Published in final edited form as:

Cytokine. 2009 April ; 46(1): 79–91. doi:10.1016/j.cyto.2008.12.019.

Interleukin-17 causes neutrophil mediated inflammation in ovalbumin-induced uveitis in DO11.10 mice

Zili Zhang^{a,1}, Wenwei Zhong^{a,1}, Doran Spencer^b, Hong Chen^a, Huiying Lu^a, Tatsushi Kawaguchi^b, and James T. Rosenbaum^b,

^a Department of Pediatrics, Oregon Health & Science University, Portland, OR 97239, USA

^b Department of Ophthalmology, Medicine, and Cell Biology, Casey Eye Institute, Oregon Health & Science University, Portland, OR 97239, USA

Abstract

T cell-mediated uveitis is strongly associated with many systemic inflammatory disorders. Th17 cells are a novel T cell subset characterized by production of interleukin (IL)-17. In this study, we used DO11.10 mice to investigate the role of IL-17 in the pathogenesis of uveitis. CD4⁺ T cells in DO11.10 mice are genetically engineered to react with ovalbumin (OVA). IL-17 expression was determined by real-time PCR and ELISPOT. Uveitis was induced by intravitreal injection of OVA, and ocular inflammation was evaluated by intravital microscopy. OVA challenge significantly induced IL-17 production by DO11.10 splenocytes *in vitro*. Next, we examined whether OVA challenge could elicit local inflammation and induce IL-17 *in vivo*. OVA elicited marked neutrophil-predominant inflammatory cell infiltration in the eyes. This leukocyte influx was mediated by CD4⁺ lymphocytes as evidenced by significant inhibition of the ocular inflammation by CD4⁺ depleting antibody. Compared to control mice, OVA treatment induced IL-17 expression. Moreover, anti-IL-17 antibody markedly reduced OVA-mediated ocular inflammation. Finally, the neutralization of IL-17 attenuated ocular expression of CXCL2 and CXCL5, two cytokines which are chemotactic for neutrophils. Our study suggests that IL-17 is implicated in the pathogenesis of this T cell-mediated model of uveitis in part through neutrophil chemotaxis as a downstream effect of IL-17.

Keywords

Inflammation; Interleukin-17; Uveitis

1. Introduction

Uveitis is a serious ophthalmologic disorder characterized by intraocular inflammation. Genetic, immunologic and environmental factors all contribute to the pathogenesis of uveitis. Uveitis has a prevalence of 115.3 per 100,000 in northern California [1]. Many systemic diseases including sarcoidosis, ankylosing spondylitis, and juvenile idiopathic arthritis (JIA)

*Corresponding author. zhangzi@ohsu.edu (Z. Zhang), zhongw@ohsu.edu (W. Zhong), rosenbaj@ohsu.edu (J.T. Rosenbaum).

¹These authors contributed equally to this manuscript.

Publisher's Disclaimer: This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues. Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit: <http://www.elsevier.com/copyright>

(formerly Juvenile rheumatoid arthritis) are commonly associated with uveitis. Uveitis is comparable to diabetes as a cause of years of visual loss [2]. Thus, our society bears significant burdens, economic as well as health-related, from uveitis. The histological changes associated with uveitis include inflammation of the intraocular space with leukocyte infiltration [3,4]. Although the etiology of uveitis is complex and multifactorial, most forms of uveitis are mediated by T lymphocytes, especially those with a CD4 positive (CD4⁺) phenotype [5,6].

Numerous studies have demonstrated that CD4⁺ T lymphocytes play an important role in the development of uveitis [7]. CD4⁺ T cells are increased in number and activated in intraocular fluids from patients with uveitis and in several animal models of uveitis [8]. Despite these studies, the mechanism of CD4⁺ T lymphocyte-mediated inflammation in uveitis is not fully understood. CD4⁺ T cells produce several critical cytokines that initiate and amplify immune responses.

Recent studies have described a unique lymphocyte population distinct from Th1 and Th2 cells. This CD4⁺ subset is called Th17 due to its characteristic production of interleukin (IL)-17 [9, 10]. Under the influence of an appropriate cytokine milieu, naïve CD4⁺ lymphocytes will differentiate to Th17 through activation of SOCS1 and ROR γ t [11,12]. Th17 lymphocytes are implicated in a variety of immune-related diseases including rheumatoid arthritis [13,14], bacterial pneumonia [15,16], asthma [17,18], multiple sclerosis [9,19], and recently in inflammatory bowel disease (IBD) [20–22]. Therefore, both IL-17 and Th17 cells have become an active research area in the autoimmune and inflammatory diseases.

IL-17 consists of two 32 kDa homodimeric protein subunits [23]. This novel cytokine has been shown to trigger the NF- κ B signaling cascade, leading to proliferation of T cells and up-regulation of numerous inflammatory mediators, including IL-1 β , IL-6, and IL-8 [24,25]. In addition, previous studies in our laboratory found that systemic overexpression of IL-17 causes granulocyte colony-stimulating factor (G-CSF)-mediated neutrophilia in mice [26]. Other studies have shown the up-regulation of CXCL2 and CXCL5 by IL-17, thereby leading to local neutrophil recruitment [23,25]. Thus, it is well established in the literature that IL-17-stimulated production of inflammatory mediators is a key element in the inflammatory cascade in a variety of pathological conditions.

Recently, Th17 and IL-17 have been found to increase in the patients with IBD, JIA and Behcet's disease, each of which may be associated with uveitis [24,27]. In addition, IL-17 can stimulate production of numerous inflammatory cytokines and chemokines observed in the patients with uveitis [28,29]. However, the role of Th17 and IL-17 in the pathogenesis of uveitis remains to be fully defined. Here, utilizing DO11.10 TCR transgenic mice, we have established an antigen-specific T cell-dependent uveitis model for better elucidating the role and mechanism of Th17 in ocular inflammation.

2. Methods

2.1. Mice and induction of uveitis

Six- to 8-week-old BALB/c and DO11.10 mice on a BALB/c background (Jackson Laboratory, Bar Harbor, Maine) were used for the experiments. The animal experimental protocols are in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and have been approved by our institutional animal care and use committee.

DO11.10 mice were injected with antigen and/or antibody in 4 μ l phosphate-buffered saline (PBS) or PBS alone as a control into the vitreous chamber of each eye. The injections were performed with ultra-thin, pulled borosilicate glass needles (outer diameter about 50 μ m) and Hamilton syringes under direct visualization through a surgical microscope. These mice

received 100 µg of OVA or equal moles of OVA_{323–339} peptide (Sigma, St. Louis, MO). Some animals received intravitreal injection of 15 µg of anti-mouse interferon-γ monoclonal antibody (eBioscience, San Diego, CA) and 20 µg of anti-mouse IL-17 monoclonal antibody (R&D Systems, Minneapolis, MN), respectively, together with 100 µg OVA in 4 µl PBS. Twenty-four hours after the OVA challenge, uveitis was evaluated by intravital microscopy.

2.2. Intravital microscopy

One hundred fifty microliters of rhodamine (0.2% in PBS) was administered intraperitoneally (i.p.) into the mice to label intravascular leukocytes right before intravital microscopy as we have previously described [30–32]. Labeled inflammatory cells in the iris and ciliary/limbal region were observed by intravital epifluorescence videomicroscopy of anesthetized animals with a modified DM-LFS microscope (Leica) and either a CF 84/NIR B&W camera from Kappa, Gleichen, Germany, or a color Optronics DEI-750CE camera (Optronics International, Chelmsford, MA). This technique has been reported in detail previously [30–32]. Real-time videos were recorded in NTSC format for 10–20 s each. Both rolling and adherent leukocytes in the iris vessels were identified as a marker for anterior chamber uveitis [30–32]. These cells were further quantified to assess the severity of the ocular inflammation [30–32].

