IL-1 and TNF Receptor–Deficient Mice Show Decreased Inflammation in an Immune Complex Model of Uveitis

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Purpose. To determine the role of interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) in the induction of uveitis by a reverse passive Arthus reaction (RPAR).

Methods. Human serum albumin (HSA) antiserum was injected into the vitreous of "knockout" or "double knockout" mice genetically deficient in IL-1 receptor type I (IL-1RI$^{-/-}$), TNF receptors p55 and p75 (TNFR p55$^{-/-}$/p75$^{-/-}$), IL-1RI and TNFR p55 (IL-1RI$^{-/-}$/TNFR p55$^{-/-}$), and controls. Twenty-four hours later, animals were challenged with intravenous HSA. Eyes were enucleated 4 hours after antigen challenge, and inflammation was quantitated by counting cells on histologic sections. Interleukin-6 in aqueous humor was measured with a B9 cell bioassay. The distribution of immune complexes in eyes was observed by immunohistochemical staining for IgG and complement component C3.

Results. Four hours after antigen challenge, immune complexes were localized at the ciliary body and iris of receptor-deficient mice. A transient uveitis was most severe at this time. A significant reduction in the median number of infiltrating cells was found in TNFR p55$^{-/-}$/p75$^{-/-}$ mice (4.8, n = 15), compared with controls (14.2, n = 20, $P < 0.05$). The median number of infiltrating cells was significantly reduced in IL-1RI$^{-/-}$ mice (knockout 2.6, n = 11; controls 7.4, n = 8, $P < 0.005$). Interleukin-1RI$^{-/-}$/TNFR p55$^{-/-}$ mice had a strong reduction in infiltrating cells (knockout 1.6, n = 11; controls 27.3, n = 12, P = 0.002). Interleukin-6 activity in aqueous humor was reduced in IL-1RI$^{-/-}$/TNFR p55$^{-/-}$ mice (P = 0.03) but not in TNFR p55$^{-/-}$/p75$^{-/-}$ (P = 0.40) mice. Most IL-1RI$^{-/-}$ mice had no detectable aqueous humor IL-6, but this group was not statistically different from controls.

Conclusions. In contrast to endotoxin-induced uveitis, both IL-1 and TNF appear to have critical roles in RPAR uveitis. When receptors for these cytokines were deleted, the severity of immune complex–induced uveitis was profoundly reduced. (*Invest Ophthalmol Vis Sci.* 1999;40: 2583–2589)

Uveitis is intraocular inflammation and a leading cause of visual impairment or blindness. It may be caused by infectious organisms or by an immune-mediated process as in sarcoidosis. Immune complex deposition could contribute to uveitis just as immune complexes have been implicated in nephritis, arthritis, and vasculitis.

The formation of immune complexes and their local deposition triggers an inflammatory cascade that leads to tissue injury and ensuing morbidity and mortality. An experimental model of immune complex–mediated pathogenesis was described nearly a century ago by Arthus.[1] It was originally characterized by edema, hemorrhage, and infiltration of acute inflammatory leukocytes, specifically neutrophils. Because of its ease and reproducibility, the experimental variant most commonly used now is the reverse passive Arthus reaction. Local injection of antibodies and intravenous injection of antigen results in the local formation of immune complexes and its consequences. Immune complex–triggered inflammation may be initiated by cell-bound Fc receptors or by activation of complement.[2,3] The response may then be amplified by paracrine mediators including several cytokines[4,5] and neuronal products such as substance P.[6] The relative importance of cell activation via immune complexes binding to Fc receptors versus complement activation is tissue specific. Complement activation appears to be dominant in the lung[7,8]; whereas signaling via Fcγ receptors is necessary in a model of autoimmune glomerulonephritis.[9] Both mechanisms are important for immune complex–induced inflammation in the skin and peritoneum.[7] There is similar evidence indicating that the cytokine requirements for immune complex–induced inflammation is also tissue specific. Both interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) are necessary in alve-

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olitis; however, in dermal vasculitis IL-1, and not TNFα, is required for full expression of tissue injury.[4,5]

Several cytokines have been described as inflammatory mediators in the eye. In particular IL-1, IL-2, IL-4, IL-6, TNF-α, and transforming growth factor-β (TGF-β) have been detected in aqueous and vitreous humors from a number of acute and chronic ocular disorders, including uveitis.[10–13] Soluble immune complexes,[14,15] aggregated IgG,[15,16] monomeric Fc fragments,[17] and IgG-coated sheep red blood cells[18] have been reported to stimulate secretion of IL-1, TNFα, IL-6, IL-10, and prostaglandin by murine or human monocytes and macrophages.

