Suppression of Immune-Mediated Ocular Inflammation in Mice by Interleukin 1 Receptor Antagonist Administration

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Objective: To evaluate the effects of an interleukin 1 receptor antagonist (IL-1RA) on the development of immune-mediated ocular inflammation in mice.

Methods: Recombinant, human, nonglycosylated IL-1RA (anakinra [kineret]) was tested for its inhibitory effects in 2 systems: (1) experimental autoimmune uveitis induced by interphotoreceptor retinoid-binding protein in B10.A mice using routine procedures and evaluated by clinical and histological examination, and (2) ocular inflammation in mice induced by transfer of hen egg lysozyme–specific T cells to hen egg lysozyme–transgenic mice. Treatment with IL-1RA included daily subcutaneous injections of the drug, at 300 and 500 mg/kg, or phosphate-buffered saline as control.

Results: Mean±SE experimental autoimmune uveitis scores of histological ocular changes of the mice at day 14 postimmunization with interphotoreceptor retinoid-binding protein were 1.5±0.3 in control mice; 1.0±0.4 in 300-mg/kg anakinra–treated mice; and 0.5±0.2 in 500-mg/kg anakinra–treated mice (P=.004). There was a corresponding decrease in the cellular immune response and cytokine production of immune cells in treated mice. Suppression of ocular inflammation by anakinra in the transfer system was also observed (P=.04).

Conclusion: Human IL-1RA suppresses immune-mediated ocular inflammation in mice, affecting both the afferent and efferent components of the pathogenic immune response.

Clinical Relevance: Systemic administration of IL-1RA may have clinical application in the management of patients with uveitis.


Ocular inflammation is one of the leading causes of eye disease, accounting for 10% to 15% of vision loss in the United States. The etiology is usually unknown and treatment remains limited. Experimental autoimmune uveoretinitis (EAU) is an ocular inflammatory model that is induced in a variety of animals by immunization with uveitogenic antigens such as S-antigen or interphotoreceptor retinoid-binding protein (IRBP). Experimental autoimmune uveoretinitis in rodents is a good model for the evaluation of ocular inflammatory mechanisms and potential therapeutic agents for uveitis.

The proinflammatory cytokine interleukin 1 (IL-1) has been implicated in the mediation of autoimmune diseases including uveitis. Interleukin 1 is up-regulated in eyes with EAU and uveitis. In transgenic IL-1 mice, which express human IL-1β in the eye, severe ocular inflammation occurs shortly after birth. Being a multipotent cytokine with a wide range of biological activities, including chemotaxis, activation of inflammatory and antigen-presenting cells, up-regulation of adhesion and costimulatory molecules on cells, and mediation of the acute phase response, IL-1 is an attractive target for therapeutic intervention.

The IL-1 receptor antagonist (IL-1RA) is a naturally occurring inhibitor of IL-1 activity. It is a 152–amino acid residue glycoprotein of 25,000 native molecular weight and has 26% amino acid homology to IL-1α and 19% homology to IL-1β. It competitively binds to IL-1 type I receptor with an affinity comparable with that of IL-1α or IL-1β but does not result in signal transduction. The amino acid sequences of IL-1RA from at least 4 species (human, rat, mouse, and rabbit) are known. There is at least 75% homology between the IL-1RA from the different species. The IL-1RA is released in vivo during experimentally induced inflammation and is thought to be part of a naturally occurring feedback mechanism to limit the extent of IL-1 activity.
Administration of IL-1RA has been shown to abrogate IL-1 activity in both in vitro and in vivo experiments. Specifically, in experimental autoimmune encephalomyelitis (EAE) in rats, it ameliorates the severity and delays the onset of the disease. Clinical trials using daily subcutaneous injections of IL-1RA have also demonstrated efficacy in symptomatic relief and improved radiographic scores in patients with rheumatoid arthritis. In the eye, topically applied IL-1RA has been found to be effective in modulating ocular surface inflammation induced by alkaline injury to the cornea in rats and in the prolongation of corneal allograft survival in mice. However, the effects of IL-1 inhibition on EAU are unknown. Herein, we evaluated the effects of treatment with an IL-1RA on the development of this disease. Our results show that subcutaneous injection of IL-1RA suppresses the development of disease and the IRBP-specific cellular immune response as compared with saline-treated controls, and support the clinical use of IL-1RA in patients with uveitis.