2.3. Histology

For histological evaluation, eyes were fixed in 3% paraformaldehyde. Then, the tissues were embedded in paraffin, sectioned, and stained with haematoxylin and eosin (H&E). Ocular inflammation was assessed by light microscopy according to the degree of cellular infiltration and other pathological change.

2.4. Ex vivo whole-mount preparation and imaging

Iris whole-mounts were prepared 24 h after intravitreal challenge with specific antigen (OVA) or control in DO11.10 mice, as described previously [33]. Briefly, eyes were enucleated, dissected to remove the posterior of the eye and lens, and fixed in 4% paraformaldehyde at 4 °C overnight. Following fixation, the remaining anterior segments were washed in tris-buffered saline (TBS) and incubated for 60 min in 2% rabbit serum. For CD4 and IL-17 costaining, anterior segments were incubated in fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD4 antibody (eBioscience) (0.5 µg/100 µl) and allophycocyanin (APC)-labeled anti-mouse IL-17 antibody (0.125 µg/100 µl) diluted in TBS containing 2% rabbit serum overnight at 4 °C.

For immunohistochemistry, anterior segments were incubated with 2.5 mg/ml rat anti-mouse Ly6G antibody (R&D systems) overnight at 4 °C. After washing in TBS 5 times, the excised eye segments were incubated with HRP-conjugated goat anti-rat IgG (1:500) at 4 °C overnight, followed by extensive washing in TBS. Then, the Ly6G staining was developed with the substrate DAB (50 ml/ml) and DAB enhancer, respectively, for 3–5 min using Liquid DAB-Plus Substrate Kit according to the manufacture's instruction (Invitrogen, Carlsbad, CA). After stopping the reaction with the rinse of distilled water, the anterior segments of the eyes were then cut into the shape of a clover leaf such that it could be flat-mounted on SuperFrost® Plus coated glass microscope slides (Fisher Scientific, Pittsburgh, PA) under a coverslip with SlowFade® (Molecular Probes, Eugene, OR) as the mounting media. Samples were imaged using a Leica DM5000B epifluorescent microscope and photographed with a Leica DC500 digital camera (Leica).

2.5. ELISPOT assay

Splenocytes from BALB/c and DO11.10 mice were obtained by crushing the spleen and removing red blood cells with Red Blood Cell Lysing Buffer (Sigma, St. Louis, MO). A

previously described ELISPOT assay was used to determine IL-17 production [34]. Briefly, 96-well Immulon II plates (Millipore, Bedford, MA) were coated with 1.5 µg/ml purified rat anti-mouse IL-17 capture antibody (eBioscience)/well in 100 µl PBS overnight at 4 °C. Plates were washed, blocked with complete RPMI, and seeded with 100 µl of lymphocytes/well starting from 3 to 5 × 10⁵ cell/well in triplicate. Cells were stimulated with and without OVA_{323–339} peptide (Anaspec, San Jose, CA) or anti-mouse CD3 antibody (7.5 µg/ml) and anti-CD28 antibody (5 µg/ml) (BD, San Jose, CA) overnight in a 37 °C humidified incubator with 5% CO₂. One hundred microliters (1.5 µg/ml) of biotinylated rat antimouse IL-17 detecting antibody (eBioscience) was added to each well the following day and incubated for 2 h at room temperature or overnight at 4 °C. Streptavidin-alkaline phosphatase conjugate (eBioscience) was added in 1:1000 dilution with PBS at room temperature for 1 h. Plates were then developed using streptavidin alkaline phosphatase and 5-bromo-4-chloro-3-indolyl phosphate toluidine p-nitro blue tetrazolium chloride substrate (KPL, Gaithersburg, MD). The reaction was stopped by the addition of distilled water. IL-17 spots were analyzed by an AID Elispot high resolution reader system EliSpot 04 HR (AID Elispot, Strassberg, Germany).

2.6. RT-PCR and Th17 pathway-focused PCR array

Total RNA from eye homogenates was isolated with RNAeasy Mini kit (Qiagen, Valencia, CA). First-strand cDNA synthesis was accomplished with oligo (dT)-primed Omniscript reverse transcriptase kit (Qiagen, Valencia, CA). Gene-specific cDNA was amplified by a hotstart touchdown PCR procedure, with Platinum Taq DNA Polymerase kit (Invitrogen, Carlsbad, CA) and mouse specific primer pairs (IL-17 sense, 5'-GTG GCG GCT ACA GTG AAG GCA-3', and IL-17 antisense, 5'-GAC AAT CGA GGC CAC GCA GGT-3', 420 bp amplicon; CXCL2 sense, 5'-GAA CAA AGG CAA GGC TAA CTG A-3', and CXCL2 antisense, 5'-AAC ATA ACA ACA TCT GGG CAA T-3', 235 bp amplicon; or CXCL5 sense, 5'-CTC AGT CATAGC CGC AAC CGAGC-3', and CXCL5 antisense, 5'-CGC TTC TTT CCA CTG CGA GTG C-3', 260 bp amplicon). PCR thermal cycler conditions were as follows: 1 × 15 min 95 °C, 35 cycles denaturation (45 s, 94 °C), annealing (45 s, 61 °C), and extension (45 s, 72 °C). A primer pair for a constitutively expressed gene, β-actin (sense, 5' ATGCCAACACAGTGCTGTCT3', and antisense, 5' AAGCACTT GCGGTGCACGAT3'), was included in each assay as an internal control. PCR products were separated by electrophoresis in a 2% agarose gel and analyzed by densitometry. RNA samples from 3 animals were pooled in the same experimental group. Th17 for Autoimmunity and Inflammation PCR Array was performed independently 3 times according to the manufacturer's protocol (Superarray, Fredrick, MD), and GEArray® Expression Analysis Suite software was used for data extraction and analysis.

2.7. Statistics

Data are expressed as the average ± SD, and a representative experiment is shown for each figure. Statistical probabilities were evaluated by Student's *t*-test, with a value of *p* < 0.05 considered significant.

3. Results

3.1. OVA induces antigen specific IL-17 production in the splenocytes of DO11.10 mice

To establish an antigen-specific and T cell-mediated uveitis model, we chose DO11.10 mice that have a transgenic TCR specifically responding to the OVA_{323–339} epitope. First, we tested whether OVA was able to elicit an antigen specific IL-17 response. Splenocytes were harvested from DO11.10 mice on the BALB/c background or BALB/c mice as a control, and IL-17 production was measured by ELISPOT assay. Following 24-h *in vitro* nonspecific T cell activation by anti-CD3 and anti-CD28 antibodies, IL-17 expression was induced at a comparable level in both DO11.10 and BALB/c mice, suggesting that these 2 strains have

comparable IL-17 response of peripheral Th17 cells to general T cell activation (Fig. 1). However after 24-h *in vitro* incubation of OVA with the splenocytes from these 2 groups, only DO11.10 splenocytes displayed an antigen specific response to OVA challenge with significant IL-17 production. This result indicates that DO11.10 mice can generate an antigen-specific IL-17 response.

Next, we defined the time course of OVA-induced IL-17 expression in the DO11.10 splenocytes. As measured by ELISPOT, OVA treatment caused a notable increase of IL-17 production as early as 6 h after the stimulation, and this induction reached a maximal level by 45 h (Fig. 2).