The biological activities of IL-1 and TNF-α suggest that each could be a key mediator in the development of uveitis. Intravitreal injection of human TNF-α or IL-1α in rabbit eyes induced leukocyte infiltration and protein leakage.[19–21] However, studies of uveitis induced by intravitreal injection of endotoxin (lipopolysaccharide) have not consistently confirmed the involvement of TNF-α and IL-1 in the initiation of this inflammatory processes. Rosenbaum and Boney[22] showed that administration of a human IL-1 receptor antagonist (IL-1ra) did not block endotoxin-induced uveitis (EIU) in rabbits. Likewise, inhibitors of TNF-α failed to block EIU.[23–26] Some of these reports indicated that TNF-α inhibition exacerbated this inflammatory response.[23,26] More recently we reported that in mice the ocular inflammatory response to intravitreal endotoxin was not affected by the lack of TNF receptors.[27,28]

The biological activities of IL-1 and TNF-α are mediated by receptors present on the cell surface. Transmembrane signaling of the type I IL-1 receptor is stimulated on binding of IL-1α or IL-β. The type II IL-1 receptor seems to act as an inhibitor of IL-1 because binding to this receptor does not appear to initiate transmembrane events.[29,30]

There are two known receptors for TNF, and they are coexpressed on most cell types. These are the 55 to 60 kDa TNFR-I (p55) and the 70 to 80 kDa TNFR-II (p75). In the past, TNF-induced cytotoxicity was attributed solely to the p55 receptor, whereas TNF-induced proliferation was attributed to the p75 receptor.[31,32] However, many recent papers have shown that p75 can greatly enhance p55-induced cell death.[33–36] In particular, membrane-bound TNF-α appears to be the primary ligand for p75 and can cause cytotoxicity in cells that are not affected by soluble TNF-α.[34]

Previous studies showed that TNFR p55$^{-/-}$/p75$^{-/-}$ mice are protected against a normally lethal regimen of D-galactosamine and endotoxin injections. Similar results were found with TNFR p55$^{-/-}$ mice but not with p75$^{-/-}$ mice.[37] However, other reports have shown that the TNFR p75$^{-/-}$ mice develop an exacerbated febrile response after high doses of endotoxin.[38] Using a pulmonary inflammation model, Peschon et al.[37] showed that the initial pulmonary influx of neutrophils in response to *M. faeni* is dramatically decreased in mice lacking either p55 or p55 and p75 TNF receptors, but exacerbated in TNFR p75$^{-/-}$ mice.[37] All these studies indicate a differential role for TNF receptors in inflammation.

The present work investigates the role of TNF and IL-1 in the pathogenesis of immune complex–induced uveitis. Leukocyte infiltration and IL-6 levels were evaluated in mice in IL-1R1$^{-/-}$, TNFR p55$^{-/-}$/p75$^{-/-}$, and IL-1R1$^{-/-}$/TNFR p55$^{-/-}$ mice and their controls. The results indicate that both cytokines, IL-1 and TNF, play an important role because the absence of receptors for either cytokine causes profound inhibition of reverse passive Arthus reaction (RPAR) uveitis.

Materials and Methods

Animals

Breeding pairs of C57BL/6x129SV random F2 hybrid mice genetically deficient in IL-1 receptor type 1 (IL-1R1$^{-/-}$), TNF receptor p55 and p75 (TNFR p55$^{-/-}$/p75$^{-/-}$), or IL-1RI and TNFR p55 (IL-1R1$^{-/-}$/TNFR p55$^{-/-}$) were a gift from Immunex (Seattle, WA).[37,39] All three lines of gene deletion mice appeared healthy and displayed no overt phenotype. TNFR p55–deficient mice are impaired in their formation of B-lymphocyte germinal centers in peripheral lymphoid organs.[40,41] C57BL/6x129SV F1 mice (The Jackson Laboratory, Bar Harbor, ME) were used as controls. Genotyping was performed by polymerase chain reaction analysis of tail DNA with the use of primer pairs specific for the natural receptor alleles and for the recombinant neomycin-receptor null-mutation alleles.

