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Autoimmune Uveitis Elicited with Antigen-Pulsed Dendritic Cells Has a Distinct Clinical Signature and Is Driven by Unique Effector Mechanisms: Initial Encounter with Autoantigen Defines Disease Phenotype

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Human autoimmune uveitis is a heterogeneous group of potentially blinding ocular diseases in which most patients who exhibit immunity recognize the same retinal Ag. It is represented by the model of experimental autoimmune uveitis (EAU) induced in mice by immunization with retinal Ag in CFA. Murine EAU is characterized by a Th1/Th17 response pattern, which may not represent all types of human uveitis. We report in this study a new model of EAU induced by injection of matured dendritic cells loaded with a uveotogenic retinal peptide. Dendritic cell-induced EAU demonstrated unique characteristics compared with traditional EAU in terms of clinical manifestations, the nature of the inflammatory infiltrating cells, the cytokine response profile, and a strict requirement for IFN-γ, whereas IL-17 appeared to play a minor role. Disease was self-limiting, but could be reinduced with the same Ag in CFA, albeit with reduced severity, suggesting postrecorvery resistance. Our study demonstrates in a disease setting that the context in which the same autoantigen is initially presented to the immune system precipitates distinct forms of pathology via a distinct pathogenic pathway on the same genetic background. These findings may shed new light on the complex biology and the heterogeneous nature of human uveitis, and provide an alternative model for uveitic diseases of immune origin. *The Journal of Immunology, 2007, 178: 5578–5587.*

Posterterior uveitis of a putative autoimmune origin affects 150,000 Americans annually and is estimated to be responsible for ~10% of the cases of severe handicap (1). Experimental autoimmune uveitis (EAU) in animals, induced by immunization with a retinal Ag in CFA serves as a model of this group of blinding diseases. EAU induced in this fashion is a Th1/Th17 dominated response to the immunizing retinal Ag in which a massive inflammatory infiltrate composed primarily of mononuclear cells causes a rapid and irreversible destruction of photoreceptor cells (D. Luger and R. R. Caspi, submitted for publication) (2–4).

Over the past 20 years, the EAU model in animals has contributed to the understanding of genetic associations, cellular mechanisms, and therapeutic regulation of clinical uveitis patients (5–7). As useful as it has been, however, this model does not accurately represent the full spectrum of human uveitic disease. Human uveitis is a heterogenous group of diseases that differ as to the disease course and severity, as well as histopathological manifestations (7). Curiously, responses to the same retinal Ag may be observed in several different uveitic diseases. For example, responses to retinal arrestin (retinal soluble Ag) are seen in birdshot retinochoroidopathy, intermediate uveitis, sympathetic ophthalmia, and Behcet’s disease, which are all clinically and histologically distinct forms of uveitis (7). We hypothesized that the context in which the retinal Ag is first presented to the immune system may determine the effector choice and result in a different form of disease. We therefore set out to establish a model of EAU induced with activated Ag-pulsed dendritic cells (DC) as a complementary approach to the traditional EAU model induced by immunization with Ag in CFA.

DC are “professional” APCs, which constitute the main APC that prime naive T cells (8). Innate stimuli from exogenous substances like microbial components or endogenous substances like CD40L and uric acid can act as danger signals to activate DC (9, 10). Upon encountering Ag in the presence of such innate stimuli, DC undergo maturation, including Ag processing, up-regulation of MHC class II molecules, induction of costimulatory activity, and migrate to lymph nodes where they interact with Ag-specific T cells and direct them to differentiate into effector or memory cells. The nature of innate stimuli is important in determining the direction and type of the resulting adaptive response (reviewed in Ref. 11). In vivo, infusion of Ag-pulsed DC has been shown to trigger autoimmune diabetes and experimental autoimmune encephalomyelitis in transgenic mouse models (12, 13) and autoimmune myocarditis in nontransgenic mice (14). However, the question whether in vitro matured DC can elicit autoimmunity to retina, an immune privileged site, has hitherto not been examined.

