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Immunology of the canine eye in health and disease: a concise review

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Abstract: The canine eye is an immune-privileged organ that is provided with systems to prevent and control the local immune response, which could have a detrimental impact. The entry of blood-derived antigens is hindered by the blood-ocular barriers and potential invading pathogens are tackled by local antimicrobial molecules. Despite the existence of numerous immune-competent cells, the anterior chamber of the eye is characterised by low responsiveness. This review is focused on the innate and adaptive immunity employed to control health and disease in the canine eye.

Keywords: dog; immunity; immune privilege

List of abbreviations

ACAID = anterior chamber-associated immune deviation; **APC** = antigen-presenting cell; **CNS** = central nervous system; **CSK/CSKC** = chronic superficial keratitis/keratoconjunctivitis; **CTL** = cytotoxic T lymphocyte; **DC** = dendritic cell; **EALT** = eye-associated lymphoid tissue; **KCS** = keratoconjunctivitis sicca; **LC** = Langerhans cells; **MALT** = mucosa-associated lymphoid tissues; **MHC** = major histocompatibility complex; **MPS** = mononuclear phagocyte system; **NOD** = nuclear-binding oligomerisation domain; **PAMP** = pathogen-associated molecular pattern; **RPE** = retinal pigment epithelium; **T_H** = T helper; **TLR** = Toll-like receptors; **T_{reg}** = regulatory T cells

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1. Introduction

The eye's surface is outward-facing, and it is thus threatened by a number of antigenic molecules and pathogens. As a mucosa, it is defended by mucosal immunity, which includes innate and adaptive systems locally found in tear film and tissue. The immune response carries out two conflicting functions: the degradation of invasive microorganisms is outweighed by the control of inflammatory reac-

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tions potentially detrimental to the delicate ocular setup. The immune system of the eye consists of an eye-associated lymphoid tissue (EALT), which is a branch of the mucosal immune system (Knop and Knop 2007).

Like in the rest of the body, the ocular immune system includes both innate and adaptive defences. Innate immunity is the first line of defence sustained by physical barriers such as blinking, the third eyelid and tear film, which contribute to protect the eye against microorganisms, chemicals or allergens. Adaptive immunity acts as the second line of defence and is promoted by both humoral and cell-mediated mechanisms. These two types of immunity are in continuous interplay with each other in order to ensure appropriate responses to different conditions: tolerance of normal microflora, exposure to environmental irritants, limited blood and lymph supply of the cornea and high sensitivity of ocular tissues to inflammatory responses (Day and Crispin 2008; Gilger 2008; de Andrade et al. 2016).

The eye is considered an immunologically privileged site because of its scarcity of resident lymphocytes and antigen-presenting cells (APCs), absence of lymphatic drainage except of the conjunctival type, and the existence of a blood-aqueous barrier and a blood-retinal barrier (BRB). Nevertheless, the eye is not forsaken by the systemic immune system, as demonstrated by the elicitation of a systemic immune response after the introduction of an antigenic molecule into the anterior chamber. However, in this privileged organ, allografted cells may evade immune detection and avoid rejection. In other words, immunological processes are tightly interrelated in order to achieve a local immunological escape, which prevents deleterious intraocular reactions (Day and Crispin 2008). A failure of this mechanism can cause infectious keratitis or, more seriously, immune-mediated disorders (Gilger 2008).

The purpose of this article is to review the existing literature on canine eye immunology and to correlate the immune privilege of the eye with the pathogenesis of some ocular diseases.

2. Ocular innate immunity

The innate branch of the immune system is the first line of protection against different types of insults (microorganisms, allergens, pollutants, chemicals and radiation) and it is not specific.

The physical action of eyelid closure (blinking) coupled to tear washout effectively hinder microorganism invasion and colonization.

The ocular surface is lined with a non-keratinized stratified epithelium (basal, middle and superficial layers) that contains many mucin-producing goblet cells; its superficial layers are in contact with microorganisms (i.e., the ocular microflora) and act as a barrier to microorganism intrusion thanks to the presence of epithelial intercellular tight junctions and to the rapid regeneration of epithelial cells with shedding of the external layers and consequently of the potentially infected cells (Lavach et al. 1977; Gilger 2008; de Andrade et al. 2016; Galletti et al. 2017).

The most recent studies have shown that the eye's surface microflora plays a part in both the defence of ocular health and in the initiation of diseases, in particular by stimulation of immune cells and control of mucin synthesis. Different studies have uncovered the role of microflora cross talk with pattern recognition receptors in immune protection and vision preservation (Miller and Iovieno 2009).

The components of the mucus gel of the tear veil help to maintain clarity, cohesion, hydration and preservation of the eye's shallow (Royle et al. 2008). The tear film plays the following important roles: providing an even surface for light refraction, lubricating the palpebrae as well as the conjunctiva and the cornea, providing the latter with nourishment, removing metabolic excretions, allowing lymphocytes to reach the cornea and conjunctiva, clearing away extraneous substances and protecting the surface of the eye from pathogens by the deployment of specific and non-specific molecules (Davidson and Kuonen 2004). The canine tear film, measuring 7–10 μm (Pinto Ribeiro et al. 2008), includes an inner mucus film mainly secreted by the epithelial cells and the conjunctival goblet cells, an intermediate watery layer generated by nictitans and lacrimal glands and an external oily layer originating primarily from the Meibomian glands (Cabral et al. 2005; Day and Crispin 2008).