METHODOLOGICAL METHODS

ANIMALS

Female B10.A mice, aged 6 to 8 weeks, were purchased from Jackson Laboratory (Bar Harbor, Me). (FVB/N × B10.BR) F1 mice used for the adoptive transfer of ocular inflammation were generated as described elsewhere. The mice were housed in a pathogen-free facility and maintained in a 12-hour light–12-hour dark cycle. Food and water were supplied ad libitum. Animals were treated in compliance with the Association for Research in Vision and Ophthalmology Statement for Use of Animals in Ophthalmic and Vision Research and the National Institutes of Health Resolution on the Use of Animals in Research.

REAGENTS

Whole IRBP was prepared from bovine retinas by concanavalin A–Sepharose affinity chromatography and fast protein liquid chromatography, as described elsewhere. Pertussis toxin was purchased from Sigma-Aldrich (St Louis, Mo). Recombinant human nonglycosylated IL-1RA, anakinra (Kineret), was purchased from Amgen (Thousand Oaks, Calif).

INDUCTION OF EAU

Experimental autoimmune uveitis was induced in mice as described by Caspi et al. Mice were immunized with 50 µg of IRBP emulsified in complete Freund adjuvant containing 2.5 mg/mL of Mycobacterium tuberculosis H37RA. The emulsion was injected subcutaneously into the base of the tail and both thighs in a total volume of 0.2 mL. Intraperitoneal pertussis toxin (0.5 µg in 0.1 mL) was also given to each mouse.

ADOPTIVE TRANSFER OF IMMUNE-MEDIATED OCULAR INFLAMMATION

Adoptively transferred ocular inflammation was carried out as described in detail by Kim et al. In brief, T-helper lymphocytes type 1 (T\textsubscript{H}1) specific against hen egg lysozyme were injected intravenously into transgenic mice expressing hen egg lysozyme in their lens. Ocular changes in recipient mice were assessed by histological examination 7 days after cell transfer.

TREATMENT WITH ANAKINRA

Anakinra was administered once daily via subcutaneous injection of 0.1 mL at doses of 300 mg/kg or 500 mg/kg. One group of mice immunized with IRBP received treatment from the day of immunization and another group was treated from 8 days postimmunization. For the adoptive transfer studies, the mice were treated from the day of adoptive transfer and killed 7 days later. The control animals received injection of 0.1 mL of phosphate-buffered saline.

ASSESSMENT OF EAU

Double-masked clinical assessments by funduscopic examinations were performed periodically from 10 days postimmunization. Disease severity was graded on a 4-point scale as described previously. Mice were killed at 14 days postimmunization. The eyes were enucleated and fixed in 4% phosphate-buffered glutaraldehyde for 30 minutes and transferred into 4% phosphate-buffered formaldehyde. Fixed tissues were embedded in methacrylate, and 4- to 6-µm sections via the papillary–optic nerve axis were stained with hematoxylin–eosin. The histological grade was evaluated in a double-masked fashion as described previously.

LYMPHOCYTE PROLIFERATION ASSAY

Lymphocytes were collected from draining lymph nodes at 14 days postimmunization, pooled within each group, and cultured in triplicate in flat-bottomed microplates at 4 × 10^6 cells in 0.2 mL of RPMI 1640 medium, supplemented with HL-1 (Bio Whittaker, Walkersville, Md), 2-mercaptoethanol (50µM), methyl-α-D-mannoside (20 mg/mL) (Aldrich Sigma, St Louis, Mo), and antibiotics, with or without IRBP, at concentrations of 0.3 to 30 µg/mL. The cells were incubated for 72 hours, and 0.3 µCi of tritiated thymidine was added to each well. The cultures were further incubated for 16 hours. Evaluation of lymphocyte proliferation was determined by tritiated thymidine incorporation measured by a MicroBeta TriLux scintillation counter (Perkin Elmer Life Science, Boston, Mass), and the data are presented as mean counts per minute minus the background counts per minute.

