Background: The normal corneal stroma is endowed with large numbers of resident dendritic cells (DCs). The purpose of this study was to examine the phenotype and distribution of these cells in inflammation.

Methods: Normal and inflamed murine corneas were excised at different time points and immunofluorescence staining with multiple antibodies was performed by confocal microscopy on whole-mounted corneal stromas to characterize and evaluate the distribution of DCs in inflammation.

Results: CD11c+CD11b+ myeloid DCs were present throughout the anterior stroma. In the periphery of the normal cornea, nearly one half were major histocompatibility complex (MHC) class II+CD80+CD86+, while they were uniformly MHC class II−CD80−CD86− in the center. In inflammation, in addition to a significant increase in the number of DCs, a majority up-regulated their expression of MHC class II, CD80, and CD86, indicating their state of maturation. The up-regulation of MHC class II and costimulatory molecules on DCs was seen as early as 24 hours after induction of inflammation or transplantation. In addition to the CD11c+DCs in the anterior stroma, a CD11c−CD11b+ population of monocytes/macrophages was present almost exclusively in the posterior stroma of the cornea. These cells were found throughout all layers of the stroma, and in increased numbers, after induction of inflammation.

Conclusion: The present study demonstrates, for the first time, the phenotypic changes and distribution of resident stromal DCs in corneal inflammation.

Clinical Relevance: These novel data suggest that the cornea is capable of participating in immune and inflammatory responses by virtue of its own heterogeneous population of DCs.

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observed in the normal corneal stroma or epithelium of several species. Various forms of trauma to the cornea, including cauterezation, placement of sutures, and injection of polystyrene beads into the stroma, have been shown to result in the presence of MHC class II+ DCs in the central cornea, which were presumed to be entirely due to de novo migration of DCs into the tissue. However, this paradigm for corneal DC recruitment has recently been profoundly revised as new data have established the presence of significant numbers of CD45+ resident BM-derived cells, including immature or precursor DCs in the normal cornea. In the epithelium, we have characterized a population of resident MHC class II+ Langerhans cells (LCs) in the center of the normal cornea that become activated after inflammation. Brissette-Storkus et al recently identified a population of resident macrophages in the normal stroma, while we have demonstrated that BM-derived cells in the stroma consist of several subsets: a large population of resident myeloid DCs in the anterior stroma, and macrophages in the posterior stroma. The presence and phenotype of leukocytic cells in the cornea, including APC and DC populations, have important implications for a variety of physiologic and pathologic responses, including wound healing, because it focuses on the cornea as a participant in immune and inflammatory responses rather than the stroma being essentially a collagen-based tissue that simply responds to infiltrating cells. This study was undertaken to determine how the distribution, phenotype, and maturation states of stromal BM-derived DCs are altered in inflammation.

**METHODS**

**EXPERIMENTAL ANIMALS**

Seven- to 14-week-old male BALB/c mice (Taconic, Germantown, NY, or from our own breeding facility) were used in these experiments. All protocols were approved by the Schepens Eye Research Institute Animal Care and Use Committee, and all animals were treated according to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

**CAUTERIZATION OF CORNEAL SURFACE**

Animals were deeply anesthetized with an intraperitoneal injection of 3 to 4 mg of ketamine hydrochloride and 0.1 mg of xylazine hydrochloride and were placed under the operating microscope. With the tip of a handheld thermal cautery (Aaron Instruments Co, St Louis, Mo), followed by application of antibiotic ointment. Transplanted corneas were excised at 2, 6, 12, and 24 hours after surgery and examined in immunohistochemical studies.

