [3]. In addition, the immunosuppressive mechanisms of the ocular microenvironment include contact-dependent suppression of T cell activation by iris, ciliary body, and retinal pigmented epithelial cells [4–6] along with the extensive expression of FasL to induce apoptosis in activated Th1 cells [7,8].

The most interesting of the ocular immunosuppressive mechanisms are the three potential pathways that mediate the induction of $CD4^+$ regulatory T (Treg) cells in mice. There is anterior chamber-associated immune deviation (ACAID), pigment epithelium-dependent activation of

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Treg cells, and aqueous humor conversion of Th1 cells into Treg cells [1]. The effects of intraocular transforming growth factor-β_2 (TGF-β_2) on F4/80$^+$ macrophages mediate ACAID [9,10]. Such APC pick up and process antigen from within the ocular microenvironment [11, 12]. The macrophages migrate to the marginal zone of the spleen, and through a series of highly regulated activities, the macrophages establish multicellular clusters from which Treg cells emerge [13–16]. This induction of regulatory immunity can be adoptively transferred using spleen APC or spleen CD3$^+$ T cells from mice with ACAID to naive mice [12,17–20]. ACAID has been long been considered an important pathway in inducing Treg cells that help in preserving tolerance to ocular autoantigens.

The activation of effector CD4$^+$ T cells that were in physical contact with pigmented epithelial cells produces TGF-β and functions as Treg cells [21]. The pigmented epithelial-dependent Treg cells can be used to suppress an adoptive transfer of DTH. The contact-dependent mechanism of the pigmented epithelial cells is unknown; however, it does not involve obvious adhesion or T cell activation-associated molecules [6]. It is speculated that the importance of this pathway of activating a Treg cell is to have a preemptive mechanism to prevent the production of inflammatory lymphokines and to ensure lymphokine production that is supportive of the ocular immunosuppressive microenvironment.

The third potential pathway of inducing Treg cells is the activity of the aqueous humor immunosuppressive factor α-melanocyte-stimulating hormone (α-MSH) [22–25]. This immunomodulating neuropeptide is constitutively expressed within the ocular microenvironment, and we have found that at its ocular physiological concentration, α-MSH converts Th1 cells into Treg cells [24,25]. We have previously shown that α-MSH-induced Treg cells are effective in suppressing antigen-specific DTH and autoimmune disease [23–25]. This induction of regulatory activity is through the melanocortin 5 receptor expressed on primed CD4$^+$ T cells [24]. This focus affected on Th1 cells suggests that the ocular microenvironment has the means to directly suppress T cells activated within the eye [2].

The mechanisms of each of these pathways overlap with the localized suppression of inflammation and affect certain immune cells and stages of immunity [26]. They are also examples of the redundancy in the ocular microenvironment to suppress and to manipulate immunity to further promote the local immunosuppression and possibly maintain antigen-specific tolerance. So, if it is possible to induce an immune response to an ocular autoantigen, there should be an induction or expansion of regulatory immunity to the autoantigen. This may very well be the case in human uveitis and rat experimental autoimmune uveoretinitis (EAU) [27,28].

In mice, EAU is a retina-specific, Th1 cell-mediated autoimmune disease, which can be induced in B10.RIII mice and models human autoimmune endogenous uveitis [29–31]. This autoimmune disease is induced by immunizing a naive B10.RIII mouse with a peptide fragment of a human retinal autoantigen interphotoreceptor retinoid-binding protein (IRBP) [32]. The clinical symptoms naturally resolve without spontaneous relapse. Therefore, this is an induced immune response to an ocular autoantigen, and in the experiments to be described, we demonstrate that there is induced immune regulation in the spleens of EAU-recovered mice that can suppress, in adoptive transfer experiments, the induction of EAU in other mice.

MATERIALS AND METHODS

Animals and reagents

Female B10.RIII (H-2^r) mice, 6 – 8 weeks old, were obtained from Jackson Laboratories (Bar Harbor, ME). Schepens Institutional Animal Care and Use Committee (Boston, MA) approved the animal use and care. Invitrogen Life Technologies (Carlsbad, CA) synthesized human IRBP

peptide (IRBPp)161–180 (SGIPYIISYLHPGNTILHVD). Complete Freund's adjuvant (CFA), incomplete Freund's adjuvant, and desiccated *Mycobacterium tuberculosis* H37RA were purchased from Difco Laboratories (Detroit, MI).