CYTOKINE PRODUCTION

Lymphocytes, prepared as described earlier, were cultured in 24-well plates at 5 × 10^6 cells per well in 1 mL of the medium described earlier, with or without stimulants, as indicated. Supernatants were collected after 48 hours of incubation and the levels of cytokines were quantitated using the multiplex SearchLight Arrays technology (Pierce Chemical Co, Rockford, Ill).

IRBP-SPECIFIED ANTIBODY DETERMINATION

Serum samples were collected by cardiac puncture following euthanasia and the levels of total IgG antibodies specific for IRBP were measured by enzyme-linked immunosorbent assay as described elsewhere. The data are presented as optical density values at 405 nm.

TOXICOLOGICAL ASSESSMENT

The mice in each group were weighed at the beginning and the end of the experiment to examine for significant weight loss. Kidneys and livers of mice from treatment and control groups were harvested, fixed, sectioned, stained, and examined for histological abnormalities.
topathological changes. Blood samples from both groups were obtained for complete cell blood counts.

STATISTICAL ANALYSIS

Data are presented as the mean±SE of each group. Experiments were repeated at least 2 times and the results were reproducible. Clinical and histological EAU scores were analyzed using the Snedecor and Cochran z test for linear trend in proportions (nonparametric, frequency based). Each mouse (average of both eyes) was treated as a statistical event. Lymphocyte proliferation and IRPB-specific antibody data were analyzed by independent t test. Probability values of \( P \leq .05 \) were considered significant.

Figure 1. Effects of anakinra (recombinant, human, nonglycosylated interleukin 1-receptor antagonist [kineret]) on the development of experimental autoimmune uveitis, days 0 to 14. Groups of mice immunized with interphotoreceptor retinoid-binding protein were treated daily with anakinra at the indicated doses. Controls were similarly injected with phosphate-buffered saline. Scoring of both clinical (A) and histological (B) changes was carried out on day 14 postimmunization. Treatment with anakinra at 500 mg/kg inhibited significantly the ocular disease by both clinical and histological changes compared with controls (asterisk indicates \( P=.004 \) for both parameters). Data of A and B were compiled from 3 separate experiments. C, Typical histological changes in the eye of a control mouse treated with phosphate-buffered saline. Note the inflammatory cells (arrows) in the retina and vitreous (V) and damage of the photoreceptor layer (P) and the outer nuclear layer (ONL). D, Remarkably reduced histological changes in the eye of a mouse treated with anakinra (500 mg/kg) (C and D, hematoxylin-eosin, original magnification \( \times 100 \)).

RESULTS

EFFECTS OF ANAKINRA ON EAU AND IMMUNE RESPONSES WHEN GIVEN FROM DAY 0

Anakinra, at 300 mg/kg or 500 mg/kg, was injected subcutaneously in mice daily from the day of immunization with IRBP. Total numbers of mice used for each group, in 3 separate experiments, were 14 for the control group, 10 for the 300-mg/kg group, and 14 for the 500-mg/kg group. All animals in the control group developed disease, whereas 2 of 10 mice in the 300-mg/kg group and 7 of 14 mice in the 500-mg/kg group failed to develop disease. The mean clinical and histological results are summarized in Figure 1A and B, respectively. There was a dose-dependent effect observed in the clinical (Figure 1A) and the histological scores (Figure 1B) at 14 days' postimmunization. The mean±SE clinical scores were 1.0±0.4 and 0.4±0.2 in the 300-mg/kg and 500-mg/kg anakinra–treated group, respectively, vs 1.5±0.3 in the phosphate-buffered saline–treated group. Similar to clinical scores, the mean±SE histological scores were 1.0±0.4 and 0.5±0.2 in the 300-mg/kg and 500-mg/kg anakinra–treated group, respectively, vs 1.5±0.3 in the saline–treated controls. The difference, for both clinical and histological scores, between the control group and the 500-mg/kg anakinra–treated group was significant (\( P=.004 \)). Figure 1C and D highlights the typical difference in histological changes between control and treated mice. To assess the effect of treatment with anakinra on the development of cellular immune response, lymphocytes har-
The levels of cytokines, interferon gamma, tumor necrosis factor α, IL-4, IL-5, IL-10, IL-13, IL-1α, IL-1β, IL-1RA, and IL-6 were measured. All cytokines, with the exception of IL-5, showed a decrease in release ranging from 34% to 70% in the supernatant of lymphocytes harvested from 500-mg/kg anakinra–treated mice as compared with controls (Figure 3).