The mucus film provides lubrication and protection of the cornea, binds the watery tear layer to the epithelium of the cornea, preserving it from physical stress, and impedes dryness and microorganism spread. The aqueous layer lubricates and protects the surface of the eye; it removes external bodies from the cornea or conjunctiva, and it harbours antiseptic molecules and antibodies. The watery

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film also contains dispersed mucin (that lowers the superficial tension, increases the diffusion of the aqueous layer and confers viscosity to the tear film), lactoferrin, lysozyme, transferrin, tear-specific prealbumin, albumin, ceruloplasmin, glycoproteins and immunoglobulins, which take part in the defence of the eye surface. The outermost oily Meibomian layer ensures an even optical shallow, prevents the dehydration of tears and reduces the spread of debris (Davidson and Kuonen 2004).

Mucins present on the ocular surface constitute the macromolecular scaffold of this hydrated gel, and glycans decorate these glycoproteins representing an abundant source of binding ligands. Both of these molecules control microbial binding, prevent bacterial colonisation, contribute to eliminating surface foreign material and modulate the physicochemical peculiarities of the gel (Royle et al. 2008). Furthermore, the transmembrane mucins dock the ocular tear veil to the external corneal epithelium allowing the transition between aqueous tear film and hydrophobic epithelium (Gilger 2008). Mucins of the natural tear film are mostly uncharacterised, but in dogs two different types of mucins, secreted and membrane-bound, have been identified; the gel layer of this pre-ocular tear film seems to have peculiar features which differentiate it from other supramucosal layers (Hicks et al. 1997). The major structural difference between the human and canine ocular glycans is that sialylated glycans prevail in human, while fucosylated glycans predominate in dogs (Royle et al. 2008). The synthesis and secretion of mucin by goblet cells are induced by different cytokines (e.g., IL-6 and IFN-γ) released by dendritic cells and inflamed conjunctival cells and are crucial in preserving eye health. In the past, tear supply and control of the inflammation of the ocular surface were the main treatments for dry eye syndrome (keratoconjunctivitis sicca, see below). Drug-based modulation of mucin secretion is now an additional therapeutic option for dry eye syndrome characterized by unstable tear layer, and has the advantage of sustaining a healthy tear layer formulation (Kim 2015).

Tear film and anatomical barriers of the eye surface (mucous and epithelium) contain several antimicrobial peptides (AMPs). These molecules are synthesised by epithelial cells of the cornea, conjunctiva, lacrimal sac and nasolacrimal surface, and they contribute to the prevention of microorganism invasion (Haynes et al. 1999; Gilger 2008). As signified by their name, these molecules have the capacity to kill or inhibit the growth of microorganisms; although the single peptides vary in their efficiencies in killing different microorganisms, as a group they are effective against both Gram-negative and Gram-positive bacteria, some fungi and certain viruses (McDermott 2004; Garreis et al. 2010). AMPs also have a spectrum of non-microbicidal functions and act as signalling molecules; they are immunomodulators, have anti-cancer activity and control vascularisation and wound healing (Mohammed et al. 2017). Mammalian defensins are typically 29- to 45-amino acid molecules containing six cysteine residues that are connected to build three disulphide bonds and a β-sheet structure. They can be distinguished into two groups, known as α and β, according to location and interaction of the cysteines. During inflammation or infection, α-defensins are produced by resident neutrophils, while β-defensins are produced by epithelial cells (McDermott 2004).

Apart from these AMPs, tears also contain other antimicrobial compounds, mainly in the aqueous film. For example, lysozyme, a molecule that acts as a first line of defence against ocular pathogens, is secreted by the tear gland and it induces bacteriolysis due to hydrolysis of the peptidoglycan in bacterial cell walls. Due to its chitinase activity, lysozyme also possesses antifungal effect (Davidson and Kuonen 2004). Tear glands also produce lactoferrin; this molecule reversibly attaches to two atoms of iron, hence diminishing the levels of this metal available for the metabolism and growth of bacteria. Some bacteria hinder the activity of lactoferrin by producing and exposing proteins that function as lactoferrin receptors on their outer membranes. These bind and "trap" lactoferrin, allowing the bacteria to use the iron attached to it. This capability to chelate iron may increase the pathogenicity of some bacteria. Furthermore, lactoferrin binds copper, immunoglobulins (IgA, IgG) and complement system proteins, in this way modulating immune system function. The α-lysin molecule (whose source in the tear film is not known) induces cell membranes to rupture by an as yet uncharacterised mechanism. The content of α-lysin in tears is higher than in plasma, serum or aqueous humour. Complement is discharged from the serum and participates in bacterial lysis (Davidson and Kuonen 2004; Knop and Knop 2007; Garreis et al. 2010). Finally, tear-specific prealbumin (lipocalin) scavenges potentially harm-

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ful bacterial molecules, and angiogenin, a lacrimal protein highly concentrated in tears, has mainly microbicidal activity in the tear layer (Paulsen et al. 2005; Knop and Knop 2007; Gilger 2008).