Induction of EAU

To induce EAU in B10.RIII mice, they were injected subcutaneously with 50 μg IRBPp161–180, emulsified in CFA containing 2.5 mg/ml heat-killed *M. tuberculosis* H37RA [25]. The severity of retinal inflammation was clinically assessed every 3 or 4 days by fundus examination and scored on a 0 –5 scale [24,25,33]. Eyes were given a score of 0 for no inflammation; eyes with only white focal lesions of vessels were given a scored of 1; eyes with linear vessel lesions, over less than half of the retina, were given a scored of 2; eyes with linear vessels lesions, over more than half of the retina, were given a score of 3; eyes with severe chorioretinal exudates or retinal hemorrhages in addition to the vasculitis were given a score of 4; and eyes with a subretinal hemorrhage or a retinal detachment were given a score of 5. The fundus examination was performed on anesthetized mice, and their pupils dilated with 1.0% Tropic-amide ophthalmic solution (Akorn Inc., Buffalo Grove, IL) before the examination.

Adoptive transfer of spleen cells

Spleens were collected from mice that had recovered from EAU (all the eyes in a group of mice had scores less than 1 on day 40 after the B10.RIII mice were immunized to induce EAU). One group of B10.RIII mice was surgically enucleated 1 week before it was immunized with IRBPp. Their spleens were collected on day 40 as if they had EAU and recovered. The post-recovery spleen cells were made into a single-cell suspension, red blood cells were lysed with lysing buffer (Sigma Chemical Co., St. Louis, MO), and 30×10^6 cells were injected intravenously (i.v.) into a naïve, syngeneic mouse on the day the mouse was immunized with IRBPp. In several experiments, we depleted specific populations of spleen cells before their adoptive transfer. $Thy1.2^-$ cells were isolated by negative selection using anti-Thy1.2-coated Dynabeads (Dynal, Lake Success, NY). The purity of this cell sorting was 99% $Thy1.2^-$ cells. The depletion of $CD11b^+$ and $CD19^+$ cells was done by staining the post-recovery spleen cells with anti-CD11b or with anti-CD19 antibody conjugated with fluorescein isothiocyanate; both antibodies were from BD PharMingen (San Diego, CA). The cells were then selected out and discarded by fluorescence-activated flow cytometry. The spleen cell preparations were 100% depleted of $CD11b^+$ or $CD19^+$ cells. The depleted, post-recovery spleen cells were collected and resuspended in physiological phosphate-buffered saline (PBS), and 30×10^6 cells were injected i.v. into each naïve, syngeneic mouse on the day the mouse was immunized with IRBPp to induce EAU.

Adoptive transfer of in vitro-activated and sorted post-recovery spleen cells

Spleens were collected as described above from mice that had recovered from EAU. The spleen cells were resuspended in serum-free media: RPMI 1640, 0.1% bovine serum albumin, 0.2% ITS+1 media supplement (Sigma Chemical Co.), and 3×10^6 cells were added to the wells of a 96-well flat-bottom culture plate with 5 μg/ml appropriate IRBPp. The cells were incubated for 24 h and collected. The cultured cells were first enriched for $CD3^+$ T cells using R&D Systems (Minneapolis, MN) mouse T cell enrichment columns. The purity of the sorted cells was assayed by flow cytometry, and it was 99% $CD3^+$ cells. Negative selection of specific populations of T cells $CD4^+$ $CD8^+$ or depletion of activated $CD25^+$ $CD4^+$ cells was done using specific SpinSep and StemSep kits (StemCell Technologies, Vancouver, BC, Canada). The purity of the cell sorting was 90% for $CD4^+$ and $CD8^+$ cells and 100% elimination of $CD25^+$ $CD4^+$ cells. The sorted post-recovery spleen cells were collected and resuspended in

physiological PBS, and 1×10^6 cells were injected i.v. into each naïve, syngeneic mouse on the day the mouse was immunized with IRBPp to induce EAU.

