·Review·

# Host immune cellular reactions in corneal neovascularization

*Nizar S. Abdelfattah[1,2], Mohamed Amgad[3], Amira A Zayed[4]*

[1]Doheny Eye Institute, University of California, Los Angeles, CA 90033, USA
[2]Ophthalmology Department, David Geffen School of Medicine, University of California, Los Angeles, CA 90033, USA
[3]Faculty of Medicine, Cairo University, Cairo 11956, Egypt
[4]Department of Surgery, Mayo Clinic, Rochester, Minnesota 55904, USA
**Correspondence to:** Nizar S. Abdelfattah. 1355 San Pablo Street, Suite 100, Los Angeles, CA 90033, USA. nizar@ucla.edu
Received: 2015-05-16 Accepted: 2015-06-29

## Abstract

**• Corneal neovascularization (CNV) is a global important cause of visual impairment. The immune mechanisms leading to corneal heme- and lymphangiogenesis have been extensively studied over the past years as more attempts were made to develop better prophylactic and therapeutic measures. This article aims to discuss immune cells of particular relevance to CNV, with a focus on macrophages, Th17 cells, dendritic cells and the underlying immunology of common pathologies involving neovascularization of the cornea. Hopefully, a thorough understanding of these topics would propel the efforts to halt the detrimental effects of CNV.**

• **KEYWORDS:** corneal neovascularization; macrophage; Th17 cells; dendritic cells; herpes simplex keratitis; keratoplasty; angiogenesis; lymphangiogenesis; contact lenses
**DOI:10.18240/ijo.2016.04.25**

Abdelfattah NS, Amgad M, Zayed AA. Host immune cellular reactions in corneal neovascularization. *Int J Ophthalmol* 2016;9(4):625-633

## INTRODUCTION

Aberrant neovascularization (NV) of the cornea is a prevalent cause of visual impairment in all age groups representing a major public health concern worldwide. Angiogenesis is defined as the process of developing new blood vessels from pre-existing ones, up till now, remaining as an incompletely understood process that involves multiple interactions between different immune cell types[1-2].

Optical quality of vascularized corneas is reduced by five mechanisms: 1) opacity caused by the circulating blood cells in the vascular channels, 2) irregular architecture of the vascular walls inducing high-order aberrations, 3) alterations in the spacing of stromal collagen between blood vessels, 4) fluid leakage, edema, and lipid deposition in the tissue surrounding permeable blood vessels, and 5) in the case of superficial pannus, corneal surface irregularity[3-4].

Understanding how different diseases can react on the cellular and molecular level to develop corneal angiogenesis requires reviewing normal immune structure inside the cornea and strategies incorporated in different disease processes.

## PHYSIOLOGIC FOUNDATIONS

### Normal Immune Cells of the Cornea

**Innate defense immunity** When an infection reaches the cornea, it is first confronted by innate immunity [5]. Components of innate immunity are nonspecific first line systems that are present since birth. Physical barriers, such as the bony orbit and the eyelids, guard against traumatic events, while infections are guarded against by more physiologically adjusted corneal elements, including tears, corneal nerves, epithelium, keratocytes, polymorphonuclear cells, and some cytokines.

**Tears** Tears are the physical barrier that flushes foreign bodies out of the corneal surface and mediates transfer of plasma anti-infective proteins (lactoferrin, lysozyme, lipocalin, and beta-lysin) and immunoglobulins to the cornea, thereby locally fighting infection. One of the major highly concentrated immunoglobulins in tears is immunoglobulin (Ig) A. Secretory IgA binds to bacteria and prevents their adherence to epithelium. Tear IgG as well as IgA can neutralize some viruses and bind bacteria and hence sharing in corneal defense[6-7].

**Epithelial cells** Corneal epithelial cells are capable of secreting cytokines to activate immune responses; thus protecting against microbial invasion. When epithelial cells are lysed by infection or trauma, they release the cytokine interleukin (IL)-α [7-9]. This capacity to secrete IL-1α is also shared by stromal keratocytes. When cell lysis occurs on a chronic basis, IL-1α secretion would lead to enhanced immune invasion, inflammation, neovascularization and hence destruction of cornea. Interestingly, in physiological conditions, the corneal epithelium can secrete the soluble and membrane-bound forms of the IL-1α receptor (IL-1RII) [10] which is a natural IL-1α antagonist.

**Keratocytes** Keratocytes have the capacity to secrete IL-6 and defensins after activation by IL-1α and tumor necrosis factor (TNF)-α [11-13]. IL-6 synergizes with these factors as immune modulators. Defensins hold therapeutic potential in ocular infections as they have a broad spectrum of antimicrobial activity (against bacteria, fungi, and viruses) and accelerate epithelial healing[14-15].

**Corneal nerves** Corneal nerves have a physical indirect barrier mechanism of the cornea by receiving sensory information leading to reflex lid closure to protect the eye[16]. Sensations of discomfort and pain may also secrete neuropeptides inducing cytokine action. Moreover, terminal ends of corneal sensory neurons have a chemical barrier mechanism of defense by secreting calcitonin gene-related peptide and substance P in response to pain [17-18]. Both chemicals can bind to epithelial cells and induce IL-8 synthesis leading to neutrophilic influx.

**Complement** The complement system is a strictly organized pathway of proteins that activate each other to generate biologically active enzymes, opsonins, anaphylotoxins, and chemotaxins. Peripheral cornea has more concentrations of all seven complement components as compared to the central cornea due to diffusion of complement components from limbal vessels into the cornea[19].

**Interferons** Interferons (IFN) are a group of cytoprotective proteins made by virally-infected cells, inducing a generalized viro-immune state in the surrounding normal cells [20]. IFN-α is secreted by leucocytes, whereas IFN-β is secreted by fibroblasts, and IFN-γ by T-cells and natural killer (NK) cells. Furthermore, IFNs enhance major histocompatibility complex (MHC) class I molecules production; thereby enhancing capacity of cells infected by viruses to present viral antigens to T-cells[20].

**Cells of innate immunity**

**Neutrophils** Neutrophils are one of the normally encountered cells in the cornea, they move through endothelial cells of the limbal vasculature by diapedesis to act as a critical factor in innate immunity through phagocytosis and microbial killing[21].

**Eosinophils** On the cell membrane of every eosinophil lie surface receptors for IgE and complement components. Activation of these eosinophils can be achieved through IL-3, IL-5, and granulocyte colony-stimulating factor. Moreover, in parasitic infestations, eosinophils release several pertinent granule proteins, such as major basic protein and cationic protein[22].

**Macrophages** Macrophages possess phagocytic and antigen presenting properties as well as the ability to secrete inflammatory cytokines. Having been thought to be residing only in the conjunctiva, macrophages have been recently found in the stroma of mice corneas, contributing to host immune responses[23].