**ANTIBODIES**

The immunohistochemical staining procedures were performed with the following antibodies: fluorescein isothiocyanate (FITC)-conjugated mouse anti–mouse class II MHC (AF6-120, anti-Iaα); FITC-conjugated mouse anti–mouse class II MHC (30-10-8, anti-Iaβ); purified hamster anti–mouse CD11c (HL3, dendritic cell marker); FITC-conjugated rat anti–mouse CD8α (53-6.7, lymphoid DC marker); FITC-conjugated hamster anti–mouse CD3-e (145-2C11, T-lymphocyte marker); FITC-conjugated rat anti–mouse CD11b (M1/70, monocye/macrophage marker); purified rat anti–mouse CD45 (30-F11, panleukocyte marker); FITC-conjugated hamster anti–mouse CD80 (16-10A1, B7-1); phycocerythrin (PE)–conjugated rat anti–mouse (GL1, B7-2); FITC-conjugated rat anti–mouse GR-1 (RB6-8C5, neutrophil marker); and rat anti–mouse CD16/CD32 (2.4G2, FcyIII/II receptor). The secondary antibodies were Cy3-conjugated goat anti–mouse F(ab')2, rhodamine-conjugated goat anti–rat IgG (Santa Cruz Biotechnology, Inc, Santa Cruz, Calif), and Cy5-conjugated goat anti–Armenian hamster IgG. Isotype controls included rat IgG2a, rat IgG2b-FITC, rat IgG1, rat IgG1-PE, rat IgG2a-FITC, hamster IgG, hamster IgG-FITC, mouse IgG1-FITC, and mouse IgG2b-FITC. All primary and secondary monoclonal antibodies (except where noted) and isotype-matched controls were purchased (Pharmingen, San Diego, Calif).

**IMMUNOFLUORESCENCE CONFOCAL MICROSCOPY AND IMMUNOHISTOCHEMISTRY**

Corneas were excised and the epithelium was removed from the stroma by a modification of a technique previously described. Briefly, freshly excised corneas were immersed in PBS containing 20mM EDTA (Sigma-Aldrich Corp, St Louis, Mo) at 37°C for 1 hour. The epithelium was then removed from the underlying stroma by means of forceps and only the stroma was subjected to immunofluorescence staining and initially washed in PBS. Corneal tissue, deprived of the epithelium, was fixed in acetone for 15 minutes at room temperature for immunofluorescence staining. Corneas were then incubated in 2% bovine serum albumin diluted in PBS for 15 minutes. To block nonspecific staining, sections were blocked with anti-FcR monoclonal antibody (CD16/CD32) for 30 minutes before they were immunostained with primary antibodies or isotype-matched control antibodies for 2 hours. Afterward, corneal tissues were incubated with a second FITC- or PE-conjugated primary antibody or with secondary antibodies. All staining procedures were performed at room temperature, and 3 thorough washings in PBS followed every step for 5 minutes each. Finally, corneas were covered with a mounting medium and analyzed by a confocal microscope (Leica TCS 4D; Lasertechnik, Heidelberg, Germany) as described before. At least 3 to 5 different corneas were examined per each double-staining experiment; representative data are presented throughout the “Results” section. Five to 8 different fields were analyzed for each specimen with the use of a grid and the numbers were averaged. The paired t test was used to compare the number of positively labeled stromal cells in different areas of the stroma. P<.05 was considered significant.
EXPRESSION OF MATURATION MARKERS DURING INFLAMMATION BY RESIDENT DCs IN THE CORNEAL STROMA

Corneas were excised 2 weeks after cauterization to assess the distribution and phenotype of stromal DCs. The epithelium was removed to deprive the cornea of epithelial LCs before staining. Immunofluorescence confocal microscopy of whole-mount normal corneas, deprived of the epithelium and stained with CD11c (DC marker) and MHC class II, demonstrated DCs throughout the periphery and center of the anterior stroma. Some DCs in the periphery expressed MHC class II (Figure 1A), while DCs in the central and paracentral areas of the stroma were uniformly MHC class II− (Figure 1B). Results similar to those for MHC class II were obtained for CD80 (B7-1) and CD86 (B7-2) (data not shown). In addition, staining with the CD11c isotype control showed no staining (Figure 1C). In inflamed corneas, the number of DCs expressing MHC class II in the periphery had increased significantly (Figure 2A), as shown by double-staining with CD11c and MHC class II. The MHC class II+ DCs were now also present in the paracentral areas and the center of the stroma (Figure 2B). In inflamed corneas, surface expression of CD80 and CD86 was similarly increased for peripheral DCs (Figure 2C) and was also present on DCs in the central areas of the stroma (Figure 2D). All CD11c+ DCs were CD11b+, but did not stain for GR-1, CD3, or CD8a, and therefore represented myeloid DCs from a monocytic lineage (lymphoid, vs myeloid, DCs are CD80+/CD86+ DCs in the inflamed cornea was significantly higher (almost twice as high) than the increase in the number of CD11c+ cells in these areas, indicating up-regulation of these markers in resident DCs in addition to the large recruitment of new DCs into the cornea. Specifically, at day 14 after cauterization, the total number of CD11c+ cells in the center increased by 60/mm² from the normal cornea to the inflamed cornea, while the number of MHC class II+ cells in the center increased by 120/mm², suggesting that recruitment of cells into the corneal stroma was not entirely responsible for changes in MHC class II expression. A conceptual model for the dis-
Maturation of Resident DCs

