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BASIC SCIENCE

Expression of Stat3 and indoleamine 2, 3-dioxygenase in cornea keratocytes as factor of ocular immune privilege

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Abstract
Purpose Ocular immune privilege is a multifactorial phenomenon evolutionally selected to prevent immunogenic inflammation from disrupting the visual axis and causing blindness. Here, we investigated the role of signal transducers and activators of transcription (Stat3) and indoleamine 2,3-dioxygenase (IDO) in ocular immune privilege in corneal stromal cells.
Methods Human keratocytes were isolated and cultured in vitro, and Stat3 and IDO expression on keratocytes was investigated by reverse transcription polymerase chain reaction (RT-PCR). The active form of Stat3 was detected by flow-cytometry, and IDO enzyme activity following IFN-γ stimulation of keratocytes was measured by tryptophan to kynurenine conversion with photometric determination of kynurenine concentration in the supernatant.
Results Stat3 was constitutively expressed in cultured keratocytes and up-regulated following IFN-γ stimulation. The active form of Stat3 was also up-regulated following IFN-γ stimulation. IDO expression and enzyme activity was markedly induced following IFN-γ stimulation, but this induction was prevented by the IDO specific inhibitor, 1-methyl tryptophan (1-MT).
Conclusions On the basis of this study, Stat3 and IDO may act as a factor of ocular immune privilege in corneal keratocytes. Thus, focus on these inhibitory molecules should be considered in studies aimed at developing therapeutic agents for controlling ocular inflammatory or immune diseases.

Keywords Indoleamine 2,3-dioxygenase · Immune privilege · Keratocyte · Signal transducers and activators of transcription · 1-methyl tryptophan

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Introduction

The eye is an organ characterized by strong immune tolerance, and employs several strategies to prevent or modify innate and adaptive immune responses. This phenomenon is termed ocular immune privilege.

Ocular immune privilege provides the eye with protection against pathogens while minimizing the risk of vision impairment [1–5]. Orthotropic corneal allograft studies have recently demonstrated that immunological ignorance is one of the mechanisms by which this process occurs. In their natural state, corneal cells do not express MHC class II antigens, and expression of MHC class I antigens is reduced [1, 6, 7]. However, when corneal stromal cells are stimulated with IFN-γ, expression of both MHC class I and II is increased [8, 9].

After recognition of MHC antigens, activation of T cells can be regulated by endogenous immunosuppressive agents. Among these immunosuppressive agents, Signal transducer and activator of transcription 3 (Stat3) and

indoleamine 2, 3-dioxygenase (IDO) have recently been reported to demonstrate strong immunosuppressive activity [10–13].

Stat3 is a key transcriptional mediator for many cytokines, and regulates T-cell activity. Expression of Stat3 is regulated by various cytokines and is upregulated in many tumors, especially associated lymphoid or myeloid tumors [10]. Stat3 activity can mediate immune evasion in tumor tissues by blocking both the production of and response to inflammatory signals by multiple components of the immune system [13]. Stat3 deficiency leads to enhanced activation of inflammatory cytokines, and causes Crohn's disease-like inflammatory bowel disease [11, 12].

IDO is the rate-limiting enzyme in the catabolism of tryptophan to N-formylkynurenine [14]. Production of IDO by syncytiotrophoblasts [15] macrophages, dendritic cells, and epithelial cells has recently been demonstrated to inhibit T-cell proliferation by tryptophan depletion. The various effects of IDO production include T-cell immune tolerance within the tumor micorenvironment, T-cell suppression and apoptosis in autoimmune disease, inhibition of T-cell reactivity, and regulation of T-cell responses in allergic asthma and organ-specific autoimmunity [16–21]. Paradoxically, IFN-γ not only induces MHC class I and II expression on antigen-presenting cells (APCs) for T-cell activation, but also induces Stat3 activation and IDO production [22, 23].

Ocular immune privilege is necessary for preventing or modifying innate and adaptive immune responses occurring in intraocular cells. However, the mechanisms mediating this phenomenon still need defining. Given that IFNγ induces similar upregulation of MHC antigens in APC and corneal stromal cells, the aim of this study was to determine if the immnosuppressive molecules Stat3 and IDO are expressed in corneal stromal cells, and to establish if corneal stromal cells play an important role in cornea immune tolerance.