Data presentation and statistical analysis

The EAU scores reported in the figures were the mean score of all the mice in a treatment group. Each treatment group was a total of 10 mice from two independent experiments of five mice each. On each day of observation, the EAU score of a mouse was calculated by averaging the clinical scores of both of its eyes. Consistency of the scoring was validated by the cooperation of a veterinarian ophthalmologist in our laboratory, Dr. Daniel J. Biros, DVM, American College of Veterinary Ophthalmologists, who randomly chose and scored the mice with no prior knowledge of our scores. We had greater than 95% concordance with scores greater than 1. Statistical analysis between treatment groups was performed using a Mann-Whitney nonparametric test.

RESULTS

Regulatory immunity in the spleens of EAU-recovered mice

If in the recovery of EAU there is an induction of regulatory immunity, we should be able to find it in the spleen, which is, for ocular immunology, the most logical place to search [26]. Also from previous work, this regulatory immunity should be transferable, suppressing the clinical symptoms of EAU in other immunized mice [19,20]. To test this possibility, we collected spleen cells from EAU-recovered B10.RIII mice at approximately Day 40. Recovery was defined as the time when the uveitis scores, described in Materials and Methods, of all the eyes in a mouse group fell to 1 or less. Like an ACAID experiment, we collected the post-recovery spleen cells and adoptively transferred them into naive B10.RIII mice (30×10^6 spleen cells per mouse), which were then immunized for EAU with IRBPp on the same day (Fig. 1). The fundus examinations showed that the mice injected with post-recovery spleen cells had a significant reduction in clinical scores, which reflects a suppressed inflammatory response compared with the course of retinal inflammation in mice not injected with post-recovery spleen cells (Fig. 1) or in mice injected with naive spleen cells (not shown). However, unlike ACAID [19,20] experiments, neither the adoptive transfer of post-recovery APC (Thy 1.2^- cells) alone nor $CD3^+$ cells alone suppressed EAU in the recipient mice (Fig. 1A). In addition, there was significant suppression in EAU when we adoptively transferred post-EAU spleen cells depleted of potential APC [13,15], $CD11b^+$ (macrophages), or $CD19^+$ (B cells; Fig. 1B). Therefore, EAU-recovered mice have in their spleens regulatory immunity, and the immune cells or interactions between immune cells are similar but not the same as reported for the adoptive transfer of ACAID.

Regulatory immunity in enucleated mice immunized with IRBPp

To examine the possibility that an immunization to the ocular autoantigen was sufficient to induce regulatory immunity in the spleen, the B10.RIII mice were enucleated 7 days before being immunized with IRBPp. The mice were kept for 40 days, the length of time the control whole mice recovered from EAU, and spleen cells were collected. The adoptive transfer of spleen cells from the IRBPp-immunized enucleated mice into whole B10.RIII mice immunized for EAU could not suppress the severity of EAU as compared with suppression seen in the adoptive transfer of post-recovery spleen cells (Fig. 2). Therefore, immunization alone cannot account for the presence of the regulatory immunity in the spleen following recovery from EAU. The results do suggest that induction of regulatory immunity in the spleens of EAU-recovered mice to ocular autoantigens requires the ocular microenvironment and possibly the autoimmune disease itself.

Post-recovery regulatory immunity by activated CD4+ T cells

As we can suppress the severity of EAU by the adoptive transfer of unfractionated spleen cells from post-recovery EAU mice, but not by the adoptive transfer of APC or T cells alone, it is suggested that we were looking at something different from ACAID. If it was ACAID, then the adoptive transfer of the post-recovery APC (Thy 1.2^{-} cells) alone should have been sufficient to mediate suppression [19,20]. The inability to suppress EAU with the post-recovery T cells alone suggested that the Treg cells need to be activated, presumably by the spleen APC presenting ocular autoantigen, which would be obtained when we immunized the mice that received the adoptive transfer of unfractionated spleen cells. To examine this possibility, we collected the post-EAU spleens and cultured them in vitro with added IRBPp. The spleen cells were incubated for 24 h, and we sorted out the $CD4^+$ and $CD8^+$ T cells and adoptively transferred these individual, activated populations of T cells into other mice immunized for EAU, and the course of uveoretinitis in these mice was observed and scored.