We report a new and distinct type of EAU in wild-type (WT) B10.RIII mice, induced by peripheral injection of retinal peptide-loaded in vitro-matured DC. This new model demonstrated unique effector mechanisms and distinct clinical manifestations compared...
with traditional EAU in terms of cytokine response profile, a non-redundant requirement for IFN-γ, the cellular composition of the inflammatory infiltrate, appearance of fundus lesions, and the severity and course of the disease. This study thus demonstrates that distinct forms of autoimmune uveitis can be induced by the same autoantigen presented in the context of different innate triggering stimuli. Our findings may help to shed light on the heterogeneous nature of uveitis in humans in the face of patient responses to the same autoantigen and provides a model for those types of uveitis that were not adequately represented by the traditional EAU model.

Materials and Methods

Mice

Six- to 8-wk-old B10.RII female mice and IFN-γ knockout (GKO) mice in C57BL/6 background supplied by The Jackson Laboratory were housed under pathogen-free conditions and were given standard laboratory chow and water ad libitum. GKO mice were backcrossed 10 generations onto B10.RII background. Animal care and use were reviewed and approved by the Institutional Animal Care and Use Committee and are in compliance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

Hydrodynamic delivery of plasmid DNA

Hydrodynamic delivery is an efficient method for in vivo expression of plasmid encoded proteins. The plasmid, pUMCv-mFLex containing the extracellular domain of murine fms-like tyrosine kinase 3 ligand (Flt-3 ligand) (secreted form), was obtained from National Gene Vector Laboratory (University of Michigan, Ann Arbor, MI). The purified plasmid DNA, referred to in this study as Flt-3 ligand DNA, was extracted from Escherichia coli and purified by using endo-free plasmid purification kit (Qiagen). Hydrodynamic delivery of the naked plasmid DNA was performed as described by Knapp and Liu (15). Briefly, 50 μg of purified plasmid was diluted in 2.0 ml of sterilized Ringer’s solution and injected into mice through the tail vein over 5–8 s. In our hands, protein encoded by a plasmid delivered in this fashion can be detected by Western blotting in the liver within 8 h (P. B. Silver and R. R. Caspi, unpublished observations).

Splenic DC isolation

Single-cell suspension of spleen was prepared from mice 7 days after injection of plasmid DNA as described by Björck (16) and Vremerč et al. (17) with modifications. Briefly, B10.RII spleen was minced into small fragments and digested with collagenase D (Roche) and DNase I (Roche) for 45 min at 37°C and passed through a 70-μm nylon cell strainer (BD Falcon) and treated with EDTA for 5 min. After red cell lysis and washing, cells were incubated with Abs to block Fc receptor interactions (FcR-blocking Ab, CD16/CD32, BD Biosciences), then incubated with anti CD11c-conjugated magnetic beads (Miltenyi Biotec) at 4–8°C for exactly 15 min, washed twice, and filtered through 40-μm cell strainer. The positive population was purified by autoMACS (Miltenyi Biotec) running the “posset” program. The purity of CD11c+ population was checked on FACSCalibur (BD Biosciences) and was typically >94% (data not shown). Some of the DC were further sorted by FACSAria (BD Biosciences) to the purity of 99%.

DC maturation and Ag pulsing

The purified CD11c+ cells were resuspended in RPMI 1640 medium supplemented with 2 mM l-glutamine, 50 μM 2-ME, 50 μg/ml penicillin/streptomycin, 1 mM sodium pyruvate, 1% nonessential amino acid, 10 ng/ml recombinant mouse GM-CSF (PeproTech) and containing 1% fresh-frozen mouse serum (complete medium). Aliquots of 2 × 106 cells/ml were delivered into 12-well plates (catalog no. 3512, Costar; Corning) and allowed to adhere for 1 h at 37°C, after which the wells were rinsed twice to remove nonadherent cells. The Ag, a 20-aa peptide sequence SLYHPNTLHVD encoded by human interphotoreceptor retinoid binding protein (IRBP) residues 161–180 (p161–180), encoding the major pathogenic epitope, was synthesized by AnaSpec using Fmoc technology (18). Ag pulsing was for 4.5 h, unless otherwise specified. For maturation the medium was additionally supplemented with 100 μg/ml P161–180 and 1 μg/ml LPS (E. coli O111:B4; Sigma-Aldrich), 5 μg/ml anti-CD40 (3/23; BD Biosciences). In some experiments a crude mycobacteria extract was used instead of LPS. The extract was made by a heat-killed freeze-dried Mycobacterium tuberculosis strain H37RA (Difco) suspended in 10 ml of PBS. After homogenization, the suspension was sonicated and repeatedly freeze/thawed 10 times, then centrifuged for 30 min at 8,000 rpm. The supernatant was collected and aliquoted. The resulting solution (OD280 = 0.91, OD260 = 0.59) was used at a final 1/20 dilution in combination with anti-CD40 to stimulate DC.

