DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Eye Institute
Bethesda, Maryland 20892

January 10th, 2025

Helen Keller Foundation Prize Selection Committee (by email)

Dear Selection Committee Members,

2026 Helen Keller prize nomination for Dr. Rachel Caspi

I am delighted to be writing in support of Dr. Rachel Caspi's nomination for the Helen Keller Prize in recognition of the totality of her contributions to vision science and the fight against blindness over the course of her illustrious career. Throughout her 40 years at the National Eye Institute, Dr. Caspi has shifted paradigms and challenged dogmas in both immunology and ophthalmology and the breadth of her contributions have substantially shaped our understanding of how the immune system and the eye interact. With almost 300 peer-reviewed papers and book chapters to her name, she has profoundly influenced the way the vision research community thinks about how immune cells maintain ocular health and drive eye diseases from the ocular surface to the retina, and she has also generated the major pre-clinical models for translation of new immune therapies to the clinic. In addition to her scientific contributions, she has been an influential opinion leader and advocate for vision research as well as a wonderful role model for women in science. Her intelligence, courage, and commitment, coupled with huge inner strength and resolve has driven both her professional success, and her personal resilience (she had a bone marrow transplant for aggressive blood cancer 6yrs ago). She remains unwaveringly dedicated to her work and the members of her lab and continues to better the lives of others. By putting Dr. Caspi forward for this prize, I strongly believe that I am nominating an outstanding individual whose nature and achievements align well with the spirit of Helen Keller's life and the goals of the Foundation, and whose recognition would unquestionably both raise public awareness and understanding of research on eye disease and serve as an inspirational example to new and future researchers in vision science.

Key discoveries that have fundamentally progressed understanding of the immune system and the eye

1. <u>Discovery of biological mechanisms that protect the eye from immune damage (self-tolerance)</u>
The very first publication demonstrating control of ocular damage from immune cells by resident cells in the eye was by Dr Caspi in 1987 in the journal Science (cited 148 times). She went on to elucidate that this depends on a soluble mediator called TGF-β and requires the presence of retinoic acid (which is abundant in the eye because of its role in vision). Dr. Caspi then demonstrated that suppression of immune responses to ocular proteins could be augmented by the forced expression of these proteins outside the eye and that there are processes in the thymus (the organ responsible for the production and selection of immune cells throughout the body) which reduce susceptibility to ocular autoimmunity.

- RR Caspi et al., Organ-resident, nonlymphoid cells suppress proliferation of autoimmune T-helper lymphocytes. Science 237, 1029-1032 (1987).
- R Zhou et al., The living eye "disarms" uncommitted autoreactive T cells by converting them to Foxp3(+) regulatory cells following local antigen recognition. J Immunol 188, 1742-1750 (2012)
- RK Agarwal et al., Retroviral gene therapy with an immunoglobulin-antigen fusion construct protects from experimental autoimmune uveitis. J Clin Invest 106, 245-252 (2000)
- DvAvichezer et al., An immunologically privileged retinal antigen elicits tolerance: major role for central selection mechanisms. J Exp Med 198, 1665-1676 (2003).

2. Discovery of the subtypes of immune cells that cause autoimmune diseases of the eye

Dr. Caspi was the first to report that immune cells called lymphocytes that produce a soluble mediator called interleukin-17 are key drivers of ocular autoimmunity (J Exp Med 2008, cited 834 times) and unraveled the relationship between these cells and interferon-γ producing lymphocytes that had been perplexing the wider field of autoimmunity across all fields of medicine for years. In addition, she was the first to describe a novel immune-cell regulatory loop that aborts induction of inflammation-causing lymphocytes and demonstrated that their production of interleukin-17 is self-limited by an autocrine mechanism.

- D Luger et al., Either a CD2517 or a CD251 effector response can drive autoimmunity: conditions of disease induction affect dominant effector category. J Exp Med 205, 799-810 (2008).
- WP Chong et al., CD25-CD25 crosstalk controls the autopathogenic CD2517 response through an innate CD25-γ/IL-27 axis. J Exp Med. 212:1739-52 (2015)
- WP Chong et al., The cytokine IL-17A limits Th17 pathogenicity via a negative feedback loop driven by autocrine induction of IL-24. Immunity, 53:384-397 (2020).

