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The good, the bad and the ugly – APCs of the eye

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Dendritic-cell (DC) populations throughout the body have a wide range of features in common, which are associated with their primary function in antigen presentation. The unique immune milieu of the anterior segment of the eye is characterized by a selective DC-dependent inability to develop delayed hypersensitivity responses following antigen invasion into the eye. Recent research papers provide evidence that different maturation stages of DC subsets are detectable at different corneal sites. Thus, the corneal DC, as well as the DC and the macrophages in the iris and ciliary body, have the potential of determining the outcome of immunity or tolerance within this organ.

The integrity of the visual system in the face of ever-changing immune challenges is an essential prerequisite for vision, and as a consequence, for human well being. Since the discovery of a unique immune homeostasis in the anterior segment of the eye, researchers have been fascinated by the special immunological status of this organ [1].

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During the past years it has been discovered that the selective inability to develop delayed hypersensitivity responses following antigen invasion into the anterior segment of the eye is highly dependent on dendritic cells (DCs), which form the basis of an extraordinary phenomenon called ‘anterior chamber-associated immune deviation’ (ACAID). This enables the eye to receive immune protection against pathogens and also to avoid inflammatory and immunological damage to the visual system [2,3].

End of a dogma: DCs are located even in the center of the cornea

Each anatomical structure in the eye, such as the cornea, anterior chamber, iris, ciliary body, vitreous, choroid, sclera or retina, has its unique way to handle immunity in the complex network of the immune system of the eye. In particular, owing to its direct contact to the environment, the cornea represents one of the ‘hot spots’ within the immune system of the eye. In the past, it had been postulated that most of the extraordinary immune behaviour of the cornea relies on the complete absence of MHC II-bearing DCs from the middle of this organ. In addition, it has been thought that DCs do not migrate to the center of the cornea unless inflammatory stimuli or mechanic damage occur at this site. This dogma has been questioned in recent research papers, in which the existence of distinct murine bone marrow derived DC (BMDC) subtypes and Langerhans cell (LC) subtypes within the corneal tissues have been demonstrated [4,5]. Altogether, the detailed phenotypical characteristics and tissue distributions of a total of six novel resident BMDC subsets at different precursor and maturation stages

within the cornea have been described. According to this new conceptional model, CD80⁺ CD86⁺ MHC II⁺ CD11c⁺ DCs and LCs are located in the periphery of the anterior cornea, whereas macrophage-like cells occur in the posterior part and CD14⁺ cells are detectable throughout the whole cornea [5–7] (Figure 1). Surprisingly, immature precursor subsets of DCs and LCs were discovered even in the central area of the cornea [5]. These DC subsets lack MHC II to any significant extent and have therefore been overlooked for a long time (Figure 1). Although these DCs of the central cornea have molecules on their cellular surface that clearly identify them as a part of the DC lineage, such as CD45, CD11b and rarely CD11c, they do not express accessory molecules for T-cell stimulation, such as CD40, CD80, CD86 or MHC II molecules, and rest in the central cornea in an immature state (Figure 1).

What have we learned from corneal graft transplantation medicine?

Recent data support the hypothesis that inflammatory stimuli determine the maturation stage of DCs and their

tolerogenic or immunogenic properties in the immune system [8]. This concept is nicely transferable onto experiences gained from human and murine corneal graft transplantation medicine [9,10]. Although the transplantation into intact corneal tissue, in which inflammatory signals are rare, achieves a highly satisfactory graft survival rate, a dramatic boost of the rejection rate can be observed following transplantation into an inflamed and vascularized recipients bed [11]. In parallel, in experimental models of uveoretinitis the increase of the number of mature MHC class II-bearing DCs in the cervical draining lymph nodes correlates directly with the degree of retinal tissue damage [12]. This implies that DCs at different maturation stages might be the main initiators of this devastating tissue destruction. These observations might provide a link to what happens in the corneal model; the complete and exclusive absence of MHC II molecules in the normal healthy cornea might be the basis of its sustained tolerance to foreign antigens. This concept is based on the notion that antigen presentation in the absence of co-stimulatory signals can lead to effective