Induction of EAU

Induction by DC injection. In vitro matured DC were washed twice in HBSS containing 1% mouse serum and 1–2 million cells were injected s.c. into the left footpads of recipient mice. An identical injection was repeated, or not, 4 days later. Some mice were also given 0.3–0.4 μg of pertussis toxin (PTX; Sigma-Aldrich) i.p. 2 days after the first DC injection. The induction regimen is shown in Fig. 1. Disease was evaluated as described below.

Induction of EAU by immunization. The “traditional” EAU model was induced by immunizing B10.RII mice s.c. in the thighs and base of the tail with 9–12 μg of p161–180 as a 1:1 (v/v) emulsion in CFA (Sigma-Aldrich) supplemented with M. tuberculosis strain H37RA to 2.5 mg/ml. Disease was evaluated as described below.

EAU scoring

Clinical EAU was evaluated by periodic fundus examination (fundoscopy) and was confirmed by histological examination of H&E-stained sections of eyes collected on day 18. Collection and processing of eyes for histopathology was performed as described (4). Pathology was scored in a masked fashion by a facility ophthalmic pathologist (Chi-Chao Chan) on a scale of 0–4, according to the severity and type of lesions, essentially as described (4), with 0 being no disease and 4 representing complete retinal destruction.

Quantitation of Ag-specific lymphocyte proliferation and cytokine release

Draining lymph nodes or spleens were extracted from mice primed with p161–180-pulsed DC or from mice immunized by the traditional method 8 or 18 days after antigenic challenge, as specified. The lymph node cells or splenocytes after red cell lysis were rendered into a single-cell suspension and were stimulated in complete medium (without GM-CSF) with 20 μg/ml p161–180. After 48 h, unless another incubation time is specified, cultures were pulsed overnight by [3H]thymidine for proliferation assay, or supernatants were collected for cytokine assay. [3H]Thymidine incorporation was measured in a beta-scintillation counter. The supernatants were stored at –20°C and were assayed by multiplex ELISA using the Search-Light Technology ( Pierce-Endogen) (19).

Preparation of ocular extracts

Both left and right eyes were enucleated from DC-infused or CFA-immunized mice on day 14 and minced into small pieces in 300 μl of PBS with protease inhibitor mixture (Calbiochem). The tissues were briefly sonicated. The soluble fraction was collected after high-speed centrifugation and stored at −80°C for cytokine assay by multiplex ELISA. All procedures were done at 4°C.

Flow cytometric analysis

For surface marker analysis, DCs were preincubated for 30 min at 4°C with Fc blocking Abs before staining with fluorochrome-labeled Abs. For intracellular cytokine staining, draining lymph nodes or primed spleen cells were cultured with IRBP p161–180 for 4 days, and then 10 ng/ml PMA/250 ng/ml ionomycin (Sigma-Aldrich) and GolgiStop (BD Biosciences) were added for 4 h. Cells were then stained with anti-CD40 to stimulate DC.
was added for the last 5 h. Cells were treated and stained using the BD Cytofix/Cytoperm kit per the manufacturer’s instructions. At least 10,000 live events (after exclusion of 7-aminoactinomycin D-positive cells) were collected on a BD FACSCalibur using CellQuest software and were analyzed using FlowJo software. Fluorescent conjugated mAbs for flow cytometry analysis of DC surface markers were CD11c (HL3), CD8α (Ly-2), CD11b (M1/70), B220 (RA3-6B2), CD40 (3/23), CD80 (16-10A1), CD86 (GL1), and MHC class II molecule I-EK (14-4-4S). Fluorescent-conjugated mAbs for surface staining and for intracellular cytokine staining were FITC-conjugated CD4 (GK1.5), PE conjugated anti-IL-17 (TC11-18H10), allophycocyanin-conjugated anti-IFN-γ (XMG1.2), and the appropriate isotype controls. All mAbs were purchased from BD Biosciences.