To confirm that MHC class II+ DCs represent resident DCs that mature in inflammation and not simply those recruited de novo from the limbal areas, we analyzed corneas at earlier time points, days 1, 3, and 7 after cautery. CD11c and CD45 (leukocyte common marker) double-staining with MHC class II showed that a subset of DCs in the center of the cornea expressed MHC class II as early as day 1 after cautery (Figure 5A), whereas the paracentral areas away from the cautery sites remained MHC class II−, indicating up-regulation of this marker in resident DCs. By days 3 and 7 after cautery, these paracentral sites also contained MHC class II+ cells. Similarly, up-regulation of B7 (CD80, CD86) costimulatory markers on resident DCs was seen around cautery sites at the paracentral areas of the cornea at day 1 when we double-stained corneas for CD11c and B7 expression (Figure 5B).

To further eliminate any doubt that the surface expression of MHC class II and B7 costimulatory molecules in inflammation is largely through up-regulation by resident cells, and not solely due to influx of new leu-

Figure 2. Expression of maturation markers by stromal dendritic cells (DCs) in the central cornea in corneal inflammation. A, In inflamed eyes, higher numbers of CD11c+ cells are seen throughout the corneal stroma, with many coexpressing major histocompatibility complex class II (yellow). B, The inflamed corneal center also contains MHC class II+ DCs. Double-staining of inflamed whole-mounted corneal stromas with CD11c (red) and CD80 (green) shows large numbers of DCs in the periphery (C) and center of the cornea that coexpress the CD80 costimulatory molecule (yellow) (D) (original magnification ×160 [A and C], ×400 [B and D]).
kocytes, we performed double-staining for the DC marker CD11c and donor-type MHC class II of C57BL/6 mice \((Iab)\) at different time points after corneal transplantation into BALB/c \((Ia\ d)\) recipients. Data showed a large number of resident CD11c+ DCs in the donor button of the ungrafted corneas that were MHC class II− (Figure 6A). At 2, 6, and 12 hours after surgery, corneas still did not show any expression for donor class II (Figure 6B). However, by 24 hours after transplantation, novel donor class II \((Ia\ b)\) expression could be detected close to the graft-host junction (Figure 6C).

**COMMENT**

For many years, the LCs of the epithelium in the limbal area were thought to be the only cells that constitutively express MHC class II molecules in the cornea. This led to the widely accepted dogma that APCs are virtually absent from the central regions of the cornea.9-18 Recently, however, data from our group demonstrated that the cornea contains resident APCs that are universally MHC class II− but are capable of migrating to draining lymph nodes of allografted hosts.32 The location, exact phenotype, and distribution of these cells in the cornea remained unknown, however, although some of these cells clearly resided in the epithelium.33 Recently, Brissette-Storkus et al34 showed that BM-derived cells also reside in the normal uninflamed murine stroma, but they identified these as macrophages and not DCs. More recent data from our laboratory suggest that the normal anterior stroma is indeed endowed with large numbers of CD11c+CD11b− resident myeloid DCs from a monocytic lineage that are immature and MHC class II−CD80−CD86− in the stroma center,35 in addition to CD11c+CD11b+ macrophage-type cells in the posterior stroma,33 similar to cells described by Brissette-Storkus et al. There are some differences in the methods of our studies that may have led to the different outcomes. First, although both studies used corneal flat-mounts, we fixed the corneas with acetone, while their laboratory used 1% paraformaldehyde for fixation. Second, our laboratories used different CD11c antibodies. In addition, while we used Cy5 as a secondary antibody, they used streptavidin–horseradish peroxidase and fluorophore tyramide. Nevertheless, our data should not be seen as necessarily refuting the observations of Brissette-Storkus et al. Indeed, it is interesting that Brissette-Storkus et al described 2 populations of BM-derived cells—half being F4/80+ and the other half, F4/ 80−. Since F4/80 is used as a marker for macrophages and is often negative on DCs, most likely the cells described by their laboratory relate to the macrophage population that we have confirmed is present in the posterior stroma.33

