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Interleukin-1 Receptor Signaling is Critical for the Development of Autoimmune Uveitis

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Abstract

Interleukin-1 beta (IL-1β) is a pro-inflammatory cytokine important for local and systemic immunity. However, aberrant production of this cytokine is implicated in pathogenic mechanisms of a number of inflammatory diseases including Behçet's disease and age-related macular degeneration. We report here the increased secretion of IL-1β in the retina by neutrophils, macrophages, and dendritic cells during ocular inflammation and show that loss of IL-1R signaling confers protection from experimental autoimmune uveitis (EAU). Moreover, the amelioration of EAU in *Il1r*-deficient mice was associated with reduced infiltration of inflammatory cells into the retina and decreased numbers of uveitogenic Th17 cells that mediate uveitis. These findings indicate the possible utility of IL-1R blocking agents for the treatment of ocular inflammatory diseases.

Introduction

Uveitis is a diverse group of potentially sight-threatening intraocular inflammatory diseases that includes Behçet's disease, birdshot retinochoroidopathy, Vogt-Koyanagi-Harada disease, sympathetic ophthalmia, and ocular sarcoidosis. The etiology of uveitis is not fully understood but may be linked to autoimmunity (1). Cytokine signaling plays critical role(s) in the pathology of uveitis. IL-17 is substantially elevated in freshly-isolated PBMC from patients with active uveitis, as well as in PBMC and lymph nodes of mice with experimental autoimmune uveitis (EAU), a model of human uveitis. The increase in IL-17 up-regulates tumor necrosis factor (TNF)-α in the retina, suggesting a mechanism by which Th17 cells contribute to ocular pathology during uveitis (1). In addition, IL-17 blocking antibodies ameliorate EAU while IL-27 and IL-35 protect mice from EAU by inhibiting the expansion of Th17 cells, underscoring the pathogenic role of IL-17 and TNF-α in intraocular inflammatory diseases (1, 2). These findings suggest that manipulation of cytokine signaling has therapeutic potential in the treatment of uveitis.

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IL-1β is mainly produced by monocytes, macrophages, dendritic cells (DCs), and neutrophils and serves a role in host defense to pathogens (3). It triggers inflammatory responses by multiple mechanisms, including the induction of cyclooxygenase-2 and inducible nitric oxide synthase, and recruitment of inflammatory cells (4). More recently, IL-1 signaling has been shown to synergize with IL-6 and IL-23 to promote Th17 cell differentiation via induction of critical regulators IRF4 and RORγt (5), and IL-1β combined with IL-23 induces production of IL-17 in γδ T cells (6). Dysregulation of IL-1β expression leads to the development of autoimmune diseases, such as rheumatoid arthritis, type 2 diabetes, and gout, as well as autoinflammatory diseases, such as Familial Mediterranean Fever and cryopyrin-associated periodic syndromes (4, 7, 8). For this reason, IL-1 receptor antagonist (IL-1RA; Anakinra), soluble decoy IL-1 receptor (Rilonacept), and IL-1β neutralizing antibody (Canakinumab) have been approved for treating these diseases, with therapeutic benefit (9).

In the eye, increased IL-1β secretion has been implicated in Behçet's disease and age-related macular degeneration (AMD) (10, 11) and intraperitoneal administration of IL-1β during priming phase of EAU increases disease severity, suggesting that IL-1β might play a pathogenic role in the eye (12). However, the exact role of IL-1β in ocular inflammatory diseases is controversial and not well defined. Here we report that myeloid cells produce IL-1β in the retina of mice during EAU, and analysis of *Il1r*-deficient mice provides direct evidence that IL-1 signaling is required for the development of uveitis. We further show that the reduced severity of intraocular inflammation in *Il1r*-deficient mice correlates with impaired Th17 cell differentiation and decreased recruitment of inflammatory cells into the retina. Our data thus indicate that agents blocking IL-1 signaling pathway may have considerable potential for treating uveitis.