3.2. OVA induces a CD4⁺ T cell-dependent uveitis in DO11.10 mice

In light of above *in vitro* findings, we went further to investigate whether OVA could provoke T cell dependent uveitis in DO11.10 mice. We have previously described a uveitis model in which OVA is injected intravitreally in mice that had passively received DO11.10 splenocytes [31]. The current model differs by virtue of the injection of OVA directly into the eyes of DO11.10 mice. OVA_{323–339} peptide or OVA was intravitreally delivered to the eyes of DO11.10 mice. Twenty-four hours later, the circulating leukocytes in these mice were labeled with rhodamine by intraperitoneal injection, and uveitis was monitored by intravital microscopy. Since our *in vitro* study showed that OVA could induce an antigen specific IL-17 response as early as 9 h, we chose the 24-h time point to evaluate *in vivo* effect of OVA in the eyes of DO11.10 mice. Compared to control eyes which received PBS injection, intravitreal administration of OVA_{323–339} peptide or OVA resulted in a marked influx of leukocytes in the eyes of DO11.10 mice (Fig. 3A). Moreover, a significant number of the leukocytes rolled or adhered to the vascular beds in the iris (Fig. 3B). Furthermore, OVA challenge also induced an iridocyclitis as evidenced by leukocyte infiltration and exudates in anterior chamber and the anatomic space adjacent to the lens and posterior to the ciliary body (Fig. 3C). We then profiled the infiltrating cell phenotype at different time points after OVA challenge by RT-PCR analysis of CD4 expression and *ex vivo* staining of cell surface marker labeling antibody against Ly6G on excised iris tissues. The PCR study showed that a marked increase of CD4 expression in the eyes at 6 h after OVA stimulation (data not shown), suggesting an ocular influx of CD4⁺ lymphocytes. However compared to bovine serum albumin treatment as a non antigen specific control, OVA-induced inflammatory cells at 24 h were predominantly positive for Ly6G, a surface marker expressed by neutrophils (Fig. 4A). Further histology analysis confirmed that the infiltrating leukocytes were mainly polymorphonuclear neutrophils (Fig. 4B). In addition, this result indicates that the neutrophil influx at 24 h is antigen specific.

Several recent studies have shown that CD4⁺ T lymphocytes play a central role in the recruitment and activation of neutrophils during inflammation [35–37]. Thus, to explore whether this ocular neutrophilic inflammation was initiated by CD4⁺ lymphocytes, we treated the DO11.10 mice intraperitoneally with GK1.5 antibody for 2 consecutive days to deplete peripheral CD4⁺ T cells. Then, these mice were intravitreally challenged with or without OVA, and the uveitis was assessed 24 h later. As shown in Fig. 5, depletion of CD4⁺ cells by GK1.5 antibody markedly reduced OVA-induced uveitis as evidenced by diminishing leukocyte infiltration. We also tested the effect of GK1.5 antibody on lipopolysaccharide (LPS)-induced uveitis, which is primarily a CD4⁺ T cell-independent model in which the ocular inflammation is mediated by innate immune response to endotoxin. Depletion of CD4⁺ cells by GK1.5 antibody did not suppress LPS-induced uveitis (data not shown). Since GK1.5 antibody specifically inhibited neutrophil infiltration in OVA-challenged animals but not in the LPS-treated group, CD4⁺ lymphocytes are instrumental in OVA-induced ocular neutrophil infiltration. This indicates that OVA is able to instigate a CD4⁺ T cell-dependent uveitis in DO11.10 mice.

3.3. Characterization of IL-17 and its related genes in OVA-induced uveitis in DO11.10 mice

To define the lineage of CD4⁺ T helper cells implicated in the ocular inflammation, we profiled gene expression in the eyes of control and OVA-challenged mice using a real-time PCR based Superarray. As shown in Table 1, the intravitreal injection of OVA markedly augmented the transcription of the Th1 cytokine (interferon- γ) but not T-bet at 24 h. The increase of interferon- γ expression is further consistent with the ELISPOT finding that *in vitro* OVA challenge induced interferon- γ production in the splenocytes from DO11.10 mice (data not shown). Despite OVA enhanced interferon- γ expression, intravitreal administration of interferon- γ neutralizing antibody did not alter the severity of uveitis at 24 h after OVA stimulation (Fig. 6). Moreover, OVA treatment did not significantly change the expression of transcripts for GATA-3 or Th2 cytokines (IL-4 and IL-5) (Table 1). Taken together, these results suggest that neither Th1 nor Th2 cells are responsible for OVA-induced uveitis in DO11.10 mice.

In light of above findings, we went on to examine the role of Th17 in this model of ocular inflammation. Recent studies have identified several IL-17 isotypes. IL-17A is currently the most studied isotype of IL-17. In addition, SOCS1 and ROR γ t are two important signaling molecules implicated in Th17 cell induction. Once differentiated, Th17 cells produce signature cytokines such as IL-17F and IL-21 in addition to IL-17A. IL-21 further exerts a propagating effect on Th17 cells in an autocrine fashion. Since our data demonstrate an indispensable role of CD4⁺ cells in the induction of OVA-induced uveitis, it is reasonable to postulate that CD4⁺ Th17 cells and IL-17 are critical mediators in the ocular inflammation. To test this assertion, we first examined transcriptional changes of IL-17A in the eyes at 24 h after OVA challenge. RT-PCR revealed strong expression of IL-17A mRNA in the ocular samples after intravitreal OVA challenge, whereas expression of an independent housekeeping gene (β -actin) was not affected (Fig. 7). In contrast, no transcription of IL-17 was detected by the RT-PCR in control mice. Moreover, to confirm IL-17 expression at a protein level, we used *ex vivo* staining of IL-17 in excised iris tissues. Immunohistochemistry with IL-17 antibody showed specific IL-17 staining in the eyes at 24 h after OVA challenge, whereas no IL-17 signal was detected in the control group (Fig. 8). However compared to Ly6G⁺ neutrophils (Fig. 4A), there were fewer IL-17⁺ cells in the OVA-treated eyes. Previously, we demonstrated that CD4⁺ cell are key to elicit OVA-induced ocular inflammation. In order to determine if CD4⁺ T cells were the major cellular source of IL-17, the iris was dissected from OVA-treated eyes and double stained with FITC-conjugated anti-CD4 and APC-labeled anti-IL-17 antibodies (eBioscience). As illustrated in Fig. 9, not every CD4⁺ lymphocytes expressed IL-17. Nevertheless, most IL-17⁺ cells were CD4 positive. The presence of these double positive cells suggests that infiltrating CD4⁺ T cells are a source of IL-17 in the OVA-induced uveitis.

Th17 cells are derived from naive CD4⁺ lymphocytes, and the findings from both CD4⁺ cell depletion and IL-17 neutralization experiments clearly suggest that the cellular source of IL-17 is OVA-specific CD4⁺ T cells. Nevertheless, recent studies found that some Natural Killer T cells (NKT) also produce IL-17 [38–40]. NKT are a heterogeneous group of T cells that share natural killer (NK) cell markers CD161 (also called NK1.1) and CD244. In order to rule out the possibility that NKT is the source of IL-17 during OVA-induced uveitis, DO11.10 mice received two intraperitoneal injections of 200 μ g anti-CD161 antibody (eBioscience) 3 days apart before intravitreal OVA challenge. The reduction of NKT was confirmed by flow cytometry of CD244 staining in peripheral blood (data not shown). However, unlike GK1.5 antibody, anti-CD161 treatment did not significantly reduce the ocular inflammation and IL-17 expression (Fig. 10), indicating that NKT are not responsible for the IL-17-mediated uveitis.