**Figure 3.** The distribution of dendritic cells (DCs) and macrophages in normal and inflamed corneas. Optically stacked sections of the anterior (A and D), middle (B and E), and posterior stromal (C and F) layers show different subsets of cells. Normal corneas double-stained with CD11c (red) and CD11b (green) show that the anterior stroma exclusively contains CD11c+CD11b− DCs (yellow) (A). The middle portions of the stroma harbor a negligible amount of DCs (B). The posterior stroma, however, contains only CD11c+CD11b− monocytes/macrophages (C) in the normal cornea. Two weeks after cautery, CD11c+CD11b− (yellow) are still limited to the anterior stroma, while CD11c+CD11b+ macrophages (green) are also present in this region (D). Monocytes/macrophages are also present in the midstroma in inflammation (E) and in increased numbers in the posterior stroma (F) (original magnification ×400).
In contrast to the DC population in the peripheral limbus, which is the focus of the present study.

Using an immunofluorescence double-staining technique applied to confocal microscopy, we herein describe alterations to BM-derived cells present in the corneal stroma. While, in the normal cornea, MHC class II and B7 molecule (CD80 and CD86)—positive cells can be found solely in the periphery and limbus, in inflammation the expression of these surface molecules is potently up-regulated in the center of the corneal stroma. This up-regulation is observed in inflamed corneas as early as 24 hours after induction of inflammation, first near the cautery sites and later at days 3, 7, and 14, throughout the cornea. Our data suggest that the increased density of MHC class II+ and B7+ cells, especially in the center of the stroma, is mostly due to up-regulation of these cell surface markers on resident DCs, although cells migrating into the cornea from the limbus also contribute to their presence, as reflected by the increase in the number of cells that express CD11c. At 24 hours after cauterization, MHC class II+ and B7+ cells were initially detected only around cautery sites in the corneal center, while the peripheral sites were still negative for these maturation markers, suggesting that cells expressing these markers were not simply due to influx of mature DCs from the peripheral limbus.

Finally, in the transplantation experiments described, we detected novel expression of donor-type MHC class II/II antigens among the graft’s CD11c+ cells as early as 24 hours after transplantation, while at earlier time points the corneas did not express donor MHC class II. This suggests that recruitment of cells from the host periphery (that would express host MHC class II) could not entirely explain the up-regulation of MHC in the graft, as previously proposed.\textsuperscript{35,46} The negative expression of donor MHC class II at early time points after transplantation and in ungrafted donor buttons rules out contamination as a cause of this expression.

The first step in the generation of inflammation is an inciting stimulus that may be microbial, traumatic, or due to introduction of a novel antigen (eg, as occurs in
tissue allotransplantation). These stimuli may lead to release of proinflammatory cytokines, nucleic acid fragments, heat shock protein, and a variety of other mediators that in the aggregate signal the host that normal physiologic conditions and the microenvironment have been violated. In response to these signals, the second step in the cascade of events occurs when local (resident) tissue cells activate signal transduction pathways (eg, nuclear factor κB) that augment or down-modulate these cells’ expression of cytokine genes and/or cytokine receptor genes, which in turn dictate the response of these resident cells to paracrine signals in the microenvironment by other cells in the proximity.57 These responses are not limited to classic immunoinflammatory mediators (eg, interleukins and interferons) but also include other molecular classes such as growth factors, chemokines, and adhesion factors, which act in a coordinated fashion to regulate the immune/inflammatory response and induce the immigration of inflammatory cells, including APCs. Resident cells of the cornea are not just targets of cytokine-mediated responses but, rather, active components of the cytokine network. Their cytokines influence surrounding cells and attract other BM-derived cell types, including DCs, to the area by controlling the expression of cell adhesion molecules and by providing directionality to leukocytes through chemotactic cytokines, also known as chemokines.