Materials and Methods

Mice and antibodies

WT and *Il1r*$^{-/-}$ mice on the C57BL/6J background were from the Jackson Laboratory. All protocols were approved by the NHLBI and NEI Animal Care and Use Committees and followed NIH guidelines for using animals in intramural research. Anti-pro-IL-1β-APC and the corresponding isotype control (rat $IgG_1\kappa$) were from eBioscience; other antibodies for flow cytometric analysis were from Biolegend.

Induction and evaluation of EAU

EAU induction was performed as previously described (2). Mice were immunized s.c. with 150 μg bovine IRBP and 300 μg human IRBP peptide (amino acids 1–20) in 0.2 ml 1:1 vol/vol emulsion with complete Freund's adjuvant containing 2.5 mg/ml *Mycobacterium tuberculosis* strain H37RA. Mice also received i.p. 0.3 μg *Bordetella pertussis* toxin at the time of IRBP immunization. Disease severity was evaluated by fundoscopy at days 14 and 20 and by histological analysis at day 21, as previously described (13).

Immune cell identification and cytokine expression in the retina

At day 21 of EAU induction, mouse retinae were isolated, cut into pieces, and incubated with 1 mg/ml collagenase D and 500 μg/ml DNase I (Roche) at 37°C for 30 min. Single cell suspension was subjected to flow cytometry analysis (FACSCanto II). To determine intracellular pro-IL-1β expression, cells were stained with surface markers, fixed and permeabilized (BD Biosciences), and stained with anti-pro-IL-1β. To assess intracellular IL-17 and IFN-γ expression, cells were seeded into a 96-well plate and re-stimulated with 50 ng/ml PMA and 1 μM ionomycin for 4 h; intracellular cytokine expression was then determined.

Cytokine analysis in the retina and lymph nodes

Mouse retinae were isolated and incubated with RIPA buffer containing protease inhibitor cocktail (100 μl/retina) at 4°C for 20 min. IL-1β levels in the retina were quantified by an ELISA kit (BD Biosciences) that uses an IL-1β-specific antibody that does not cross-react with pro-IL-1β (14). For analysis of cytokine secretion by peripheral T cells, cervical and draining lymph nodes cells were isolated and stimulated (5×10^6 cells) with $IRBP_{1–20}$ peptide (10 μg/ml) for 3 d. IL-17, IFN-γ, and IL-1β levels in culture supernatant was determined by ELISA (BD Biosciences).

Statistical analysis

Statistical comparison between samples was done by unpaired *t* test; $P < 0.05$ was considered as statistically significant.

Results and Discussion

Myeloid cells produce IL-1β in the retina during EAU

Chronic uveitis in humans is characterized by repeated cycles of remission and recurrent inflammation and is often accompanied by vascular, fibrotic, and neurodegenerative changes (15). A recent report showed that intraperitoneal injection of recombinant IL-1β protein at the priming phase (days 0–6), but not in the effector phase, enhanced the severity of acute uveitis in mice (12), consistent with a possible pathogenic role for this cytokine in uveitis. In this study, we induced uveitis in C57BL/6J WT mice and show here that compared to retina from un-immunized mice, IL-1β secretion was increased in the retina during EAU (Fig. 1A). Our detection of more than 100 pg IL-1β in the retina of mice with EAU raises the possibility that in situ secretion of IL-1β in the retina may promote the development of severe uveitis. We then investigated which cell populations in the retina potentially produced the IL-1β, as assessed by intracellular cytokine staining and flow cytometric analysis. Because microglial cells, resident macrophage-like cells that reside in the retina, play essential roles in regulating ocular inflammation, we examined whether IL-1β was produced by myeloid cells in the retina during EAU. We used cell-surface expression of CD11b and Ly6G to identify macrophages/DCs ($CD11b^{hi}$ $Ly6G^{low}$, R1) and $CD11b^{hi}$ $Ly6G^{hi}$ for neutrophils ($CD11b^{hi}$ $Ly6G^{hi}$, R2) (Fig. 1B, upper panel). Macrophages and DCs in the retina were identified as $CD11c^{+}$ MHC II^{low} (R3) and $CD11c^{hi}$ MHC II^{hi} (R4) subpopulations, respectively, of the R1 population (Fig. 1B, lower panel). Neutrophils, macrophages, and DCs (R2, R3, and R4) all expressed pro-IL-1β, with most neutrophils

(R2) and the CD11c^{hi} MHC II^{hi} DCs (R4) expressing the greatest amount of this protein (Fig. 1C, 1D). These results indicate that the secretion of IL-1β in the retina during EAU was by myeloid cells.