The effect of anakinra treatment on the production of IRBP-specific antibodies was evaluated by measurement of the IRBP-specific IgG in the serum samples of treated and control mice. Measurement of the IRBP-specific antibody production did not reveal a significant difference between anakinra-treated mice compared with the controls (Figure 4) (P = .46).

**EFFECTS OF ANAKINRA ON EAU WHEN GIVEN FROM 8 TO 14 DAYS’ POSTIMMUNIZATION**

To evaluate the effect of IL-1 suppression on the efferent limb of the immune response, we gave anakinra at 500 mg/kg daily on days 8 to 14 postimmunization. All mice in both the treated and control groups developed disease. The mean±SE clinical scores in the control (n = 10) vs the treated group (n = 10) were 1.6±0.3 and 1.2±0.3, respectively (Figure 5A), while the mean±SE histological scores were 1.8±0.3 and 1.2±0.3, respectively (Figure 5B). These differences did not reach significance (P = .60). There was no difference in the lymphocyte proliferation assay, IRBP-specific antibody production, and cytokine expressions between the treatment and control groups in the efferent immune response experiment (ie, treatment started on 8 days’ postimmunization).

**INHIBITORY EFFECTS OF ANAKINRA ON ADOPTIVE TRANSFER OF OCULAR INFLAMMATION**

To further study the effect of IL-1 blockage on the efferent limb of immune response, we tested the effect of anakinra on the adoptive transfer of activated TH1 cells into susceptible mice, as described elsewhere. There was a significant reduction of disease scores (Figure 6) (control [n = 6] vs treatment [n = 6], mean ± SE, 3.74±1.17 vs 1.8±1.48) between controls and those treated with 500 mg/kg of anakinra (P = .04). Thus, anakinra suppresses ocular inflammation induced by adoptively transferred activated TH1 cells. Figure 6B and C demonstrates the typical difference in the histopathological changes in eyes of the 2 groups.

**TOXICOLOGY STUDIES**

Weight gain was recorded for all groups of mice. There was no decrease in weight or reduced weight gain in the anakinra-treated mice to suggest toxic effects of the drug. Mean weight increase over 14 days was 9% for controls and 15% for the 500-mg/kg anakinra–treated mice. There was no difference in histopathological changes observed in the kidneys and livers harvested from both the anakinra-treated mice and the controls. Complete blood cell counts performed for anakinra-treated mice and controls also did not reveal any decrease in leukocyte or erythrocyte counts to indicate toxic effects of the drug. No injection site infection or reaction was observed throughout all of our experiments.

The results of these experiments show that daily subcutaneous injection of anakinra at a dose of 500 mg/kg can suppress EAU in mice. Our results are consistent with results from similar experiments in EAE in rats, which showed delay of onset and amelioration of disease in anakinra-treated rodents. In support of the proinflammatory role of IL-1 and the antagonistic role of IL-1RA in the eye, Rosenbaum and Boney have demonstrated in rabbits that intraocular inflammation induced by intravitreal injection of IL-1 can be suppressed by an injection of IL-1RA. The elevated intraocular and serum levels of IL-1 and IL-1RA in EAU in mice and in pa-
tients with uveitis are also indicative of their possible roles in the pathogenesis and resolution of ocular inflammation. Herein, we demonstrated that the systemic administration (via subcutaneous injection) of IL-1RA suppresses EAU in mice. The results of our study support the therapeutic application of anakinra injections in patients with uveitis.