Candidate molecules that are known to up-regulate expression of MHC class II and costimulatory molecules, and induce the maturation and migration of DCs, include IL-1, granulocyte-macrophage colony-stimulating factor (GM-CSF), TNF-α, CD40L, and lipopolysaccharide.1,48-50 While GM-CSF and IL-3 enhance DC differentiation,1,52 TNF-α and CD40L stimulate the final maturation of DCs.1,46 Interleukin 1 signals IL-1 receptors to up-regulate GM-CSF receptors, establishing responsiveness of DCs to GM-CSF for growth, viability, and function.31 Interleukin 1 and TNF-α are cross-regulated closely in multiple models of inflammation, and stimulation of central corneal tissue results in both IL-1 and TNF-α expression from resident corneal cells.52 Moreover, both IL-1 and TNF-α regulate GM-CSF gene expression in corneal cells.53 Previous studies from our group showed that IL-1 and TNF-α are up-regulated after inflammation; they induce migration of DCs, including LCs, into the cornea; and suppression of these cytokines down-modulates DC and LC migration in the cornea.41,93,94 establishing the critical roles IL-1 and TNF-α in corneal inflammation and transplantation immunity.34,56,58-61 Interleukin 1 and TNF-α also induce intercellular adhesion molecule 1 (ICAM-1) and chemokine expression in the cornea that provides the directionality to infiltrating leukocytes. ICAM-1 is an important and ubiquitous adhesion molecule that mediates leukocyte binding to vascular endothelium during acute inflammation.62

Subsequent to the transendothelial migration of leukocytes into the inflamed tissue, cells require recruitment to the primary site of inflammation, and directionality is provided to leukocytes, including APCs, by chemokines. Chemokines are the specific cytokines that direct cell migration in development, homeostasis, and defense against foreign antigens. The central role of ICAM-1 and chemokines in corneal immune and inflammatory disease is now well established by our laboratory.63-66 In our previous studies, we showed that suppressing IL-1 and TNF-α can significantly suppress LC migration from the limbus into the corneal epithelium, and we proposed that this is a principal mechanism by which immunity in the cornea can be regulated. However, in light of the data presented herein, it is possible that an additional mechanism by which suppression of these cytokines can down-modulate corneal immunity is by suppressing the maturation of already resident stromal DCs in addition to suppressing the de novo recruitment of cells into the cornea.

As far as we know, DCs survive for weeks or months, depending on their type and anatomic site of origin, and need to be replenished. Skin myeloid DCs, for example, have a turnover rate of around 30 days.60 Although we have no exact data on the survival time of corneal DCs, they probably need to be replenished, as most other DCs do. Since the cornea is the only tissue to date described to be universally MHC class II− compared with other sites, where the number of MHC class II+ DCs is very low, it is tempting to speculate that the cornea is keeping these DCs in...
an immature state. The phenotype of the DCs in the center of the anterior normal stroma fits a precursor or highly immature DC, since these DCs have negligible to absent MHC class II or costimulatory molecule expression under normal conditions.69 While, as briefly mentioned in the 2 preceding paragraphs, the role of proinflammatory cytokines in promoting DC maturation has been elucidated, very little is known about the molecular mechanisms that suppress DC maturation. Several candidates include prostaglandin E2 and cytokines such as transforming growth factor β and IL-10 that are known to have a profound capacity to down-regulate MHC class II expression.70-74 Interestingly, previous studies have demonstrated that the corneal endothelium produces basal levels of endogenous prostaglandin E2—a potential mechanism that could implicate the down-regulation of MHC class II by stromal cells.75