**Natural killer cells** Apart from other T and B-lymphocytes, NK cells lack membrane bound antigen recognition molecules [24]. However, MHC class I molecules are bound to NK cells through surface receptors delivering inhibitory signals to NK cells. Thereby, target cells that lack MHC class I molecules are destroyed immediately by NK cells, as frequently occurs in virally-infected cells, antibody-coated cells, undifferentiated cells, and tumor cells [24-25]. In addition, NK cells can secrete TNF-α and IFN-α.

**Acquired Defenses Immunity** If a microorganism was able to bypass and challenge innate immunity with persistence of infective antigens, cell mediated immunity would take over and bring microbial replication under control. This can be achieved *via* Langerhans cells action and the release of cytokines.

**Langerhans Cells** Langerhans cells are antigen presenting cells of the cornea that are responsible for recognition, processing, and presentation of antigens [26]. They were previously thought to be residing only in the periphery of the cornea and characterized by carrying MHC class II antigens. However, Langerhans cells have been isolated in the central cornea of human infants [27] and, recently, MHC class II-negative Langerhans cells have been demonstrated in the central cornea of BALB/c mice[28] and at the basal epithelium of donor human corneal tissue [26]. Generally speaking, when Langerhans cells are urgently required, recognition and identification of non-self antigen is carried out. Consequently, antigen is processed and is transported to the surface by MHC molecules, either class I or II [29]. T-cell receptor binding to antigen on MHC molecule leads to activation of T-cells. This way maturating T-cells into effector cell, which is CD4 positive if the MHC molecules were class II, or CD8 positive if the MHC were class I. And thereby the T-cells either directly kill foreign microorganisms (CD8 positive T-cytotoxic cells) or secrete cytokines (CD4 positive T-helper cells) calling for chemotaxis of other effector cells, mainly macrophages, leading to lysis of pathogens and activating other inflammatory cascades.

**Cytokines** Cytokines release varies according to the secreting T-cell. Two major subsets of T helper cells, Th1 and Th2, have been described with differential cytokine production profiles [30-31]. A third subset, Th17 has only been recently discovered [32]. Table 1 summarizes the known types and functions of Th cells. The rate limiting factor controlling type of immune response produced is cytokine expression controlled by specific cellular chemotaxis at sites of cellular immune response.

**Immune Privilege and Angiogenic Versus Antiangiogenic Proteins** Cornea has been designated as an "angiogenesis privileged site", that requires low levels of angiogenic factors and high levels of anti-angiogenic factors to maintain its avascularity and hence transparency. Rupture of this

**Table 1 Types of T-helper cells**

| Types | T-helper 1 | T-helper 2 | T-helper 17 |
|---|---|---|---|
| Secretion | IL-2 and IFN-α | IL-4 and IL-5 | IL-17, IL-17F, IL-21, IL-22 |
| Cytolysis | Cytolytic | Non-cytolytic | N/A |
| Antibody production | IgA, IgM, IgG, but not IgE | IgA, IgM, IgG, and IgE | N/A |
| Immune selectivity | Cases of delayed type hypersensitivity and low antibody production | Cases of allergy and persistent antibody production. | Have a role in transplant rejection and autoimmune diseases |

homeostasis in a wide variety of diseases leads significantly to the occurrence of corneal neovascularization[3].

Angiogenesis occurs in tissues when the balance between angiogenic and anti-angiogenic factors is disturbed in favor of angiogenic molecules. It has to be clear that neovascularization requires not only up-regulation of angiogenic factors, but also the down-regulation of anti-angiogenic factors[33].

**Steps of Corneal New Blood and Lymph Vessel Formation** Corneal NV consists of the formation of new vascular structures in previously avascular areas. In an *in-vivo* experimental corneal model, the growth of a capillary involves an ordered sequence of events: the release of angiogenic factors, vascular endothelial cell activation, lysis of the basement membrane of a parent venule, vascular endothelial cell proliferation, migration of capillary endothelial cells towards the angiogenic stimulus, lumen formation, development of branches, and anastomosis of the tip of one tube with another to form a loop[34].

Lymphatic capillaries are blind-ended vessels that are made of a single layer of lymphatic endothelial cells (LEC's), have no basement membrane, are not surrounded by smooth muscles or pericytes and have inter-junctional gaps to allow for entry of immune cells [35-36]. Collecting lymph vessels, on the other hand, are surrounded by smooth muscles, have continuous inter-endothelial junctions, and contain valves to prevent backflow of lymph[35-37].

Whereas blood vessels are responsible for delivering oxygen and nutrients to tissues and disposing of the products of cellular respiration and metabolism, lymphatic vessels return excess fluid, colloid and extravasated leukocytes back into circulation. The presence of lymph nodes along the lymphatic circuit allows for immune clearance of pathogens and provides a niche where antigen-presenting cells interact with vast numbers of lymphocytes, allowing for sensitization against foreign antigens.

Corneal NV can be derived from stroma, which is mainly associated with stromal keratitis. It can also develop from the superficial corneal periphery, which is mainly associated with ocular surface disorders, such as Stevens-Johnson syndrome, ocular pemphigoid, and thermal or chemical burns [38-39]. Although NV may involve several corneal layers, a study has demonstrated that the main locations of vascularized corneal buttons are in the upper and middle third areas of the anterior stroma [40]. Similarly, induced lymphatic vessels are localized to the corneal sub-epithelium and stromal layers in the wounded cornea.

**Selected Immunologic Topics of Particular Relevance to Corneal Neovascularization**

**Role of macrophages in hemangiogenesis** Macrophages are derived from monocytes that exit the bloodstream and niche into peripheral tissues [41]. They are either classically-activated by the Th1 cytokines (M1) or alternatively-activated by Th2 pathway (M2) [42]. M1 macrophages are mostly involved in eliciting the inflammatory response through secretion of matrix metalloproteinases, NO and TNF-α, while M2 are involved in the removal of cell debris, resolving inflammation and wound healing. Thus, it is M2 macrophages which are most relevant to angiogenesis[43-44].

There are three primary mechanisms by which macrophages promote hemangiogenesis: 1) macrophages drill tunnels to facilitate subsequent growth of new capillaries by removing debris (phagocytic function) and degrading connective tissue, thus providing a temporary scaffold for the new vessels[45]; 2) macrophages provide paracrine support for vascular networks that is VEGF-independent ("non-canonical") and physically-interact with blood vessels during vascular remodeling [46]; 3) macrophages act as a major source of epithelial growth factors and angiogenic factors[47].