In this study, we chose to use anakinra, a human, recombinant, nonglycosylated form of IL-1RA, because of its previous use in EAE studies in rats and clinical trials in patients with rheumatoid arthritis. Unlike earlier reports on EAE, we did not show significant suppression of the efferent limb, although the histological and clinical scores were lower in the treatment group. Such discrepancy could be owing to different target tissues (the retina in EAU vs central nervous system myelin in EAE) and different animal species used (mice vs rats). There was a dose response demonstrated in our studies, showing greater suppression of disease with increasing dose (Figure 1A and B). The effective dose of anakinra for clinical trials of rheumatoid arthritis is 1 to 1.5 mg/kg per day, and for EAE in rats, 27000 mg/kg per day. In this study, we treated the mice with different doses, 300 and 500-mg/kg per day. The 500-mg/kg per day anakinra treatment regime was found to suppress EAU effectively and without any detectable toxic effects. The significantly higher dose of anakinra required for animal studies to demonstrate efficacy suggests that human IL-1RA may have less effect when used in other species.

To further evaluate the role of IL-1 in EAU, we started the anakinra treatment in mice 8 days postimmunization to attempt to inhibit selectively the efferent immune response. There was a decrease in clinical and histological scores of treated mice compared with phosphate-buffered saline controls (Figure 4). However, this decrease was not significant and was smaller as compared with those mice treated from the day of immunization. To further test the role of IL-1 in the efferent immune response, we tested the blockage of IL-1 on a different model, using adoptive transfer of activated T, cells. In this system, we demonstrated significant amelioration of disease by anakinra, indicating therefore that IL-1RA can suppress the efferent arm of the immune response. Because uveitis in patients represents postsensitization and the efferent immune response, any potential drug for uveitis has to demonstrate an effect on the efferent immune response to be useful in the treatment of active uveitis.

The 70% to 95% suppression of lymphocyte proliferation assay in mice treated with anakinra (Figure 3) demonstrates the inhibitory effects of IL-1RA on the cellular immune response. There was no significant decrease in IRBP-specific antibody production in the treated group (Figure 5). This may signify a relatively lesser effect of IL-1RA on the humoral than the cellular immune response. Because EAU is predominantly mediated by a cellular immune response rather than the humoral immune response, our result showing the suppression of cellular...
mediated immune response is consistent with the inhibition of disease. We also demonstrated a decrease in the expression of cytokines such as interferon gamma, tumor necrosis factor \( \text{TNF-}\alpha \), IL-1\( \alpha \), and IL-6, indicating suppression of cellular immune response by anakinra. No suppression of IL-5 and low suppression of IL-4 were observed, suggesting that T\(_h\)1-associated cytokines were more affected than T\(_h\)2-related cytokines by this therapy. Our results indicate that human IL-1RA given at 500 mg/kg daily can suppress disease development in EAU in mice and specific cellular immune response, but it does not significantly affect the IRBP-specific antibody levels.

During EAU in mice, levels of IL-1RA in the eye are naturally elevated up to 17 times.\(^6\) It is possibly part of a natural feedback mechanism to regulate inflammation and restore homeostasis within the tissue milieu. In human studies, no serious toxic effects attributed to anakinra have been observed when administered at mean calculated doses of up to 35 times the dose given to patients with rheumatoid arthritis during a 72-hour treatment period.\(^8\) There were no significant toxic effects attributable to the anakinra given in our study.

In conclusion, our data support the notion that IL-1 plays a role in both the afferent and efferent immune responses of immune-mediated ocular inflammation in mice. Interleukin-1 receptor antagonist suppresses disease in EAU and in the adoptive transfers system without significant toxic effects; hence, this study supports the use of anakinra as a potential therapy for patients with uveitis.

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