In this model, OVA caused IL-17 production and uveitis within 24 h. This finding is consistent with other reports that IL-17 can be rapidly induced as early as 12 h after microbial challenge [16]. However, it is unclear whether OVA-specific transgenic T cells in DO11.10 mice undergo expansion process after the antigen stimulation. IL-23 is a heterodimeric cytokine including a

specific subunit p19. IL-23 is crucial for promoting Th17 cell proliferation. To determine whether Th17 cell expansion was implicated in the rapid kinetics of OVA-induced uveitis in DO11.10 mice, we treated the mice intraperitoneally with 100 µg anti-IL-23 p19 antibody (R&D Systems) right before intravitreal OVA challenge, and uveitis was assessed at 24 h when the inflammation reached its peak. Neutralization of IL-23 by anti-IL-23 p19 antibody did not lessen the ocular influx of inflammatory cells (Fig. 11). This result implies that IL-23-driven Th17 cell proliferation is dispensable in OVA-induced uveitis in DO11.10 mice.

Then we employed Th17-specific real-time PCR microarray (Superarray) to probe the transcriptional changes of other IL-17-related genes. As shown in Table 2, OVA markedly augmented the expression of transcripts for IL-17 signal transduction molecules (SOCS1 and RORγt), Th17-derived signature cytokines, namely IL-17F and IL-21, and other IL-17 downstream cytokines including IL-1β, IL-6 and TNF-α. Thus, this result implicates IL-17 in antigen-specific T cell-dependent uveitis.

Using the same Th17-specific real-time PCR microarray technique, we also explored the change of IL-17 downstream gene products. Among IL-17 effector molecules, mRNA for CXCL2 and CXCL5 chemokines that attract neutrophils were up-regulated 621- and 165-fold in the eyes with uveitis, respectively. This was confirmed by independent RT-PCR analysis showing more CXCL2 and CXCL5 mRNA in OVA-challenged eyes. This is particularly relevant because the inflammatory cells in the eyes at 24 h after OVA stimulation were predominantly neutrophils, as noted above. Thus, coincident expression of CXCL2 and CXCL5 could explain the neutrophil infiltration as a mechanism of IL-17-mediated uveitis.

3.4. IL-17. neutralizing antibody inhibits OVA-induced ocular inflammation in DO11.10 mice

To further confirm the role of IL-17 in OVA-induced uveitis, we compared the severity of OVA-induced uveitis in mice treated with and without anti-IL-17 monoclonal antibody (R&D Systems). The IL-17 antibody was administered intravitreally along with OVA upon the induction of uveitis. Twenty-four hours later, ocular inflammation was assessed by intravital microscopy. Compared to OVA stimulation alone, anti-IL-17 monoclonal antibody significantly reduced rolling and adherent leukocytes in the vasculature of the eyes (Fig. 12A and B). Since we previously showed that inflowing cells at the 24 h time point were largely neutrophils in OVA-induced uveitis, we examined the expression of neutrophilic chemokines (CXCL2 and CXCL5) in the eyes treated with anti-IL-17 antibody. As illustrated in Fig. 12C, the IL-17 antibody markedly attenuated the transcription of CXCL2 and CXCL5. This study suggests that IL-17 mediates OVA-induced uveitis in DO11.10 mice in part through dependence on chemokines that attract neutrophils.

4. Discussion

Uveitis is one of the most common ocular disorders that potentially cause severe visual impairment. Many autoimmune/rheumatologic diseases present with uveitis as a manifestation of multiple organ inflammation, and the majority of these diseases are mediated by an aberrant T cell response. Recent research has identified a pathogenic subpopulation of CD4⁺ lymphocytes called Th17 cells. These cells characteristically elicit IL-17-dependent inflammation in many autoimmune diseases. Recent studies have showed that IL-23 supports the proliferation of Th17 cells. Current data suggest that IL-23 plays an important role in maintaining long-term survival of Th17 cells as well as inducing IL-17 production in memory T cells [37,41]. Interestingly, variants of the IL-23 receptor (IL-23R) gene are implicated in the susceptibility to IBD, ankylosing spondylitis and psoriasis, each of which can present as uveitis. Recently, our laboratory found polymorphisms of IL-23R also predispose to acute anterior uveitis (Martin, unpublished). These findings highlight the pivotal role of the novel IL-23 and IL-17 axis in the pathogenesis of systemic inflammatory diseases and uveitis.

IL-17 was initially discovered by Golstein and coworkers [42]. IL-17 shows 58% homology with an open reading frame of the T-lymphotropic herpes virus samirii, and is the founding member of an IL-17-like cytokine family named IL-17A-F [43,44]. Secreted IL-17 consists of two 32 kDa homodimeric protein subunits [45]. It appears that IL-17 family members signal through the IL-17 receptor [43], a Type I transmembrane protein that is expressed in many tissues. IL-17 has been reported to activate all three classes of MAP kinases, including ERK1 and ERK2, JNK, and p38 [45–47]. The IL-17–induced activation of the MAP kinase pathway results in the production of IL-6 and the CXC chemokines, thereby causing the recruitment of neutrophils in the local tissues [48,49]. In addition, IL-17 can up-regulate granulopoietic factors such as G-CSF that can lead to an increase in both mature neutrophils and neutrophil progenitors in the spleen and bone marrow [16]. More recently, the role of IL-17 in regulating inflammation has been better defined through neutralization studies or using mice with a homozygous deletion of the ligand [50] or receptor [16]. Mice with a homozygous deletion of the IL-17 receptor have a markedly diminished recruitment of neutrophils into the lung in response to a challenge with a gram-negative pathogen [16]. This is likely due to decreased CXC chemokine expression, as well as the production of granulopoietic factors such as G-CSF and stem cell factor [16,26]. In this model system, IL-17 is induced relatively rapidly with detectable mRNA levels by 12 hours after bacterial challenge and detectable protein in the local tissue by 16 hours. In addition, IL-17 has been shown to play a role in antigen-induced adaptive immune responses [51]. These published reports are consistent with our finding of rapid *in vitro* expression of IL-17 and *in vivo* development of OVA-induced uveitis. Interestingly, neutralization of IL-23 did not alter the course of ocular inflammation after OVA stimulation. This suggests that at least the early phase of Th17 cell activation is not IL-23-dependent in DO11.10 mice. Since nearly all CD4⁺ T cells in DO11.10 mice are homogenous in terms of their reactivity to OVA, the sheer number of reactive transgenic CD4⁺ cells may alter the normal kinetics of T cell activation and proliferation. As a result, these DO11.10 lymphocytes may not undergo regular T cell priming or expansion as observed in a non-transgenic model.

IL-17 has been found to be elevated in a variety of inflammatory conditions including rheumatoid arthritis [13,46] and recently in IBD [20–22]. All these diseases are well known for their association with uveitis. Recently, Amadi-Obi et al. demonstrated that Th17 cells are expanded during active uveitis in human patients [49]. These authors further confirmed the role of IL-17 in ocular inflammation using a rodent experimental autoimmune uveitis (EAU) model [52]. In line with this report, Peng et al. recently demonstrated that T cells autoreactive to interphotoreceptor retinoid-binding protein express IL-17 [53]. These Th17 cells are further expanded by IL-23, and uveitogenic in the EAU model [53,54]. While the EAU model is well characterized, we chose to study a model dependent on local antigen injection for several reasons. As a T cell-mediated uveitis model, EAU requires immunization of mice with retinal-derived antigens. Thus, it usually takes more than 2 weeks to develop EAU, which mainly exhibits retinitis. In addition, B10.RIII mice are usually employed for the study EAU. Their dark pigment in the iris as well as posterior retinal inflammation hinder the intravital microscopic imaging, a sensitive technique to study ocular leukocyte migration and activation [30–32].