Methods

Isolation and culture of human corneal stromal cells

Human keratocytes were isolated from fresh human corneal stroma using a collagenase digestion protocol. The mean age of donor patients was 35.65±12.55 years old, and all contents were obtained with informed consent. All protocols involving human cornea were approved by the Institutional Review Board at the Inje University, and were consistent with the guidelines of the Declaration of Helsinki.

A total of six peripheral cornea were obtained from brain-dead patients after keratoplasty, and were soaked in antibiotic solution before tissue culture. Cornea tissues were washed twice in a 60 mm cell culture dish containing 3 ml of DMEM culture media (Gibco, Carlsbad, CA, USA). The corneal tissues were then placed in 10-mm cell-culture dishes with 0.2 ml DMEM culture media to prevent desiccation. With two 21-G surgical blades, the cornea was cut into small pieces, transferred to a 100-mm cell-culture dish (Corning Life Sciences, Rochester, NY, USA) with a 1 ml pipette, and digested with 100 U collagenase type IV (Sigma, St Louis, MO, USA) for 1 hour at 37°C, 5% CO_2. Digested cornea tissue was then placed between glass slides, and washed with 10 ml of DMEM to remove the collagenase. Isolated cells were cultured in serum-free DMEM for maintenance of keratocytes in culture. Cells that underwent three passages were used in RT-PCR and cells passed five to eight times were used for FACS analysis. In some instances, cells were treated with recombinant human IFN-γ (500 U/ml, R&D Systems, Minneapolis, MN, USA) for 3 days.

Antibodies

PE-conjugated anti-human CD45 , PE-conjugated anti-human CD1a, PE-conjugated anti-human CD11, PE-conjugated anti-human CD11c, PE-conjugated anti-human CD34, PE-conjugated anti-human CD34 (BD Bioscience, CA, San Diego) and PE-conjugated streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) monoclonal antibodies were used for analysis via flow cytometry.

Molecular analysis with flow cytometry

1×10^5 cells were put into each FACS tube (Falcon), and washed with FACS buffer (PBS, 0.05% NaN3, 1% FBS). To prevent non-specific molecular binding, the cell pellet was resuspended in human Ig, and incubated at 4°C for 15 minutes. PE-conjugated or biotin-conjugated monoclonal primary antibody (0.5 μg/tube) was then added to the suspended cells, incubated at 4°C for 30 minutes, and washed three times in FACS buffer. To detect biotin-conjugated monoclonal primary antibodies, cells were further incubated with the secondary antibody PE-conjugated streptavidin, at 4°C for 30 minutes. Each antibody-conjugated cell was analyzed using FACSort flow cytometer (BD Biosciences, San Diego, CA, USA). Activated Stat3 was detected with a PE-conjugated anti-phospho-Stat3 monoclonal antibody using intracellular staining. 1×10^6 cells were permeabilized with cytofix/cytoperm solution (BD Biosciences) at room temperature, for 20 minutes. After fix/perm, cells were washed with perm-washing solution two times, and stained with PE-anti-phospho-Stat3 monoclonal antibody

for 30 minutes. After staining, cells were washed with perm-washing solution two times and analyzed by flow cytometry.

RT-PCR

Total RNA of corneal stromal cells treated with or without IFN-γ was extracted with Trizol. cDNA was synthesized with 1 μg of each total RNA using a reverse transcriptase kit (Promega). PCR was performed using human Stat3 and IDO primers, at 94°C, for 1 minute, at 55°C for 1 minute, and at 72°C, for 1 minute. A total of 35 cycles was performed. The PCR primers are as follows: Stat3, forward 5′-AAGTG ATGAACATGGAGGAG-3′, reverse 5′-TTTCTTTGCA GAATTTAGCC-3′; IDO, forward 5′-GCGCTGTTGGAAAT AGCTTC-3′, reverse 5′-TTTGGGTCTTCCCAGAACC-3′; β-actin, forward 5′-TTGGCAATGAGCGGTTCC-3′, reverse 5′-GTACGCCAACACAGTGCT-3′.