3. Discovery that bacteria in the gut can cause inflammation in the eye and that ocular surface resident bacteria are essential for eye health

Dr. Caspi's recent pioneering contributions have demonstrated for the first time that bacteria in the gut can trigger autoimmunity in the eye by mimicking retinal proteins, whereas on the ocular surface bacteria play a central role in defense against harmful infections. Linking this back to her expertise in immune cell subtypes, she has also demonstrated that rare groups of lymphocytes on the surface of the eye protect themselves from harmful bacteria by expressing interleukin-17.

- R Horai et al., Microbiota-dependent activation of an autoreactive T cell receptor provokes autoimmunity in an immunologically privileged site. Immunity. 43:343-53. 2015. (Horai et al., Immunity 2015)
- AJ St. Leger et al., An ocular commensal protects against corneal infection by driving an Interleukin 17 response from mucosal γδ T cells. Immunity, 47, 148–158, 2017.
- AJ St Leger et al., STAT-3-independent production of IL-17 by mouse innate-like alphabeta T cells controls ocular infection. J Exp Med, 215:1079-90. 2018

The creation of murine models for ocular immunology which continue to improve the treatment of inflammatory eye diseases

In 1988, Dr. Caspi generated a new model of autoimmune disease, experimental autoimmune uveitis (EAU) which unquestionably transformed the field of ocular immunology (Journal of Immunology, cited 567 times). This has been refined over subsequent years to identify susceptible mouse strains and component peptides which have been adopted by eye researchers worldwide and have now been used in literally thousands of research studies. Her lab went on to develop "humanized" and spontaneous models of uveitis which together have furthered mechanistic understanding of clinical uveitis and have also served to inform the development of potential new therapeutic approaches.

- RR Caspi et al., A new model of autoimmune disease. Experimental autoimmune uveoretinitis induced in mice with two different retinal antigens. J Immunol 140, 1490-1495 (1988).
- G. Pennesi et al., A humanized model of experimental autoimmune uveitis in HLA class II transgenic mice. J Clin Invest 111, 1171-1180 (2003).
- R. Horai et al., Breakdown of immune privilege and spontaneous autoimmunity in mice expressing a transgenic T cell receptor specific for a retinal autoantigen. J Autoimmun 44, 21-33 (2013).

Translational impact

Uveitis currently affects circa 2 million Americans and is responsible for 10-15% of blindness and over the past 2 decades Dr. Caspi's murine models have become the standard platform for testing new uveitis treatments for uveitis (such as adalimumab) and proof-of-principle for the off-label use of other immunosuppressant therapies that were originally developed for non-ocular indications (eg, tacrolimus and mycophenolate mofetil). Consequently, many hundreds of thousands of patients around the world have recovered from the sight-threatening effects of uveitis, or avoided the complications caused by historical treatments, either because Dr. Caspi's murine models were used to demonstrate that these new drugs were effective or because the biological target(s) of the drug have been shown in her models to be critical in the pathogenesis of the disease. This success has massively increased interest in the discovery of new drugs for inflammatory eye diseases and currently there are more than 60 novel medicines under pre-clinical development for uveitis, the majority of which are using Dr. Caspi's animal models for testing. Although there are substantial barriers to bringing new medicines to market, it is highly likely that the tools she has developed will further prevent blindness and improve lives. Indeed, Roche are currently running Phase 3 clinical trials of an intravitreal injection for uveitis that blocks the action of an immune mediator called interleukin-6 which was originally tested in Dr. Caspi's experimental models. Although I am focusing on the translational impact of some of these mice, the downstream benefits of her more recent discoveries is also likely to be substantial, and the importance of bacteria in the gut and on the ocular surface in maintaining the immune health of the eye and driving disease are yet to be realized in new approaches to treating patients. Nonetheless, it is highly probable that these will also lead to novel ways of combating immune and infectious damage to the eye, some of which are likely to apply beyond classical inflammatory eye diseases to also benefit patients with other blinding conditions known to involve the immune system..

Scientific standing, citizenship and mentorship

A further indicator of the eminence of Dr. Caspi's career-long body of work is the journals in which her seminal papers have featured, none of which are limited to the fields of ophthalmology or vision science, and all of which are among the most prestigious publications in general science and immunology. This reflects that her research is of the highest possible scientific standing and is evidence that she is an ambassador for vision research in the most lofty circles of science. Over the past 30+ years she has mentored more than 60 trainees, many of whom are now independent investigators with their own research groups in major academic institutions in the US and around the world. This is an ongoing career legacy to the fields of both ocular immunology and vision research as a whole that will continue to grow.