All mice were provided food and water ad libitum and were kept on a 12 hour light-dark cycle. All experiments were conducted in accordance with the ARVO Statement on the Use of Animals in Opthalmic Research.

RPAR-Induced Uveitis

Uveitis was induced in 6- to 8-week-old IL-1R1$^{-/-}$, TNFR p55$^{-/-}$/p75$^{-/-}$, and IL-1R1$^{-/-}$/TNFR p55$^{-/-}$ mice and controls by bilateral intravitreal injection of 2 μl of heat-inactivated serum (57°C, 30 minutes) from mice immunized earlier with 100 μl of an emulsion containing 50% 10 mg/ml human serum albumin (HSA; Buminate 25%; Baxter Healthcare Corporation, Glendale, CA) in saline and 50% TiterMax Gold (CytRx, Norcross, GA). Blood was collected 4 to 6 weeks after immunization by cardiac puncture to minimize endotoxin contamination of the serum. The HSA antiserum contained less than 0.05 U/ml of endotoxin (E-Toxate; Sigma, St. Louis, MO). The intravitreal injections were given with a 27-gauge needle. Twenty-four hours later, animals were challenged intravenously with HSA (1.5 mg/g of body weight). Eyes were enucleated for quantification of inflammation after 4 hours. One eye from each animal was fixed in 10% neutral-buffered formalin (Richard-Allan, Richland, MI), embedded in paraffin, and sectioned for histologic analysis. Aqueous humor was collected from the contralateral eye and centrifuged briefly at 10,000*g*. Two-microliter aliquots of the supernatants were diluted in 60 μl of saline containing 0.25% HSA and kept frozen until they were assayed for IL-6 bioactivity. Mice receiving saline instead of HSA antiserum or HSA were used as internal controls.

Inflammation was quantified by determining the mean number of infiltrating cells in the aqueous and vitreous humors of 5 hematoxylin and eosin–stained sections per each eye. One sagittal section including the optic nerve head and two sections at either side of it were selected from each eye. Sections from these locations usually contained the highest number of infiltrating cells.

IL-6 Bioassay

Interleukin-6 activity in aqueous humor was determined by measuring [^{3}H]thymidine incorporation in a murine B9 cell bioassay as described.[42,43] The sensitivity of the assay was