Toll-like receptors (TLRs) are innate immune receptors that sense the presence of microorganisms. They are normally expressed on cells that are most likely to initially encounter microbes, such as dendritic cells, mast cells, neutrophils and macrophages (Gilger 2008). Epithelial surface cells of the cornea lack expression of surface TLRs; they are mainly intracellular or exposed by the basal epithelial cells, thereby impeding pathogen recognition by TLRs (Ueta et al. 2004). TLRs are transmembrane proteins with an extracellular domain which binds to the ligand (pathogen-associated molecular pattern, PAMP), a transmembrane domain and an intracellular domain which transduces the signal through a cascade resulting in the production of pro-inflammatory cytokines. In this way, TLRs also play a crucial role in stimulating inflammation and in eliciting and modulating the adaptive immune response. Different invaders (bacteria, viruses, fungi and also worms) can harbour PAMPs and stimulate ocular TLRs (Chang et al. 2006; Turin and Riva 2008). The response of TLRs to PAMPs depends on the specific receptor and cell type and can include microbial phagocytosis, reactive nitrogen and oxygen species production, inflammatory cytokine synthesis and expression of co-stimulatory molecules (Gilger 2008). TLRs play an important role in the immune response of the ocular surface, in particular by facilitating the interplay between the innate and adaptive immune responses; they promote the immunological tolerance of the ocular surface to environmental antigens in order to hinder needless inflammatory responses to normal flora (the so-called "immunosilent condition") (Ueta et al. 2004; Gilger 2008). Moreover, the ocular surface is characterised by a shift to a pro-inflammatory environment when the eye is closed. Indeed, the closure of the eyelids during sleep markedly decreases oxygen exchange and tear secretion; the complement in tears increases during the first hours of sleep, and many neutrophils reach the site; furthermore, hypoxia increases the synthesis of TLRs in conjunctival epithelium, and blinking causes exfoliation of corneal cells, which subsequently ends (Sack et al. 2000; Galletti et al. 2017).

Nuclear-binding oligomerisation domain (NOD) proteins are cytoplasmic signalling receptors of the innate immune system that sense intracellular pathogens by recognising their PAMPs. Similar to TLRs, the binding of the ligand to NOD proteins triggers the stimulation of the transcription factor nuclear factor-κβ, which induces the transcription of pro-inflammatory cytokine genes. NOD proteins (NOD1 and NOD2) are expressed in canine basal corneal epithelium, corneal endothelium and conjunctival epithelium but not in goblet cells. The presence of NOD proteins at the barrier regions of the eye suggests a role in the detection of ocular germs and the induction of antimicrobial defences (Scurrell et al. 2009).

3. Ocular adaptive immunity

The immune components of the eye surface are known as eye-associated lymphoid tissue (EALT), which is part of the mucosa-associated lymphoid tissues (MALT). EALT represents a functional mucosal immunological system consisting of lacrimal glands, lacrimal drainage-associated lymphoid tissue and conjunctiva-associated lymphoid tissue. MALT and hence EALT have peculiarities that differentiate them from the components of the central immune system. In particular, immunological tolerance, ignorance or immunosuppression of the peripheral microenvironment abrogate reactivity and induce anti-inflammatory immune responses (Knop and Knop 2005a; Knop and Knop 2007). On the other hand, the eye should maintain the ability to effectively respond to multiple invaders.

EALT (as well as MALT) includes two types of structures, an "organised" lymphoid tissue made of immune cells organised in follicles, and a vast "diffuse" lymphoid tissue. The follicular organised form serves as an afferent branch of mucosal immunity, since antigenic molecules are captured from the environment by the specialised overlying follicle-associated epithelium, processed by antigen-presenting cells and presented to lymphocytes, which are activated and then proliferate and differentiate into effector cells (T or B lineage) and finally migrate from follicles to afferent lymphatic vessels, the bloodstream and effector organs (lacrimal gland and conjunctiva). Conversely, the diffuse form of lymphoid tissue serves as an efferent branch of mucosal immunity and it includes the arising effector cells delivered via post-capillary blood vessels that are diffused throughout the mu-

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cosae and the associated glands. Lymphoid cells are found in the connective tissue as lamina propria lymphocytes and plasma cells or within the basal layers of the epithelium as intraepithelial lymphocytes, while effector cytotoxic T lymphocytes (CTLs) are found in the epithelium, where they are more abundant than T helper lymphocytes, whose role is to modulate the differentiation of other cells (Knop and Knop 2005a; Knop and Knop 2005b; Knop and Knop 2007; Gilger 2008). Studies on the localisation of EALT in the conjunctiva demonstrated its presence in the superficial top of the cornea, particularly when the eye is shut. This localisation, along with the fact that the cornea lacks its own lymphoid cells, and the fact that in the shut-eye situation (e.g. during night-time) the corneal integrity is preserved by a large influx of leukocytes and their mediators, strongly suggests a crucial role for EALT in maintaining corneal health and integrity (Knop and Knop 2005b).