Il1r-deficient mice develop less severe EAU

We next used mice that cannot respond to IL-1β to directly examine whether IL-1β is indeed necessary for the development of severe uveitis. We induced EAU in C57BL/6J or *Il1r*$^{-/-}$ mice and monitored disease progression by fundoscopy. Fundus images show that WT mice developed severe inflammation characterized by blurred optic disc margins and enlarged juxtapapillary area, retinal vasculitis with moderate cuffing, vitreitis, choroiditis and yellow-whitish retinal and choroidal infiltrates (Fig. 2A). In contrast, disease in the *Il1r*$^{-/-}$ mice was significantly suppressed, with an average clinical score of approximately 1.0 compared to 3.0 for WT eyes (Fig. 2B). Histological analysis further revealed cardinal pathological features of severe uveitis, including the infiltration of inflammatory cells into the retina, development of granulomas, and retinal folds in WT mice, whereas no evidence of retina folding or the other features of severe uveitis were observed in the eyes of *Il1r*$^{-/-}$ mice (Fig. 2C). The histological score for *Il1r*$^{-/-}$ mice was markedly decreased compared to WT mice (Fig. 2D). Furthermore, *Il1r*$^{-/-}$ mice had fewer neutrophils (Fig. 3A, B), macrophages and DCs (Fig. 3A, C), and lymphocytes (CD4^{+} and CD8^{+}) (Fig. 3D–3F), suggesting that IL-1R signaling might be critical for immune cell infiltration into the eyes during EAU.

Loss of IL-1R signaling inhibits expansion of Th17 cells and the secretion of IL-17

IL-1β together with IL-6 and IL-23 promotes the differentiation of IL-17-producing Th17 cells in the absence of TGF-β signaling (16), and IL-17 has been implicated in the pathogenesis of uveitis in humans and mice (1). Consistent with published reports (1, 17), we found a significant increase in IL-17-producing Th17 cells in the retina during EAU (Fig. 4A, 4B). The much lower level of IL-17-producing cells in the retina of the *Il1r*$^{-/-}$ mice (Fig. 4A, 4B) correlated with reduced severity of EAU, suggesting that IL-1R-dependent signaling plays a crucial role in driving IL-17 production during EAU. Interestingly, loss of IL-1β signaling had no significant effect on the levels of IFN-γ-producing Th1 cells in the retina during EAU (Fig. 4C), consistent with reports that EAU pathology is primarily mediated by Th17 cells (1, 17). This is also consistent with a report that Th17-induced ocular inflammation is driven by recruitment of neutrophils into the retina, whereas Th1 cells in the retina mainly induce higher proportions of CD8 cells (18). Thus, the marked reduction in neutrophils in the retina during EAU (Fig. 3B) is in line with reduction of Th17 cells in the retina. However, in contrast to the retina, the loss of IL-1R signaling in peripheral lymphoid tissues was associated with a significant reduction in both Th1 and Th17 cells, underscoring the requirement of IL-1R signaling in promoting inflammatory responses (Fig. 4D, 4E). These results also suggest that additional cytokine signal(s) may be involved in inducing IFN-γ expression in retinal CD4^{+} T cells during EAU. Finally, unlike the production of IL-17 and IFN-γ, pro-IL-1β and IL-1β were minimally produced in the cervical and draining lymph nodes during EAU, and the levels were not altered in *Il1r*$^{-/-}$ mice (Fig. 4F and Supplemental Fig. 1), and correspondingly, IL-1β was not detected in the serum during EAU (Supplemental Fig. 2).