**Results**

**Characterization of Flt-3 ligand-elicited, in vitro-matured splenic DC**

DCs are a rare population in spleen and lymphoid organ. Repeated injections of Flt-3 ligand can increase the number of functional DCs (21). In the present study, we used a single hydrodynamic injection of Flt-3 ligand DNA rather than repeated injections of Flt-3 ligand protein. Hydrodynamic delivery of plasmid DNA has been reported as an efficient approach to express exogenous genes in vivo (15). A single hydrodynamic injection of 50 μg of Flt-3 ligand DNA into B10.RIII mice elicited a 10-fold increase of splenic CD11c+ cells on day 7. This response was composed of a 5-fold increase in the ratio of CD11c+ cells in spleen, combined with a doubling of spleen size compared with untreated mice (Fig. 2, A and B, and data not shown). CD11c+ cells coexpressing CD8α, CD11b, or B220 were significantly expanded, with most of the expansion occurring in CD8α- and B220-positive populations (Fig. 2B, compare ratio of upper to lower right quadrants in...
naive vs. Flt-3L-treated cells). Expression of costimulatory molecules (CD40, CD80, CD86) and MHC class II molecules were not markedly altered (Fig. 2C). After being pulsed ex vivo with p161–180 and reinjected into naive mice, the Flt-3 ligand-elicited DC appeared functionally similar to “normal” resident splenic DC in terms of their ability to prime an Ag-specific proliferative response in the draining lymph node (Fig. 2D).

One of the important properties of DC is to release cytokines upon innate stimulation. We therefore examined inflammatory cytokine production by Flt-3 ligand-elicited DC under different stimulation conditions. When stimulated overnight by LPS alone or LPS plus anti-CD40 MACS-isolated CD11c+ cells expressed MHC class II (99%; data not shown) and produced a variety of cytokines (Fig. 2E). These included IL-1, IL-6, IL-12, TNF-α, IFN-γ, as well as the anti-inflammatory cytokine IL-10. Under the conditions of stimulation used in this study, near-maximal amounts of all the tested cytokines were seen with LPS stimulation alone, but maximal IL-12p70 production required stimulation with both LPS and anti-CD40 (Fig. 2F). Unlike cytokine production, expression of CD80 and CD86 and of MHC class II was up-regulated by explanation into culture alone, even in the absence of LPS and anti-CD40 (data not shown). Possible change in expression of CD40 after the maturation process could not be examined due to masking of surface CD40 molecules by the anti-CD40 present in the culture.

Because in the traditional method of EAU induction, by immunization in CFA, DC resident in the lymph node encounter mycobacterial components, we compared production of IL-12 and IL-23 by Flt-3 ligand-elicited DC stimulated by LPS or by a crude mycobacterial lysate (at 1/20 dilution). Purified Flt-3 ligand-elicited CD11c+ DC were stimulated by LPS and anti-CD40 or by mycobacterial lysate and anti-CD40 overnight, and production of IL-12 and IL-23 was measured by ELISA. Either LPS or mycobacterial extract combined with anti-CD40 induced IL-12p70 and IFN-γ secretion. However, only mycobacterial lysate was able to induce detectable IL-23 production (Fig. 2F). To confirm that the IFN-γ produced was of DC origin and not by any residual NK cells, which are major producers of IFN-γ, the experiment was repeated after sorting CD11c+ DC on a FACSAria to 99% purity, with similar results (data not shown). IL-17 was not produced under these conditions of stimulation.

Peripheral injection of retinal peptide-loaded in vitro-matured splenic DC induce uveitis