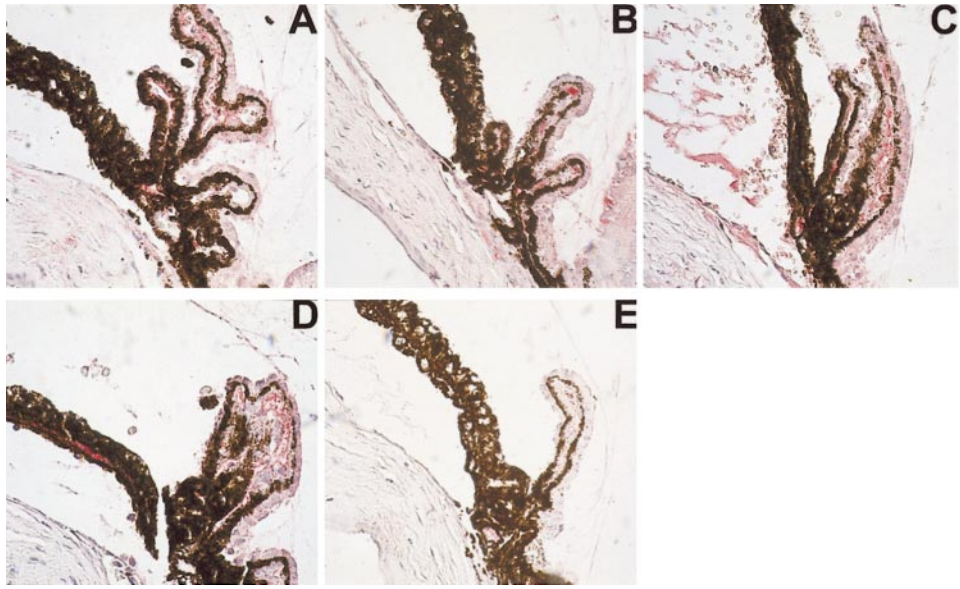

FIGURE 1. Deposition of complement component C3 is not altered by lack of IL-1 or TNF receptors. Eyes were enucleated 4 hours after intravenous HSA challenge to induce immune complex deposition, and sections were immunohistochemically stained for C3 (*red*). Counterstaining was with hematoxylin (*blue*). Positive staining is observed in the ciliary body and iris of mice from all four lines. (Original magnification, ×200.) (**A**) C57BL/6x129SV F1 genetic control; (**B**) IL-1R1$^{-/-}$mouse; (**C**) TNFR p55$^{-/-}$/p75$^{-/-}$ mouse; (**D**) IL-1R1$^{-/-}$/TNFR p55$^{-/-}$ mouse; (**E**) negative control, C57BL/6x129SV F1 mouse that had not been injected with anti-HSA or HSA.

approximately 0.2 pg/ml for recombinant mouse IL-6 (specific activity of 1.2×10^7 U/mg; PharMingen, San Diego, CA).

Immunohistochemistry

Five-micrometer tissue sections were immunostained for immune complexes with anti-mouse–IgG polyclonal antibody (1:100,000; Sigma Chemical) and anti-C3c polyclonal antibody (1:1000; NORDIC Immunologic Laboratory, Tilburg, The Netherlands) with an alkaline phosphatase detection system. Briefly, sections were deparaffinized and incubated with blocking normal serum of the same species as the secondary antibody. Sections were incubated with the primary antibody overnight at 4°C, washed in TBS (50 mM Tris, pH 7.5, 0.15 M NaCl) plus 0.5% Tween-20 and incubated for 1 hour at room temperature with biotinylated anti-IgG secondary antibody. After washing with TBS, the sections were incubated with avidin-biotin conjugated to alkaline phosphatase and then washed again. Fast Red (Biogenex, San Ramon, CA) was used as chromogen. Counterstaining was done with hematoxylin.

Statistical Analysis

Statistical significance was evaluated by using a one-tailed unpaired Mann–Whitney test. $P < 0.05$ was considered to be statistically significant. Results are presented as the median of the sample group.

RESULTS

Immune Complex Immunohistochemistry

Deposits of HSA–anti-HSA immune complexes are expected in the eyes of mice receiving an intravitreal injection of HSA antibodies and an intravenous injection of HSA protein. Immune complexes can be visualized by the colocalization of immunohistochemical staining for IgG and complement component C3. Positive staining for both proteins was seen in the ciliary body, iris, and Bruch's membrane in eye sections taken 4 hours after intravenous HSA challenge and, thereby, indi-