Macrophages were shown to have a role in angiogenesis in the granulation tissue during the early phases of the repair process. Furthermore, they participate in vascular maturation and stabilization during the latter stages [48]. In fact, M2 macrophages participate in both vascular network formation and neural development [49]. However, macrophages are heterogenic in nature and can express pro as well as anti-angiogenic factors. In a study by Chen *et al*[50], depletion of macrophages as a whole was found not to have apparent effects on alkali-induced CNV; because CCR2- and CX3CR1-expressing macrophages exhibited opposite effects on angiogenesis [51]. Also, accumulation of CX3CR1-positive macrophages intraocularly was found to dampen alkali-induced CNV by producing antiangiogenic factors such as TSP-1 and ADAMTS-1[52].

**Role of macrophages in lymphangiogenesis** Macrophages are known to express a number of markers used for their characterization and localization during experimental assays. These include F4/80, CD 11b and CD 68 [53-55]. LEC's also are

characterized by a number of markers, including Lymphatic Vascular Endothelial Hyaluran Receptor (LYVE-1), a transmembrane receptor first described by Kaipainen *et al*[56]. Other specific LEC markers include Prox-1 [57], which is a transcription factor and Podoplanin, a membrane glycoprotein [58-59]. Having said that, there are three main mechanisms by which macrophages promote lymphangiogenesis: 1) macrophages transdifferentiate into endothelial cells, thus participate-structurally- in lymphatic vessels. This is evidenced by the fact that some F4/80 positive, CD11b positive murine macrophages have been shown to express LYVE-1 *in vitro* and in tumor granulation tissue [60-61]. Moreover, mesenchymal cells co-expressing CD45 (a leukocyte marker) and LYVE and Prox-1 (LEC markers) have been detected at areas of lymphangiogenesis [62-63]. In fact, macrophages alone can form LYVE positive/Podoplanin positive tube-like structures. Nonetheless, some experimental accounts negate this structural role. For example, Runx1-targeted mice (which have defective hematopoeisis) display normal development of lymphatic sacs [63-64]; 2) macrophages secrete paracrine factors, most importantly VEGF-C and VEGF-D which bind to VEGF receptor 3 (VEGFR-3) and activate nuclear factor kappa-B signaling (NFκ-B) in sprouting lymphatics [65]. They also secrete VEGF-A, which acts to promote lymphangiogenesis, both directly (by acting on VEGFR-2) or indirectly by recruiting more macrophages into the site [66-67]; 3) macrophages are found at the tips of sprouting lymphatics and act as "bridge cells" that guide tip cells into finding and anastomosing with tip cells from other sprouting lymphatics. This process is VEGF-independent [68-69].

**Role of Th17 in corneal neovascularization** Th17 is a distinct set of T-helper cells that has been recently discovered and found to have a role in a variety of immune events, including transplant rejection [70]. Th17 cells secrete IL-21, IL-22, IL-17F and, most relevant to our discussion here, IL-17[71-72]. $CD4^+CD25^+Foxp3^+$ Tregs (T-regulatory cells) have a role in "tuning down" the immune response and are thus one of the factors opposing rejection of transplanted organs. Indeed, a higher level of Foxp3 expression in Tregs is correlated with longer graft survival[73].

One mechanism by which Th17 cells participate in transplant rejection is through shifting of the Th1: Treg axis towards the Th1 side [74]. Indeed, IL-17 promotes the recruitment of Th1 cells by inducing the expression of chemokines[75]. Moreover, dual regulation between Th17 cells and Treg cells has been reported in the literature [76-77]. IL-17 has a similar regulatory effect on Th1 cells, by influencing the secretion of IL-12 by antigen-presenting cells [74]. Overall, Th17 cells were found to be more important during the early stages of corneal allograft rejection while Th1 cells were responsible for the late stages[74].

IL-17 stimulates both hemangiogenesis and lymphangiogenesis. It causes macrophages to secrete IL-1β, TNF-α and stromelysin[78]. IL-1β, thereafter, causes migration of vascular endothelial cells and the development of microvessel-like structures [79]. Furthermore, IL-17 has been shown to increase the expression of IL-8, IL-6 and PGE2 and intracellular adhesion molecule-1 (ICAM-1) by fibroblasts and keratinocytes. IL-17 also upregulates the expression of VEGF, KC, MIP-2, PG's and NO by fibroblasts, further supporting the development of new blood vessels[79-83].

More importantly, IL-17 increases VEGF-D secretion and VEGFR-3 expression. Although VEGF-D expression is inhibited by IL-1B (whose secretion is also stimulated by IL-17), the net result of VEGF-D, A and C stimulation of VEGFR-3 is pro-lymphangiogenic. VEGFR-3 stimulation induces LEC proliferation and tube formation[84].

**A Closer Look at Herpes Stromal Keratitis** With most strains of HSV-1, live virus is cleared from the corneal surface within 1wk of infection. During this period, the innate immune system, including neutrophils, NK cells, and γδ T-cells, are activated and recruited to the site of infection within the cornea. Upon entry into the cornea, these cells release cytokines that can inhibit viral replication, but can also cause tissue damage and attract further immune cells to the site[85].

Corneal stromal disease begins after most viral antigens are cleared from the epithelium. Herpes simplex keratitis (HSK) begins to develop 7-10d after murine corneal infection, as indicated by corneal opacity, blood vessel growth into the avascular cornea, and substantial infiltration of leukocytes[86]. Early studies demonstrated that T-cell-deficient mice do not develop HSK, and T-cell adoptive transfer could reconstitute the disease[87-88]. Subsequent studies demonstrated a major role for CD4 T-cells and their Th1 cytokines in mediating HSK [89-90]. Costimulatory interactions, including B7.1/B7.2 on antigen-presenting cells with CD28 on T-cells, in the cornea are required for efficient HSK immunopathology, while OX40-OX40 ligand and CD40-CD40 ligand interactions appear to be dispensable for disease development[91-93].

In addition, CD8 T-cells were shown to mediate a transient form of HSK in the absence of CD4 T-cells, or when mice are infected with certain strains of HSV-1[94-95]. Progression of herpes simplex-1 stromal keratitis immune response following infection runs through the following stages [86]: 1) stage 1: right after infection, innate immune cells are recruited and secrete cytokines and chemokines; infected epithelial cells secrete VEGF; and antigen-presenting cells capture viral antigens before trafficking to draining lymph nodes; 2) stage 2: in response to chemokines, CD4 T-cells infiltrate the cornea and orchestrate a more chronic inflammation dominated by neutrophils and facilitated by ingrowth of blood and lymphatic vessels; 3) stage 3:

recurrent bouts of corneal inflammation result in stromal scarring and subsequent visual loss; 4) stage 4: corneal transplantation may be attempted once scarring occurs, but immunomediated graft rejection is common in hosts with previous HSK. Ideally, future treatments will block HSK pathogenesis before scarring occurs.