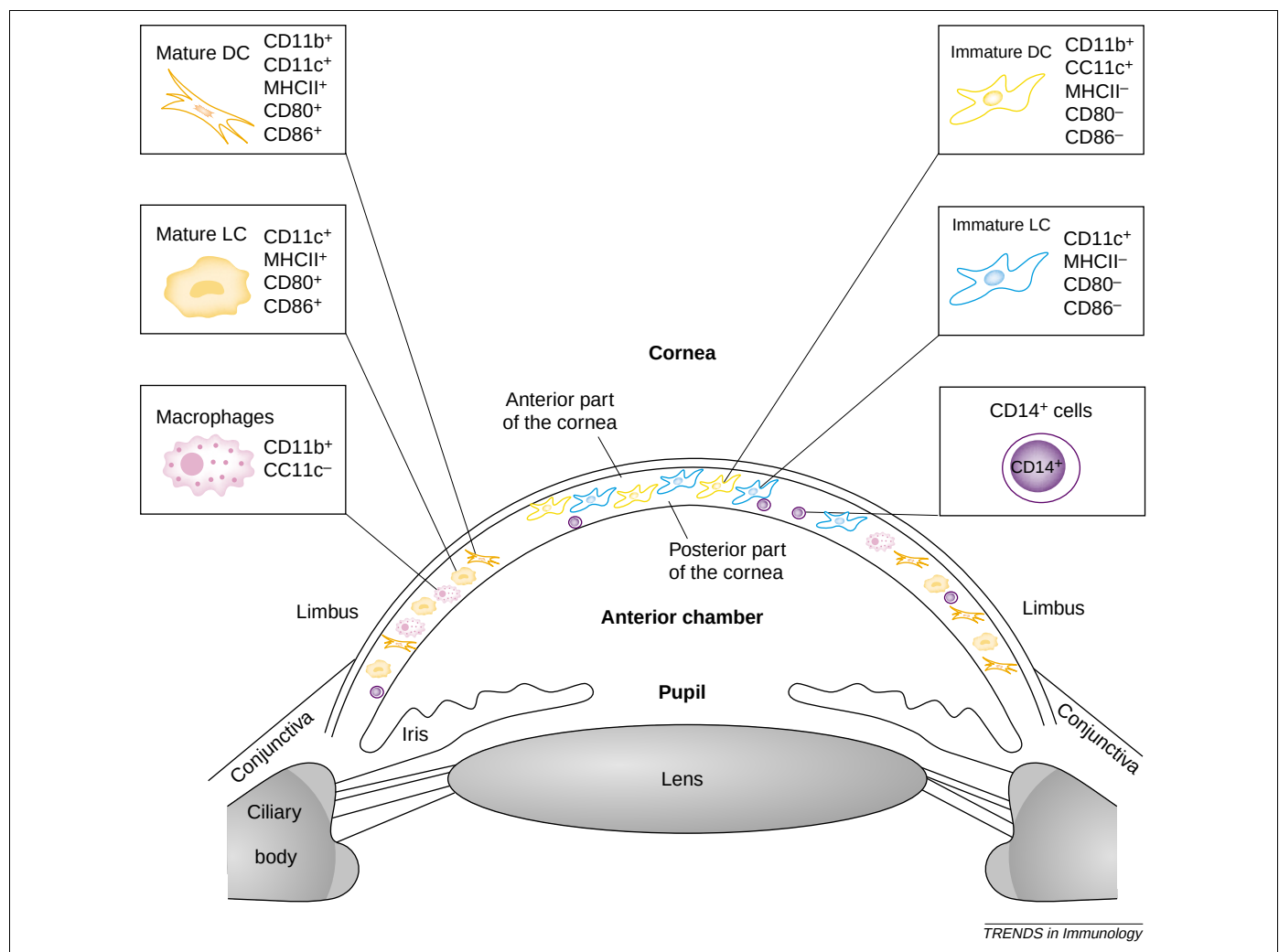


Figure 1. Localization of the recently described subsets of APCs within the corneal tissue. So far, six different APC subtypes have been described in the corneal tissue. High numbers of DCs and LCs, which do not express MHC II molecules and co-stimulatory molecules are located in the center of the cornea. By contrast, the density of mature, MHC II, co-stimulatory molecule-expressing DCs and LCs is high in the periphery and decreases from the periphery toward the center. Further, CD14⁺ cells are located throughout the whole corneal tissue, whereas macrophages are detectable in the posterior part of the cornea. Abbreviations: APC, antigen-presenting cell; DC, dendritic cell; LC, Langerhans cell; Mac, macrophage.

tolerance and T cells primed by DCs lacking co-stimulatory molecules are rapidly anergized in order to protect the tissue from any further damage [13].