Endotoxin-induced uveitis (EIU) is another popular model that can quickly elicit ocular inflammation [55,56]. However, EIU involves an acute inflammatory response and T cells are not the initiating component in its pathogenesis. Indeed, we are unable to detect IL-17 expression in EIU (unpublished data). In light of the significance of IL-17 in T cell-mediated autoimmunity and noninfectious uveitis, we aimed to develop a uveitis model without time-consuming systemic priming/sensitization to better study Th17 cells in ocular inflammation. In this study, we chose DO11.10 mice on the BALB/c background. The absence of iris pigmentation in these mice allows us to characterize the ocular inflammation using intravital

microscopy. We have shown that DO11.10 mice exhibit a strong and specific IL-17 response to OVA stimulation. In addition, OVA-induced uveitis in DO11.10 mice is antigen specific and T cell-dependent. Most importantly, this uveitis is mediated by IL-17 as evidenced by the activation of IL-17 signaling and the blocking effect of anti-IL-17 antibody. Although neutrophils could theoretically produce IL-17, we believe that T cells are the primary source of IL-17 production in this unique model based upon the following observations. First, we are unable to detect IL-17 expression in neutrophil-predominant EIU (unpublished observations), suggesting that neutrophils are not major IL-17 producing leukocytes in eye inflammation. Second, immunohistochemistry showed a significant disparity of Ly6G⁺ and IL-17⁺ cells in terms of their number and distribution in the inflamed eye. Third, anti-IL-17 antibody mitigated certain chemokines, thereby attenuating ocular neutrophil infiltration in OVA-induced uveitis. This indicates that IL-17 is an upstream cytokine rather than an effector molecule of neutrophil activation. In this study, we also found that neutralization of NKT cells did not alleviate OVA-induced uveitis. Most importantly, the experiment of CD4 and IL-17 co-staining clearly indicates that infiltrating CD4⁺ lymphocytes are an important source of IL-17 in the OVA-evoked uveitis.

Under a presumption that OVA elicits a Th2 reaction, DO11.10 mice have been commonly used to study atopic diseases such as asthma [57]. However, Wilder and her colleagues reported that compared to OVA-primed BALB/c mice, DO11.10 mice do not develop eosinophilia or OVA specific IgE in response to OVA aerosol exposure [58]. In fact, these DO11.10 mice display an early T cell activation and neutrophil infiltration in the lung [59]. This study suggests that OVA evokes a T cell response distinct from Th2 in DO11.10 mice. These observations are consistent with our results showing that DO11.10 mice develop Th17-driven inflammation. We found that 24-h OVA stimulation induced interferon- γ but not IL-4 in the splenocytes and eyes of DO11.10 mice, suggesting that OVA could elicit a Th1 response in this model. However, our experiments showed that interferon- γ neutralizing antibody did not attenuate the OVA-induced ocular inflammation. Thus, it indicates that the Th1 T cell subset is not primarily responsible for the development of this unique uveitis model.

Since Superarray analysis showed that mRNA for interferon- γ was up-regulated in OVA-induced uveitis, one speculation is that activation of macrophages may play a role in this model. Nevertheless, we and others clearly demonstrated neutrophil dominant inflammation as a feature of OVA-induced uveitis and airway inflammation in DO11.10 mice [58]. Our study has convincingly demonstrated that CD4⁺ lymphocytes, especially Th17 cells, play an inciting role in the inflammatory process. Furthermore, neutrophilia is a well documented downstream event of IL-17 action [27]. Our results provide an insight into the mechanism of IL-17 action. Consistent with Wilder's finding, OVA challenge resulted in a rapid neutrophil influx in the eyes of DO11.10 mice. This neutrophil-dominant inflammation coincided with the expression of transcripts for CXCL2 and CXCL5. It is well documented that CXCL2 and CXCL5 are two potent cytokines which are chemotactic for neutrophils [59–61]. They are mainly produced by monocytes and epithelial cells, respectively [60–62]. CXCL2 and CXCL5 interact with CXCR2 on the surface of neutrophils to exert their effects [61–63]. IL-17 has been shown to up-regulate the production of these two chemokines [61–64]. In this study, we found that reduction of ocular CXCL2 and CXCL5 expression correlated with inhibition of neutrophil infiltration by IL-17 neutralizing antibody. This result suggests that IL-17 initiates neutrophilic inflammation through induction of neutrophil chemotactic mediators. Studies to evaluate the effect of neutralization of CXCL2 and CXCL5 in this model are in progress.

In summary, we have developed a time-efficient uveitis model that is ideal for the evolving and important research on the role of IL-17 in immune-mediated disease. This report is the first to describe this model, although we have previously described a model in which DO11.10 splenocytes are stimulated in vitro, passively transferred to BALB/c mice, and then uveitis is

induced by intraocular injection of antigen. The passive transfer model allows the investigator to fluorescently label a specific population of T cells, while the current model produces more robust ocular inflammation. Our data suggest that IL-17 plays a critical role in the pathogenesis of OVA-induced uveitis in DO11.10 mice, and neutrophil chemotaxis is a key mechanism mediating the downstream effect of IL-17. Finally, this study provides additional rationale to target IL-17 for treating uveitis and related diseases.

Acknowledgments

The authors thank Judie McDonald for assistance with manuscript preparation.

Supported by National Eye Institute Grants (HD033703-10, EY016788, EY013093, and EY006484).

References

1. Gritz DC, Wong IG. Incidence and prevalence of uveitis in Northern California; the Northern California Epidemiology of Uveitis Study. *Ophthalmology* 2004;111(3):491–500. [PubMed: 15019324] discussion 500
2. Nussenblatt RB. The natural history of uveitis. *International Ophthalmology* 1990;14(5–6):303–8. [PubMed: 2249907]
3. Becker MD, Adamus G, Davey MP, Rosenbaum JT. The role of T cells in autoimmune uveitis. *Ocular Immunology & Inflammation* 2000;8(2):93–100. [PubMed: 10980681]
4. Martin TM, Smith JR, Rosenbaum JT. Anterior uveitis: current concepts of pathogenesis and interactions with the spondyloarthropathies. *Current Opinion in Rheumatology* 2002;14(4):337–41. [PubMed: 12118164]
5. Atalla L, Linker-Israeli M, Steinman L, Rao NA. Inhibition of autoimmune uveitis by anti-CD4 antibody. *Investigative Ophthalmology & Visual Science* 1990;31(7):1264–70. [PubMed: 1973156]
6. Kilmartin DJ, Fletcher ZJ, Almeida JA, Liversidge J, Forrester JV, Dick AD. CD69 expression on peripheral CD4+ T cells parallels disease activity and is reduced by mycophenolate mofetil therapy in uveitis. *Investigative Ophthalmology & Visual Science* 2001;42(6):1285–92. [PubMed: 11328741]
7. Streilein JW. Ocular immune privilege: the eye takes a dim but practical view of immunity and inflammation. *Journal of Leukocyte Biology* 2003;74(2):179–85. [PubMed: 12885934]
8. de Smet MD, Chan CC. Regulation of ocular inflammation—what experimental and human studies have taught us. *Progress in Retinal & Eye Research* 2001;20(6):761–97. [PubMed: 11587917]
9. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *Journal of Experimental Medicine* 2005;201(2):233–40. [PubMed: 15657292]
10. Bettelli E, Oukka M, Kuchroo VK. T(H)-17 cells in the circle of immunity and autoimmunity. *Nature Immunology* 2007;8(4):345–50. [PubMed: 17375096]
11. Tanaka K, Ichiyama K, Hashimoto M, Yoshida H, Takimoto T, Takaesu G, et al. Loss of Suppressor of Cytokine Signaling 1 in Helper T Cells Leads to Defective Th17 Differentiation by Enhancing Antagonistic Effects of IFN-gamma on STAT3 and Smads. *Journal of Immunology* 2008;180(6):3746–56.
12. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* 2006;126(6):1121–33. [PubMed: 16990136]
13. Chabaud M, Durand JM, Buchs N, Fossiez F, Page G, Frappart L, et al. Human interleukin-17: A T cell-derived proinflammatory cytokine produced by the rheumatoid synovium. *Arthritis & Rheumatism* 1999;42(5):963–70. [PubMed: 10323452]
14. Lubberts E, Joosten LA, Oppers B, van den Bersselaar L, Coenen-de Roo CJ, Kolls JK, et al. IL-1-independent role of IL-17 in synovial inflammation and joint destruction during collagen-induced arthritis. *Journal of Immunology* 2001;167(2):1004–13.
15. Ye P, Rodriguez FH, Kanaly S, Stocking KL, Schurr J, Schwarzenberger P, et al. Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor

