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# Breakdown of immune privilege and spontaneous autoimmunity in mice expressing a transgenic T cell receptor specific for a retinal autoantigen

Reiko Horai [a,*], Phyllis B. Silver [a,5], Jun Chen [a,5], Rajeev K. Agarwal [a,1,5], Wai Po Chong [a], Yingyos Jittayasothorn [a], Mary J. Mattapallil [a], Sonia Nguyen [a], Kannan Natarajan [b], Rafael Villasmil [c], Peng Wang [a,2], Zaruhi Karabekian [a,3], Simon D. Lytton [a,4], Chi-Chao Chan [a], Rachel R. Caspi [a,*]

[a] Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, MD 20892-1857, USA
[b] Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA
[c] Flow Cytometry Core, National Eye Institute, National Institutes of Health, Bethesda, MD, USA

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ABSTRACT

Despite presence of circulating retina-specific T cells in healthy individuals, ocular immune privilege usually averts development of autoimmune uveitis. To study the breakdown of immune privilege and development of disease, we generated transgenic (Tg) mice that express a T cell receptor (TCR) specific for interphotoreceptor retinoid-binding protein (IRBP), which serves as an autoimmune target in uveitis induced by immunization. Three lines of TCR Tg mice, with different levels of expression of the transgenic R161 TCR and different proportions of IRBP-specific $CD4^+$ T cells in their peripheral repertoire, were successfully established. Importantly, two of the lines rapidly developed spontaneous uveitis, reaching 100% incidence by 2 and 3 months of age, respectively, whereas the third appeared "poised" and only developed appreciable disease upon immune perturbation. Susceptibility roughly paralleled expression of the R161 TCR. In all three lines, peripheral $CD4^+$ T cells displayed a naïve phenotype, but proliferated *in vitro* in response to IRBP and elicited uveitis upon adoptive transfer. In contrast, $CD4^+$ T cells infiltrating uveitic eyes mostly showed an effector/memory phenotype, and included Th1, Th17 as well as T regulatory cells that appeared to have been peripherally converted from conventional $CD4^+$ T cells rather than thymically derived. Thus, R161 mice provide a new and valuable model of spontaneous autoimmune disease that circumvents the limitations of active immunization and adjuvants, and allows to study basic mechanisms involved in maintenance and breakdown of immune homeostasis affecting immunologically privileged sites such as the eye.

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## 1. Introduction

The healthy eye resides behind a protective blood-retinal barrier that prevents free movement of cells and even large molecules into and out of the globe. This sequestration of the eye from the immune system is part of the phenomenon known as immune privilege of the eye, which protects the delicate ocular structures that are critical to vision from collateral damage as a consequence of environmental inflammatory insults [1]. However, this separation from the immune system arguably also impedes efficient induction of peripheral tolerance to eye-specific antigens, allowing persistence in the circulation of non-tolerized eye-reactive T cells. This may help to explain why, despite immune privilege, the eye is subject to destructive autoimmunity manifesting as uveitis [2].

Uveitis is a group of blinding inflammatory diseases that result in destruction of the light-sensitive photoreceptor cells of the

*Abbreviations:* Ag, antigen; IRBP, interphotoreceptor retinoid binding protein; TCR, T cell receptor; CFA, complete Freund's adjuvant; EAU, experimental autoimmune uveitis; HEL, hen egg lysozyme; RAG, recombination activating gene; WT, wild type; PMA, phorbol myristate acetate; SP, single positive; AIRE, autoimmune regulator.

* Corresponding authors. Tel.: +1 301 435 4555, +1 301 435 4573.
*E-mail addresses:* hreiko@mail.nih.gov (R. Horai), caspir@mail.nih.gov, rcaspi@helix.nih.gov (R.R. Caspi).
[1] Present address: Translational Research Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Rockville, MD, USA.
[2] Present address: Beijing Biobank of Clinical Resources, Capital Medical University, Beijing, China.
[3] Present address: The George Washington University, Washington DC, USA.
[4] Present address: SeraDiaLogistics, 81545 Munich, Germany.
[5] These authors contributed equally to this work.