The severity of EAU was suppressed in mice injected with post-recovery spleen $CD4^+$ T cells that were stimulated in vitro with IRBPp before they were adoptively transferred into the mice immunized for EAU (Fig. 3A). In addition, the severity of EAU could not be suppressed in mice injected with IRBPp-stimulated $CD4^+$ T cells sorted from spleens of naive mice (Fig. 3A) nor could $CD8^+$ T cells, sorted from spleens of naive or EAU-recovered mice, suppress EAU (Fig. 3B). Moreover, depleting the $CD25^+$ cells from the in vitro-stimulated, post-recovery $CD4^+$ T cells eliminated the suppressive activity of the adoptively transferred $CD4^+$ T cells (Fig. 4), demonstrating that the regulatory immunity is mediated by the post-EAU, splenic $CD4^+$ T cells, which have been antigen stimulated by post-EAU, splenic APC. The results also demonstrate that such a population of Treg $CD4^+$ cells cannot be detected or stimulated from spleens of mice that had not suffered EAU. Therefore, EAU-recovered B10.RIII mice have in their spleens regulatory immunity mediated by $CD4^+$ T cells that are awakened by post-EAU spleen APC presenting exogenously provided ocular autoantigen from the immunization of the recipient injected with whole spleen cells or from in vitro culturing with autoantigen before sorting and adoptive transfer of the activated post-EAU, splenic $CD4^+$ T cells.

DISCUSSION

Our results, at first blush, suggest that during EAU, there is the induction of ACAID, which previous publications predicted [19,20]. However, what we have found are some of the elements of ACAID in our results. We found that like ACAID, the adoptive transfer of post-EAU, unfractionated spleen cells transferred immune regulation to ocular autoantigen in naive recipient mice. Unlike ACAID, the post-EAU spleen APC on their own couldn't transfer immune regulation. It is interesting that neither could the post-EAU spleen $CD3^+$ T cells on their own transfer immune regulation. Unlike the published methods for adoptively transferring ACAID, we adoptively transferred the T cells without APC. Another contrast to ACAID is that the immune regulation was not associated with $CD8^+$ T cells but solely with $CD4^+$ T cells [34].

One of the other similarities with ACAID is the need for the post-EAU spleen APC to present antigen [13,15] to activate the $CD4^+$ T cells that will mediate the suppression. Our evidence for this comes from the need to transfer unfractionated, post-EAU spleen cells or to provide antigen to the post-EAU spleen cells in vitro and then transfer the activated $CD4^+$ T cells into IRBPp-immunized mice. The APC in the unfractionated, post-EAU spleen cell adoptive transfer must be processing IRBP from the immunization of the naive recipient. It is interesting to see that the immunization scheme to induce EAU in the recipient could not mediate the activation of suppressive activity by the post-EAU $CD4^+$ T cells when they are transferred alone. This suggests that there is a unique relationship between the presentation of IRBPp by

the post-EAU APC and the post-EAU $CD4^+$ T cells that cannot be replicated by the APC mediating the immune response to the immunization. Also, it appears that the post-EAU spleen APC, required for the activation of the Treg cells, can be filled by macrophages or B cells.

In ACAID, two types of APC, macrophages and B cells, engage in a communal sharing of the ocular antigens, and without the B cells, ACAID is not induced [15,35]. In contrast, we were able to suppress EAU when the macrophages or the B cells, but not when both, were depleted from the adoptively transferred, post-recovery spleen cells. Our results demonstrate that it is required to adoptively transfer from post-EAU spleens the macrophages or the B cells with the T cells to suppress EAU in the recipient mouse. There is a possibility that the adoptively transferred, post-EAU macrophages or B cells recruit other types of host APC to help in activating the Treg cells [36]. Our results also demonstrate that this must be a property of ocular immune privilege, as we cannot find such regulatory immunity in the spleens of enucleated mice immunized with IRBPp. These findings are constant with the mechanisms of inducing regulatory immune cells by the ocular immune-privileged microenvironment [1].