**A Closer Look at Corneal Graft Versus Host Disease** Boisgérault *et al* [96] based their work on the fact that corneal allografts are naturally devoid of MHC class II+APCs and minor Ag-mismatched corneal grafts are more readily rejected than their MHC-mismatched counterparts. Accordingly, it has been hypothesized that these transplants do not trigger direct T-cell alloresponse, but that donor Ags are presented by host APCs, *i.e.* in an indirect fashion. So in their study, they determined the Ag specificity, frequency, and phenotype of T-cells activated. They found that in rejecting mice the T-cell response was mediated by two T-cell subsets: 1) CD4 positive T-cells that recognize alloantigens exclusively through indirect pathway and secrete IL-2, and 2) IFN-γ producing CD8 T-cells recognizing donor MHC in a direct fashion. Surprisingly, CD8 positive T-cells activated directly were not required for graft rejection. They concluded that only CD4 positive cells *via* indirect allorecognition have the ability to reject allogeneic corneal grafts. Although alloreactive CD8 positive T-cells activated T-cells via the direct pathway, they are not fully competent and cannot contribute to the rejection unless they receive an additional signal provided by professional APCs in the periphery.

Chen *et al* [50] investigated the role of very late antigen 1 (VLA-1) (also known as integrin receptor α1β1) in CNV, and found that corneal angiogenesis and lymphangiogenesis were both significantly suppressed in VLA-1 knockout mice. After transplantation, both blood [CD31 positive vessels (CD31 positive LYVE-1 negative LYVE-1 positive)] and lymph were significantly decreased in the VLA-1 knockout recipients; improving corneal graft survival. The surprisingly high survival rate in VLA-1-blockade or VLA-1-deficient conditions may be explained by the fact that this molecular pathway is involved in both innate and adaptive aspects of corneal transplantation immunity, since both innate (neutrophil and macrophage) and T-cell infiltrations are suppressed.

Barcia *et al* [97] studies suggest that endothelial destruction during graft rejection may be due to apoptotic cell death. Furthermore, increased expression of anti-apoptotic genes in the corneal endothelium is a potential approach for improving allograft survival. They found that Bcl-xL protected cultured corneal endothelial cells from apoptosis and that lentiviral delivery of Bcl-xL to the corneal endothelium of donor corneas significantly improved the survival of allografts. They observed a significant increase in the survival rate despite a relatively modest (15%) transduction efficiency. Given that stress induces corneal endothelial cells to secrete pro-apoptotic cytokines such as TNF-α, INF-c and IL-1, it is possible that Bcl-xL overexpressing cells do not generate these cytokines and thereby reduce the overall intensity of the apoptotic insult.

Hanson *et al* [98] showed that when human embryonic stem cells were transplanted onto a human corneal button (without limbus) with the epithelial layer partially removed for up to 9d; the transplanted cells established and expanded on Bowman's membrane, forming a 1-4 cell layer surrounded by host corneal epithelial cells. Expression of the corneal marker PAX6 appeared 3d after transplantation, and after 6d, the cells were expressing both PAX6 and CK3, showing that it is possible to transplant cells originating from hESCs onto Bowman's membrane with the epithelial layer partially removed and to get these cells to establish, grow and differentiate into corneal epithelial-like cells *in vitro*.

Shen *et al* [99] showed that PD-L1, but not PD-L2, is constitutively expressed at high levels by the corneal epithelial cells, and at low levels by corneal CD45 positive cells in the stroma, whereas it is undetectable on stromal fibroblasts and corneal endothelial cells. Inflammation induces PD-L1 up-regulation by corneal epithelial cells, and infiltration of significant numbers of PD-L1 positive CD45 positive CD11b positive cells. Blockade with anti-PD-L1 mAb dramatically enhances rejection of C57BL/6 corneal allografts by BALB/c recipients. BALB/c grafts placed in PD-L1-/- C57BL/6 hosts resulted in pronounced T cell priming in the draining lymph nodes, and universally underwent rapid rejection.

**Role of Draining Lymphatics in Corneal Graft Versus Host Disease** Yamagami *et al* [100] transplanted corneas in mice that had their cervical lymph nodes (CLN) excised before transplantation, and compared their IFN-γ and IL-2 expressing cells with mice that retained their CLN. Additionally, they evaluated splenectomized mice (Sp-), and hosts without either CLN or spleen. As a result; 100% of high-risk grafts among CLN positive hosts were rejected, while 92% of CLN negative hosts accepted their high-risk allografts, and demonstrated suppressed allospecific delayed type hypersensitivity response. Moreover, significantly lower numbers of IFN-γ and IL-2 expressing cells were infiltrating corneal grafts in CLN negative group. All Sp- hosts rejected corneal allografts, whereas 86% of CLN-Sp- hosts accepted their allografts. This suggests the idea that draining CLN plays a critical role in alloimmunity and rejection of high-risk corneal grafts.

Afterwards, Jin *et al* [101] proved that passage of corneal APCs through draining LNs is a significant inducer of immune responses. The investigation involved expression and function of chemokine receptor CCR-7 in controlling corneal

APC migration during inflammatory responses. Results have shown that CCR-7 and its ligand CCL-21 expressed significant upregulation in corneal inflammation. According to Geissmann *et al* [102], immature CCR-7 positive dendritic cells (DC) mostly will be poor in stimulating T-cells and thereby can induce a state of immune tolerance. This area of association between CCR-7-mediated corneal DC trafficking and immune stimulation versus tolerance needs supplementary studying. Although normal cornea lacks lymphatic vessels in all its layers, it can easily allow lymphatic growth once exposed to inflammatory stimulation [103]. This neolymphangiogenesis accompanied by normally present conjunctival lymphatics facilitates admission of APCs to corresponding submandibular lymph node [104]. Their finding of CCR7 positive DC close to CCL21 positive and LYVE-1 positive lymphatics suggests that CCL21-CCR7 interactions promote DC access to the lymphoid compartments.

**Role of Dendritic Cells in Keratoplasty and Herpes Simplex Keratitis** Antigen-presenting cells, such as DCs and macrophages, which were previously thought to be absent in the cornea, are now known to be located in the basal layer of the corneal epithelium and throughout the corneal stroma, respectively[105].

When inflammation takes place, maturation of dendritic cells to express MHC class II and B7 (CD80/CD86) co-stimulatory molecules occurs. In keratoplasty, dendritic cells of donor cornea migrate to host cervical lymph nodes through inflamed bed lymphatics, thereby activating host T-cells. This shows clearly the corneal diverse capabilities of antigen presentation methods[106].