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neuroretina [3]. Uveitic diseases are estimated to underlie 10–15% of legal blindness in the developed world [4,5]. In most cases of human uveitis the pathogenic trigger is unknown. A notable exception is sympathetic ophthalmia, in which a penetrating trauma to one eye is followed, weeks or months later, by a destructive inflammation in the uninjured, "sympathizing" eye. This can be facilitated by inflammation that often accompanies such an injury. Available evidence, including recall responses to eye-derived antigens often seen in such patients, led to the notion that normally sequestered antigens from the eye drain into the regional lymph node and elicit a systemic autoimmune response, which then precipitates the autoimmune attack on the uninjured eye. Many human patients exhibit responses to retinal arrestin (also known as the retinal soluble antigen, or S–Ag) and some respond to interphotoreceptor retinoid-binding protein (IRBP) and other retinal proteins [6–10].

Experimental autoimmune uveitis (EAU) induced in susceptible animal models by systemic immunization with retina-derived antigens or their peptides emulsified in complete Freund's adjuvant (CFA) mimics this systemic autoimmunization response [11]. While in humans the major retinal antigen being recognized appears to be arrestin, mice preferentially develop uveitis with IRBP. The reason for this species-specific preference may be connected to the MHC and presentation of appropriate epitopes, as "humanized" HLA transgenic mice become susceptible to arrestin-induced EAU [12]. While immunization-induced EAU has been a valuable tool for modeling uveitis and the cellular mechanisms that drive it, the vast majority of uveitis cases present without evidence of trauma to the eye that could cause autoimmunization [3], and may not be adequately represented by this model. In addition, induction of EAU by immunization is dependent on massive stimulation of the immune system by the mycobacteria in CFA, which again may not be representative of human disease. We, therefore, set out to establish an alternative model of uveitis by generating mice transgenic for a retina-specific T cell receptor (TCR).

TCR Tg mice have been helpful in studying tissue-specific autopathogenic responses in other autoimmune diseases including models of type 1 diabetes, autoimmune gastritis and multiple sclerosis [13–21]. However, TCR Tg mice that respond to native retinal antigens have not been available to study ocular autoimmunity. Double-Tg mice, in which hen egg lysozyme (HEL) or β-galactosidase were expressed as retinal neo-self antigens under different retina-specific promoters, and combined with the corresponding TCR transgene, have yielded confusing results. Namely, levels of retinal and of thymic expression of the neo-self Ag, and most importantly, the ability of the transgenic host to develop uveitis were variable, and did not seem to be easily explained either by the identity of the retina-specific promoter or of the neo-Ag placed under its control [22–25].

Because the level and pattern of expression of neo-self antigens may vary, and may not mimic the endogenous Ag, we generated TCR Tg mice expressing a TCR specific to the endogenous uveitogenic antigen IRBP. Three lines expressing different levels of the same TCR displayed different susceptibilities to spontaneous disease, which could be correlated to transgene copy numbers and levels of peripheral expression of the transgenic TCR, for an analyzable effect on disease pathogenesis. These mice constitute the first model for a spontaneous uveitis directed at a native retinal Ag and promise to shed light on how T cells reactive to retina become primed and cause autoimmunity.

## 2. Materials and methods

### 2.1. Mice

B10.RIII mice were from Jackson Laboratory (B10.RIII-H2$^{r}$ H2-T18$^{b}$/(71NS)SnJ). B10.A RAG2$^{-/-}$ mice were from Taconic Farms (Taconic Farms, Inc.), and were backcrossed to B10.RIII. IRBP TCR Tg (R161) mice were generated in house on the B10.RIII background (see ahead), and were maintained on the B10.RIII background or were crossed to B10.RIII RAG2$^{-/-}$ mice. All animals were maintained under specific pathogen free conditions. Animal care and use followed Institutional guidelines, animal study protocol #NEI-581.