IDO activity analysis

5×10^3 cultured corneal stromal cells passed three times were treated with 500 U/ml recombinant human IFN-γ (R&D Systems) and cultured for 3 days. Then, 75 μl of culture supernatant was reacted with 125 μl methylene blue reaction buffer at 37°C for 1 hour. Reaction was stopped with 30% trichloroacetic acid. Then, 125 μl of reaction solution was mixed with same volume of Ehrlich reagent at room temperature for 10 minutes. Concentrations of kynurenine were measured with HPLC (high performance liquid chromatography**).**

Results

Isolation and culture of human corneal stromal cells

To examine the role corneal stromal cells play in mediating ocular immune privilege, six peripheral cornea were obtained from brain-dead patients after keratoplasty. Corneal stromal cells, located in the corneal stromal cell layer were isolated by collagenase digestion and cultured with serum-free DMEM (Fig. 1a). These cells exhibited a fibroblastic morphology with a branched cytoplasm (Fig. 1b). To exclude the possibility of contamination with blood and immune cells, flow cytometric analysis was performed. Isolated cells did not stain positive for the surface antigens CD11b (mononuclear cell), CD11a (dendritic cell), CD45 (polynuclear cell), and CD34 (endothelial cell), and this remained unchanged after culture with recombinant IFN-γ (Fig. 2). Therefore, this data demonstrates the protocol used for isolating and culturing corneal stromal cells resulted in a pure population without immune cell contamination.

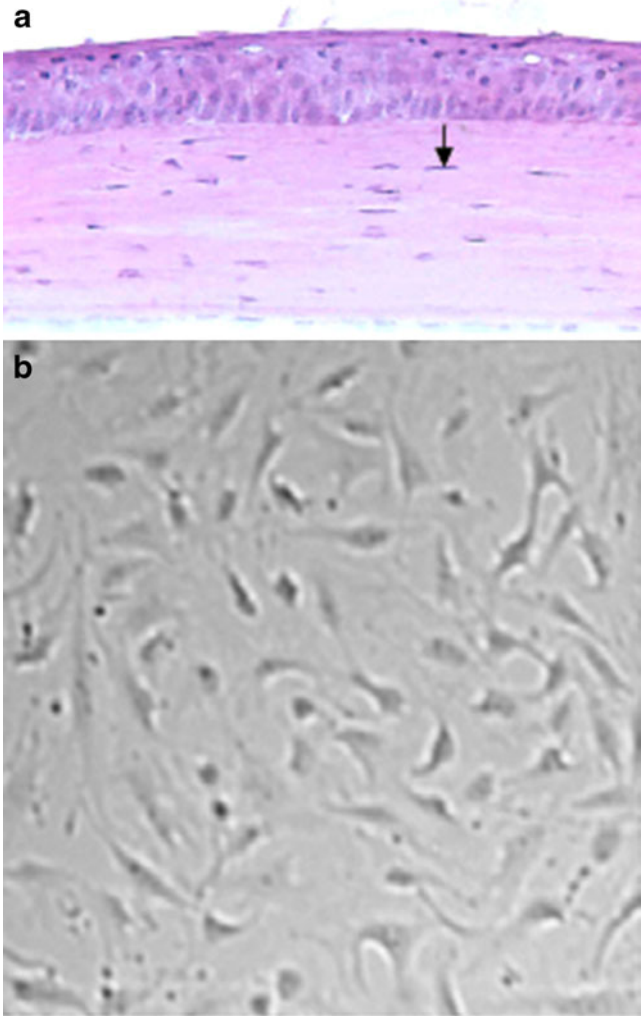

Fig. 1 Isolation and culture of human keratocytes. **a** Histological analysis of human corneal tissue, and arrow indicates keratocyte (hematoxylin and eosin, ×100). **b** Keratocytes grew out of the corneal stroma, and these were subcultured three times to expand the number of cells and to ensure their purity (×200)

Phenotype of corneal stromal cells

If corneal stromal cells are to play a similar role in ocular immune privilege as APC do in other organs, these cells should express similar surface antigens. To test the immunological phenotype of corneal stromal cells, flow cytometry was used to analyze the cell surface antigens MHC class I, CD40, CD86 (B7-2), and 4-1BBL because these molecules are important in initiating and controlling the immune response. Corneal stromal cells expressed MHC class I, but did not express CD86. CD40 and 4-1BBL were expressed at low levels (Fig. 3 top). Stimulation with IFN-γ increased the expression of MHC class I and CD40,but the expression of CD86 and 4-1BBL was not changed (Fig. 3 bottom). Thus, corneal stromal cells exhibit a phenotype similar to APC, and should therefore