During HSV-1 infection of the epithelium, these DCs may play a role in priming the immune response by acquiring viral antigens from infected epithelial cells in the cornea, and directly presenting them to naive T-cells in the lymph nodes, or by becoming infected and migrating to the lymph nodes, where resident lymph node DCs could cross-present viral antigens to T-cells, as seen in the cutaneous HSV-1 infection model [107]. An initial DC infiltration from the limbus at approximately 5d postinfection is followed by a second massive DC infiltration into the cornea at 10d postinfection, coincident with HSK onset[91]. Studies that involved depletion of DCs suggest a role for these cells in the presentation of HSV-1 antigens to CD4 T-cells, which also infiltrate the cornea during HSK. An HSK reactivation model showed a direct correlation between the quantity of DCs in the cornea and corneal opacification[108].

Furthermore, bilateral HSV-1 infection of mice following monocular DC depletion showed HSK development in the non-depleted eye only, indicating that DCs may be involved in the effector phase of the inflammatory response [109]. However, one caveat to these early depletion experiments is uncertainty about the specificity of depletion for DCs and the efficacy in depleting all DC subpopulations. Therefore, further studies employing more specific methods of DC depletion are needed to clarify the role of DCs in HSK. Despite extensive investigations into the mechanisms of CD4 T-cell mediated HSK, the nature of the stimulus/stimuli that activate CD4 T-cells within the corneal stroma remains controversial[86].

## CONCLUSION

Recent discoveries about the roles of immune cells such as macrophages, dendritic cells and Th17 cells in CNV, as well as the underlying immune mechanisms of common CNV-related pathologies uncover exciting and promising new potentials. A thorough understanding of the immunological cells and interactions involved in neovascularization of the cornea is needed to develop better targeted and more potent treatments against CNV.

## ACKNOWLEDGEMENTS

**Conflicts of Interest: Abdelfattah NS,** None; **Amgad M,** None; **Zayed AA,** None.

## REFERENCES

1 Abdelfattah NS, Amgad M, Zayed AA, Salem H, Elkhanany AE, Hussein H, Abd El-Baky N. Clinical correlates of common corneal neovascular diseases: a literature review. *Int J Ophthalmol* 2015;8(1):182-193.

2 Ellenberg D, Azar DT, Hallak JA, Tobaigy F, Han KY, Jain S, Zhou Z, Chang JH. Novel aspects of corneal angiogenic and lymphangiogenic privilege. *Prog Retin Eye Res* 2010;29(3):208-248.

3 Azar DT. Corneal angiogenic privilege: angiogenic and antiangiogenic factors in corneal avascularity, vasculogenesis, and wound healing (an American Ophthalmological Society thesis). *Trans Am Ophthalmol Soc* 2006;104:264-302.

4 Hsu CC, Chang HM, Lin TC, Hung KH, Chien KH, Chen SY, Chen SN, Chen YT. Corneal neovascularization and contemporary antiangiogenic therapeutics. *J Chin Med Assoc* 2015;78(6):323-330.

5 Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. *Nat Immunol* 2015;16(4):343-353.

6 McDermott AM. Antimicrobial compounds in tears. *Exp Eye Res* 2013; 117:53-61.

7 Pearlman E, Sun Y, Roy S, Karmakar M, Hise AG, Szczotka-Flynn L, Ghannoum M, Chinnery HR, McMenamin PG, Rietsch A. Host defense at the ocular surface. *Int Rev Immunol* 2013;32(1):4-18.

8 Niederkorn JY, Peeler JS, Mellon J. Phagocytosis of particulate antigens by corneal epithelial cells stimulates interleukin-1 secretion and migration of Langerhans cells into the central cornea. *Reg Immunol* 1989;2 (2): 83-90.

9 Garreis F, Gottschalt M, Paulsen FP. Antimicrobial peptides as a major part of the innate immune defense at the ocular surface. *Dev Ophthalmol* 2010;45:16-22.

10 Cubitt CL, Lausch RN, Oakes JE. Synthesis of type II interleukin-1 receptors by human corneal epithelial cells but not by keratocytes. *Invest Ophthalmol Vis Sci* 2001;42(3):701-704.

11 Cubitt CL, Lausch RN, Oakes JE. Differences in interleukin-6 gene expression between cultured human corneal epithelial cells and keratocytes. *Invest Ophthalmol Vis Sci* 1995;36(2):330-336.

12 Gottsch JD, Li Q, Ashraf MF, O'Brien TP, Stark WJ, Liu SH. Defensin gene expression in the cornea. *Curr Eye Res* 1998;17(11):1082-1086.

13 Nishida T. Commanding roles of keratocytes in health and disease. *Cornea* 2010;29:S3-S6.

14 Wilson SS, Wiens ME, Smith JG. Antiviral mechanisms of human defensins. *J Mol Biol* 2013;425(24):4965-4980.

15 Zhao L, Lu W. Defensins in innate immunity. *Curr Opin Hematol* 2014; 21(1):37-42.

16 Shaheen BS, Bakir M, Jain S. Corneal nerves in health and disease. *Surv Ophthalmol* 2014;59(3):263-285.

17 Tran MT, Ritchie MH, Lausch RN, Oakes JE. Calcitonin gene-related peptide induces IL-8 synthesis in human corneal epithelial cells. *J Immunol* 2000;164(8):4307-4312.

18 Tran MT, Lausch RN, Oakes JE. Substance P differentially stimulates IL-8 synthesis in human corneal epithelial cells. *Invest Ophthalmol Vis Sci* 2000;41(12):3871-3877.

19 Mondino BJ, Brady KJ. Distribution of hemolytic complement in the normal cornea. *Arch Ophthalmol* 1981;99(8):1430-1433.

20 Hoffmann HH, Schneider WM, Rice CM. Interferons and viruses: an evolutionary arms race of molecular interactions. *Trends Immunol* 2015;36 (3):124-138.

21 Kruger P, Saffarzadeh M, Weber AN, Rieber N, Radsak M, von Bernuth H, Benarafa C, Roos D, Skokowa J, Hartl D. Neutrophils: Between host defence, immune modulation, and tissue injury. *PLoS Pathog* 2015;11(3): e1004651.

22 Trocme SD, Aldave AJ. The eye and the eosinophil. *Surv Ophthalmol* 1994;39(3):241-252.

23 Brissette-Storkus CS, Reynolds SM, Lepisto AJ, Hendricks RL. Identification of a novel macrophage population in the normal mouse corneal stroma. *Invest Ophthalmol Vis Sci* 2002;43(7):2264-2271.