Consistent with her standing as the world's pre-eminent ocular immunologist, Dr.Caspi has previously received both the ARVO Friedenwald award (2010) and the Alcon Research Institute award (2012). To follow these with the Helen Keller award, which would be the most substantial recognition of her contribution to vision research to date, would inspire all of those in our field, especially those at formative stages of their careers. Her life and work are truly aligned with the legacy of Helen Keller and the values and goals of the Foundation, and I cannot commend her strongly enough to the selection committee. She could not be a more apt and deserving recipient.

Please do not hesitate to contact me if I can provide any further information in support of Dr. Caspi's nomination.

Yours sincerely,

Richard Lee MD, PhD
Clinical Director, National Eye Institute

UCL INSTITUTE OF OPHTHALMOLOGY
PROFESSOR ANDREW D. DICK
DIRECTOR

Helen Keller Prize for Vision Research Panel

28 January 2025

Dear ARVO/Brightfocus panel

Re: Dr Rachel Caspi nomination for Helen Keller Prize for Vision Research

It is my utmost pleasure to nominate Dr Rachel Caspi for the Helen Keller Prize. Dr Caspi is the world's pre-eminent and highly cited basic scientist in ocular immunology. Her work has throughout her career and continues to be of outstanding quality in its discovery, understanding of immune system related to the eye and forming experimental models and platforms for many laboratories to utilise and translate for the benefit of patients. I unreservedly recommend her for this award.

I write as Director of UCL Institute of Ophthalmology, Duke-Elder Chair of Ophthalmology at UCL and Chair and Professor of Ophthalmology, University of Bristol. My work over the past 30 years, as a clinician scientist, has been in the field of ocular immunology, translational science of immune mediated and degenerative diseases of the eye, including Uveitis and as a clinician treating retinal disorders and uveitis. I have known Dr Caspi's work for all this time and was introduced to her work through her seminal paper in 1998 that introduced the world to an articulate mouse model of uveitis, murine experimental autoimmune uveoretinitis (EAU). She continued to describe antigens, peptides and epitopes promoting inflammation and as a result could be used to induce immune tolerance. This body of work remains pivotal to many and paved the way for intricate interrogation of immune responses in intraocular inflammation from mouse to man. Whilst I have never collaborated or worked with Dr Caspi, she has been a support to me during my early career, and as I will describe later, a terrific mentor to many in our field worldwide.

The strengths and excellence of Dr Caspi's work, and outputs are a result of her scientific rigour, original discovery science and unwavering commitment to the unanswered questions. Her work has impacted the wider immunological field. While for many years we perceived the eye as an immune privileged site (thought at that time to be due to immune sequestration) Dr Caspi was pivotal to understanding (and relating to clinical disease) that the immune system, through T cells, reacted and drove autoimmune responses and inflammation in the eye. Her science paper in 1987, documented the role of non-canonical immune cells in the retina (Muller cells) that regulate the T cell expansion and inflammation. This finding, followed by the generation of a murine model of

Prof Andrew D. Dick, BSc, MBBS(Lond), MD(Aberd), MRCP(UK), FRCS(Lond), FRCS(Edin), FRCP(Edin), FRCOphth, FMedSci
Director of UCL-Institute of Ophthalmology
UCL Duke Elder Professor of Ophthalmology
UCL INSTITUTE OF OPHTHALMOLOGY
11-43 Bath Street, London EC1V 9EL
Tel: +44 (0)20 7608 6808 Fax: +44 (0)20 7608 4011
Email: a.dick@ucl.ac.uk
www.ucl.ac.uk/ioo

uveitis as mentioned above, paved the way to recognise the eye is not immune sequestered but has active immune regulatory mechanisms to regulate inflammation. Such an approach was further developed and provide human relevance by creating a humanised and spontaneous model of EAU. Together these models have opened the opportunity for many laboratories around the world. Nevertheless, Dr Caspi continued to *lead* the field. Her work identified the nuances of T cell activation, the cytokines they produce and how they either protect or promote inflammation. For example, her laboratory was the first to describe that TH17 T cells promoted inflammation (J Exp Med 2008), whereas TH1 cell secretion of IFNgamma attenuated disease depending on when secreted during the evolution of uveitis in the mouse (J Exp Med 1998). Her lab has produced a library of outputs to understand this further. In particular, the relevance that TH17 cells can also regulate if the inflammation is more innate driven. This is important as it may offer reason as to why the translation of IL-17 blockade was unsuccessful in man. Dr Caspi has worked with clinicians to provide the basic science rationale for the potential of 'tolerance' therapy in man.