In summary, our studies reveal that IL-1β is produced in the retinae during EAU by myeloid cells and indicate a potential pathogenic role for IL-1R signaling in uveitis. Analysis of the retinae of *Il1r*$^{-/-}$ mice with EAU further suggests that IL-1-dependent recruitment of uveitogenic Th17 cells into the retinae is critical for developing severe EAU. Our studies thus indicate that IL-1/IL-1R-blocking agents might be useful for treating uveitis as well as potentially other ocular inflammatory diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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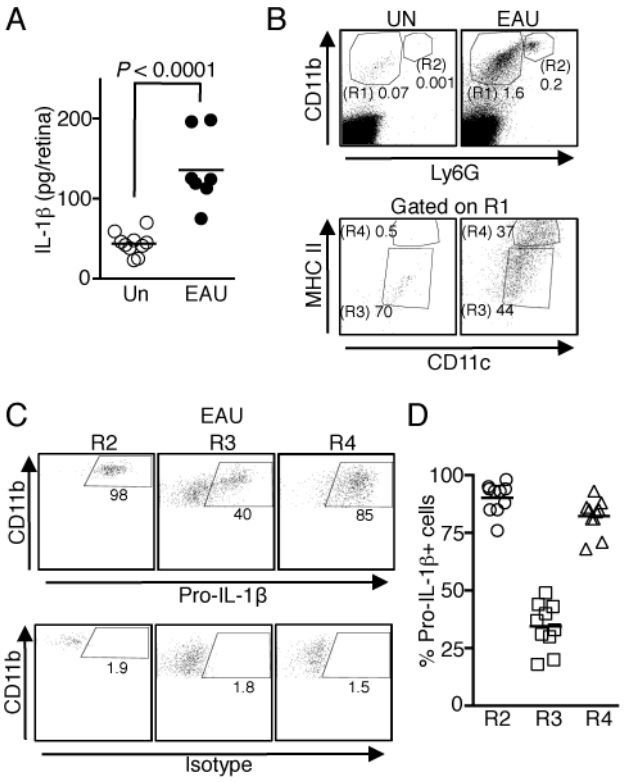

FIGURE 1.

IL-1β is expressed in the retina during EAU. Retinae of WT mice without immunization (Un) and with EAU were isolated on day 21 post-immunization, digested with collagenase and analyzed by ELISA (**A**) or flow cytometry (**B–D**). (**A**) Analysis of IL-1β secretion by ELISA. Data are from 2 independent experiments with a total of 7–10 mice. (**B**) Macrophages/DCs ($CD11b^+$ $Ly6G^-$, R1) and neutrophils ($CD11b^+$ $Ly6G^+$, R2) that had infiltrated into the retina were first identified by CD11b and Ly6G staining. Macrophages and DCs were defined as $CD11c^+$ MHC II^{low} macrophages (R3) and $CD11c^{hi}$ MHC II^{hi} DCs (R4) (**C and D**) Expression of intracellular pro-IL-1β in the immune cells identified in Fig. 1B after EAU induction. Shown are representative flow cytometric plots (**C**) and as well as data from 10 individual mice (**D**).