The function of the lymphocytes in the eye surface is not to induce inflammation but, on the contrary, to inhibit inflammatory processes which may cause destruction of the ocular surface tissue. The activation and modulation of the mucosal immune response is controlled via antigen presentation by different types of APCs and is carried out by various populations of effector T helper lymphocytes resulting from this priming process (Dana 2004; Knop and Knop 2005b).

3.1 APCs, lymphocytes and plasma cells

Similar to the innate immune response, the acquired immune response branches out into cell-mediated defence, which is directly operated by T lymphocytes, and humoral defence, which is sustained by soluble antigen receptors (immunoglobulins) produced by plasma cells of the mucosa. In contrast to innate immunity, acquired immunity involves lymphoid cells, and it is more specific and variable (Knop and Knop 2007). This antigen-specific second line of defence is characterised by memory, and therefore it reacts more rapidly upon subsequent exposure to the same antigen (Gilger 2008).

In eye immunology, there is an "afferent" immunological pathway of antigen capture, processing and presentation and an "efferent" immunological pathway of diffusion and activity of effector cells; in the middle is antigen recognition and presentation, which connects innate and adaptive immunity (Knop and Knop 2007).

The adaptive immune system of the eye includes APCs, different cytokine-secreting T lymphocytes (cell-mediated immune response) and antibody-secreting B lymphocytes once they have become plasma cells (humoral immune response) (Gilger 2008).

3.1.1 The ocular APCs

The APCs process the antigen and display its epitopes complexed with the major histocompatibility complex (MHC) class II (MHC-II) molecules on their surface (in a process known as "antigen presentation") and then migrate via draining lymph nodes to prime naïve T helper lymphocytes. These cells constitutively express MHC-II, and APCs are considered "professional" if they express high levels of the MHC-II tetramer and co-stimulatory molecules and thus efficiently prime T lymphocytes. This is the case of macrophages, dendritic cells (DCs), B lymphocytes and epithelial Langerhans cells (LCs); the latter are found in the epithelial layer of the peripheral cornea, but they are not present in the central cornea; this is considered the main reason for corneal immune privilege (see below). However, during inflammatory processes (e.g., infectious keratitis), APCs are attracted to the cornea from the limbus after infiltration of neutrophils and macrophages and after release of cytokines (particularly IL-1 and TNF-α). Conversely, "nonprofessional" APCs are characterized by a low potential to stimulate T lymphocytes as a result of low constitutive expression of MHC-II and co-stimulatory molecules; nonetheless, under some conditions (e.g., inflammation) they may also produce signals capable of priming T lymphocytes. Vascular endothelial and some mesenchymal cells, for example, are nonprofessional APCs with stimulatory potential. As in other tissues, the most potent APCs on the ocular surface are DCs and LCs. Corneal LCs are bone marrow-derived cells similar to skin LCs. Under healthy conditions, LCs are the only cells that constitutively express the MHC-II tetramer in the corneal epithelium and skin (Dana 2004; Gilger 2008).

During the last years, the search for corneal APCs based on their MHC-II expression has resulted in the conclusion that the normal central cornea lacks APCs, although MHC-II-positive cells have

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occasionally been found. Even without these cells, multiple corneal stimuli (e.g., trauma, infection) are capable of inducing migration of LC from the limbus to the cornea. The normal uninflamed cornea is indeed equipped with numerous immature MHC-II-negative LC-type DCs. Conversely, the inflamed cornea harbours activated LCs that express high levels of MHC-II and co-stimulatory molecules (Hamrah et al. 2002). It has recently been shown that the normal corneal epithelium is characterised by $CD11c^+$ $Langerin^+$ cells that are LCs, while the stroma is equipped with a different population of (non-LC) $Langerin^+$ DCs. Langerin is a c-type lectin produced by specialised DCs, and it senses glycosylated patterns on pathogenic microorganisms (e.g., mycobacteria). In the past, Langerin was thought to be expressed uniquely by Langerhans cells. Recently, however, DC ($CD11c^+$) populations other than LCs were detected that are able to produce Langerin (the so-called "(non-LC) $Langerin^+$ DCs"); these cells have been described to be present in the dermis, lung, gut, kidney, liver and now in the ocular cornea too (Hattori et al. 2011). The central cornea is indeed populated by heterogeneous epithelial and stromal DCs, which act as APCs. While the corneal periphery includes immature and mature resident bone marrow-derived $CD11c^+$ DCs, the central cornea contains only immature and precursor DCs, both in epithelium and stroma, where Langerhans cells and monocytic DCs are located, respectively. When inflammation occurs, most resident DCs undergo maturation and overexpress MHC-II and B7 (CD80/CD86) co-stimulatory molecules. Besides DCs, macrophages are also found in the posterior corneal stroma. These observations modified the thesis that the cornea is immune-privileged because of the absence of resident lymphoreticular cells and indicate that cornea is equipped with different cellular mechanisms for antigen presentation (Hamrah et al. 2003).

All epithelial layers of the canine cornea harbour $CD45^+$ and $CD11c^+$ immunocompetent DCs. These are more numerous at the periphery and their numbers diminish when moving towards the central area; these cells appear to be inactive since they do not express MHC-II. Their presence all over the corneal epithelium may represent an effective mechanism to inhibit or resolve inflammatory diseases caused by microorganisms on the ocular surface (Carvalho et al. 2009).