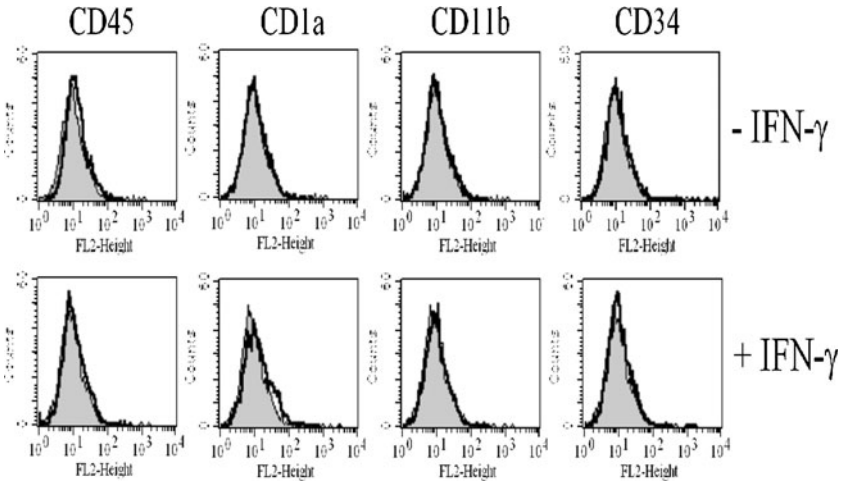

Fig. 2 Analysis of purity on cultured human keratocytes. Cultured keratocytes were stimulated with IFN-γ (500U/ml) for 0 and 3 days and stained with PE-conjugated anti-human CD45, CD1a, CD11b, CD34 and analyzed by flow cytometry

be capable of interacting with effector T cells and modifying the immune response

IDO and Stat3 mRNA expression

Two immune modulators important in inhibiting the immune response are Stat3 and IDO. Given the role of these inhibitory molecules in mediating immune privilege in other organs, the expression of Stat3 and IDO was evaluated in ocular srtomal cells. RT-PCR was performed on cells cultured in DMEM (non-inflammatory condition) or with the addition of, 500 U/ml of IFN-γ for 3 days (inflammatory condition). Stat3 was expressed during non-inflammatory conditions, but the expression level was increased after IFN-γ stimulation (Fig. 4 lanes 3 & 4). IDO, however, was not expressed during non-inflammatory conditions, but was induced following IFN-γ stimulation (Fig. 4 lanes 5 & 6) This data demonstrates that under inflammatory conditions, corneal stromal cells express the inhibitory molecules Stat3 and IDO.

Activity of Stat3 in corneal stromal cells

Upon phosphorylation, Stat3 is capable of translocating to the nucleus and activating Stat responsive genes. Constitutive expression of Stat3 in tumors results inhibits the expression of inflammatory cytokines and chemokines, as well as preventing DC maturation [13]. To determine if corneal stromal cells display similar Stat3 activity, a PE-conjugated anti-phospho-Stat3 antibody was used to analyze the expression of activated Stat3 by flow cytometry. Normal peripheral blood mononuclear cells stimulated with or without IFN-γ were not stained by anti-phospho-Stat3 antibody. However, corneal stromal cells expressed activated Stat3 (72.5%) without IFN-γ stimulation and with IFN-γ stimulation, the percentage of phospho-Stat3 positive cells increased to 84.5% (Fig. 5). Thus, corneal stromal cells constitutively express activated Stat 3, and this expression is increased under inflammatory conditions. This data suggests that activated Stat3 in corneal stromal cells may inhibit the immune response and promote ocular immune privilege similar to that observed in the tumor microenvironment.

IDO activity induced by IFN-γ

IDO has potent inhibitory effects on effector T cells. To identify whether IDO activity is induced in corneal stromal cells by IFN-γ, IDO-mediated kynurenine formation was analyzed with HPLC (high performance liquid chromatog-