- expression, neutrophil recruitment, and host defense. *Journal of Experimental Medicine* 2001;194(4):519–27. [PubMed: 11514607]
16. Ye P, Garvey PB, Zhang P, Nelson S, Bagby G, Summer WR, et al. Interleukin-17 and lung host defense against *Klebsiella pneumoniae* infection. *American Journal of Respiratory Cell & Molecular Biology* 2001;25(3):335–40. [PubMed: 11588011]
 17. Molet S, Hamid Q, Davoine F, Nutku E, Taha R, Page N, et al. IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. *Journal of Allergy & Clinical Immunology* 2001;108(3):430–8. [PubMed: 11544464]
 18. Linden A. Role of interleukin-17 and the neutrophil in asthma. *International Archives of Allergy & Immunology* 2001;126(3):179–84. [PubMed: 11752873]
 19. Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, Garren H, et al. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nature Medicine* 2002;8(5):500–8.[see comment]
 20. Fujino S, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y, et al. Increased expression of interleukin 17 in inflammatory bowel disease. *Gut* 2003;52(1):65–70. [PubMed: 12477762]
 21. Nielsen OH, Kirman I, Rudiger N, Hendel J, Vainer B. Upregulation of interleukin-12 and -17 in active inflammatory bowel disease. *Scandinavian Journal of Gastroenterology* 2003;38(2):180–5. [PubMed: 12678335]
 22. Zhang Z, Zheng M, Bindas J, Schwarzenberger P, Kolls JK. Critical role of IL-17 receptor signaling in acute TNBS-induced colitis. *Inflammatory Bowel Diseases* 2006;12(5):382–8. [PubMed: 16670527]
 23. Langrish CL, McKenzie BS, Wilson NJ, Malefyt R, Kastelein RA, Cua DJ. IL-12 and IL-23: master regulators of innate and adaptive immunity. *Immunological Reviews* 2004;202:96–105. [PubMed: 15546388]
 24. Aggarwal S, Ghilardi N, Xie MH, de Sauvage FJ, Gurney AL. Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. *Journal of Biological Chemistry* 2003;278(3):1910–4. [PubMed: 12417590]
 25. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages see comment. *Nature Immunology* 2005;6(11):1123–32. [PubMed: 16200070]
 26. Schwarzenberger P, Huang W, Ye P, Oliver P, Manuel M, Zhang Z, et al. Requirement of endogenous stem cell factor and granulocyte-colony-stimulating factor for IL-17-mediated granulopoiesis. *Journal of Immunology* 2000;164(9):4783–9.
 27. Kolls JK, Linden A. Interleukin-17 family members and inflammation. *Immunity* 2004;21(4):467–76. [PubMed: 15485625]
 28. De Vos AF, Hoekzema R, Kijlstra A. Cytokines and uveitis, a review. *Current Eye Research* 1992;11(6):581–97. [PubMed: 1505201]
 29. Wakefield D, Lloyd A. The role of cytokines in the pathogenesis of inflammatory eye disease. *Cytokine* 1992;4(1):1–5. [PubMed: 1617154]
 30. Lim LL, Hoang L, Wong T, Planck SR, Ronick MB, Gould RR, et al. Intravital microscopy of leukocyte-endothelial dynamics using the Heidelberg confocal laser microscope in scleritis and allergic conjunctivitis. *Molecular Vision* 2006;12:1302–5. [PubMed: 17110913]
 31. Dullforce PA, Seitz GW, Garman KL, Michael JA, Crespo SM, Fleischman RJ, et al. Antigen-specific accumulation of naive, memory and effector CD4 T cells during anterior uveitis monitored by intravital microscopy. *Cellular Immunology* 2006;239(1):49–60. [PubMed: 16712823]
 32. Becker MD, Crespo S, Martin TM, Planck SR, Naramura M, Rosenbaum JT. Intraocular in vivo imaging of activated T-lymphocytes expressing green-fluorescent protein after stimulation with endotoxin. *Graefes Archive for Clinical & Experimental Ophthalmology* 2001;239(8):609–12.
 33. Rosenbaum JT, Ronick MB, Song X, Choi D, Planck SR. T cell-antigen-presenting cell interactions visualized in vivo in a model of antigen-specific inflammation. *Clin Immunol.* 2007Epub ahead of print
 34. Hofstetter HH, Toyka KV, Tary-Lehmann M, Lehmann PV. Kinetics and organ distribution of IL-17-producing CD4 cells in proteolipid protein 139–151 peptide-induced experimental autoimmune encephalomyelitis of SJL mice. *Journal of Immunology* 2007;178(3):1372–8.