It is interesting that the Treg cells were not found in naive mice, as they have been found for other autoimmune diseases [37]. Recently, two publications suggest that retinal antigen is expressed in a manner that promotes systemic tolerance to retinal antigens under nonperturbed, ocular conditions [38,39]. It has been demonstrated that central immunity is responsible for the generation of Treg $CD4^+$ cells that are reactive to retinal autoantigen, although this central tolerance is not strong enough to prevent EAU by immunization with retinal autoantigen [39]. If central tolerance is responsible for the induction of Treg cells in EAU-susceptible mice, then it must be the ocular inflammation itself that promotes the expansion of Treg $CD4^+$ cells or their accumulation in the spleen. Gershon and colleagues [40] first suggested the potential for the spleen to be the homing site of naturally induced Treg cells. They found that mice, which were splenectomized, lost a population of T cells that localized to the spleen and mediated suppression of cellular immunity. Our results further support these early observations of spleen-homing Treg cells. However, this does not explain the need to provide antigen before we can see the immunosuppressive activity in the adoptively transferred, post-EAU spleen cells. Our results suggest that these T cells are lying-in-wait in the spleen for re-exposure to ocular autoantigen. Also, the presentation of antigen must be done by the post-EAU APC, or else, the solo adoptive transfer of post-EAU spleen $CD4^+$ T cells into mice being immunized for EAU would have been sufficient to activate the transferred Treg cells. Therefore, the post-EAU spleen $CD4^+$ Treg cells are rescued, anergic, IRBPp-specific T cells or an expanded population of Treg cells, two possibilities that can be mediated by ACAID-ogenic or TGF-β_2-treated APC [41,42]. There is also the possibility that the post-EAU Treg cells are generated, but inactivate, because of the lack of peripheral expression of ocular autoantigen following resolution of EAU. There are classical and recent publications that demonstrate some level of antigen sequestration in the eye and low expression of ocular autoantigens in the periphery [38,39,43–46].

There are few publications describing the natural induction of Treg cells following autoimmune disease. There is only one publication describing the emergence of post-recovery $CD4^+$ Treg cells in rats with EAU [27]. This work described the presence of $CD4^+$ Treg cells in the spleens of EAU-recovered rats. These $CD4^+$ Treg cells, when adoptively transferred into other rats, could only suppress EAU if the recipient rats were immunized to induce EAU, but not if the EAU in the recipient rat was passively induced. This suggests that they also needed to reactivate the post-EAU spleen $CD4^+$ Treg cells in the rat, just as this requirement was for our post-EAU spleen $CD4^+$ Treg cells in mice, but unlike our findings that the rat Treg cells could be activated without the need for the antigen to be presented by post-EAU spleen APC. Post-recovery, regulatory immune cells are also seen in the rat model of experimental autoimmune encephalomyelitis (EAE) [47], although this immune regulation differs from our own

discoveries in the EAU-recovered mice. Rats that have recovered from EAE have $CD8^+$ Treg cells in their spleens [48]. There are $CD4^+$ Treg cells in the post-recovery EAE rats, but they are found in the lymph nodes and may be induced by B cells [49,50]. This further supports the finding that the mechanisms of immunoregulation are different in the eye and the brain [51], and it suggests that a natural result of autoimmune disease in these immune-privileged tissues is the induction of Treg cells. The role and fate of these Treg cells in the post-autoimmune disease animal are unknown, but it is reasonable to speculate that they may regulate any further immune responses to autoantigen and therefore, provide some degree of re-established immune tolerance to the autoantigen.

The immune-privileged eye has the potential to induce regulatory immunity besides local mechanisms of immunosuppression, suggesting that the mechanisms of immune privilege reimpose themselves during autoimmune uveoretinitis and reestablish tolerance to retinal autoantigens. If this is the case, then it is possible that it is one of the known mechanisms of ocular immune privilege that mediates the induction of Treg cells in the spleen. In healthy eyes, it is known that the immunosuppressive factors TGF-β_2 and α-MSH can mediate induction of ACAID-ogenic APC and $CD25^+$ $CD4^+$ Treg cells, respectively [23,52]. It remains to be seen whether these factors are also involved in the induction of the regulatory immunity we have found in the post-EAU spleen.

Our experimental results demonstrate regulatory immunity in the spleens of EAU-recovered mice. This regulatory immunity requires the ocular microenvironment and is mediated by post-EAU $CD4^+$ Treg cells. The post-EAU $CD4^+$ Treg cells require activation by post-EAU spleen APC to mediate suppression. These findings indicate that when recovering from autoimmune uveoretinitis, the ocular microenvironment influences systemic immunity to retinal autoantigen in a manner that may help prevent a reoccurrence of the ocular autoimmune disease.