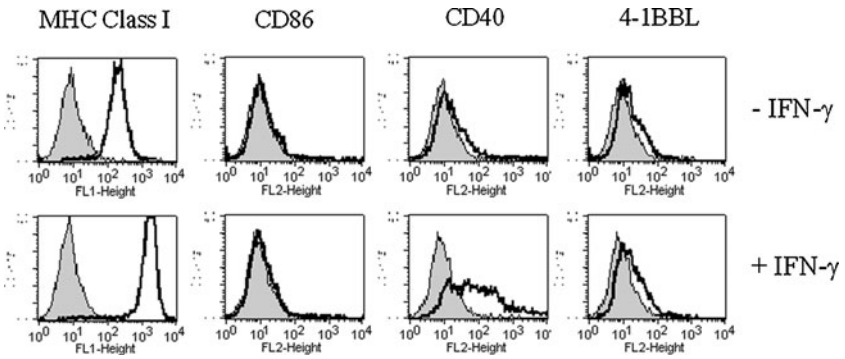

Fig. 3 Phenotypic analysis of cultured human keratocytes. Cultured keratocyte were stimulated with IFN-γ (500U/ml) for 0 and 3 days, and stained for cell surface antigens with and analyzed by flow cytometry

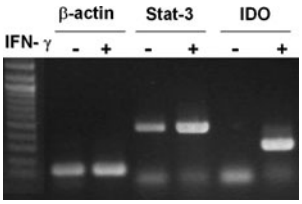

Fig. 4 Expression of Stat3 and IDO in human keratocytes. Keratocyes were cultured without or with IFN-γ(500U/ml) for 72 hrs. Total RNA was isolated and reverse-transcribed into cDNA. PCR was performed using IDO-specific primers, and the products were analyzed by agarose gel electrophoresis

raphy). Without IFN-γ, kynurenine formation was not detected in the supernatant of cultured corneal stormal cells, However, treatment with IFNγ induced appreciable levels of IDO (Fig. 6). To determine the specificity of IDO production, 1-methyl tryptophan (1-MT) was added to the cell culture media. Kynurenine production by corneal stromal cells after IFN-γ stimulation was blocked by the addition of 1-methyl tryptophan (1-MT) (Fig. 6). These data suggest that IFN-γ induced active IDO expression by corneal stromal cells.

Discussion

Every organ and tissue is subject to invasion by environmental pathogens. For the skin and mucous membranes, this threat is real, direct, diverse, and constant [1]. For internal organs (such as heart, kidney and muscle), the threat is equally real, but less direct, less diverse, and intermittent, depending on chance spread of microorganisms in the blood [1]. Ocular immune privilege — an experiment of natural selection and evolution — provides the eye, especially corneal stromal cell and pigment epithelial cell, with immune protection against pathogens that have minimal risk to the integrity of vision [1]. Compared with rejection of other types of graft, corneal allograft rejection is delayed. This indicates that the cornea is an immune privilege area.

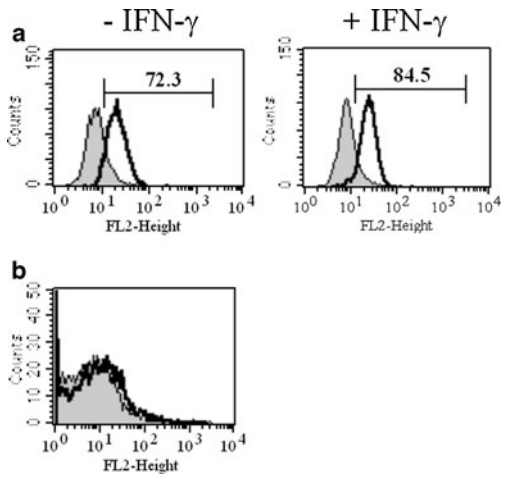

Fig. 5 Constitutive Stat3 activity in human keratocytes. **a** Keratocyes were cultured without or with IFN-γ (500U/ml) for 3 days. Cells were harvested and stained with PE-conjugated anti-phospho-Stat3, and analyzed by flow cytometry. **b** Human peripheral blood mononuclear cell were isolated from peripheral whole blood and stained with PE-conjugated anti-phospho-Stat3, and analyzed by flow cytometry

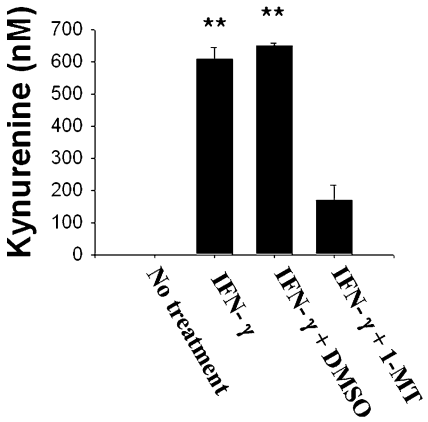

Fig. 6 IDO-mediated trytophan degradation in human keratocytes. Cells were cultured without or with IFN-γ or IFN-γ plus 1-MT for 3 days. Culture supernatants were collected and set up for analysis of IDO activity and concentration (nM) of kynurenine determined by HPLC