Depending on their maturation status, DC can initiate an immune response or induce tolerance (22, 23). To trigger autoimmunity, B10.RIII mice were given two s.c. injections 4 days apart of Flt-3 ligand-elicited DC, matured in vitro with LPS and anti-CD40 and pulsed with p161–180 and a single PTX administration on day 2 (protocol shown in Fig. 1). Histopathological examination of eyes obtained 18 days after the first DC injection showed that this regimen reproducibly yielded retinal pathology with scores up to 2 (Fig. 3). The pathology induced by DC challenge was typically milder than that induced by immunization with Ag in CFA. The full induction regimen of two administrations of LPS and CD40-treated DC and PTX was required to consistently obtain disease induction. Significantly reduced severity and incidence was seen when PTX was omitted from the induction regimen. The effect of PTX on the immune system is complex and includes permeabilization of the blood-organ barrier, innate stimulation (TLR4 activation) with consequences on the adaptive immune response phenotype and intensity, as well as inhibition of regulatory T cell function (24–27). Combined stimulation by both LPS and the agonistic anti-CD40 appeared to be required for DC to acquire uveitogenicity, consistent with the notion that a CD40-CD40L interaction is critical for the development of a productive immune response in vivo (28–30). Similarly, both doses of uveitogenic DC were required for EAU induction, because mice failed to develop EAU after only one DC dose even though all the other elements of the regimen were present. We hypothesize that after a single dose of DC, insufficient numbers of EAU effector cells were generated, and a second dose of Ag-pulsed DC could serve to prime new, as well as expand the existing, uveitogenic effectors induced by the first DC dose (Fig. 3). Thus, both the intensity of the innate stimulation, as well as the duration of the antigenic stimulus appeared to play a role. No uveitis was induced by DC without p161–180 pulsing (data not shown). Notably, no uveitis was induced by a full uveitogenic regimen of allogeneic B10.BR DC pulsed with p161–180 (this peptide is bound and presented by I-Ab) and injected into hosts pretreated with asialo-GM1 Ab (to prevent elimination of the injected allogeneic DC by NK cells) (data not shown). This result suggests that the injected DC are in fact the APC that directly initiate the adaptive response in this regimen, and passive transfer of Ag to host APC for indirect presentation is insufficient.

DC-induced uveitis is accompanied by a mixed Th1/Th2 response with minimal IL-17 production

To characterize the initial T cell response triggered by uveitogenic DC and the association with uveitis development, B10.RIII mice were injected with the full uveitogenic DC regimen or were immunized with the traditional regimen of p161–180 in CFA. We first examined the immune response pattern of responses to Ag in lymph node cells draining the sites of immunization. Multiplex ELISA of supernatants from lymph node cells collected 8 days after start of the respective induction regimens (before disease onset), revealed that mice primed by uveitogenic DC produced large amounts of IFN-γ and IL-4, whereas IL-17 production was marginal. In contrast, traditionally immunized mice produced large amounts of both IFN-γ and IL-17, but marginal amounts of IL-4 (Fig. 4A). This result was maintained in lymph node cells harvested on day 18, well after disease onset, which typically occurs around day 11 (Fig. 4B). Intracellular cytokine staining for IFN-γ and IL-17 confirmed the cytokine response pattern seen by ELISA (Fig. 4C). Because PTX is important for DC-triggered uveitis, we examined whether PTX administration altered the cytokine pattern.
DC-triggered uveitis is characterized by a mixed Th1/Th2 response with minimal IL-17 production. B10.RIII mice were injected with uveitogenic DC using the full disease induction regimen or were immunized with 9 μg of IRBP p161–180 in CFA. Draining lymph nodes were stimulated in culture with or without 20 μg/ml IRBP p161–180 for 48 h. A, Cytokine production was assayed in 48 h supernatants by multiplex ELISA in DC-induced vs immunization-induced EAU on day 8. B, Cytokine production and EAU scores (by fundoscopy) on day 18 after immunization. C, Intracellular cytokine production. Draining lymph node cells were stimulated with Ag in culture for 4 days, with PMA and ionomycin added in the last 5 h. The cells were then stained by FITC anti-CD4, PE anti-IL-17, and allophycocyanin anti-IFN-γ and were assayed by flow cytometry. The data show the population gated on CD4 cells. D, Effect of PTX on the immune response profile. Mice were injected twice with uveitogenic DC with or without PTX administration. On day 18, the draining lymph nodes were restimulated with Ag as described in A, and the proliferation and cytokine production were assayed.
triggered by uveitogenic DC. The results revealed that PTX enhanced the magnitude of the response without changing its pattern. Thus, both IFN-γ and IL-4 production were enhanced, but IL-17 production remained low (Fig. 4D).

We next examined the local cytokine milieu in the uveitic eyes on day 14, which represents the midpoint of uveitis development. At this point, the maximal incidence has been reached and the severity is still increasing. Uveitic eyes were collected and extracts were prepared as described in Materials and Methods and analyzed by multiplex ELISA. Extract from eyes with DC-elicited uveitis (Fig. 5) tested positive for the Th1 cytokine IFN-γ and for the Th2 cytokines IL-5 and IL-4, but had no detectable titers of IL-17. In contrast, immunization of p161–180 in CFA-elicited EAU revealed large amounts of intraocular IFN-γ in addition to IL-18 and IL-17, but minimal amounts of Th2 cytokines IL-4 and IL-5. It should be pointed out that because DC-primed mice often tend to develop lower disease scores, in this experiment the traditionally immunized mice were purposely immunized with a reduced dose of p161–180 so that the EAU scores were similar in both groups. Therefore, this difference in the cytokine response

FIGURE 5. Intraocular cytokines in mice with DC induced uveitis. Eyes from mice with uveitis elicited by uveitogenic DC or by active immunization with Ag in CFA were collected on day 14. Extracts were prepared and pooled from six eyes of mice with uveitis or from naive control mice. The intraocular cytokine content was assayed by multiplex ELISA.