'Danger' signals are able to activate DCs in the center of the cornea

In view of recent observations, new evidence suggests that even though these subtypes of DCs, which lack MHC molecules, might ensure a state of tolerance in the uninfamed cornea, they have the capacity to induce an immune response in the case of inflammation (Figure 2). Then specific 'danger' signals wake up these 'sleeping dogs' of the immune system causing the rapid maturation of the DC population at the center of the cornea by induction of co-stimulatory molecules and MHC II on their surface (Figure 2). This might facilitate the direct face-to-face confrontation of graft antigens with host T cells and provoke a fateful immune reaction, which in the worst case can lead

to the rapid rejection of the graft in terms of transplantation.

Which other factors determine the nature of the immune privilege of the eye?

Indeed, there are some more remarkable mechanisms that contribute to the flexibility of the immunological responsiveness or unresponsiveness of the eye to antigens.

The normal cornea is devoid of blood and lymphatic vessels. However, in response to mechanical damage or infectious stimuli, the neoangiogenesis and lymphangiogenesis induced by these signals provides a direct vascular port of entry for leukocytes and considerably shortens the way of antigen-presenting cells (APCs) through the lymphatics to the host lymph node [14]. Therefore, the prevention and control of neovascularization represents a useful strategy to maintain the quiescence of the immune status of the corneal graft and indeed the eye [14,15].