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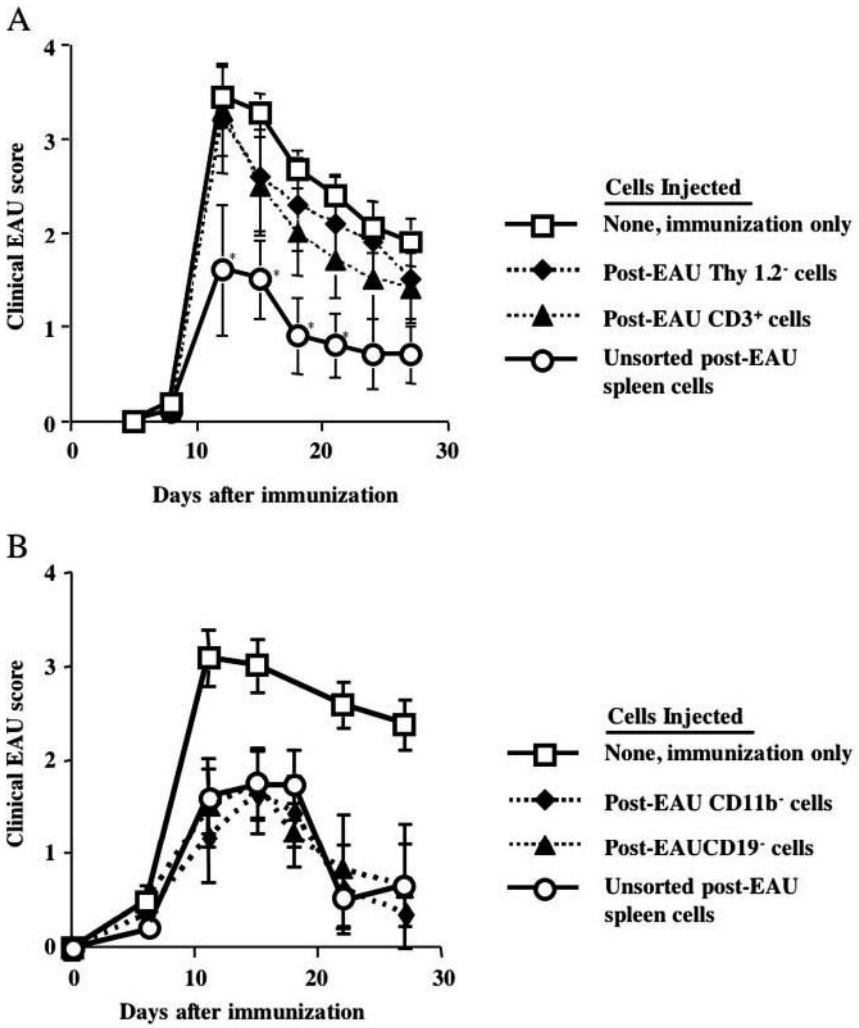

Fig. 1.

The effects of post-EAU spleen cells adoptively transferred into naive mice immunized for EAU. Spleen cells isolated from B10.RIII mice, which recovered from EAU, were collected, sorted, and adoptively transferred i.v. into syngeneic mice immunized to induce EAU. The retinas of the immunized mice were clinically examined, and the inflammation was scored to assess the effects of injecting post-EAU spleen cells (see Materials and Methods). (A) The recipient mice were injected with unsorted, post-EAU spleen cells (30×10^6 cells/mouse), post-EAU spleen Thy1.2$^-$ cells (20×10^6 cells/mouse), post-EAU CD3$^+$ T cells (10×10^6 cells/mouse), or only immunized to induce EAU. (B) The recipient mice were injected with unsorted, post-EAU spleen cells (30×10^6 cells/mouse), post-EAU spleen CD11b$^-$ cells (30×10^6 cells/mouse), post-EAU spleen CD19$^-$ cells (30×10^6 cells/mouse), or only immunized to induce EAU. The results are the mean clinical EAU score ± SEM over time as described in Materials and Methods. *, Results are significantly ($P \leq 0.05$) different from the clinical EAU scores of mice immunized with IRBPp but not injected with post-EAU cells. (B) All groups are significantly ($P \leq 0.05$) different from the clinical EAU scores of mice only immunized with IRBPp to induce EAU.