FIGURE 6. Recipient produced IFN-γ is needed for development of DC induced uveitis. A, B10.RIII WT or GKO mice were given a full uveitogenic regimen of DC. EAU was scored by fundoscopy on day 18. B–D, Splenocytes from WT or GKO recipients were cultured with IRBP p161–180 for 48 h. Proliferation (B) was assayed by tritiated thymidine uptake. Cytokine secretion into the supernatants (C) and intracellular cytokines in CD4+ cells (D) were assayed as described in Fig. 4.
pattern could not be due to any difference in severity of disease. The differential cytokine pattern between DC uveitis and CFA uveitis was consistent with the Ag-specific cytokine profile produced by draining lymph node cells. This finding is consistent with the notion that the effector T cells in the eye maintain the cytokine response pattern acquired in the peripheral lymph nodes where they were primed. In addition, uveitic eyes also contained a number of proinflammatory cytokine and chemokines, including IL-1β, RANTES, MIP-1α, and MIP-2. The cellular origin of these chemokines is currently not clear, and is likely to include both the infiltrating leukocytes and ocular resident cells.

Elicitation of EAU by uveitogenic DC is dependent on recipient-produced IFN-γ

In the traditional EAU model in which IFN-γ and IL-17 are coproduced, systemic neutralization of IFN-γ or a genetic deficiency in IFN-γ (GKO) results in exacerbated EAU scores, indicating that IFN-γ is not an essential effector cytokine in EAU (31 and data not shown). We asked whether this is also true in the DC-induced
model, in which only minimal IL-17 is induced. To address this question, we injected matured and Ag-pulsed DC from WT B10.RIII donors into WT or into GKO recipients that had been fully backcrossed onto the B10.RIII background. Fundus examination on day 18 revealed that GKO recipients were protected from uveitis, whereas most WT recipients developed disease (Fig. 6A). The inability of GKO recipients to develop EAU was not due to insufficient induction of immunity, as their splenocytes restimulated with p161–180 in vitro proliferated vigorously (Fig. 6B). As expected, although WT splenocytes produced ample IFN-γ, production of IFN-γ by GKO mice was lower than baseline. Notably, IL-17 secretion by GKO splenocytes as measured by ELISA (Fig. 6C) and by intracellular cytokine staining gated on CD4 T cells (Fig. 6D) was much higher than in WT, indicating that 1) presence of an IFN-γ response may be inhibitory to generation of an IL-17 response in vivo and 2) an IL-17 response in the absence of IFN-γ is insufficient to support induction of EAU in the DC model.

Clinical and histologic features of DC-triggered EAU

The traditional EAU model induced in B10.RIII mice by immunization with IRBP (or peptide) in CFA is a monophasic disease with onset that occurs ~10–12 days after immunization, peaking at 14–21 days and remaining active for 2–3 wk. The kinetics of the basic model in the B10.RIII strain have been extensively characterized by us and by others in previous studies (2, 3). Although inflammation eventually recedes, destruction of photoreceptors and retinal scarring remain. Depending on the immunization dose, disease can be very severe, reaching scores of up to 4 and causing widespread retinal destruction. The typical inflammatory cell infiltrate in WT mice from a number of EAU susceptible strains consists mostly of mononuclear cells (lymphocytes and monocytes) with a minority of granulocytes, throughout the entire course of the disease (3, 32).