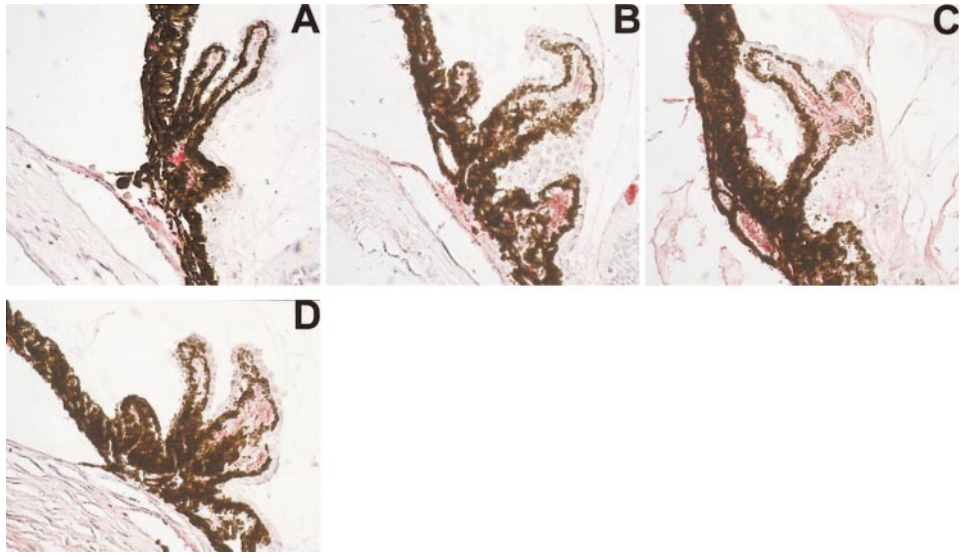

FIGURE 2. Deposition of IgG is not altered by lack of IL-1 or TNF receptors and has the same distribution as C3. Sections are from the same eyes seen in Figure 1 and have been immunohistochemically stained for IgG (*red*). Counterstaining was with hematoxylin (*blue*). Positive staining is observed in the ciliary body and iris of mice from all four lines. (Original magnification, ×200.) (**A**) C57BL/6x129SV F1 genetic control; (**B**) IL-1R1$^{-/-}$mouse; (**C**) TNFR p55$^{-/-}$/p75$^{-/-}$ mouse; (**D**) IL-1R1$^{-/-}$/TNFR p55$^{-/-}$ mouse.

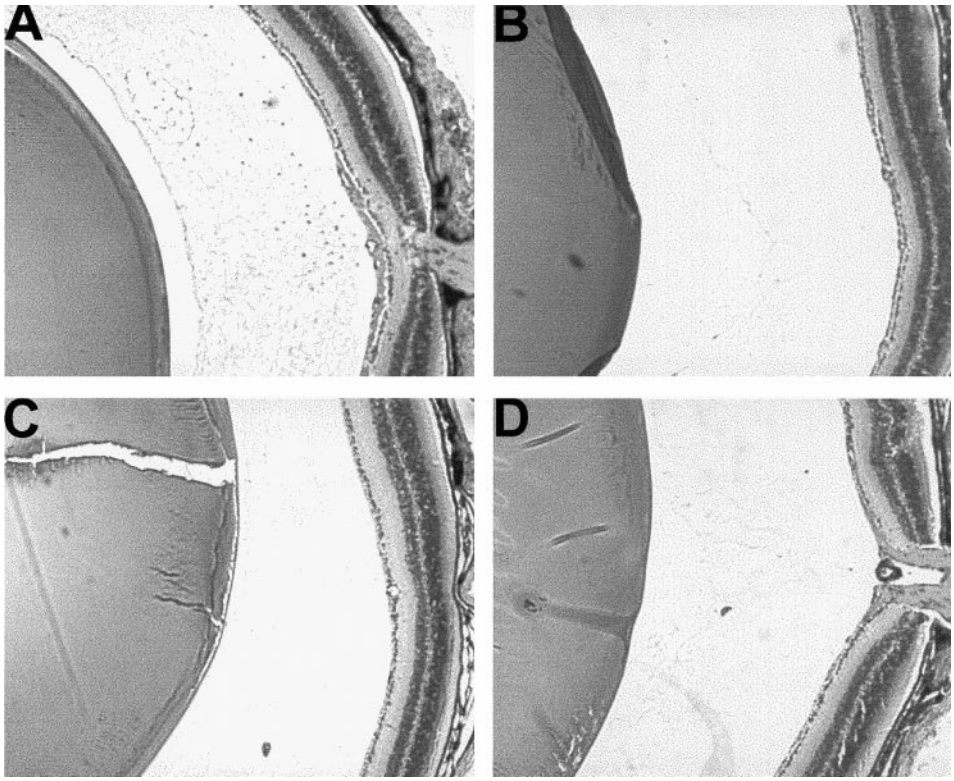

FIGURE 3. Gene deletion of TNF, IL-1 receptors, or both reduces inflammation associated with RPAR uveitis. Hematoxylin and eosin–stained sections 4 hours after HSA injection. Many infiltrating cells can be seen in the vitreous humor of eyes of control mice but not of receptor-deficient mice. (Original magnification, ×200.) (**A**) C57BL/6x129SV F1 genetic control; (**B**) IL-1R1$^{-/-}$mouse; (**C**) TNFR p55$^{-/-}$/p75$^{-/-}$ mouse; (**D**) IL-1R1$^{-/-}$/TNFR p55$^{-/-}$ mouse.