Her scientific diligence from her acute observation is exemplified by another seminal paper that described the Rd8 mutation in mice (IOVS 2008) that led to a degenerative phenotype and questioned many papers studying degenerative disorders in mice to translate to man.

Taken that Dr Caspi has been at the forefront of understanding and being the first to describe pathways of immune privilege, autoimmune disease, she has most recently been at the forefront of understanding the role of commensal microbiome in autoimmunity and ocular inflammation. The discovery is immense to our field. Firstly, gut commensals trigger autoimmunity as opposed to ocular surface commensals in healthy eye that protect through IL-17 secretion from gamma-delta T cells. The work now expands as to discovering the commensal culprits and driving translational potential.

Whilst I have, at a high level, described her prolific contribution, the depth and quality of her high impact factor outputs are supported by her high citation rate and are testament to her leadership in the field. Her supervision of many doctoral students, her mentorship of many worldwide and her collaboration within the field and in wider immunology field further exemplifies her pre-eminent standing acknowledged by us all, her quality of work and her contribution to the field of ocular immunology. To acknowledge her immense contribution, Dr Caspi has received numerous awards from NEI Director, previous recipient of ARVO Friedenwald Award and Recipient of Alcon Research Institute Award for contributions to vision research in 2011.

Her five most prestigious papers in include:

WP Chong, MJ Mattapallil*, K Raychaudhuri*, SJ Bing, WW Wang, S Wu, Y Zhong, PB Silver, Y Jittayasothorn, CC Chan, J Chen, R Horai, RR Caspi. The cytokine IL-

17A limits Th17 pathogenicity via a negative feedback loop driven by autocrine induction of IL-24. Immunity, 53:384-397 (2020).

Horai R, Z.rate-Blad.s CR, Dillenburg-Pilla P, Chen J, Kielczewski JL, Silver PB, Jittayasothorn Y,Chan CC, Yamane H, Honda K, Caspi RR. Microbiota-dependent activation of an autoreactive T cellreceptor provokes autoimmunity in an immunologically privileged site. Immunity. 43:343-53. 2015.

Wai Po Chong, Nicolas van Panhuys, Jun Chen, Phyllis B Silver, Chi-Chao Chan, Ronald N. Germain, Rachel R Caspi. CD25-CD25 crosstalk controls the autopathogenic CD2517 response through aninnate CD25-γ/IL-27 axis. J Exp Med. 212:1739-52 (2015)

R. R. Caspi et al., A new model of autoimmune disease. Experimental autoimmune uveoretinitis induced in mice with two different retinal antigens. J Immunol 140, 1490-1495 (1988).

R. R. Caspi, F. G. Roberge, R. B. Nussenblatt, Organ-resident, nonlymphoid cells suppress proliferation of autoimmune T-helper lymphocytes. Science 237, 1029-1032 (1987).

I offer my utmost and unconditional support for Dr Caspi and recommend her most highly for this award. Her work over her career has been wonderful to observe and learn from and has led the ocular immunology field.

Yours faithfully

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Caspi, Rachel

eRA COMMONS USER NAME (agency login): Intramural

POSITION TITLE: Senior Investigator with Tenure (rank equivalent to Full Professor)

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Bar-Ilan University, Ramat Gan	PhD	1984	Immunology (summa cum laude)
National Institutes of Health, Bethesda, Maryland	Postdoctoral Fellow	1989	Immunology, autoimmunity

A. Personal Statement

I am a tenured senior investigator at the NEI. I serve as Head of the Immunoregulation Section and Chief of the Laboratory of Immunology, National Eye Institute, NIH. I also hold an Adjunct Professorship at the University of Pennsylvania School of Medicine. Over the years, my work contributed seminally to shaping the current understanding of fundamental mechanisms in ocular immunity and autoimmunity, and has instructed new approaches to therapy of blinding ocular inflammatory disease.