Investigations over the past decade have added to our knowledge of the cellular components of the mononuclear phagocyte system (MPS), which comprises DCs, monocytes and macrophages, but MPS functionality is still an open question. The tissue-specific activation of MPS cells which stimulate the acquired immune response is strictly regulated, and immune-privileged site is poorly understood. It is likely that MPS has different ways of triggering acquired immune reactions in the immune-privileged environment of eye. Evidence suggests that lymphangiogenesis in the cornea improves the migration of antigen-laden DCs to the lymph nodes and consequently amplifies the T lymphocyte responses. In addition, some corneal nerves can produce neuropeptides that block corneal immune privilege and allow stimulation of T lymphocytes. Moreover, commensal-derived antigens from non-privileged distal sites may trigger the stimulation of T lymphocytes, which subsequently target host cells in immune-privileged tissues. Finally, germs can activate immune reactions via innate lymphoid cells or via γδ T lymphocytes, and gut commensal microorganisms can alter the central nervous system (CNS) by disturbing the functionality of microglia (Reyes et al. 2017).

3.1.2 Cell-mediated immunity: immune tolerance or inflammatory response?

Ocular surface APCs migrate towards the draining lymphoid compartments and stimulate naïve T cells, which consequently peripheralise and home in to the ocular surface. Evidence suggests that regulatory T cells (T_{reg}) play an important role in limiting immune damage caused by autoreactive T lymphocytes (Barabino et al. 2012). Antigen presentation in the absence of co-stimulation leads to anergy or disappearance of the reactive T lymphocytes or appearance of functional immunosuppressive T_{reg} lymphocytes, both resulting in non-reactivity (immune tolerance) (Knop and Knop 2007). Tolerance is evoked towards the multitude of non-pathogenic environmental antigens that reach the ocular surface and it is triggered by T_{regs} via the expression of immunosuppressive cytokines such as IL-10 and TGF-β. Tolerance should, of course, also be sustained versus the body's own tissue antigens (Knop and Knop 2005a).

Co-stimulation in the presence of IL-4 directs T helper (T_H) lymphocytes towards T_H2 cells, which synthesise IL-4 and IL-5 cytokines that subsequently trigger the differentiation of antibody-producing

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plasma cells normally producing anti-inflammatory IgA. When other immunoglobulin isotypes (e.g. IgE) are generated, allergic diseases may arise. Co-stimulation in the presence of IL-12 results in T_H1 cells that synthesise inflammatory cytokines (e.g. IFN-γ, TNF-α) and trigger an inflammatory reaction potentially harmful to the surface of the eye. The presence of antigens from pathogenic microorganisms usually leads T_H1 lymphocytes to mount an inflammatory cellular response, because the potential tissue destruction that may occur is preferable to the risk of infection (Knop and Knop 2005a; Knop and Knop 2007).

In follicles, the antigen is presented by APCs to naïve lymphocytes; subsequently, the lymphocytes undergo proliferation, activation and differentiation into effector cells (e.g., B or T lymphocytes). Primed lymphocytes migrate to the bloodstream and to effector organs such as lacrimal glands and conjunctiva. Diffuse lymphoid tissue (intraepithelial lymphocytes and plasma cells) is interspersed along the lacrimal gland and conjunctiva. Lymphocytes are primarily cytotoxic T cells; a few are T helper cells. Plasma cells are implicated in secretory immunity through synthesis of specific antibodies (e.g., IgA), which cross the overlaying epithelium and are liberated in the tear film and ocular surface (see below) (Knop and Knop 2005a).

3.1.3 Humoral immunity: secretory IgA and other immunoglobulins

Plasma cells in mucosal tissues are crucial for the synthesis of specific immunoglobulins (mostly IgA), which cross the overlying epithelium and form a surface layer of secretory IgA. In dogs, IgA-secreting plasma cells predominate in lacrimal glands and conjunctiva, and secretory IgA is released into the tear film and ocular surface (Gilger 2008). The secretory component protects them from the proteolysis carried out by lacrimal enzymes (Schlegel et al. 2003). The secretion of IgA by lacrimal tissue is controlled by immune factors, hormones and neural networks (Davidson and Kuonen 2004; de Andrade et al. 2016).

Secretory IgA molecules protect the eye from bacterial adsorption and colonisation, viral infection and parasite infestation (Sullivan 2000; Davidson and Kuonen 2004) by coating microorganisms and therefore impeding their attachment to the corneal epithelium and by promoting bacterial agglutination, antimicrobial neutralisation and lysis. IgA is dispersed in tears as a soluble glycoprotein or linked to eye mucus. IgG molecules are normally present at very low concentrations in tears; their levels are increased during inflammation and they take part in phagocytosis and complement-mediated bacterial lysis. IgM molecules are expressed in tears too, at very low concentrations (Davidson and Kuonen 2004). In dogs, as in humans, IgA is the prevalent Ig in tears, followed by IgG and IgM, with large variations in concentrations between individual dogs (German et al. 1998; Day and Crispin 2008).