cated the deposition of immune complexes in those sites (Figs. 1 and 2). Only faint background staining was seen in the eyes of control mice not injected with anti-HSA and HSA (Fig. 1E). IgG and C3 immunostaining patterns in mice from each line of receptor-deficient mice were indistinguishable from the controls (Figs. 1 and 2), indicating that these cytokine receptors are not critical for immune complex deposition.

RPAR-Induced Uveitis

Cell infiltration into the anterior and posterior segments was evident in histologic sections of most eyes enucleated 4 hours after intravenous HSA injection of animals primed 24 hours before with intravitreal HSA-antiserum (Fig. 3A). This RPAR uveitis was evident as early as 2 hours after the HSA challenge, persisted for a few hours, and subsided by 24 to 48 hours. Most eyes had fewer inflammatory cells than normally seen with intravitreal EIU, but a few eyes developed fibrinous membranes with many infiltrating cells. The trauma from intravitreal saline injection alone did not cause appreciable inflammation.

Dependence of RPAR Uveitis on IL-1 and TNF Activity

The induction of uveitis by RPAR may be mediated by IL-1, TNF-α, or both. To test these possibilities, intraocular RPAR was induced in mice genetically deficient in receptors for one or both of these cytokines. The severity of uveitis was scored by histologic examination 4 hours after the intravenous injection of HSA. Mice lacking TNFR p55 and p75 showed significantly less inflammation than controls (Figs. 3 and 4). The median score decreased from 14.2 cells/section ($n = 20$) in the controls to 4.8 cells/section ($n = 15$, $P < 0.05$) in knockout mice.

In the absence of IL-1R1, inhibition of the RPAR-induced uveitis was also observed (Figs. 3 and 5). The median number of infiltrating cells was significantly reduced from 7.4 cells/section ($n = 8$) in controls to 2.6 cells/section ($n = 11$, $P < 0.005$) in IL-1R1$^{-/-}$ mice.

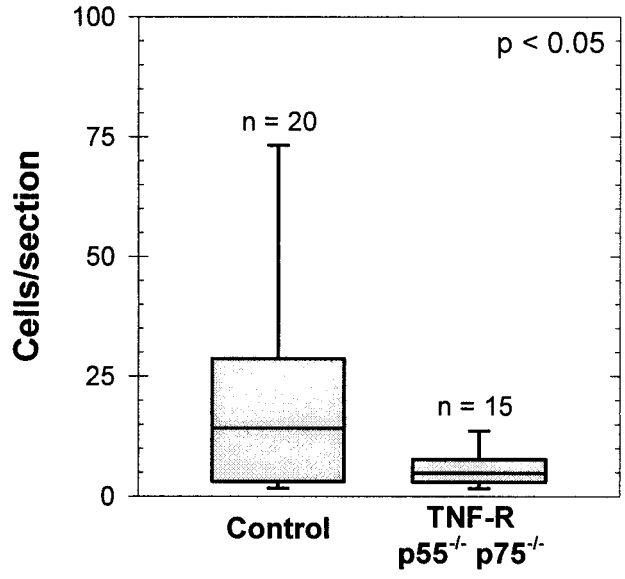

FIGURE 4. Mice with homozygous null mutation of the TNFR p55 and p75 genes are resistant to RPAR uveitis. Median values are indicated by a *line within a box*. The box boundaries indicate the 25th and 75th percentiles, and the *whiskers* below and above the boxes indicate the 10th and 90th percentiles. The numbers of animals in each group and the probability values are indicated.