Although the absence of lymphatic-drainage pathways in the eye is important for shielding ocular antigens from the immune system, this anatomical specialization alone does not account fully for the low immunogenicity of antigens placed within or arising from the eye [1]. Immunological ignorance is achieved because corneal cells do not express MHC class II antigens, and MHC class I expression is reduced. However, during an active immune response involving phagocytosis and IFN-γ production, antigen-presenting cells (APCs) expressing MHC class I and II, and costimulatory molecules (such as B7-1, CD40, 4-1BBL), are increased [8, 9]. This study demonstrated that a similar phenomenon can occur in the ocular stroma. Extracted and cultured corneal stromal cells exhibited increased expression of MHC class I and CD40 after IFN-γ stimulation (Fig. 3).

T cells have T-cell receptors (TCRs) that can recognize MHC class I or II molecules, and these cells are important mediators of immune reaction. But T-cell activation can be regulated by endogenous immunosuppressive agents such as Stat3 and IDO. Stat3 plays a critical role in the development and regulation of innate immunity, and deletion of Stat3 during hematopoiesis results in abnormalities in myeloid cells and causes Crohn's disease-like pathogenesis [12]. Stat3 activity in tumors inhibits the production of multiple proinflammatory cytokines and chemokines, while inducing the release of factors that

inhibit antigen-presenting cell (APC) maturation through activation of Stat3 in antigen-presenting cells (APCs) [13].

IDO is the rate-limiting enzyme in the catabolism of tryptophan and transformation to N-formylkynurenine [14]. The IDO action mechanism has been reported recently, which includes T-cell immune tolerance in tumor cell, T-cell suppression and apoptosis in autoimmune disease, inhibiting T-cell reactivity and inducing a T-cell response regulating allergic asthma and organ-specific autoimmunity [16–21].

Using isolated human corneal tissues, this study identifies constitutively active Stat3 and inducible IDO expression in human corneal stromal cells. High Stat3 concentration was maintained without IFN-γ, and increased in the presence of IFN-γ. This indicates that corneal immune privilege may be mediated by constitutive Stat3 activation in corneal stromal cells and can be increased following IFN-γ exposure. IDO was not expressed by these cells in normal culture media, but was highly induced following stimulation with IFN-γ. Furthermore, by analyzing kynurenine production we demonstrate that active IDO is expressed by corneal stromal cells and induced after IFN-γ stimulation. These results reveal that IFN-γ induces Stat3 and IDO in corneal stromal cells. Thus, when inflammatory reaction occurs in cornea following bacterial or viral infections, Stat3 and IDO may play a pivotal role in inhibiting inflammation and inducing immune tolerance.

Our study limitation was a one-off experiment, because we cannot take many human corneas easily, so representative study will be needed.

This study reveals that human corneal cells express Stat3 and IDO, inhibitory molecules shown to be necessary for promoting immune tolerance. Thus, these cells most probably play a crucial role in mediating ocular immune privilege. Given the difficulty in obtaining human cornea tissue, more studies are needed to determine if these cells are able to inhibit T-cell responses; however, these results lay an exciting foundation for developing therapeutic agents that will control ocular immune diseases.