24 Mandal A, Viswanathan C. Natural killer cells: in health and disease. *Hematol Oncol Stem Cell Ther* 2015;8(2):47-55.

25 Moretta L, Bottino C, Pende D, Mingari MC, Biassoni R, Moretta A. Human natural killer cells: their origin, receptors and function. *Eur J Immunol* 2002;32(5):1205-1211.

26 Knickelbein JE, Buela KA, Hendricks RL. Antigen-presenting cells are stratified within normal human corneas and are rapidly mobilized during ex vivo viral infection. *Investig Ophthalmol Vis Sci* 2014;55(2):1118-1123.

27 Chandler JW, Cummings M, Gillette TE. Presence of Langerhans cells in the central corneas of normal human infants. *Invest Ophthalmol Vis Sci* 1985;26(1):113-116.

28 Hamrah P, Zhang Q, Liu Y, Dana MR. Novel characterization of MHC class II-negative population of resident corneal Langerhans cell-type dendritic cells. *Invest Ophthalmol Vis Sci* 2002;43(3):639-646.

29 Nakayama M. Antigen Presentation by MHC-Dressed Cells. *Front Immunol* 2015;5:672.

30 Koyasu S. Natural helper cells and TH2-Type innate immunity. *Cornea* 2012;31:S20-S24.

31 Zhang Y, Zhang Y, Gu W, He L, Sun B. Th1/Th2 cell's function in immune system. *Adv Exp Med Biol* 2014;841:45-65.

32 Bedoya SK, Lam B, Lau K, Larkin J 3rd. Th17 cells in immunity and autoimmunity. *Clin Dev Immunol* 2013;2013:986789.

33 Folkman J, Shing Y. Angiogenesis. *J Biol Chem* 1992;267 (16): 10931-10934.

34 Perlman JI. Ocular disease: mechanisms and management. *JAMA J Am Med Assoc* 2011;306(1):101.

35 Baluk P, Fuxe J, Hashizume H, Romao T, Butz S, Vestweber D, Corada M, Molendini C, Dejana E, McDonald DM. Functionally specialized junctions between endothelial cells of lymphatic vessels. *J Exp Med* 2007; 204(10):2349-2362.

36 Tammela T, Saaristo A, Holopainen T, Lyytikkä J, Kotronen A, Pitkonen M, Abo-Ramadan U, Ylä-Herttuala S, Petrova TV, Alitalo K. Therapeutic differentiation and maturation of lymphatic vessels after lymph node dissection and transplantation. *Nat Med* 2007;13(12):1458-1466.

37 Alitalo K, Tammela T, Petrova TV. Lymphangiogenesis in development and human disease. *Nature* 2005;438(7070):946-953.

38 Tsai RJ, Li LM, Chen JK. Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. *N Engl J Med* 2000; 343(2):86-93.

39 Zheng M, Schwarz MA, Lee S, Kumaraguru U, Rouse BT. Control of stromal keratitis by inhibition of neovascularization. *Am J Pathol* 2001;159 (3):1021-1029.

40 Cursiefen C, Maruyama K, Jackson DG, Streilein JW, Kruse FE. Time course of angiogenesis and lymphangiogenesis after brief corneal inflammation. *Cornea* 2006;25(4):443-447.

41 Coffelt SB, Hughes R, Lewis CE. Tumor-associated macrophages: effectors of angiogenesis and tumor progression. *Biochim Biophys Acta* 2009;1796(1):11-18.

42 Edwards JP, Zhang X, Frauwirth KA, Mosser DM. Biochemical and functional characterization of three activated macrophage populations. *J Leukoc Biol* 2006;80(6):1298-1307.

43 Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. *Immunity* 2010;32(5):593-604.

44 Mosser DM. The many faces of macrophage activation. *J Leukoc Biol* 2003;73(2):209-212.

45 Moldovan NI, Goldschmidt-Clermont PJ, Parker-Thornburg J, Shapiro SD, Kolattukudy PE. Contribution of monocytes/macrophages to compensatory neovascularization: the drilling of metalloelastase-positive tunnels in ischemic myocardium. *Circ Res* 2000;87(5):378-384.

46 Okuno Y, Nakamura-Ishizu A, Kishi K, Suda T, Kubota Y. Bone marrow-derived cells serve as proangiogenic macrophages but not endothelial cells in wound healing. *Blood* 2011;117(19):5264-5272.

47 Chung ES, Chauhan SK, Jin Y, Nakao S, Hafezi-Moghadam A, van Rooijen N, Zhang Q, Chen L, Dana R.Contribution of macrophages to angiogenesis induced by vascular endothelial growth factor receptor-3-specific ligands. *Am J Pathol* 2009;175(5):1984-1992.

48 Lucas T, Waisman A, Ranjan R, Roes J, Krieg T, Müller W, Roers A, Eming SA. Differential roles of macrophages in diverse phases of skin repair. *J Immunol* 2010;184(7):3964-3677.

49 Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG. Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. *J Neurosci* 2009;29(43):13435-13444.

50 Chen L, Huq S, Gardner H, de Fougerolles AR, Barabino S, Dana MR. Very late antigen 1 blockade markedly promotes survival of corneal allografts. *Arch Ophthalmol* 2007;125(6):783-788.

51 Lu P, Li L, Liu G, van Rooijen N, Mukaida N, Zhang X. Opposite roles of CCR2 and CX3CR1 macrophages in alkali-induced corneal neovascularization. *Cornea* 2009;28(5):562-569.

52 Lu P, Li L, Kuno K, Wu Y, Baba T, Li YY, Zhang X, Mukaida N. Protective roles of the fractalkine/CX3CL1-CX3CR1 interactions in alkali-induced corneal neovascularization through enhanced antiangiogenic factor expression. *J Immunol* 2008;180(6):4283-4291.

53 Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. *Cancer Res* 2006;66(2):605-612.

54 Ahn GO, Tseng D, Liao CH, Dorie MJ, Czechowicz A, Brown JM. Inhibition of Mac-1 (CD11b/CD18) enhances tumor response to radiation by reducing myeloid cell recruitment. *Proc Natl Acad Sci U S A* 2010;107 (18):8363-8368.

55 Holness CL, Simmons DL. Molecular cloning of CD68, a human macrophage marker related to lysosomal glycoproteins. *Blood* 1993;81(6): 1607–1613.

56 Kaipainen A, Korhonen J, Mustonen T, van Hinsbergh VW, Fang GH, Dumont D, Breitman M, Alitalo K. Expression of the fms–like tyrosine kinase 4 gene becomes restricted to lymphatic endothelium during development. *Proc Natl Acad Sci USA* 1995;92(8):3566–3570.

57 Wigle JT, Oliver G. Prox1 function is required for the development of the murine lymphatic system. *Cell* 1999;98(6):769–778.