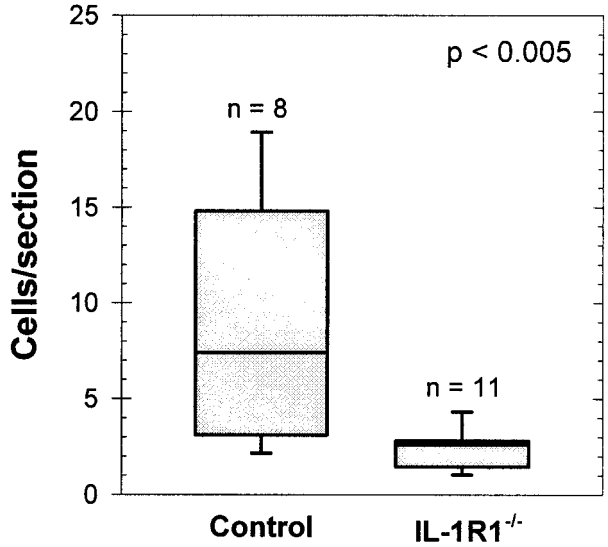

FIGURE 5. Homozygous null mutations in IL-1RI genes inhibit RPAR uveitis. Number of animals per group and the probability values are indicated.

Interleukin-1R $^{-/-}$/TNFR 55$^{-/-}$ mice had a strong reduction in the number of infiltrating cells during the RPAR inflammatory processes (Figs. 3 and 6). The median number of infiltrating cells went from 27.3 cells/section (n = 12) in controls to 1.6 cells/section (n = 11, P = 0.002) in knockout mice.

IL-6 Levels in Aqueous Humor

IL-6 bioactivity was measured in TNFR p55$^{-/-}$/p75$^{-/-}$, IL-1R1$^{-/-}$, IL-1R$^{-/-}$/TNFR p55$^{-/-}$, and control mouse aqueous humor samples collected 4 hours after the intravenous HSA challenge. Interleukin-6 levels in the aqueous humor of control mice with RPAR uveitis were low and near the limit of detectability in the assay. However, we were able to demonstrate that the absence of both IL-1 and TNF receptors resulted in a reduction in detectable IL-6. Similar reduction could not be proven when receptors for only IL-1 or TNF were deleted (Table 1). Although IL-6 was undetectable in aqueous humor of most IL-1R1$^{-/-}$ mice, a statistically significant difference between knockouts and controls could not be shown because one animal had a high (1.2 ng/ml) IL-6 level.

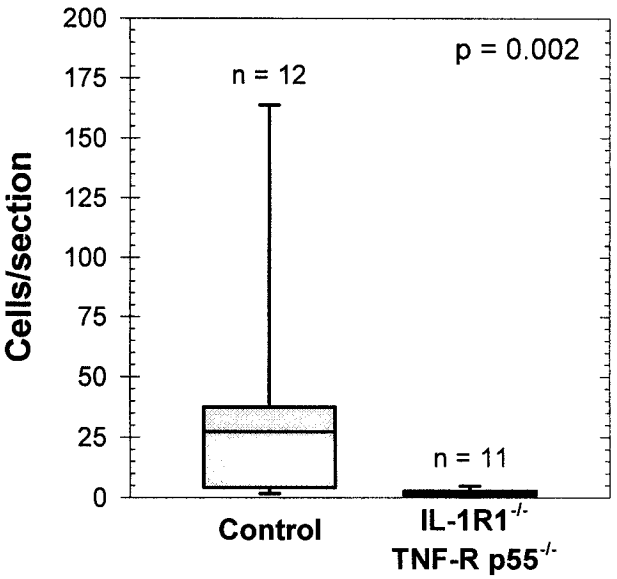

FIGURE 6. Mice with homozygous null mutations in both the IL-1RI and TNFR p55 genes have markedly reduced RPAR uveitis. Number of animals per group and the probability values are indicated.

TABLE 1. IL-6 Levels in Aqueous Humor during RPAR Uveitis

	IL-6 Levels (pg/ml)	No. of Eyes	*P*
IL-1R1$^{-/-}$	0 (0, 8)	11	
			0.08
Controls	15 (0, 70)	8	
TNFR p55$^{-/-}$/p75$^{-/-}$	30 (0, 110)	16	
			0.42
Controls	15 (0, 61)	18	
IL-1R1$^{-/-}$/TNFR p55$^{-/-}$	0 (0, 0)	11	
			0.02
Controls	40 (0, 1,680)	10	

Homozygous null mutation of IL-1R1/TNFR significantly reduced IL-6 levels. Interleukin-1R1$^{-/-}$ animals also tended to have lower IL-6 levels. The data shown are the median (25th, 75th percentiles) values calculated from the mean of IL-6 (in picograms per mililiter) for the number of eyes indicated.