Because of the unique location of the cornea, the cornea and ocular surface have constant contact with foreign antigens. The retention of the cornea as a clear tissue likely requires high clearance of these antigens without “unnecessary” inciting of T-cell activation that can endanger sight. The resident immature APCs described herein are perfectly suited for this task because of their high phagocytic and low T-cell stimulatory capacity, compared with mature APCs, which have a low phagocytic and high T-cell stimulatory capacity. Furthermore, the constitutive presence of APCs in the cornea can have significant implications for the mechanisms involved in sensitizing hosts to a variety of antigens, including donor (graft) antigens in corneal transplantation. Specifically, since the presence of resident corneal DCs was unknown until very recently, many investigators had proposed that the priming of recipient T cells in transplantation relies almost exclusively on migration of host APCs into the graft, where they can take up and process antigen and stimulate T cells in the context of host (“self”) MHC, a process known as the indirect pathway of sensitization.95,76 However, more than 40 years ago, Snell suggested that donor-derived leukocytes present in transplanted tissues were a major source of tissue immunogenicity, as they can present donor/graft MHC molecules directly to host naive T cells, a process known as the direct pathway of sensitization.77,78 As yet, we do not have data directly implicating the role of corneal DCs described herein as functional APCs in transplantation, but our data demonstrating that these DCs can significantly up-regulate MHC class II and costimulatory molecule expression suggest that, under certain conditions, perhaps when grafts are placed in highly inflamed “high-risk” beds, the direct pathway of sensitivity may also become operative. There is, in fact, some indirect evidence for this hypothesis in the literature, where experimentally manipulated MHC-disparate corneal grafts, enriched with donor APCs, are rejected in an accelerated fashion, suggesting that donor corneal APCs may become operative as passenger leukocytes.79

In summary, our data provide evidence that immature resident myeloid DCs in the corneal stroma mature as a result of corneal inflammation. The presence of MHC class II+ DCs in inflammation is therefore not only due to migration of these cells into the corneal center as previously thought. Moreover, our data suggest significant plasticity in these cells’ capacity to express class II antigen, depending on the microenvironment in which they reside. Whether it is possible to prevent the maturation process of resident DCs, and thereby mitigate or influence immunogenic disease outcome, is so far unknown and deserves further investigation.