58 Breiteneder–Geleff S, Soleiman A, Kowalski H, Horvat R, Amann G, Kriehuber E, Diem K, Weninger W, Tschachler E, Alitalo K, Kerjaschki D. Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: podoplanin as a specific marker for lymphatic endothelium. *Am J Pathol* 1999;154(2):385–394.

59 Schacht V, Ramirez MI, Hong YK, Hirakawa S, Feng D, Harvey N, Williams M, Dvorak AM, Dvorak HF, Oliver G, Detmar M. T1alpha/podoplanin deficiency disrupts normal lymphatic vasculature formation and causes lymphedema. *EMBO J* 2003;22(14):3546–3556.

60 Schledzewski K, Falkowski M, Moldenhauer G, Metharom P, Kzhyshkowska J, Ganss R, Demory A, Falkowska–Hansen B, Kurzen H, Ugurel S, Geginat G, Arnold B, Goerdt S. Lymphatic endothelium–specific hyaluronan receptor LYVE–1 is expressed by stabilin–1+, F4/80+, CD11b+ macrophages in malignant tumours and wound healing tissue in vivo and in bone marrow cultures in vitro: implications for the assessment of lymphangiogene. *J Pathol* 2006;209(1):67–77.

61 Ji RC, Eshita Y, Kato S. Investigation of intratumoural and peritumoural lymphatics expressed by podoplanin and LYVE–1 in the hybridoma–induced tumours. *Int J Exp Pathol* 2007;88(4):257–270.

62 Buttler K, Kreysing A, von Kaisenberg CS, Schweigerer L, Gale N, Papoutsi M, Wilting J. Mesenchymal cells with leukocyte and lymphendothelial characteristics in murine embryos. *Dev Dyn* 2006;235 (6):1554–1562.

63 Buttler K, Ezaki T, Wilting J. Proliferating mesodermal cells in murine embryos exhibiting macrophage and lymphendothelial characteristics. *BMC Dev Biol* 2008;8:43.

64 Srinivasan RS, Dillard ME, Lagutin OV, Lin FJ, Tsai S, Tsai MJ, Samokhvalov IM, Oliver G. Lineage tracing demonstrates the venous origin of the mammalian lymphatic vasculature. *Genes Dev* 2007;21(19):2422–2432.

65 Handa O, Naito Y, Takagi T, Shimozawa M, Kokura S, Yoshida N, Matsui H, Cepinskas G, Kvietys PR, Yoshikawa T. Tumor necrosis factor–alpha–induced cytokine–induced neutrophil chemoattractant–1 (CINC–1) production by rat gastric epithelial cells: role of reactive oxygen species and nuclear factor–kappaB. *J Pharmacol Exp Ther* 2004;309 (2): 670–676.

66 Hong YK, Lange–Asschenfeldt B, Velasco P, Hirakawa S, Kunstfeld R, Brown LF, Bohlen P, Senger DR, Detmar M. VEGF–A promotes tissue repair–associated lymphatic vessel formation via VEGFR–2 and the alpha1beta1 and alpha2beta1 integrins. *FASEB J* 2004;18 (10): 1111–1113.

67 Cursiefen C, Chen L, Borges LP, Jackson D, Cao J, Radziejewski C, D'Amore PA, Dana MR, Wiegand SJ, Streilein JW. VEGF–A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment. *J Clin Invest* 2004;113(7): 1040–1050.

68 Fantin A, Vieira JM, Gestri G, Denti L, Schwarz Q, Prykhozhij S, Peri F, Wilson SW, Ruhrberg C. Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF–mediated endothelial tip cell induction. *Blood* 2010;116(5):829–840.

69 Schmidt T, Carmeliet P. Blood–vessel formation: Bridges that guide and unite. *Nature* 2010;465(7299):697–679.

70 Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM. Th17: an effector CD4 T cell lineage with regulatory T cell ties. *Immunity* 2006;24(6):677–688.

71 Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT. Transforming growth factor–beta induces development of the T (H)17 lineage. *Nature* 2006;441(7090):231–234.

72 Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, Levy DE, Leonard WJ, Littman DR. IL–6 programs T (H)–17 cell differentiation by promoting sequential engagement of the IL–21 and IL–23 pathways. *Nat Immunol* 2007;8(9):967–974.

73 Chauhan SK, Saban DR, Lee HK, Dana R. Levels of Foxp3 in regulatory T cells reflect their functional status in transplantation. *J Immunol* 2009; 182(1):148–153.

74 Chen H, Wang W, Xie H, Xu X, Wu J, Jiang Z, Zhang M, Zhou L, Zheng S. A pathogenic role of IL– 17 at the early stage of corneal allograft rejection. *Transpl Immunol* 2009;21(3):155–161.

75 Khader SA, Bell GK, Pearl JE, Fountain JJ, Rangel–Moreno J, Cilley GE, Shen F, Eaton SM, Gaffen SL, Swain SL, Locksley RM, Haynes L, Randall TD, Cooper AM. IL–23 and IL–17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during Mycobacterium tuberculosis challenge. *Nat Immunol* 2007;8(4):369–377.

76 Yang XO, Nurieva R, Martinez GJ, Kang HS, Chung Y, Pappu BP, Shah B, Chang SH, Schluns KS, Watowich SS, Feng XH, Jetten AM, Dong C. Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. *Immunity* 2008;29(1):44–56.

77 Osorio F, LeibundGut–Landmann S, Lochner M, Lahl K, Sparwasser T, Eberl G, Reis e Sousa C. DC activated via dectin–1 convert Treg into IL–17 producers. *Eur J Immunol* 2008;38(12):3274–3281.

78 Jovanovic DV, Di Battista JA, Martel–Pelletier J, Jolicoeur FC, He Y, Zhang M, Mineau F, Pelletier JP. IL–17 stimulates the production and expression of proinflammatory cytokines, IL–beta and TNF–alpha, by human macrophages. *J Immunol* 1998;160(7):3513–3521.

79 Numasaki M, Fukushi J, Ono M, Narula SK, Zavodny PJ, Kudo T, Robbins PD, Tahara H, Lotze MT. Interleukin–17 promotes angiogenesis and tumor growth. *Blood* 2003;101(7):2620–2627.

80 Yao Z, Fanslow WC, Seldin MF, Rousseau AM, Painter SL, Comeau MR, Cohen JI, Spriggs MK. Herpesvirus Saimiri encodes a new cytokine, IL–17, which binds to a novel cytokine receptor. *Immunity* 1995;3 (6): 811–821.