The concentration of albumin in tears significantly correlates with IgG, but not with IgM or IgA; IgM and IgA concentrations in tears correlate with each other. These relationships hint at a similar passage for IgG and albumin into secretions and a different one for IgM and IgA. Because albumin passively diffuses from serum into secretions, serum is presumably also the source of IgG found in mucosal secretions. Conversely, IgM and IgA are synthesised and secreted by the plasma cells of local glandular tissue (German et al. 1998).

3.2 The retina and its immunity

The retina is the third and inner ocular coat (after the cornea and lens); it is a highly specialised tissue crucial for vision. Injury to any of the sophisticated components of the retina may cause visual loss (Detrick and Hooks 2010). The retina contains several types of immune cells, most of which represent the so-called "glia", which are particularly sensitive and easily activated after infection or injury (Garden and Moller 2006). Furthermore, a particular structure, the retinal pigment epithelium (RPE), plays a crucial role in defending the eye (Detrick and Hooks 2010).

3.2.1 The glia cells

Three kinds of glial cells are primarily found in the mammalian retina, where they contribute to maintaining homeostasis: Müller cells (the prevalent ones), astrocytes and microglia (Vecino et al. 2016). A fourth type of glial cells, the oligodendrocytes, is found only sporadically in the retina in association with myelinated ganglion cell axons in

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the nerve fibre layer of a few animal species (Fischer et al. 2010; Vecino et al. 2016).

Glial cells maintain structure, contribute to metabolism and potassium uptake, perform phagocytosis of neuronal debris and produce particular transmitters and trophic stimuli. Astrocytes reside mainly in the nerve fibre layer and their processes, similar to Müller cells, envelope the blood vessels creating the BRB; they also perform a crucial function in ion homeostasis. In addition, microglia can be triggered to function as macrophages, and can interact with neurons and other glial cells via production of growth factors. The secretion of IFN-γ by infiltrating stimulated T lymphocytes first triggers Müller cells, astrocytes and microglial cells to synthesise class I and class II MHC molecules and prime these cells for further cytokine expression; the final result is initiation and/or spread of immune reactions. The intrinsic signalling pathways of glial cells propagate through Ca^{2+} waves (correlated with glutamate release). These cells play a crucial role in immunity, neuroprotection and angiogenesis. In the case of malfunction, they become primary pathogenic factors, and then the neuroglia responds to injury in order to restore homeostasis (Genini et al. 2014; Vecino et al. 2016).

Similar to astrocytes, Müller cells are capable of wrapping around axons and of forming glial connections between the retina and blood vessels (Hollander et al. 1991). Moreover, both Müller cells and astrocytes contribute to the glial sheaths surrounding neurons. In dogs, retinal axons are packed and interposed between the end-feet of the Müller cells in nest-like systems (Vecino et al. 2016). The "strategic" location of Müller cells makes them a structural and functional connection between the retinal neurons and the compartments with which they interchange molecules (sub-retinal space, retinal blood vessels and vitreous chamber), fulfilling the important roles of promoting neuronal development, survival and the relay of information (Reichenbach and Bringmann 2010; Vecino et al. 2016).

Astrocytes are mostly present only in the deepest retinal layers where they provide structural support for degenerating axons, neurotrophic supplements and sustain the BRB. As a reaction to disease or damage, the astrocytes produce proteins that jeopardise the integrity of the BRB via up-regulation of the expression of genes coding for cytokines, chemokines and complement cascade elements, which contribute to retinal degeneration (Kim et al. 2006; Vecino et al. 2016).

The CNS immune privilege (see below) was thought to be linked to the presence of the blood-brain barrier and the lack of lymph or of tissue-resident competent macrophages. The most recent studies have provided evidence of robust interactions between the immune system and the CNS and proved that microglia play a crucial role in regulating immune functions (Carson et al. 2006; Vecino et al. 2016). The CNS responds through both innate and adaptive immune reactions involving different populations of immune-competent cells such as microglia, astrocytes, macrophages and infiltrating T lymphocytes, which communicate by direct contact or by paracrine signals (Colton 2009; Ransohoff and Brown 2012; Corraliza 2014; Vecino et al. 2016). Reactivity of microglia is considered a symptom of different retinal inflammatory and degenerative diseases (Karlstetter et al. 2015). Microglia include local macrophages of the CNS, which carry out immune functions and contribute to the development and sustenance of the neural network and tissue homeostasis (Ransohoff and Brown 2012; Fernandes et al. 2014; Gertig and Hanisch 2014; Vecino et al. 2016; Reyes et al. 2017). Resident microglial cells act as immunological "watchdogs" of the retina and brain. They sense the neuronal microsurroundings and quickly react to stimuli by turning into activated phagocytes (Karlstetter et al. 2015). Microglia interact with different resident cells and neurons by secreting cytokines and chemokines (defined as their secretome) that represent signature factors for certain functional or pathological states (e.g. reactive oxygen species, nitric oxide, brain-derived neurotrophic factor, phospholipase A2 IIA type, insulin-like growth factor, glial cell line-derived neurotrophic factor, fibroblast growth factor 2 and lipocortin 1; Jha et al. 2013; Vecino et al. 2016). Canine retinal microglia also enagages in phagocytosis and reactive oxygen species production and exhibits the characteristic $CD11b^{high}$ $CD45^{low}$ immunophenotype (Genini et al. 2014).