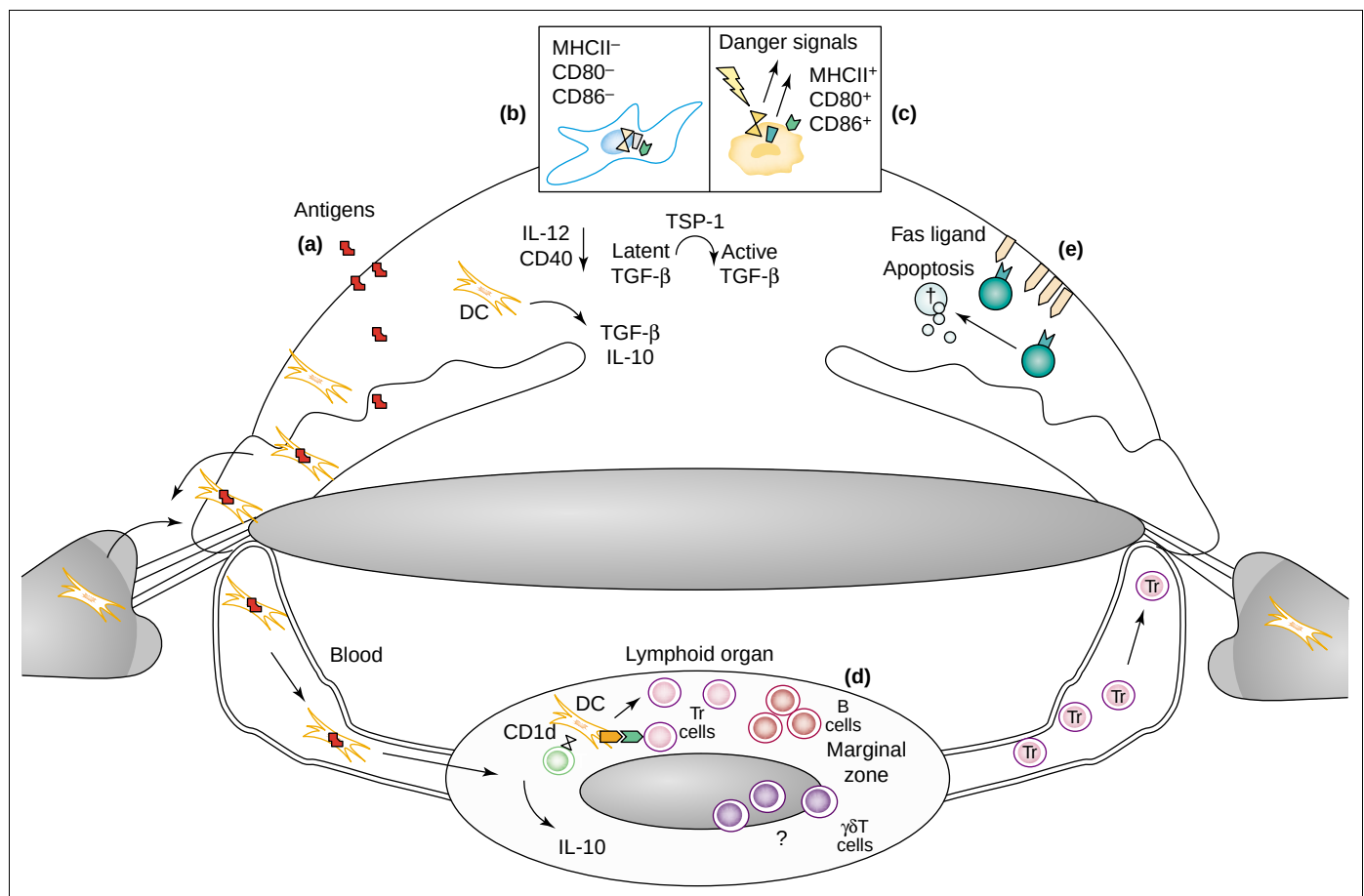


Figure 2. Interplay of factors contributing to ACAID. (a) High levels of TGF- β , IL-10 and immunomodulators, such as α -MSH, VIP, CGRP, somatostatin and TSP-1 are detectable in the aqueous humor. Inactive TGF- β is transformed into the active form by TSP-1 in the anterior segment of the eye. After antigen uptake, DCs migrate to the peripheral lymphoid tissue where they induce Tr cells, which both result in peripheral tolerance and regulation of Th1 and Th2 effector responses in the lymphoid organs (b) In the absence of danger signals, immature DCs, which do not have MHC class II molecules or co-stimulatory molecules, reside in the center of the cornea. (c) Danger signals, such as inflammatory stimuli, lead to the rapid activation and maturation of these DCs. (d) In the periphery, the inhibition of the development of CD8⁺ CTL responses by $\gamma\delta$ T cells in the spleen or the induction of antigen-specific Tr cells by CD1d-reactive, IL-10-producing NKT cells or marginal zone B cells result in the effective induction of peripheral tolerance. (The question marks indicate that the exact localization of $\gamma\delta$ T cells in the spleen is unknown.) (e) Further, Fas ligand, which lines the anterior chamber of the eye, induces apoptosis of inflammatory cells, such as lymphocytes, which invade into the eye. Abbreviations: ACAID, anterior chamber-associated immune deviation; α -MSH, α -melanocyte stimulating hormone; B, marginal zone B cell; CGRP, calcitonin gene related-peptide; CTL, cytotoxic T-lymphocyte; DC, dendritic cell; IL-10, interleukin-10; NKT, natural killer T; TGF- β , transforming-growth-factor- β ; Tr, regulatory T; TSP-1, thrombospondin-1; VIP, vasoactive intestinal peptide. Parts of figure reproduced with permission from Ref. [5].

Another reason for the unique immune deviation of the eye is soluble factors within the aqueous humor. It comprises high levels of the immunosuppressive cytokines transforming-growth-factor- β 2 (TGF- β 2), as well as other immunomodulators, such as α -melanocyte stimulating hormone (α -MSH), vasoactive intestinal peptide (VIP), calcitonin gene related-peptide (CGRP), somatostatin and thrombospondin (TSP)-1 (Figure 2). It is supposed that intraocular antigen-capturing DCs, after contact with aqueous humor of the anterior chamber, migrate through the trabecular meshwork through blood vessels to the spleen [16]. Additionally, they begin to secrete interleukin-10 (IL-10) and TGF- β in an autocrine fashion, thereby creating a microenvironment that is rich in tolerance-inducing mediators [16]. Furthermore, these DCs do not sustain a sufficient IL-12 secretion and fail to upregulate the co-stimulatory molecule CD40, even after T-cell contact (Figure 2). Most importantly, they promote the effective suppression of both Th1- and Th2-dependent inflammatory reactions in the lymphoid organs and induce a sufficient level of tolerance in this way [16–18]. Yet another mechanism of tolerance could be the induction of apoptosis leading to the immediate cell death of invading inflammatory cells by Fas-ligand, which, as found in mice, constitutively lines the iris, ciliary body and corneal endothelium [19] (Figure 2).

...mechanism of tolerance could be the induction of apoptosis leading to the immediate cell death of invading inflammatory cells by Fas-ligand...