35. Stoppacciaro A, Melani C, Parenza M, Mastracchio A, Bassi C, Baroni C, et al. Regression of an established tumor genetically modified to release granulocyte colony-stimulating factor requires granulocyte-T cell cooperation and T cell-produced interferon gamma. *Journal of Experimental Medicine* 1993;178(1):151–61. [PubMed: 7686211]
36. Caldwell CC, Okaya T, Martignoni A, Husted T, Schuster R, Lentsch AB. Divergent functions of CD4+ T lymphocytes in acute liver inflammation and injury after ischemia-reperfusion. *American Journal of Physiology - Gastrointestinal & Liver Physiology* 2005;289(5):G969–76. [PubMed: 16002566]
37. Zwacka RM, Zhang Y, Halldorson J, Schlossberg H, Dudus L, Engelhardt JF. CD4(+) T-lymphocytes mediate ischemia/reperfusion-induced inflammatory responses in mouse liver. *Journal of Clinical Investigation* 1997;100(2):279–89. [PubMed: 9218504]
38. Grajewski RS, Hansen AM, Agarwal RK, Kronenberg M, Sidobre S, Su SB, Silver PB, et al. Activation of invariant NKT cells ameliorates experimental ocular autoimmunity by a mechanism involving innate IFN-gamma production and dampening of the adaptive Th1 and Th17 responses. *Journal of Immunology* 2008;181(7):4791–7.
39. Yoshiga Y, Goto D, Segawa S, Ohnishi Y, Matsumoto I, Ito S, et al. Invariant NKT cells produce IL-17 through IL-23-dependent and -independent pathways with potential modulation of Th17 response in collagen-induced arthritis. *International Journal of Molecular Medicine* 2008;22(3):369–74. [PubMed: 18698497]
40. Coquet JM, Chakravarti S, Kyriarissoudis K, McNab FW, Pitt LA, McKenzie BS, et al. Diverse cytokine production by NKT cell subsets and identification of an IL-17-producing CD4-NK1.1- NKT cell population. *Proceedings of the National Academy of Sciences of the United States of America* 2008;105(32):11287–92. [PubMed: 18685112]
41. McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T, et al. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology see comment. *Nature Immunology* 2007;8(12):1390–7. [PubMed: 17994024]
42. Hunter CA. New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. *Nature Reviews Immunology* 2005;5(7):521–31.
43. Rouvier E, Luciani MF, Mattei MG, Denizot F, Golstein P. CTLA-8, cloned from an activated T cell, bearing AU-rich messenger RNA instability sequences, and homologous to a herpesvirus saimiri gene. *Journal of Immunology* 1993;150(12):5445–56.
44. Yao Z, Fanslow WC, Seldin MF, Rousseau AM, Painter SL, Comeau MR, et al. Herpesvirus Saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. *Immunity* 1995;3(6):811–21. [PubMed: 8777726]
45. Aggarwal S, Gurney AL. IL-17: prototype member of an emerging cytokine family. *Journal of Leukocyte Biology* 2002;71(1):1–8. [PubMed: 11781375]
46. Laan M, Lotvall J, Chung KF, Linden A. IL-17-induced cytokine release in human bronchial epithelial cells in vitro: role of mitogen-activated protein (MAP) kinases. *British Journal of Pharmacology* 2001;133(1):200–6. [PubMed: 11325811]
47. Spriggs MK. Interleukin-17 and its receptor. *Journal of Clinical Immunology* 1997;17(5):366–9. [PubMed: 9327335]
48. Awane M, Andres PG, Li DJ, Reinecker HC. NF-kappa B-inducing kinase is a common mediator of IL-17-, TNF-alpha-, and IL-1 beta-induced chemokine promoter activation in intestinal epithelial cells. *Journal of Immunology* 1999;162(9):5337–44.
49. Laan M, Cui ZH, Hoshino H, Lotvall J, Sjostrand M, Gruenert DC, et al. Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. *Journal of Immunology* 1999;162(4):2347–52.
50. Hata K, Andoh A, Shimada M, Fujino S, Bamba S, Araki Y, et al. IL-17 stimulates inflammatory responses via NF-kappaB and MAP kinase pathways in human colonic myofibroblasts. *American Journal of Physiology - Gastrointestinal & Liver Physiology* 2002;282(6):G1035–44. [PubMed: 12016129]
51. Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, et al. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity* 2002;17(3):375–87. [PubMed: 12354389]

52. Hellings PW, Kasran A, Liu Z, Vandekerckhove P, Wuyts A, Overbergh L, et al. Interleukin-17 orchestrates the granulocyte influx into airways after allergen inhalation in a mouse model of allergic asthma see comment. *American Journal of Respiratory Cell & Molecular Biology* 2003;28(1):42–50. [PubMed: 12495931]
53. Amadi-Obi A, Yu CR, Liu X, Mahdi RM, et al. TH17 cells contribute to uveitis and scleritis and are expanded by IL-2 and inhibited by IL-27/STAT1. *Nat Med* 2007;13:711–8. [PubMed: 17496900]
54. Peng Y, Han G, Shao H, Wang Y, Kaplan HJ, Sun D. Characterization of IL-17+ interphotoreceptor retinoid-binding protein-specific T cells in experimental autoimmune uveitis. *Investigative Ophthalmology & Visual Science* 2007;48(9):4153–61. [PubMed: 17724201]
55. Luger D, Caspi RR. New perspectives on effector mechanisms in uveitis. *Semin Immunopathol*. 2008Epub ahead of print
56. Yang P, Smith JR, Damodar KS, Planck SR, Rosenbaum JT. Visualization of cell death in vivo during murine endotoxin-induced uveitis. *Investigative Ophthalmology & Visual Science* 2003;44(5):1993–7. [PubMed: 12714634]
57. Becker MD, Nobiling R, Planck SR, Rosenbaum JT. Digital video-imaging of leukocyte migration in the iris: intravital microscopy in a physiological model during the onset of endotoxin-induced uveitis. *Journal of Immunological Methods* 2000;240(1–2):23–37. [PubMed: 10854598]
58. Archer AJ, Cramton JL, Pfau JC, Colasurdo G, Holian A. Airway responsiveness after acute exposure to urban particulate matter 1648 in a DO11.10 murine model. *American Journal of Physiology - Lung Cellular & Molecular Physiology* 2004;286(2):L337–43. [PubMed: 14660482]
59. Wilder JA, Collie DD, Bice DE, Tesfaigzi Y, Lyons CR, et al. Ovalbumin aerosols induce airway hyperreactivity in naive DO11.10 T cell receptor transgenic mice without pulmonary eosinophilia or OVA-specific antibody. *Journal of Leukocyte Biology* 2001;69(4):538–47. [PubMed: 11310839]
60. Wolpe SD, Sherry B, Juers D, Davatilis G, Yurt RW, Cerami A. Identification and characterization of macrophage inflammatory protein 2. *Proceedings of the National Academy of Sciences of the United States of America* 1989;86(2):612–6. [PubMed: 2643119]
61. Persson T, Monsef N, Andersson P, Bjartell A, Malm J, Calafat J, et al. Expression of the neutrophil-activating CXC chemokine ENA-78/CXCL5 by human eosinophils. *Clinical & Experimental Allergy* 2003;33(4):531–7. [PubMed: 12680872]
62. He R, Oyoshi MK, Jin H, Geha RS. Epicutaneous antigen exposure induces a Th17 response that drives airway inflammation after inhalation challenge. *Proceedings of the National Academy of Sciences of the United States of America* 2007;104(40):15817–22. [PubMed: 17893340]
63. Ruddy MJ, Shen F, Smith JB, Sharma A, Gaffen SL. Interleukin-17 regulates expression of the CXC chemokine LIX/CXCL5 in osteoblasts: implications for inflammation and neutrophil recruitment. *Journal of Leukocyte Biology* 2004;76(1):135–44. [PubMed: 15107456]
64. Liang SC, Long AJ, Bennett F, Whitters MJ, Karim R, Collins M, et al. An IL-17F/A heterodimer protein is produced by mouse Th17 cells and induces airway neutrophil recruitment. *Journal of Immunology* 2007;179(11):7791–9.

Fig. 1.

OVA specifically induces IL-17 production in the splenocytes from DO11.10 mice. Splenocytes were isolated from DO11.10 and BALB/c mice. They were divided into 3 groups ($n = 5$): Control, OVA, and anti-CD3 and anti-CD28 antibodies. After 24-h stimulation, IL-17 production was measured by ELISPOT. IL-17 activity is defined as number of spots per 400,000 input cells (* $p < 0.01$).

Fig. 2.

OVA induces IL-17 production in a time-dependent manner. Splenocytes were isolated from DO11.10 mice. These cells were further stimulated with OVA for 3, 9, 21, 27, 33, 45 and 51 h, respectively ($n = 3$). IL-17 production was determined by ELISPOT.

Fig. 3.

Intravitreal injection of OVA elicits uveitis in DO11.10 mice. OVA or PBS as a control vehicle was administered intravitreally ($n = 12$). In addition, a group of mice received intravitreal injection of OVA₃₂₃₋₃₃₉ peptide ($n = 4$). Twenty-four hours later, circulating leukocytes were labeled with rhodamine by intraperitoneal injection, and ocular inflammation was assessed by intravital microscopy. (A) Representative image of a single frame from intravital microscopy videos taken of the iris at 24 h during the course of inflammation induced by OVA. Note: Influx and adhesion of rhodamine-labeled leukocytes in OVA-challenged eye. (B) Quantization of rolling and adherent cells in the vascular bed of the iris ($*p < 0.05$). (C) Representative histology of mouse eyes challenged with and without OVA. The ocular tissue was harvested for H&E staining. The inflammatory change was evaluated by light microscopy. Anterior chamber

inflammation and a leukocytic infiltrate posterior to the ciliary body and exudates (arrows) were observed in the eye treated with OVA compared to control group.