EAU induced with uveitogenic DC was typically less severe than EAU induced by immunization, with fundoscopic and histological scores typically not exceeding 2. Onset occurred on day 11 after initial infusion of uveitogenic DC. On day 18, fundoscopic examination revealed focal infiltrates and white linear lesions in the chorioretinal layer. The blood vessels were engorged and cuffed. However, in contrast to the traditional EAU model, the lesions in DC induced EAU seldom appeared as a thick diffuse infiltration cuffing the entire length of the retinal vessel, but rather as small spots and short linear lesions dispersed in the fundus, resulting in a distinct clinical appearance (Fig. 7A). Histological examination of the eyes showed inflammation in the posterior pole including inflammatory cell infiltration, retinal folds, and small serous detachments (Fig. 7B). The most prominent distinguishing feature of DC-induced EAU was the predominantly granulocytic nature of the inflammatory infiltrate with a minor proportion of eosinophils (~1%). Quantitation of the different cell types comprising the inflammatory infiltrate is shown in Fig. 7D.

Uveitis in the human is often a chronic, or a relapsing disease. We monitored the kinetics of DC-induced uveitis by fundoscopic examination (Fig. 8). The kinetics of clinical scores showed that the disease onset occurred on day 11 after first injection of uveitogenic DC. Scores and incidence peaked on day 16–18, after which scores and incidence gradually diminished. The disease did not relapse spontaneously, but a challenge of the same mice on day 60 with IRBP p161–180 in CFA reinduced EAU in 100% of the mice. Notably, however, disease scores in recovered mice did not attain the level of scores in similarly challenged control mice, which had initially received DC not pulsed with Ag and did not develop EAU, suggesting a level of postrecovery tolerance (Fig. 8). This result is consistent with the report of Kitaichi et al. (33) in the traditional EAU model.

Discussion

Over the last 20–30 years the traditional EAU model, induced by immunization with retinal Ag emulsified in CFA, contributed greatly to the understanding of cellular, molecular, and immunogenetic mechanisms involved in uveitis. The present study describes a new model of EAU induced in B10.RIII mice by infusion of matured and Ag-pulsed DC rather than by active immunization. We believe that this new model offers some advantages over the traditional model. Firstly, it avoids the complexity caused by a persisting depot of mycobacteria and Ag, which continues to prime new effector T and B cells well into the efferent phase of disease. Second, the strongly biased Th1/Th17 nature of the immune response in the traditional model may also be a consequence of the persistent and potent innate stimulation provided by the Ag/CFA depot, which may be much more intense than the transient and usually unnoticed exposure to mimic Ag that is believed to be the uveitogenic trigger in most cases of clinical uveitis. Third, the new model offers the possibility to genetically and/or phenotypically manipulate DC to be used for disease induction, and thus to help dissect and better define the conditions and stimuli that contribute to uveitogenicity. As an example, a combination of several innate stimulii in vitro and in vivo was needed to bring out the full uveitogenic potency of DC in this study. This finding might suggest that a combined action of multiple independent stimuli creates a “multiple hit” situation that is needed to pass the threshold of disease induction. Our data are consistent with the notion that the injected DC themselves act as the proximal APC that prime the uveitogenic effector T cells in this model. This notion is supported by the finding that a full uveitogenic regimen of allogeneic DC pulsed with Ag did not result in EAU, indicating that transfer of Ag and cross-presentation by host-derived APC is insufficient.

DC-induced EAU noticeably differed from the traditional model by several attributes. It was characteristically mild to moderate disease and did not result in total destruction of the retina. The duration of active disease was shorter, possibly reflecting the lack of a persisting Ag depot. The type of immune response elicited was characterized by a low to undetectable Ag-specific IL-17 response and a high IFN-γ response combined with a detectable IL-4 and IL-5 production. It should be emphasized that in the traditional
EAU model IFN-γ is completely dispensable. In fact, a more severe form of EAU develops in GKO mice or in mice treated with neutralizing IFN-γ Abs, indicating that IFN-γ is not an essential effector cytokine in the traditional EAU model, but rather plays a protective role. We speculate that this role may be due to its ability to inhibit the IL-17 effector response, as GKO mice as well as mice treated with anti-IFN-γ Abs exhibit an enhanced IL-17 response to IRBP (D. Luger, R. S. Grajewski, and R. R. Caspi, submitted for publication) (Fig. 6, C and D). Notably, in the DC-induced EAU model infusion of WT DC into GKO mice failed to induce disease, suggesting that an IFN-γ producing effector was necessary and an IL-17 response in the absence of IFN-γ was unable to support EAU pathogenesis.