Other features of ocular immune privilege include the inhibition of the development of CD8⁺ cytotoxic T-lymphocyte (CTL) responses by $\gamma\delta$ T cells in the spleen and the induction of antigen-specific regulatory T (Tr) cells by CD1d-reactive, IL-10 producing natural killer T (NKT) cells, which both result in the effective induction of peripheral tolerance [20–24] (Figure 2). A recently described model has shown that the site for effective induction of peripheral tolerance in the spleen is in the marginal zones (and not in the expected T-cell areas) where marginal zone B cells are required for the induction of Tr cells [25].

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Taken together, studies of the past decade have advanced our understanding of the complex mechanisms

of immunity and tolerance, which are the basis of ACAID. With regard to this sophisticated network, it has become obvious that in ocular diseases, such as uveitis, a deficit in one or more of the mechanisms described here can cause the loss of ocular immune privilege and lead to unwanted self-destruction. It will be a challenge in the future to unravel whether or not plasmacytoid DCs, which have a pivotal role in tolerance induction, are involved in ACAID. In addition, a better understanding of the ACAID phenomenon and its mechanisms of tolerance induction would enable us to protect the anterior segment of the eye from the potentially blinding consequences of inflammatory damage in diseases, such as uveitis, but also in terms of corneal graft transplantation medicine.

Conclusion

In order to elucidate the secret of the immune privileged state of the eye, research work has figured out a DC-dependent immunological paradigm, which has gained general importance and relevance throughout the whole immune system. It consists of a balance between immature, tolerogenic DCs on the one hand and mature, immunogenic DCs on the other hand. Nevertheless, the data appraised suggest that more complex mechanisms are involved. It has become clear that the homeostasis of ACAID represents a more dynamic than static interplay, which is flexibly directed by additional tolerogenic and immunogenic maturation stimuli delivered by the environment. Therefore, a key issue for the future will be a better understanding of the factors that modulate the maturation state of APCs and their capacity to either stimulate or tolerize T cells in response to antigens invading the eye. Further, the relevance of the mechanisms occurring in the murine system have to be analyzed for their clinical implication in the human immune system. From the model described here, evidence arises that the immune privilege of the eye, and at the same time the integrity of vision, is controlled by the dichotomic nature of DCs, which can act as the good, bad or ugly players in the complex game of the immune system.

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Is innervation an early target in autoimmune diabetes?

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In the non-obese diabetic (NOD) mouse, a spontaneous model of type 1 diabetes (T1D), recent evidence suggests that Schwann cells (Scs) and neurons surrounding insulin-producing β cells of the islets of Langerhans are destroyed before β cells. During normal perinatal development, macrophages (M Φ) are involved in phagocytosis of apoptotic neurons. Pertinently, M Φ are already present at birth in NOD pancreata. Their possible abnormal control of nerve phagocytosis, together with transient β -cell hyperactivity and lymphocyte anomalies, might conjointly participate in T1D pathogenesis.

Type 1 diabetes (T1D) is thought to result from autoimmune β -cell destruction. Despite extensive investigation, mainly in non-obese diabetic (NOD) mice, the etiology of T1D remains unknown [1]. However, recent data support the notion that innervation is an early target in its pathogenesis, with β -cell targeting occurring later [2,3]. The first immune cells, M Φ and dendritic cells (DCs), also known as antigen-presenting cells (APCs), accumulate around NOD islets and ducts at weaning (three weeks

of age) (Figure 1a). Subsequently, T cells migrate to the pancreas and home around ducts (periductulitis) and islets (peri-insulitis), followed by M Φ , DCs and T-cell infiltration into the islets (insulitis) (Figure 1b). Destructive insulitis coincides with scavenger M Φ influx and leads to β -cell destruction [1].

Are β cells the only targets in T1D?

The fact that β cells are thought to be specifically targeted is puzzling in light of: (i) the peri-insular location of the first infiltrating immunocytes because β cells are located in the center of the islet and the islet blood flow follows a $\beta \rightarrow \alpha$ direction [4] (Figure 1a and Figure 2a); (ii) the periductular infiltrate, which develops concomitantly with peri-insulitis and insulitis (Figure 1b); (iii) the undisturbed progression of insulitis in NOD mice, whose β cells lack MHC class I, with no or little progression to hyperglycemia [3]; (iv) the non- β -cell specificity of some islet-cell antigens (ICAs), which include glutamate decarboxylase (GAD); (v) the various autoantibodies (anti-GAD, anti-ICA512 and anti-glioma 38) directed against proteins shared by islet and neuronal cells [5]; and (vi) the appearance of autoantibodies to GAD before those to