Our long-standing interest is the role of adaptive and innate immune responses in homeostasis and pathogenesis of autoimmunity to the neuroretina, using animal models developed in our lab that represent autoimmune uveitis in humans. Early on, I had established a model of experimental autoimmune uveitis (EAU) in mice, by immunizing them with a retinal protein. The EAU model is now in use worldwide, and has generated thousands of publications. A “humanized” version, in HLA-transgenic mice, validates its relevance to human disease. Through the use of this model, and more recently also a genetically engineered *spontaneous* uveitis model, we have elucidated fundamental mechanisms governing immune responses affecting the eye. Our studies defined critical checkpoints in breakdown of self-tolerance and development of disease, driven by adaptive and innate immune components, which have led to a better understanding of the pathogenesis of uveitis and provided a platform for the study of clinically relevant immunotherapeutic approaches.

Our second field of research is the commensal microbiome and its effects on local ocular immunity and autoimmunity. In early studies, we showed that tolerance to orally administered antigen can protect from uveitis, but exposure to an antigen through the gut may under some conditions have deleterious consequences. We found that development of spontaneous uveitis is highly dependent on gut commensal microbes, which act as a trigger of autoimmune uveitis by providing activation signals to retina-specific uveitogenic lymphocytes. We are also exploring he role of commensals on the ocular surface and studying their role in regulating local mucosal immunity and host defense. In this area, we have provided the first rigorous proof of concept that *bona fide* commensal microbes colonize the ocular surface for the long term as resident flora, and have functional consequences for local immune homeostasis and host defense.

Our studies are pertinent not only to uveitis, but also to autoimmune and inflammatory diseases in general.

In recognition of my scientific contributions, I have received the ARVO Friedenwald award 2010 and the Alcon Research Institute award 2012, as well as 3 NIH Bench-to-bedside awards (2011, 2017 and 2019). I have organized and co-organized scientific meetings and events including 2 Keystone conferences, 2 FASEB summer conferences on Autoimmunity, 2 ARVO summer research conferences on ocular immunology and inflammation, a Midwinter Conference of Immunologists (MCI), a number of minisymposia, and have authored and co-authored 280 publications.

Over the past >30 years, I have mentored more than 60 trainees, of these >35 at the postdoctoral level. Five of them are still at NIH as Staff Scientists and Core Heads, 10 have gone on to teaching or Biopharma industry positions, and 15 have developed into independent researchers or physicians, who hold leadership positions at prestigious Institutions in the US and abroad. This includes: Duke University, Cleveland Clinic, Univ of

Pittsburgh, George Washington University at Washington DC, the Institute Pasteur of Shanghai, Zhongshan Ophthalmology Center at the Sun-Yat-Sen University, Beijing Capital Medical University, the Baptist University of Hong-Kong, Teaching and Research Institute of the Albert Einstein Israelite Hospital in Saō Paulo, Brazil and the Federal University of Santa Catarina in Florianopolis, Brazil. Additionally, I have or am serving on numerous selection Committees, the NIH Central Tenure Committee, study sections and Editorial **b**oards.

Highlighted Research Support:

- NEI/NIH Intramural funding, Project # EY000184 "Molecular, cellular and genetic mechanisms in uveitis" to RR Caspi – ongoing since 1989
- Binational Science Foundation (BSF) USA–Israel grant to Caspi and Sredni
- Alcon Research Institute Award to RR Caspi - 2012 – non-expiring
- K99/R00 Pathway to Independence Award to AS St. Leger. 2016
- NIH Bench-to-Bedside Award: "Microbiome and Uveitis" Awarded to HN Sen and RR Caspi, 2017-2019
- NIH Bench to Bedside Award to RR Caspi "Commensal microbiota as possible pathobiont in autoinflammatory disease" 2019-2021
- NEI Intramural project development award 2024 to X. Xu
- 2 Prevention of Blindness Society of Metropolitan Washington (POB) awards to lab staff
- Numerous NIH FARE, travel awards and other competitive grants to Fellows in the lab

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2017-	Adjunct Professor, Department of Pathology, University of Pennsylvania, Philadelphia, PA
2017 -	Chief, Laboratory of Immunology, NEI, Bethesda, MD
2016-2017	Acting Chief, Laboratory of Immunology, NEI, Bethesda, MD
2005-	NEI Flow Cytometry Core Supervisor, Laboratory of Immunology, NEI, Bethesda, MD
2001-	Adjunct Professor, Department of Pathology, University of Pennsylvania, Philadelphia, PA
1999 - 2016	Deputy Chief, Laboratory of Immunology, NEI, Bethesda, MD
1990-	Chief, Section of Immunoregulation, Laboratory of Immunology, Bethesda, MD
1987 - 1989	Visiting Associate, Laboratory of Immunology, NEI, Bethesda, MD
1984 - 1986	Visiting Fellow, Laboratory of Immunology, NEI, Bethesda, MD
1980 - 1983	Teaching Instructor, Bar-Ilan University, Department of Life Sciences, Ramat Gan