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REFERENCES

21. Catly R, Van den Oord J, Foets B, Missotten L. Morphologic and immunophe-
notypic heterogeneity of corneal dendritic cells. Graefes Arch Clin Exp Ophthal-
22. Williams KA, Ash JK, Coster DJ. Histocompatibility antigen and passenger cell con-
23. Williams KA, Mann TS, Lewis M, Coster DJ. The role of resident accessory cells in corneal allo-
24. Vantrappen L, Geboes K, Missotte L, Maudgal PC, Desmer V, Lymphocytes and
26:220-225.
25. Fujikawa LS, Colvin RB, Bhan AK, Fuller TC, Foster CS. Expression of HLA-A/B/C
and DR focus antigens on epithelial, stromal, and endothelial cells of the hu-
26. Treseler PA, Sanfilippo F. The expression of major histocompatibility complex and
27. Wang H, Kaplan HJ, Chan WC, Johnson M. The distribution and ontology of MHC
28. Pels E, van der Gaag R, HLA-A,B,C, and HLA-DR antigens and dendritic cells in
29. Williamson JP, DiMarco S, Streilein JW. Immunobiology of Langerhans cells on the
cellular surface of the cornea. Langerhans cells within the central cornea interfere
with induction of anterior chamber associated immune deviation. Invest Ophthal-
30. Sano Y, Ksander BR, Streilein JW. Fate of orthopic corneal allografts in eyes that
cannot support anterior chamber-associated immune deviation induction. Invest
31. Niederkorn JY, Peeler JS, Mellon J. Phagocytosis of particulate antigens by cor-
neal epithelial cells stimulates interleukin-1 secretion and migration of Langer-
32. Liu Y, Hamrah P, Zhang Q, Taylor AW, Dana MR. Draining lymph nodes of cor-
neal transplant hosts exhibit evidence for donor major histocompatibility com-
xplex (MHC) class II-positive dendritic cells derived from MHC class II-negative
33. Hamrah P, Liu Y, Zhang Q, Dana MR. The corneal stroma is endowed with sig-
ificant numbers of resident dendritic cells. Invest Ophthalmol Vis Sci. 2003;44:
581-589.
34. Brissette-Storkus CS, Reynolds SM, Lepisto AJ, Hendricks RL. Identification of
a novel macrophage population in the normal mouse corneal stroma. Invest Ophthal-
35. Hamrah P, Zhang Q, Liu Y, Dana MR. Novel characterization of MHC class II
negative population of resident Langerhans cell-type dendritic cells. In-
36. Liu Y, Hamrah P, Taylor AW, Dana MR. Resident corneal dendritic cells that mi-
grate from corneal explants may mediate alloreactivity [ARVO abstract]. Invest
37. O'Brien TP, Li Q, Ashraf MT, Matteson DM, Stark WJ, Chan CC. Inflammatory
response in the early stages of wound healing after eximer keratokeratotomy. Arch
38. Li J, Xie L, Dong X. An experimental study on epikeratophakia [in Chinese]. Zhon-
ghua Yan Ke Za Zhi. 1996;32:233-238.
39. Hartlet LD. Corneal and ocular surface histochemistry. Prog Histochem Cyto-
40. Yamagami S, Dana MR. The critical role of draining lymph nodes in corneal al-
41. Dekaris I, Zhu SN, Dana MR. TNF-a regulates corneal Langerhans cell migra-
42. Metley JP, Witmer Pack MD, Agger R, Crowley MT, Lawless D, Steinman RM. The
distinct leukocyte integrins of mouse spleen dendritic cells as identified with
43. Maraskovsky E, Brasel K, Teepe M, et al. Dramatic increase in the numbers of
functionally mature dendritic cells in flt3 ligand–treated mice: multiple dendritic
45. Dana MR, Qian Y, Hamrah P. Twenty-five-year panorama of corneal immuno-
lology: emerging concepts in the immunopathogenesis of microbial keratitis, pe-
1997;159:532-536.
Inflammation: Basic Principles and Clinical Correlates. Philadelphia, Pa: Lippincott
Williams & Wilkins; 1991:9-50.
48. Caux C, Dezutter-Dambuyant C, Schmidt D, Banchereau J. GM-CSF and TNF-
alpha cooperate in the generation of dendritic Langerhans cells. Annu Rev
49. Colletti CL, Lausch RN, Oakes JE. Differential regulation of granulocyte-
macrophage colony-stimulating factor gene expression in human corneal cells
50. Dana MR, Yamada J, Streilein JW. Topical interleukin-1 receptor antagonist
cells from mouse bone marrow cultures supplemented with granulocy-
52. Sekine-Okan investigated M. Luca, Rungger D, et al. Expression and release of
 tumor necrosis factor-alpha by explants of mouse cornea. Invest Ophthalmol Vis
53. Cubitt CL, Lausch RN, Oakes JE. Differential regulation of granulocyte-
macrophage colony-stimulating factor gene expression in human corneal cells
54. Dana MR, Zhu SN, Dana MR. Topical soluble tumor necrosis factor
receptor type I suppresses ocular chemokine gene expression and rejection of
55. Lamina J, Dana MR, Zhu SN, Alard P, Streilein JW. Interleukin 1 receptor an-
tagonist suppresses allosensitization in corneal transplantation. Arch Ophthal-
56. Zhu S, Dekaris I, Ducker G, Dana MR. Early expression of proinflammatory cy-
tokines interleukin-1 and tumor necrosis factor-alpha after corneal transplanta-
57. Keane-Myers AM, Miyazaki D, Liu G, Deksas I, Oso S, Dana MR. Prevention of al-
terine eye disease by treatment with IL-1 receptor antagonist. Invest Ophthal-
58. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigra-
60. Zhu SN, Dana MR. TNF-a modulates Langerhans cell activity and promotes ocular immune privilege.
61. Finkelman DF, Barnstable CM. Immunobiology of the immune system. 4th ed. New York: Garland
Science; 1999:122-129.
62. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigra-
63. Zhu SN, Dana MR. Expression of nuclear factor-kappaB and specific tolerance by interleukin-10 in vivo.

References...
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The fundus photographs and photomicrographs depicted in Figures 1, 2, and 3 were not white balanced when they were taken 28 years ago. These images have been digitally altered to rectify fading and changes in color of the background that have occurred with the passage of time. No other alterations have been made in the content of the illustrations.

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REFERENCES

9. Duke JR. Granuloma of the choroid of unknown etiology. Case presented at the annual meeting of the Ophthalmic Pathology Club; March 20, 1961; Washington, DC.