3.2.2 The retinal pigment epithelium

The retinal pigment epithelium is a cellular monolayer inserted between the blood-rich choroid and the photoreceptor layer. The latter and the RPE are necessary for vision. RPE cells accomplish multi-

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ple functions that are physiologically important to sustain the neural retina (Bok 1993) and immunologically important in the immune defence. Indeed, the RPE cells synthesise TLRs (1–7, 9 and 10 (Kumar et al. 2004)), complement proteins and Fc-γ receptors; they react to IFN-γ administration and up-regulate the synthesis of both MHC class I and II molecules, and they serve as local retinal APCs. Moreover, these cells produce proinflammatory cytokines (IL-6, IL-8, and MCP-1), chemokines and growth factors, which play an important role in pathological processes. Nonetheless, the eye is also equipped with systems capable of suppressing immune reactions (e.g., TGF-β, IL-11 and IFN-β). Finally, dysfunction or damage of RPE cells may result in ocular pathologies (Detrick and Hooks 2010).

4. Ocular immunity in the elderly

In the elderly, the body tends to lose many homeostatic mechanisms. Susceptibility to infection, neoplasia and autoimmunity is enhanced due to debilitation or dysregulation of the immune system and loss of immune privilege (Dorshkind et al. 2009; Forrester and Xu 2012). These changes ("immunosenescence") involve both systemic and mucosal compartments, as well as both innate and adaptive immunity in humans (Shaw et al. 2013; Mashaghi et al. 2017) and in dogs (Strasser et al. 2000; Blount et al. 2005). The benefit of vaccination in preventing infectious diseases is hampered both in aged humans (Goronzy and Weyand 2013) and dogs (HogenEsch et al. 2004), mainly because of an inability to maintain long-lasting adaptive immunity.

Also, the defence mechanisms of the eye (tear layer, lacrimal and Meibomian glands, corneal and conjunctival epithelia) are impaired with aging. Furthermore, a breakdown of the immunological balance of resident corneal APCs, T_{regs} and T helper 1 cells (T_H1) in the elderly can cause dysfunction of the eye and impaired vision (Mashaghi et al. 2017). Therefore, immunosenescence is associated with a number of ocular pathologies, including increased occurrence of bacterial, viral and fungal conjunctivitis and keratitis, higher frequency of some neoplasia (e.g., limbal melanoma, eyelid squamous cell carcinoma; da Conceicao et al. 2010) and local immune-mediated diseases or complications of systemic autoimmunity.

5. Ocular immune privilege

The ocular anterior chamber is characterised by a unique immune reaction system, referred to as "immune privilege". This special immunologic status promotes tolerance of foreign antigens in order to avoid an enormous immune response. The "story" of immune privilege begins in 1873 with the findings of the Dutch ophthalmologist van Dooremaal, who inserted various extraneous objects and tissues into the eyes of rabbits and dogs with the aim of identifying the aetiology of cataracts. He did not uncover the cause of cataracts, but he noticed an extended survival of mouse skin grafts implanted into the ocular anterior chamber of the dog (Van Dooremaal 1873). Seventy-five years later, the British biologist Medawar "rediscovered" the protracted survival of extraneous tissue grafts inserted into the ocular anterior chamber of rabbits and proposed the term "immune privilege" for this phenomenon (Medawar 1948). The apparent lack of lymphatic drainage in the anterior chamber was considered responsible for the antigen trapping in the eye, resulting in the phenomenon of "immunological ignorance". For the discovery of acquired immunological tolerance, Medawar (and his colleague Burnet) shared the Nobel prize in Physiology or Medicine 1960 (www.nobelprize.org/nobel_prizes/medicine/laureates/1960/). In the late 1970s, Kaplan and Streilein observed that antigens inserted into the ocular anterior chamber not only reached the peripheral lymphoid tissues but also stimulated a systemic downregulation of antigen-specific cell-mediated immune reactions (Kaplan and Streilein 1977; Kaplan and Streilein 1978), a phenomenon subsequently termed anterior chamber-associated immune deviation (ACAID) (Streilein and Niederkorn 1981). ACAID plays a crucial role in protecting the ocular anterior chamber from antigenic stimulation and uncontrolled inflammatory response (Streilein 1990). A similar immune deviation occurs also in the brain and it is known as "brain-induced immune deviation". In this case, similar to ACAID, the CNS is neither disconnected nor inert in its interplay with the immune system; indeed, local immune cells can pass through the intact blood-brain barrier, and CNS neurons and glia stimulate lymphocyte and macrophage reactions (Carson et al. 2006).

Generally, immune privilege results from various immune regulatory, anatomical and physiological

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mechanisms that protect vulnerable and vital tissues or organs (such as the eye) with a very limited regenerative capacity (Niederkorn 2002; Niederkorn 2012).