Other Experience and Professional Memberships

2015	Organizer, FASEB Summer Research Conference-Autoimmunity
2014	Organizer, ARVO Summer Education Conference-Ocular immunity, autoimmunity, and inflammation
2013	Co-organizer, FASEB Summer Research Conference-Autoimmunity
2008	Organizer, ARVO Summer Conference-Ocular Autoimmunity and Inflammation
2006	Co-organizer, Keystone Conference-Autoimmunity & Tolerance
2003	Co-organizer, Keystone Conference-Autoimmunity & Tolerance
2008 -	Editorial Board, Autoimmunity
2004 -	Editorial Board, Journal of Leukocyte Biology
2000 - 2002	Editorial Board, Ocular Immunology and Inflammation
2000 -	Editorial Board, Journal of Autoimmunity
1990 -	Editorial Board, Cellular Immunology
2015 – 2016	Israel-USA Binational Science Foundation Advisory Board member (BSF)
2008 – 2012	Council Member, Society for Leukocyte Biology
2006 -	Contributing Member, Faculty 1000
2006 -	Council Member, Midwinter Conference of Immunologists
2002 - 2006	Central Tenure Committee, NIH
2001 - 2002	Program Committee Chair, ARVO
1999 - 2002	Program Committee, ARVO
1997 - 2000	Advisor, HHMI/NIH Research Scholars Program
2017 -	Steering Committee Member, NIH Immunology Interest Group (IIG)
2003 – 2005	Steering Committee Member, NIH Immunology Interest Group (IIG)
1998 -	Steering Committee member, NIH Cytokine Interest Group (CIG)

Honors

2022	NEI Director's award for mentoring
2020	NIH Cytokine Interest Group award for Best Cytokine Paper of the Year given to Wai Po Chong, Immunity 2020.
2017	Society for Leukocyte Biology Women and Diversity "Paper of the Year" Award given to Horai, Zárate-Bladés et al, Immunity 2015
2014	NIH Director's Award for discovery of the rd8 mutation contaminating C57BL/6N strain
2012	Contributions to vision research, Alcon Research Institute
2010	Gold Fellow, ARVO
2010	Friedenwald Award for seminal contributions to the field, ARVO
1995	Award for special Achievement, NIH Director
1984	Fogarty Postdoctoral Fellowship, NIH

C. Contributions to Science

Development and characterization of animal models of human disease.

Autoimmune (noninfectious) uveitis is a heterogeneous group of diseases that affect ~2 million Americans annually and are responsible for 10-15% of blindness. To study basic mechanisms and develop therapeutic approaches, animal models must be used. My work has resulted in development of different models of uveitis in mice, induced and spontaneous, which can be used to study different aspects of human disease, and which have catapulted forward basic uveitis research. The "classical" EAU model is induced by immunization with IRBP in complete Freund's adjuvant (CFA-EAU) **(*1*)** and subsequent work characterized several peptide epitopes that can be used in place of whole IRBP to elicit disease. A variant on the "classical" theme is the "humanized" EAU model induced by immunization with retinal arrestin (S-Ag) of HLA-transgenic mice **(*2*).** DC-EAU is induced by infusion of IRBP-pulsed dendritic cells and differs from CFA-EAU by its dependence on the Th1 rather than the IL-17 response **(*3*).** Finally, the spontaneous uveitis model that develops in IRBP-TCR transgenic mice (R161H, L and M) permits to study natural triggers of the disease **(*4*).** Our study, published in the prestigious journal Immunity, demonstrated a critical role for commensal microbiota in triggering uveitis in this model, implicating commensals in the etiology of uveitis (see ref # 14, ahead).

1. R. R. Caspi *et al.*, A new model of autoimmune disease. Experimental autoimmune uveoretinitis induced in mice with two different retinal antigens. *J Immunol* 140, 1490-1495 (1988).
2. G. Pennesi *et al.*, A humanized model of experimental autoimmune uveitis in HLA class II transgenic mice. *J Clin Invest* 111, 1171-1180 (2003).
3. J. Tang *et al.*, 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. *J Immunol* 178, 5578-5587 (2007).
4. R. Horai *et al.*, Breakdown of immune privilege and spontaneous autoimmunity in mice expressing a transgenic T cell receptor specific for a retinal autoantigen. *J Autoimmun* 44, 21-33 (2013).