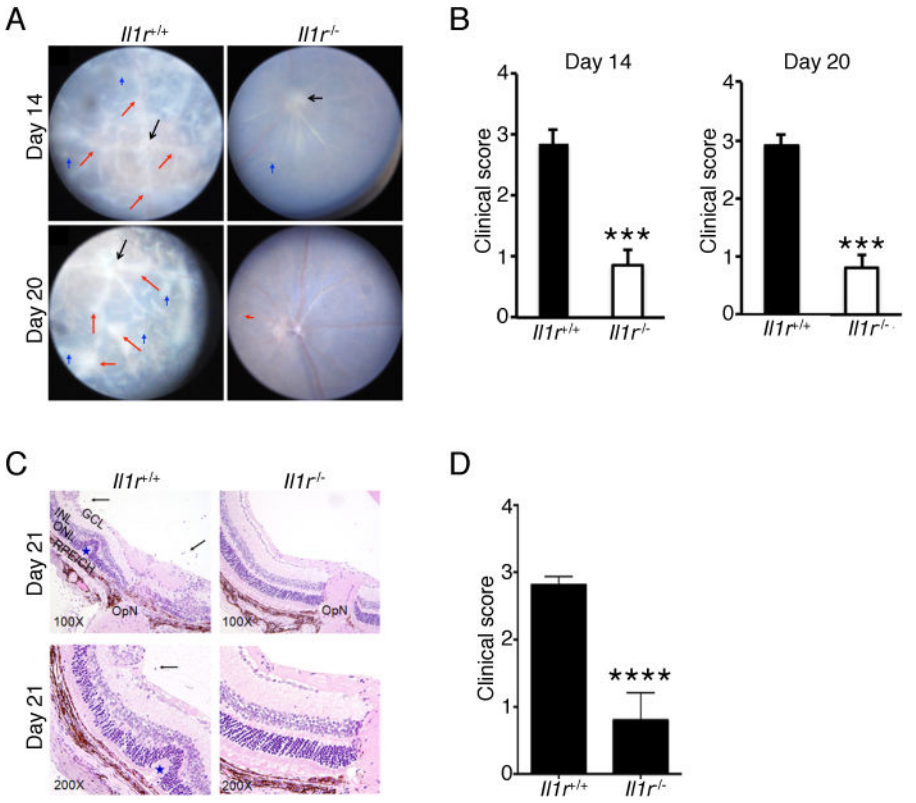

FIGURE 2.

Il1r-deficient mice develop less severe EAU. EAU was induced in WT and $Il1r^{-/-}$ mice. (**A**) Fundus images of the eyes at days 14 and 20 after EAU induction. Compared to the $Il1r^{-/-}$ mice, the retinae of WT mice reveals obvious inflammation with blurred optic disc margins and enlarged juxtapapillary area (black arrows), retinal vasculitis with moderate or severe cuffing (red arrows), and yellow-whitish retinal and choroidal infiltrates (blue arrows). (**B**) Clinical score and assessment of disease severity were based on changes at the optic nerve disc or retinal vessels and retinal and choroidal infiltrates as described in the text. ***, $P < 0.001$. (**C**) Histological analysis of the retina at day 21 after EAU induction shows increased numbers of inflammatory cells in the vitreous (black arrows), retinal folds (blue asterisk) in retina of WT compared to $Il1r^{-/-}$ mice. (**D**) Significant reduction of histological score in $Il1r^{-/-}$ mice compared to WT mice. Data are representative of 2 independent experiments (total of 10 mice/group). ****, $P < 0.0001$. Sections were stained with H&E staining. V, vitreous, black arrow, infiltrated for inflammatory cells, Blue asterisk, retinal folds; OpN, optic nerve; GCL, ganglion cell layer; INL, inner nuclear layer; ONL outer nuclear layer; RPE/CH retinal pigment epithelial cell layer; and choroid.