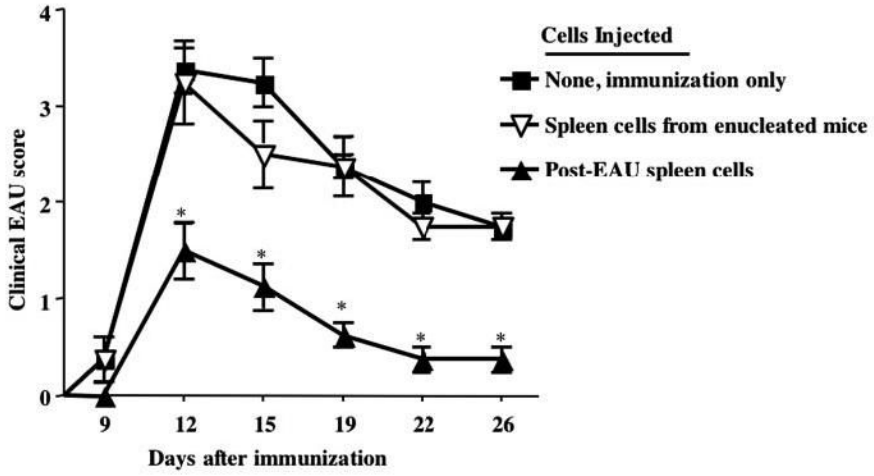

Fig. 2.

The effects of enucleating mice on regulatory immunity in the spleen. The B10.RIII mice were enucleated 7 days before IRBPp immunization. Their spleen cells were isolated 40 days after immunization. Recipient naive B10.RIII mice were immunized for EAU and injected with spleen cells (30×10^6 cells/mouse) from enucleated IRBP-immunized mice or spleen cells from intact post-EAU mice (30×10^6 cells/mouse) or were not injected with cells. The retinas of the immunized mice were examined, and the inflammation was scored. The results are the mean clinical EAU score ± SEM over time as described in Materials and Methods. *, Results are significantly ($P \leq 0.02$) different from the clinical EAU scores of mice immunized with IRBPp to induce EAU.