Establishment and maintenance of self-tolerance to retina: peripheral vs. central tolerance mechanisms and the role of immune privilege

Self-tolerance to tissue antigens is achieved by a combination of central (thymic) and peripheral mechanisms. In the case of the eye, there is also the specialized and highly complex phenomenon known as ocular immune privilege. We demonstrated that expression of IRBP in the thymus reduces susceptibility to EAU by culling discrete specificities of IRBP-responsive T cells from the repertoire **(*5*)**. However, many retina-specific T cells escape thymic deletion. Immune privilege helps minimize the possibility of uveitis by sequestering the retinal antigens behind the blood-retinal barrier (BRB), by limiting cell trafficking into the eye and by converting naïve retina specific T cells to Tregs. We showed that this TGF-β-dependent process *occurs in the living eye* and requires *in situ* antigen recognition and retinoic acid (which is abundant in the eye due to its role in vision) **(6** & Zhou et al, JI 2011). Ocular resident cells also contribute to the inhibitory ocular environment: the very first publication demonstrating control of uveitogenic lymphocytes by ocular resident cells was a paper by Caspi et al, published in the journal Science in 1987 **(7)**. However, ocular immune privilege has limitations: antigen-experienced T cells that had already been activated in the periphery can actively traverse the BRB and are not controlled by the immunoinhibitory ocular environment. In addition, the BRB impedes peripheral tolerance to retinal antigens by sequestering them within the eye. Indeed, forced expression of IRBP outside the eye results in profound and long-term resistance to EAU **(8**, Xu et al, EJI 2000 & Silver et al., JI 2007). These studies help to explain how uveitis can occur despite ocular immune privilege, and suggest that augmentation of peripheral tolerance has potential as a therapeutic approach.

Current work being prepared for publication explores the fate of retina-specific T cells that encounter their antigen within the immune privileged eye, by using single-cell RNA-Seq analysis.

5. D. Avichezer *et al.*, An immunologically privileged retinal antigen elicits tolerance: major role for central selection mechanisms. *J Exp Med* 198, 1665-1676 (2003).
6. R. Zhou *et al.*, The living eye "disarms" uncommitted autoreactive T cells by converting them to Foxp3(+) regulatory cells following local antigen recognition. *J Immunol* 188, 1742-1750 (2012).
7. R. R. Caspi, F. G. Roberge, R. B. Nussenblatt, Organ-resident, nonlymphoid cells suppress proliferation of autoimmune T-helper lymphocytes. *Science* 237, 1029-1032 (1987).
8. R. K. Agarwal *et al.*, Retroviral gene therapy with an immunoglobulin-antigen fusion construct protects from experimental autoimmune uveitis. *J Clin Invest* 106, 245-252 (2000).

Adaptive and innate effector responses in pathogenesis and regulation of ocular autoimmunity

Our work on effector and regulatory cells involved in uveitis toppled dogmas and shifted paradigms, and has helped unravel the balance between effector and regulatory mechanisms driving ocular autoimmunity. When IFN-γ-producing Th1 cells were believed to be responsible for autoimmune pathology, we demonstrated that IFN-γ, inhibits disease induction (Caspi et al., JI 1994, Jones et al., JI 1997, Tarrant et al., JEM 1999, Grajewski/Hansen et al, JI 2008). This paradox was also reported in other autoimmune disease models and perplexed researchers for many years. We finally unraveled the mechanism in 2015, by demonstrating that the "protective" IFN-γ was derived from NK cells interacting with IL-27-producing dendritic cells (DC), thus forming a novel innate IFN-γ/IL-27 axis that controls the adaptive Th17 response and limits autoimmunity **(9)**. After the discovery of Th17 cells, which promptly supplanted Th1 as "the" effectors of autoimmunity, we demonstrated that EAU can be driven either by Th1 or by Th17 effector responses (depending on the model), that both responses are independently pathogenic, and that effector dominance is determined by the innate immune environment present during initial antigen exposure **(10).** Additional studies, which were also paradigm-shifting at the time, revealed that the pathogenic effector response is secondarily controlled in the adaptive phase by T regulatory (Treg) cells, which are in part thymically derived **(11a)** and in part induced during the disease process itself **(11b).** These cells could also be elicited by directed manipulation, such as antigen-induced oral or hydrodynamic vaccination (Rizzo et al. JCI 1994, Silver et al, JI 2007), emphasizing the clinical potential of therapeutic Treg amplification. Our more recent high-profile addition to the understanding of effector T cell responses in uveitis demonstrated that the pathogenic cytokine Th17 also has a regulatory function, and dampens the production of Th17 lineage-specific inflammatory cytokines via autocrine induction of inhibitory IL-24 **(12).** We propose that the failure of clinical trials of IL-17 neutralization as therapy for uveitis may have been due to loss of this regulatory pathway.