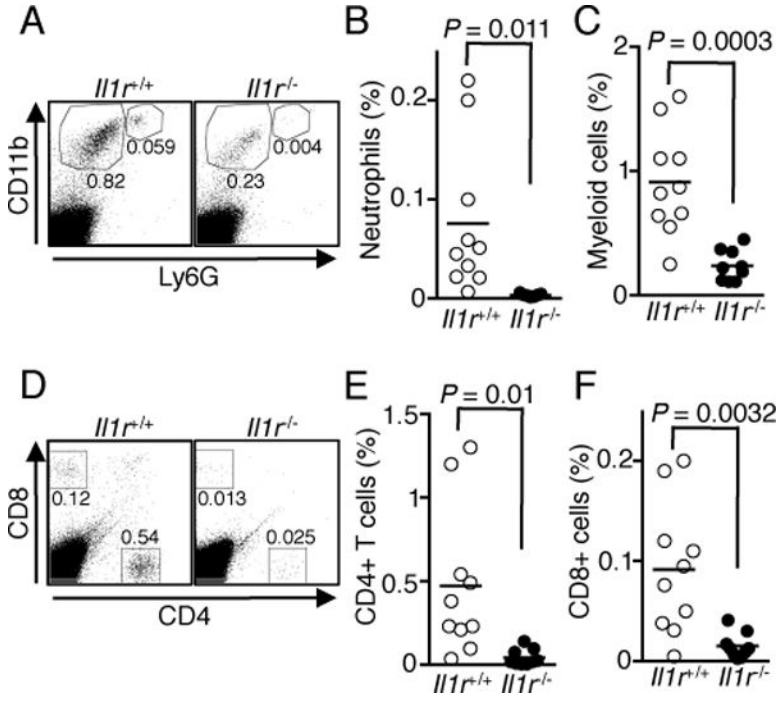

FIGURE 3.

Il1r$^{-/-}$ mice have fewer immune cells infiltrating into the retina during EAU. EAU was induced in WT and *Il1r*$^{-/-}$ mice, retinae were isolated at day 21 post-immunization, digested with collagenase and analyzed by flow cytometry. (**A**) Shown are representative blots of CD11b versus Ly6G staining. (**B and C**) Percentages of neutrophils (**B**) and macrophages/DCs (**C**) from individual mice. (**D**) Shown are representative blots of CD4 and CD8 staining. (**E and F**) Percentages of CD4^{+} (**E**) and CD8^{+} (**F**) T cells. Data shown are from 2 independent experiments with a total of 10 mice/group.

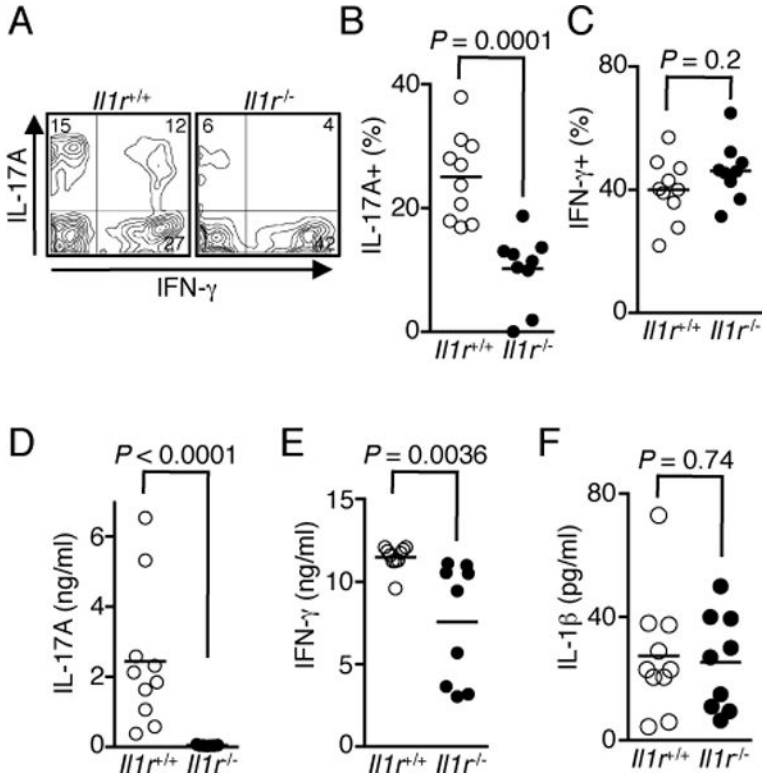

FIGURE 4.

IL-1R-dependent signaling is required for Th17 cell differentiation. (**A–C**) EAU was induced in WT and *Il1r*$^{-/-}$ mice, retinae were isolated on day 21 post-immunization and analyzed by the intracellular cytokine staining assay. Shown are representative flow cytometric plots (**A**) and percentages of CD4^{+} T cells expressing IL-17 (**B**) and IFN-γ (**C**) from individual mice. (**D and E**) Cervical and draining lymph node cells were re-stimulated with IRBP$_{1–20}$ peptide for 3 d. Amounts of IL-17 (**D**), IFN-γ (**E**), and IL-1β (**F**) in the culture were determined by ELISA. Data shown are from 2 independent experiments with a total of 10 mice/group.