### 2.2. Cloning of IRBP-specific TCR and generation of TCR Tg mice

TCR α and β chains were cloned from a highly uveitogenic T cell line specific to the IRBP$_{161–180}$ peptide [26]. The line was first fused with the BW5147α$^{-}$β$^{-}$ cells [27] (a gift from Dr. Joan Goverman, University of Washington) and a hybridoma clone was selected that showed the best growth inhibition upon IRBP$_{161–180}$ peptide stimulation. TCR α and β cDNA sequences were cloned by the 5′ RACE system (Rapid Amplification of cDNA Ends, Invitrogen), and were identified as Trav16 and Trbv5, respectively (NCBI database, http://blast.ncbi.nlm.nih.gov/Blast.cgi). The TCR α and β sequences were placed under control of the promoter and locus control region of the human CD2 gene, and the mouse H-2K promoter, respectively, using previously described plasmids [28,29] (Suppl. Fig. 1C). The TCR α and TCRβ constructs were microinjected together into B10.RIII embryos. Founder mice carrying both TCR genes were bred to B10.RIII wild type (WT) mice and expanded into transgenic lines.

### 2.3. Flow cytometric analysis

Thymus, spleen, submandibular (eye-draining) lymph nodes, non-draining (inguinal, axillary and brachial) lymph nodes and eyes were isolated to prepare single cell suspensions. Splenic red blood cells were lysed using ACK lysing buffer (Quality Biological, Inc). For analysis of eye-infiltrating cells, enucleated eyes were minced and treated with 10 μg/ml of collagenase D (Roche) for 30 min at 37 °C. Cells were washed and resuspended in culture media for *ex vivo* stimulation (see ahead), or in PBS containing 2% FBS for staining. Fc receptors were blocked using CD16/32 (2.4G2), and the following anti-mouse monoclonal antibodies (mAbs) with various fluorochromes (FITC, PE, PerCP-Cy5.5, PE-Cy7, APC, APC-Cy7, APC Alexa Fluor 780, eFluor 450, Brilliant Violet 421, V500, Brilliant Violet 605) were used: CD4 (RM4-5), CD8α (53-6.7), CD11b (M1/70), CD25 (PC61), CD44 (IM7), CD62L (Mel-14), CD45R/B220 (RA3-6B2), CD49b (DX5), NK1.1 (PK136), Ly-6C/6G (Gr-1), TCRβ (H57-597). The Abs were purchased from BD Bioscience, BioLegend or eBioscience and were used based on the availability of clones and fluorochromes from the respective vendors. Where appropriate, 7-AAD (BD Bioscience) or propidium iodide (PI; Miltenyi Biotech) was used to exclude dead cells. IRBP$_{161–180}$-specific T cells were detected using an IRBP$_{161–180}$-IA$^{r}$-IgG1 dimer reagent (p161 dimer) [30] after direct conjugation with Alexa Fluor 647 (Invitrogen), or in combination with anti-mouse IgG secondary Ab (FITC or PE-conjugated, BD Bioscience). For analysis of the Vβ repertoire, lymph node cells were collected and incubated for 20 min at 4 °C with anti-CD16/CD32, anti-TCRβ-APC, CD8α-PE and anti-CD4-PerCP-Cy5.5, and one of the 15 mAbs against TCR Vβx labeled with FITC (BD Bioscience). For intracellular cytokine staining, cells were stimulated in the complete RPMI-10% FBS with 10 ng/ml phorbol myristate acetate (PMA) and 500 ng/ml ionomycin (Calbiochem) for 4 h in the presence of Brefeldin A (Golgi Plug, BD Biosciences), fixed in 4% paraformaldehyde and permeabilized with Triton buffer (0.5% Triton X-100 and 0.1% BSA in PBS). Abs used for intracellular cytokine staining were the following; anti-mouse IL-4 (11B11), IFN-γ (XMG1.2), and IL-17A (TC11-18H10.1) conjugated with various fluorochromes as described above. Intracellular Foxp3 staining was performed following the manufacturer's protocol (eBioscience). Samples were acquired on a FACSCalibur or a FACSAria (BD Bioscience) and were analyzed using FlowJo software (TreeStar).