9. Wai Po Chong, Nicolas van Panhuys, Jun Chen, Phyllis B Silver, Chi-Chao Chan, Ronald N. Germain, Rachel R Caspi. CD25-CD25 crosstalk controls the autopathogenic CD2517 response through an innate CD25-γ/IL-27 axis. *J Exp Med.* 212:1739-52 (2015)
10. D. Luger *et al.*, Either a CD2517 or a CD251 effector response can drive autoimmunity: conditions of disease induction affect dominant effector category. *J Exp Med* 205, 799-810 (2008).
11. (a) R. S. Grajewski *et al.*, Endogenous IRBP can be dispensable for generation of natural CD4+CD25+ regulatory T cells that protect from IRBP-induced retinal autoimmunity. *J Exp Med* 203, 851-856 (2006).
    (b) P. Silver *et al.*, Retina-specific T regulatory cells bring about resolution and maintain remission of autoimmune uveitis. *J Immunol* 194, 3011-3019 (2015).
12. WP Chong, MJ Mattapallil*, K Raychaudhuri*, SJ Bing, WW Wang, S Wu, Y Zhong, PB Silver, Y Jittayasothorn, CC Chan, J Chen, R Horai, RR Caspi. The cytokine IL-17A limits Th17 pathogenicity via a negative feedback loop driven by autocrine induction of IL-24. *Immunity,* 53:384-397 (2020).

The role of commensal microbiota, and of the responses they elicit, in ocular immunity and autoimmunity

Commensal microbiota affect physiological and immunological processes in the entire body, but their role in relation to the eye has been relatively unexplored. The eye is a complex organ structurally and immunologically. While its surface is a mucosal tissue exposed to the environment, the inside of the globe is an immune privileged and sterile environment. Microbiota affect both, in very different ways. Our pioneering studies in the spontaneous uveitis model revealed that autoimmune uveitis may be triggered by commensal bacteria in the intestine through recognition by retina-specific T cells of a bacterial surrogate antigen. This "mimic" antigen activates retina-specific T cells through their clonotypic TCR, which allows them to penetrate the BRB and cause uveitis **(13).** The implications from these data are far-reaching, because if bacteria can mimic a retinal antigen, it is conceivable that they could also mimic antigens that trigger development of other autoimmune disorders. This

was subsequently confirmed by publications that identified molecular mimics for other self-antigens in the gut. Our most recent study on the gut-eye axis uncovered evidence that (microbiota-dependent) intraepithelial lymphocytes (IELs) may control activation of retina-specific T cells in the gut **(14).**

In contrast to the gut, we showed that commensals on the ocular surface have a beneficial role. The existence of a resident ocular surface microbiome was until recently a highly contentious issue. Our data demonstrated for the first time that the surface of the eye harbors resident commensals which tune the local immune response, affording protection from pathogenic organisms. We isolated, identified, and fulfilled Koch's postulates for *Corynebacterium mastitidis* as a *bona fide* ocular surface commensal that confers a protective ocular phenotype by eliciting IL-17 from γδ T cells **(15).** This study provides important proof of concept that a commensal flora exists on the ocular surface and contributes to local mucosal homeostasis.

Finally, we uncovered a STAT3-independent pathway to IL-17 production, which is utilized by some innate-like T cells (likely including γδ T cells), and which by itself affords protection from pathogen infection at the ocular surface **(16).** This finding is novel and may help explain why Jobs' Syndrome patients, who have a hypomorphic STAT3 and underproduce IL-17, are not known to suffer from ocular surface inflammation. This finding also has implications for STAT3 targeting to inhibit inflammation ("Jakinibs") and may help alleviate possible deleterious effects of this type of immunosuppressive therapy on host defense.

Current work explores the ability of human gut microbes introduced into germ-free mice to trigger autoimmune uveitis, and examines signals involved in sensing of ocular surface commensals by γδ T cells (17).

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