DISCUSSION

Inflammatory injury can be initiated by immune complexes, as modeled by the reverse passive Arthus reaction. In this study we have used RPAR to induce uveitis characterized by the deposition of immune complexes in the eye. Taking advantage of mice genetically deficient in IL-1R1, TNFR p55 and p75, or IL-1RI and TNFR p55, we demonstrated an involvement of these receptors in the RPAR uveitis model. Our results implicate both IL-1 and TNF-α in the ocular Arthus reaction. Mice from all three knockout lines had significantly fewer (less than a third) infiltrating cells than their corresponding controls. The dependency on both IL-1 and TNF-α is similar to that reported for immune-complex induced alveolitis but not dermal vasculitis.[4,5]

Control groups in studies shown in Figures 4, 5, and 6 showed differences in median cellular response. These differences reflect the biological variability inherent in the model and are not statistically significant. Receptor-knockout animals and their controls are tested simultaneously such that variables such as antiserum lot, injection technique, or stress in the animal care facility are well matched. In our studies, however, if one elects to combine all results for controls, one still finds a statistically significant reduction in inflammation for each knockout line studied. The C57BL/6x129sv F1 mice used for this work are not ideal controls for the genetically altered mice, which are progeny of random C57BL/6x129sv F2 hybrids. Nonetheless, other laboratories have published results with the F1 controls,[39] and they were used in our EIU studies discussed below. The reduction of inflammation in all three knockout lines tested, despite their differing mix of C57BL/6 and 129sv genes, supports the contention that this reduction is due to the receptor deficiency and not genetic background.

Our results with RPAR uveitis differ from those previously obtained for uveitis induced by intravitreal injection of endotoxin in these same receptor-deficient mouse lines.[28] The severity of uveitis measured in those EIU studies was not altered by deletion of TNFR p55 and p75 and was inconsistently reduced by deletion of IL-1R1. Lack of both IL-1R1 and TNFR p55 had the most profound effect. The data from the two studies support the hypothesis that uveitis resulting from different agents can be differentially dependent on the action of an individual cytokine. These differences suggest that RPAR uveitis and EIU follow some different pathways during the inflammatory response in the eye.

The deposition of immune complexes was confirmed by the detection of IgG and C3 component, with the characteristic distribution in the ciliary body, iris, and Bruch's membrane. Although the production of other complement factors, such as C5a, was not measured, there is no a priori reason to suspect that they would be directly altered in these mice. A major question in the induction and progression of uveitis is how foreign antigens get into the uveal tissue and induce disease. A central event may be the deposition of antigen from the circulation into the eye. It has been proposed that uveitis may result from an anatomic predisposition of the uveal tract to in situ immune complex formation. Fenestrated capillaries and anionic sites within the ciliary body, ciliary processes, and Bruch's membrane provide a system that, like the glomerulus, is selectively permeable, retaining molecules of a given size and charge. Circulating antibodies may exit the vasculature within the ciliary body and choroid and then selectively combine with antigens to form immune complexes.[44–46] Formation of the immune complexes within the eye may be an important factor. Mice that had been injected with preformed immune complexes, some of which were deposited in ocular vessels, did not get uveitis.[47] Likewise, only a small percentage of patients with systemic lupus erythematosus develop uveitis.[48]

Clinically anterior uveitis is a diverse collection of diseases. For example, the uveitis associated with ankylosing spondylitis differs from that associated with juvenile rheumatoid arthritis, which in turn differs from that associated with inflammatory bowel disease.[49] Different mediators are likely to contribute to clinically distinct subsets of anterior uveitis. Our results strongly support this hypothesis by demonstrating a role for TNF in immune complex–dependent anterior uveitis, whereas five previous studies have failed to show a role for TNF in endotoxin-induced anterior uveitis.[23–26,28]

Acknowledgment

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