81 Fossiez F, Djossou O, Chomarat P, Flores–Romo L, Ait–Yahia S, Maat C, Pin JJ, Garrone P, Garcia E, Saeland S, Blanchard D, Gaillard C, Das Mahapatra B, Rouvier E, Golstein P, Banchereau J, Lebecque S. T cell interleukin–17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *J Exp Med* 1996;183(6):2593–2603.

82 Yao Z, Painter SL, Fanslow WC, Ulrich D, Macduff BM, Spriggs MK, Armitage RJ. Human IL–17: a novel cytokine derived from T cells. *J Immunol* 1995;155(12):5483–5486.

83 Aarvak T, Chabaud M, Miossec P, Natvig JB. IL–17 is produced by some proinflammatory Th1/Th0 cells but not by Th2 cells. *J Immunol* 1999;162(3):1246–1251.

84 Chauhan SK, Jin Y, Goyal S, Lee HS, Fuchsluger TA, Lee HK, Dana R. A novel pro–lymphangiogenic function for Th17/IL–17. *Blood* 2011;118 (17):4630–4634.

85 Biswas PS, Rouse BT. Early events in HSV keratitis–setting the stage for a blinding disease. *Microbes Infect* 2005;7(4):799–810.

86 Knickelbein JE, Buela K, Hendricks RL. Herpes stromal keratitis: erosion of ocular immune privilege by herpes simplex virus. *Future Virology* 2010;5(6):699-708.

87 Metcalf JF, Hamilton DS, Reichert RW. Herpetic keratitis in athymic (nude) mice. *Infect Immun* 1979;26(3):1164-1171.

88 Russell RG, Nasisse MP, Larsen HS, Rouse BT. Role of T-lymphocytes in the pathogenesis of herpetic stromal keratitis. *Invest Ophthalmol Vis Sci* 1984;25(8):938-944.

89 Hendricks RL, Tumpey TM. Contribution of virus and immune factors to herpes simplex virus type I-induced corneal pathology. *Invest Ophthalmol Vis Sci* 1990;31(10):1929-1939.

90 Hendricks RL, Tumpey TM, Finnegan A. IFN-gamma and IL-2 are protective in the skin but pathologic in the corneas of HSV-1-infected mice. *J Immunol* 1992;149(9):3023-3028.

91 Chen H, Hendricks RL. B7 costimulatory requirements of T cells at an inflammatory site. *J Immunol* 1998;160(10):5045-5052.

92 Lepisto AJ, Xu M, Yagita H, Weinberg AD, Hendricks RL. Expression and function of the OX40/OX40L costimulatory pair during herpes stromal keratitis. *J Leukoc Biol* 2007;81(3):766-774.

93 Xu M, Lepisto AJ, Hendricks RL. CD154 signaling regulates the Th1 response to herpes simplex virus-1 and inflammation in infected corneas. *J Immunol* 2004;173(2):1232-1239.

94 Ghiasi H, Cai S, Perng GC, Nesburn AB, Wechsler SL. Both CD4+ and CD8+ T cells are involved in protection against HSV-1 induced corneal scarring. *Br J Ophthalmol* 2000;84(4):408-412.

95 Lepisto AJ, Frank GM, Xu M, Stuart PM, Hendricks RL. CD8 T cells mediate transient herpes stromal keratitis in CD4-deficient mice. *Invest Ophthalmol Vis Sci* 2006;47(8):3400-3409.

96 Boisgérault F, Liu Y, Anosova N, Ehrlich E, Dana MR, Benichou G. Role of CD4+ and CD8+ T cells in allorecognition: lessons from corneal transplantation. *J Immunol* 2001;167(4):1891-1899.

97 Barcia RN, Dana MR, Kazlauskas A. Corneal Graft Rejection Is Accompanied by Apoptosis of the Endothelium and Is Prevented by Gene Therapy With Bcl-xL. *Am J Transplant* 2007;7(9):2082-2089.

98 Hanson C, Hardarson T, Ellerström C, Nordberg M, Caisander G, Rao M, Hyllner J, Stenevi U. Transplantation of human embryonic stem cells onto a partially wounded human cornea in vitro. *Acta Ophthalmol* 2013;91 (2):127-130.

99 Shen L, Jin Y, Freeman GJ, Sharpe AH, Dana MR. The function of donor vs recipient programmed death lignad 1 in corneal allograft survival. *J Immunol* 2007;179(6):3672-3679.

100 Yamagami S, Dana MR, Tsuru T. Draining lymph nodes play an essential role in alloimmunity generated in response to high-risk corneal transplantation. *Cornea* 2002;21(4):405-409.

101 Jin Y, Shen L, Chong EM, Hamrah P, Zhang Q, Chen L, Dana MR. The chemokine receptor CCR7 mediates corneal antigen- presenting cell trafficking. *Mol Vis* 2007;13:626-634.

102 Geissmann F, Dieu-Nosjean MC, Dezutter C, Valladeau J, Kayal S, Leborgne M, Brousse N, Saeland S, Davoust J. Accumulation of immature Langerhans cells in human lymph nodes draining chronically inflamed skin. *J Exp Med* 2002;196(4):417-430.

103 Dana R. Corneal antigen presentation: molecular regulation and functional implications. *Ocul Surf* 2005;3(4 Suppl):S169-S172.

104 Liu Y, Hamrah P, Zhang Q, Taylor AW, Dana MR. Draining lymph nodes of corneal transplant hosts exhibit evidence for donor major histocompatibility complex (MHC) class II-positive dendritic cells derived from MHC class II-negative grafts. *J Exp Med* 2002;195(2):259-268.

105 Wilson NS, Villadangos JA. Lymphoid organ dendritic cells: beyond the Langerhans cells paradigm. *Immunol Cell Biol* 2004;82(1):91-98.

106 Hamrah P, Huq SO, Liu Y, Zhang Q, Dana MR. Corneal immunity is mediated by heterogeneous population of antigen-presenting cells. *J Leukoc Biol* 2003;74(2):172-178.

107 Allan RS, Waithman J, Bedoui S, Jones CM, Villadangos JA, Zhan Y, Lew AM, Shortman K, Heath WR, Carbone FR. Migratory dendritic cells transfer antigen to a lymph node-resident dendritic cell population for efficient CTL priming. *Immunity* 2006;25(1):153-162.

108 Miller JK, Laycock KA, Nash MM, Pepose JS. Corneal Langerhans cell dynamics after herpes simplex virus reactivation. *Invest Ophthalmol Vis Sci* 1993;34(7):2282-2290.

109 Hendricks RL, Janowicz M, Tumpey TM. Critical role of corneal Langerhans cells in the CD4- but not CD8-mediated immunopathology in herpes simplex virus-1-infected mouse corneas. *J Immunol* 1992;148(8): 2522-2529.