Fig. 4. Predominant neutrophil infiltration occurs in the eye of DO11.10 mice at 24 h after OVA challenge. OVA or PBS as a control vehicle was administered intravitreally ($n = 4$). (A) Twenty-four hours later, the eyes were enucleated, dissected, fixed in paraformaldehyde and the anterior segment was incubated with Ly6G antibody. Note: Majority of infiltrating cells were Ly6G positive (arrows). (B) Further histology showed that infiltrating leukocytes were mainly polymorphonuclear neutrophils (arrows).

Fig. 5.

Depletion of CD4⁺ cells inhibits OVA-induced uveitis in DO11.10 mice. DO11.10 mice received 100 µg of GK1.5 antibody intraperitoneally for consecutive 2 days to deplete peripheral CD4⁺ cells ($n = 3$). Then, OVA was delivered intravitreally to induce uveitis. Twenty-four hours later, ocular inflammatory cells were assessed by intravital microscopy. (A) Representative image of a single frame from intravital microscopy videos taken of the iris at 24 h during the course of inflammation induced by OVA. Note: GK1.5 antibody prevented OVA-induced inflammatory cell infiltration in the eye. (B) Quantization of rolling and adherent cells in the vasculature of the iris treated with and without GK1.5 antibody ($*p < 0.05$).

Fig. 6. Interferon- γ neutralizing antibody does not inhibit OVA-induced ocular inflammation in DO11.10 mice. Anti-interferon- γ antibody (15 μ g per eye) was injected intravitreally along with OVA into the eye of DO11.10 mice ($n = 4$). Twenty-four hours later, ocular inflammatory cells were assessed by intravital microscopy. Then, total RNA was harvested for RT-PCR. (A) Representative image of a single frame from intravital microscopy videos taken of the iris at 24 h during the course of inflammation induced by OVA. Note: Anti-interferon- γ antibody did not suppress OVA-induced inflammatory cell infiltration in the eye. (B) Quantization of rolling and adherent cells in the vasculature of the iris treated with and without anti-interferon- γ antibody.

Fig. 7. OVA induces IL-17 gene expression in the eye of DO11.10 mice. OVA or PBS as a control vehicle was administered intravitreally. Twenty-four hours later, the eyes were harvested and total RNA was collected. RT-PCR analysis revealed upregulation of IL-17 in OVA-treated eyes ($n = 6$).

Fig. 8. Infiltration of IL-17⁺ cells occurs in the eye of DO11.10 mice at 24 h after OVA challenge. OVA or PBS as a control vehicle was administered intravitreally ($n = 3$). Twenty-four hours later, the iris was dissected and fixed in paraformaldehyde. Immunohistochemical analysis was performed to detect IL-17 protein expression in the eye. Note: IL-17⁺ cells (arrows) were only detected in OVA-treated eye but not in the control group.

Fig. 9.

Infiltrating CD4⁺ T cells express IL-17 in the eye of DO11.10 mice at 24 h after OVA challenge. OVA or PBS as a control vehicle was administered intravitreally ($n = 4$). Twenty-four hours after OVA stimulation, the iris was dissected, and fixed in paraformaldehyde. Two-color immunofluorescent staining was used to determine CD4 (FITC), green color: A) and IL-17 (APC, red color: B). The expression of CD4 and IL-17 was further analyzed by colocalization of both fluorescence colors (C). Note: Most IL-17-expressed cells were CD4⁺ lymphocytes.

Fig. 10.

Depletion of NKT cells does not inhibit OVA-induced uveitis in DO11.10 mice. DO11.10 mice received 200 μ g of anti-CD161 antibody intraperitoneally twice 3 days apart before uveitis induction ($n = 3$). Then, OVA was delivered intravitreally to induce uveitis. Twenty-four hours later, ocular inflammatory cells were assessed by intravital microscopy. (A) Representative image of a single frame from intravital microscopy videos taken of the iris at 24 h during the course of inflammation induced by OVA. Note: anti-CD161 antibody did not reduce OVA-induced inflammatory cell infiltration in the eye. (B) Quantization of rolling and adherent cells in the vasculature of the iris treated with and without anti-CD161 antibody.

Fig. 11.

IL-23p19 neutralizing antibody did not inhibit OVA-induced ocular inflammation in DO11.10 mice. Anti-IL-23p19 antibody (100 μ g per mouse) was injected intraperitoneally into DO11.10 mice when they received intravitreal OVA challenge ($n = 3$). Twenty-four hours later, ocular inflammatory cells were assessed by intravital microscopy. (A) Representative image of a single frame from intravital microscopy videos taken of the iris at 24 h during the course of inflammation induced by OVA. Note: Anti-IL-23p19 antibody did not suppress OVA-induced inflammatory cell infiltration in the eye. (B) Quantization of rolling and adherent cells in the vasculature of the iris treated with and without anti-IL-23p19 antibody.

Fig. 12.

Anti-IL-17 antibody inhibits OVA-induced ocular inflammation in DO11.10 mice. Anti-IL-17 antibody (20 μ g per eye) was injected intravitreally along with OVA into the eye of DO11.10 mice ($n = 7$). Twenty four hours later, ocular inflammatory cells were assessed by intravital microscopy. Then, total RNA was harvested for RT-PCR. (A) Representative image of a single frame from intravital microscopy videos taken of the iris at 24 h during the course of inflammation induced by OVA. Note: Anti-IL-17 antibody inhibited OVA-induced inflammatory cell infiltration in the eye. (B) Quantitation of rolling and adherent cells in the vasculature of the iris treated with and without anti-IL-17 antibody ($p < 0.05$). (C) RT-PCR analysis of CXCL2 and CXCL5 revealed up-regulation of these 2 chemokines in the eyes treated with OVA. This up-regulation was reduced by anti-IL-17 antibody.

Table 1

Expression of Th1 and Th2 related genes in the eyes at 24 h after OVA challenge (Average of three independent arrays). Fold changes in gene expression between control and OVA-treated groups were calculated using $\Delta\Delta C_t$ method in the PCR Array Data Analysis template (<http://www.SABiosciences.com/pcrarraydataanalysis.php>). The value of 1 indicates no change of the expression of a gene of interest between experimental and control groups. If the fold change was greater than 1, then the result was reported as a fold up-regulation. If the fold change was less than 1, it represents a down-regulation.

Categories	Gene	Fold change (Compared to control group)
Th1 transcription factor	T-bet	1.0 $\pm$ 0.0
Th1 cytokine	Interferon- γ	58.6 $\pm$ 16.9
Th2 transcription factor	GATA-3	1.0 $\pm$ 0.0
Th2 cytokines	IL-4	0.8 $\pm$ 0.4
	IL-5	0.57 $\pm$ 0.9

Table 2

Expression of selected IL-17-related genes in the eyes at 24 h after OVA challenge (Average of three independent arrays).

Categories	Genes	Fold increase (Compared to control group)
Th17 signaling	SOCS1	6.1 ± 1.3
	ROR γ t	1.1 ± 0.4
Th17 unique cytokines	IL-17F	8.9 ± 1.7
	IL-21	2.7 ± 0.6
IL-17 downstream cytokines	IL-1 β	51.3 ± 22.1
	IL-6	16.7 ± 7.7
	TNF- α	7.25 ± 1.6