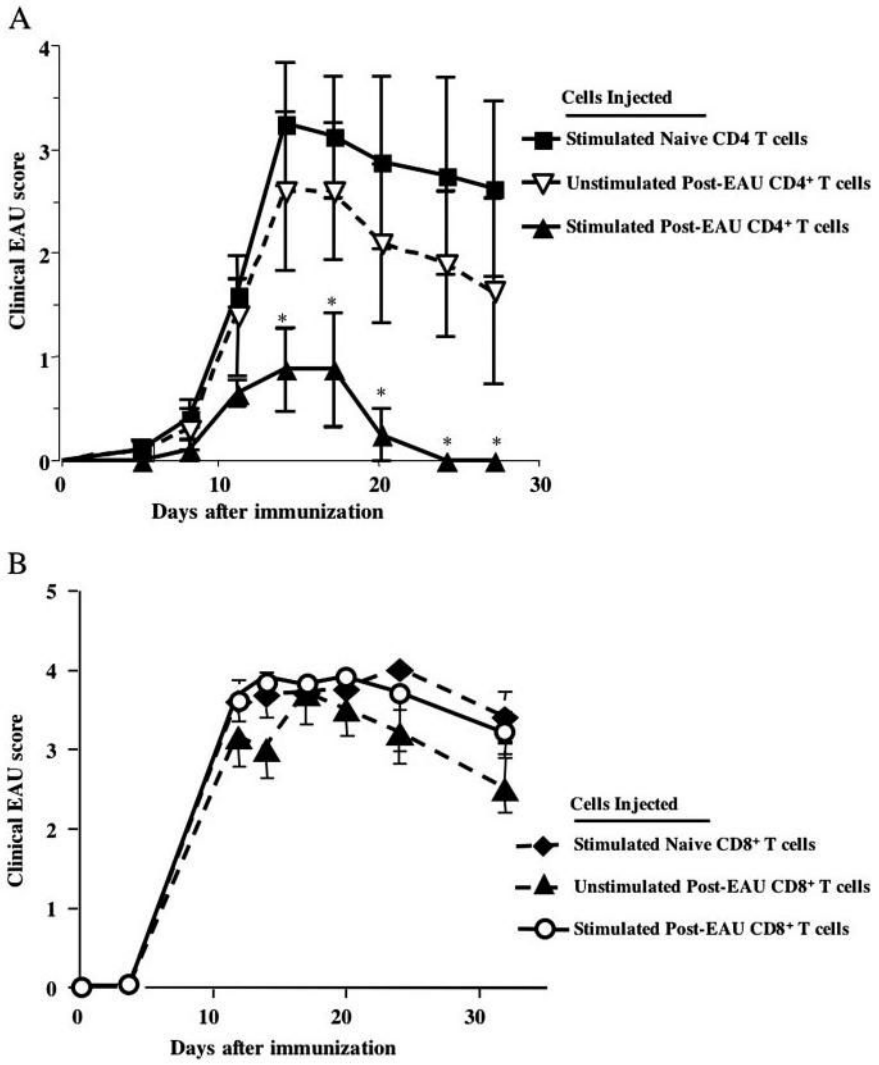

Fig. 3.

The effects of adoptively transferring antigen-stimulated post-EAU $CD4^+$ T cells. Spleen cells isolated from B10.RIII mice that recovered from EAU were collected and placed in culture with added IRBPp (5 μg/ml) for 24 h as described in Materials and Methods. The cultured cells were negatively selected for (A) post-EAU $CD4^+$ T cells or (B) post-EAU $CD8^+$ T cells. Recipient naive B10.RIII mice were immunized for EAU and injected with culture-stimulated post-EAU T cells (1×10^6 cells/mouse), culture-unstimulated, post-EAU T cells (1×10^6 cells/mouse), or culture-stimulated, naive spleen T cells (1×10^6 cells/mouse). The results are the mean clinical EAU score ± SEM over time as described in Materials and Methods. *, Results are significantly ($P \leq 0.02$) different from the clinical EAU scores of IRBPp-immunized mice injected with stimulated, naive T cells.

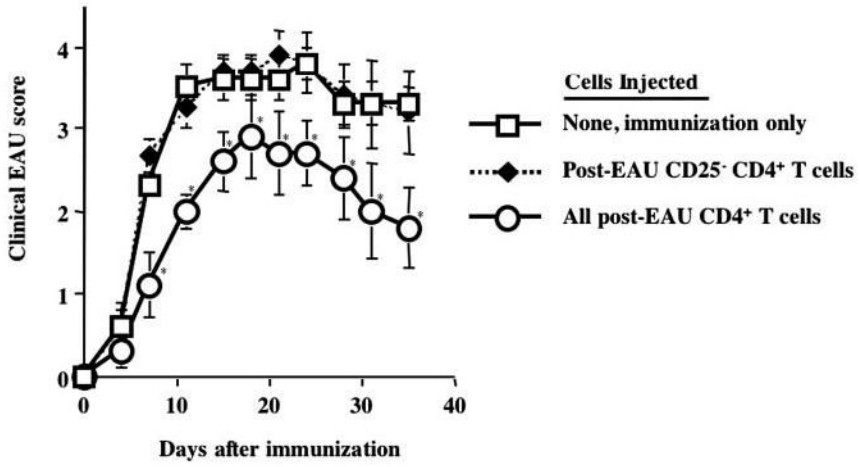

Fig. 4.

The effects of adoptively transferring culture-stimulated, post-EAU spleen T cells devoid of the activated $CD4^+$ T cells. Spleen cells isolated from B10.RIII mice that recovered from EAU were collected and placed in culture with added IRBPp (50 μg/ml) for 24 h as described in Materials and Methods. The cultured, post-EAU $CD4^+$ T cells were selected, and the $CD25^+$ T cells were depleted. Recipient naive B10.RIII mice were immunized for EAU and injected with culture-stimulated, post-EAU $CD25^-$ $CD4^+$ T cells (1×10^6 cells/mouse) or culture-stimulated, post-EAU $CD4^+$ T cells (1×10^6 cells/mouse) or were not injected with cells. The results are the mean clinical EAU score ± SEM over time as described in Materials and Methods. *, Results are significantly ($P \leq 0.05$) different from the clinical EAU scores of IRBPp-immunized mice not injected with cells.