Last but not least, the inflammatory infiltrate in the eyes of mice with DC-induced EAU was characterized by a predominance of granulocytes, in clear contrast to the predominantly lymphocytic infiltrate typical of the traditional EAU model. IL-17 rather than IFN-γ is known for its ability to recruit granulocytes (34), therefore in view of the dependence of DC-induced EAU on IFN-γ and the typically low IL-17 production, this predominantly granulocytic response was surprising. There are a number of reports in the literature of the ability of IFN-γ and IL-18 to recruit granulocytes (35, 36) and IFN-γ has been reported to facilitate granulocyte infiltration into inflamed corneas (37). However, the underlying molecular mechanisms remain obscure. A significant IL-4 and IL-5 response in the DC EAU model, not normally seen in B10.RII mice under active immunization conditions in CFA, could also have contributed to the granulocytic nature of the inflammation. Due to differences in multiple cytokines and chemokines between the traditional and the DC-induced model, it is difficult to point to a particular factor as primarily responsible for this effect.

A difference between clinical uveitis and the EAU model, both DC and immunization-induced, is that the former tends to be chronic and relapsing, whereas the latter tends to be monophasic. This difference might reflect the fact that development of uveitis in humans occurs against the backdrop of a dysregulated immune system in which normal regulatory mechanisms have failed. In contrast, in the EAU model we are inducing uveitis in an animal with a healthy immune system that is not genetically prone to autoimmunity, and that to the best of our knowledge would not develop uveitis without our active intervention. Therefore the ability to reinduce EAU is as close as we can get to a relapsing situation. This limitation of the EAU model can, perhaps, help to understand the regulatory mechanisms that have failed in clinical uveitis.

In the traditional EAU model, recovery is accompanied by induction of active regulatory mechanisms that help to restore homeostasis and limit reinduction of the disease (33). The milder and self-limiting nature of DC-induced inflammation compared with the active immunization model, which leaves a persistent Ag depot, raised the possibility that Ag availability might be a limiting factor and might potentially account for remission of DC-induced EAU in the absence of active resolution mechanisms. Rechallenge of recovered mice with a dose of the same uveitogenic peptide in CFA resulted in reinduction of disease, albeit with reduced severity compared with controls. Two conclusions stem from this finding: 1) reduced disease scores compared with control suggest a level of postrecovery tolerance; additional studies are needed to distinguish between depletion or anergy of the available effector precursors from induction of regulatory mechanisms, and 2) re-exposure to the same Ag epitope can re-ignite disease, putting in question epitope spreading as a critical component of relapse, as was suggested in autoimmunity to CNS Ags (38).

Uveitis is a clinically heterogeneous disease. Although the antigenic triggers of autoimmune uveitis are still under discussion, there is a large body of evidence implicating responses to retinal Ags in the etiology and/or progression of the disease. Many, though certainly not all, uveitis patients have detectable immunological responses to retinal Ags, most often to retinal arrestin (soluble Ag) (7). One of the explanations that had been offered for this clinical heterogeneity is a difference in the Ags being recognized. However, strong responses to soluble Ag (retinal arrestin) as the only detectable specificity are seen in diverse uveitic diseases. This includes uveitides limited to the eye, such as birdshot retinochoroidopathy and sympathetic ophthalmia, as well as systemic diseases where the eye is one of a number of targets involved, such as Behcet’s disease and Vogt-Koyanagi-Harada disease. Another explanation, which has experimental support, suggests that differences in the genetic background of the individual affect expression of disease induced with the same Ag. Indeed, strains of mice and rats genetically predisposed to a predominantly Th1 (and as we now know also Th17) response vs a balanced or predominantly Th2 response, differ in the severity of EAU that they develop (39, 40). Our present data, showing two forms of EAU on the same genetic background, suggest yet another explanation. Namely, on the same genetic background the effector response phenotype and the characteristic manifestations of disease can be a consequence of the context in which the particular autoantigen is first seen by the immune system, i.e., the specific combination of innate stimuli at the time when initial Ag presentation and recognition take place, as well as other conditions such as the type of APC and the duration of exposure to Ag.

In summary, we report in this study a new model of EAU that differs from the traditional model and presents certain unique advantages for the study of the processes affecting pathogenesis. The new model sheds light on the variability seen in clinical uveitis by demonstrating how the same Ag presented in a different context can result in different clinical and immunological manifestations even in individuals on the same genetic background. This model of EAU may represent types of uveitis that are not adequately represented by the traditional model.

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Disclosures
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