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References

1. Streilein JW (2003) Ocular immune privilege: therapeutic opportunities from an experiment of nature. Nat Rev Immunol 3:879–889
2. McBride BW, McGil JI, Smith JL (1998) MHC class I and II antigen expression in normal human corneas and in corneas from cases of herpetic keratitis. Immunology 65:583–587
3. Seo SK, Gebhardt BM, Lim HY, Kang SW, Higaki S, Varnell ED, Hill JM, Kaufman HE, Kwon BS (2001) Murine keratocyte function as antigen-presenting cells. Eur J Immunol 31:3318–3328
4. Takeda K, Clausen BE, Kaisho T, Tsujimura T, Terada N, Förster I, Akira S (1999) Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. Immunity 10:39–49
5. Cheng F, Wang HW, Cuenca A, Huang M, Ghansah T, Brayer J, Kerr WG, Takeda K, Akira S, Schoenberger SP, Yu H, Jove R, Sotomayor EM (2003) A critical role for Stat3 signaling in immune tolerance. Immunity 19:425–436
6. Streilein JW (1999) In: Streilein JW (ed) Immune responses and the eye: Chemical immunology 73. Karger, Basel, pp 186–206
7. Niederkorn JY (2002) A review of the role of immune privilege in the high rate of acceptance of corneal transplants without immunosuppression, including an analysis of the immunopathogenic mechanisms that can destroy cornea grafts in diseased eyes. Immunology and immunomodulation of corneal transplantation. Int Rev Immunol 21:173–196
8. Taylor AA (2003) Review of the influence of aqueous humor on immunity. Ocul Immunol Inflamm 11:231–241
9. Streilein JW, Masli S, Takeuchi M, Kezuka T (2002) The eye's view of antigen presentation. Hum Immunol 63:435–443
10. Calo V, Migliavacca M, Bazan V, Macaluso M, Buscemi M, Gebbia N (2003) STAT proteins: from normal control of cellular events to tumorigenesis. Cell Physiol 197:157–168
11. Takeda K, Clausen BE, Kaisho T, Tsujimura T, Terada N, Forster I (1999) Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. Immunity 10:39–49
12. Welte T, Zhang SS, Wang T, Zhang Z, Hesslein DG, Yin Z, Kano A, Iwamoto Y, Li E, Craft JE, Bothwell AL, Fikriq E, Koni PA, Flavell RA, Fu XY (2003) STAT3 deletion during hematopoiesis causes Crohn's disease-like pathogenesis and lethality: a critical role of STAT3 in innate immunity. Proc Natl Acad Sci USA 100:1879–1884
13. Wang T, Niu G, Kortylewski M, Burdelya L, Shain K, Zhang S, Bhattacharya R, Gabrilovich D, Heller R, Coppola D, Dalton W, Jove R, Pardoll D, Yu H (2004) Regulation of the innate and adaptive immune responses by Stat3 signaling in tumor cells. Nat Med 10:48–54
14. Taylor MW, Feng G (1991) Relationship between interferon-g, indoleamine 2,3-dioxygenase, and tryptophan catabolism. FASEB J 5:2516
15. Munn DH, Zhou M, Attwood J, Bondarev I, Conway S, Marshall B, Brown C, Mellor A (1998) Prevention of allogeneic fetal rejection by tryptophan catabolism. Science 281:1191
16. Grohmann U, Orabona C, Fallarino F, Vacca C, Calcinaro F, Falorni A, Candeloro P, Belladonna ML, Bianchi R, Fioretti MC, Puccetti P (2002) CTLA-4-lg regulates tryptophan catabolism in vivo. Nat Immunol 3:1097–1101
17. Babcock TA, Carlin JM (2000) Transcriptional activation of indoleamine dioxygenase by interleukin 1 and tumor necrosis factor alpha in interferon-treated epithelial cells. Cytokine 12:588–594
18. Hwu P, Du MX, Lapointe R, Do M, Taylor MW, Young HA (2000) Indoleamine 2,3-dioxygense production by human dendritic cells results in the inhibition of T cell proliferation. J Immunol 164:3596–3599
19. Munn DH, Sharma MD, Lee JR, Jhaver KG, Johnson TS, Keskin DB, Marshall B, Chandler P, Antonia SJ, Burgess R, Slinqluff CL Jr, Mellor AL (2002) Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. Science 297:1867–1870
20. Mellor AL, Baban B, Chandler P, Marshall B, Jhaver K, Hansen A, Koni PA, Iwashima M, Munn DH (2003) Cutting edge: Induced

indoleamine 2,3-dioxygenase expression in dendritic cell subsets suppresses T cell clonal expansion. J Immunol 171:1652–1655
21. Fallarino F, Grohmann U, Hwang KW, Orabona C, Vacca C, Bianchi R, Belladonna ML, Fioretti MC, Aleqre ML, Puccetti P (2003) Modulation of tryptophan catabolism by regulatory T cells. Nat Immunol 4:1206–1212
22. Munn DH, Shafizadedh E, Attwood JT, Bondarev I, Pashine A, Mellor AL (1999) Inhibition of T cell proliferation by macrophage tryptophan catabolism. J Exp Med 189:1363–1372
23. Yamazaki F, Kuroiwa R, Takikawa O, Kido R (1985) Human indoleamine 2,3-dioxygenase. Its tissue distribution, and characterization of the placental enzyme. Biochem J 230:635–638