First of all, physical barriers such as the blood-aqueous barrier and the lack of efferent lymphatic vessels prevent the passage of large molecules and cells into and out of the eye (Zhou and Caspi 2010). The cornea itself is considered an immune-privileged site as a result of multiple active and passive mechanisms involving both innate and acquired immunity. The lack of TLRs on the epithelial surface of cornea cells is primarily responsible for minimising antigen recognition (impaired innate immunity) (Ueta et al. 2004). Moreover, the scarcity of corneal vasculature and lymphatic tissue makes it naturally non-responsive (impaired adaptive immunity) (Knop and Knop 2005a). Soluble and cell-bound immunosuppressive agents of the ocular microenvironment (e.g., neuropeptides, TGF-β, Fas-ligand, vasoactive intestinal peptide and others) inhibit the activity of immune-competent cells (Zhou and Caspi 2010). For example, TGF-β and macrophage migration inhibitory factor are present in the aqueous humour at concentrations sufficient to inhibit natural killer cell-mediated cytotoxicity. This is very important, as both corneal endothelial cells and lens epithelial cells synthesise little or no MHC class I antigen presentation molecules and thus are highly sensitive to natural killer-mediated lysis (Apte et al. 1998; Niederkorn 2006). Besides inhibiting T lymphocytes, the RPE and the iris/ciliary body also triggers them to turn into T_{reg} lymphocytes. Lastly, the eye actively modulates systemic immune responses, as for example in ACAID (Streilein 2003; Caspi 2006; Caspi 2008; Caspi 2010; Zhou and Caspi 2010; Perez et al. 2013; de Andrade et al. 2016).

After inoculation into the anterior chamber, soluble antigens are locally processed by mechanisms controlled by intraocular cytokines such as TGF-β and then translocated via the bloodstream to the spleen (ACAID does not occur in absence of spleen) (Streilein 2003; Day and Crispin 2008). In this case, the humoral immune response is favoured, along with a simultaneous inhibition of the delayed type hypersensitivity reaction together with preferred stimulation of T_H2 cells or IL-10-producing T_{reg} lymphocytes. If cell-associated antigens are inoculated into the anterior chamber, a different form of ACAID occurs. Helper and cytotoxic T lymphocytes are stimulated, and they recirculate to the inoculated eye (penetrating via the uveal tract); however, they do not undergo functional differentiation, presumably because of local activity of TGF-β (Day and Crispin 2008). Alternatively, they synthetize Fas, which binds to Fas-ligand, ubiquitously expressed in the ocular cells (e.g. cornea, iris, ciliary body and retina), and induce apoptosis. The synthesis of Fas-ligand is considered helpful in creating a barrier which encompasses the eye and protects it from the activity of inflammatory cells (expressing Fas) that are recruited into the eye (Griffith et al. 1995; Day and Crispin 2008; de Andrade et al. 2016).

Programmed death receptor-1 (PD-1 or CD279) and its ligand PDL-1 belong to the B7/CD28 family expressed by helper and cytotoxic T lymphocytes as well as by natural killer cells and APCs. The binding of PD-1 to its ligand (PDL-1) induces suppression of T lymphocyte activation, proliferation and cytokine secretion due to alteration of T cell receptor signalling. Therefore, these molecules play a crucial role in sustaining local tolerance and in preserving immune-privileged microenvironments, including the ocular one (Coy et al. 2017; Wang et al. 2017).

Whenever these mechanisms are impaired due to different causes (e.g., injury of the surface epithelium, decrease of IgA or of the production of immunosuppressive molecules), inflammatory cytokines are expressed and the immune system is granted uncontrolled access to the antigen (Gilger 2008).

6. Ocular immunity in disease

Both microbial antigens and "danger signals" such as tissue disruption or an altered cytokine milieu can bias the normal way of antigen presentation towards inflammation and, therefore, alteration of the physiologically protective mucosal immunity (Knop and Knop 2005a). Mechanical destruction and increased production of inflammatory cytokines is characteristic, for instance, of several types of conjunctivitis and keratoconjunctivitis as well as uveitis both in humans and in dogs. Below, we briefly describe some examples of immune-mediated ocular diseases.

Keratoconjunctivitis sicca (KCS), also known as "dry eye disease", is a common syndrome caused by a deficiency in the aqueous tear layer due to the damage of the lacrimal and the nictitans glands. Besides quantitative, also qualitative defects of

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the tear film result in KCS. Mucin abnormalities include mucus accumulation, altered pathways of mucus secretion, diminished conjunctival goblet cell frequency and aberrant mucin glycosylation (Davidson and Kuonen 2004). In the conjunctival epithelium of canine patients with KCS, the population of goblet cells is significantly decreased, and in the lacrimal glands there is a lymphocyte infiltration, mainly by B and T helper lymphocytes (Izci et al. 2015). KCS may occur as a secondary effect of periorbital (lacrimal) injury, reaction to drugs (especially sulphonamides) or acute lacrimal inflammation; nevertheless, more frequently it is the result of infiltration of lymphocytes into the lacrimal gland, followed by atrophy and fibrosis. These features are similar to the ones involved in the human ocular autoimmune disease, Sjögren's syndrome. Genetic predisposition to KCS is reported for the English Bulldog, Lhasa Apso, Shih Tzu and West Highland White Terrier (Day and Crispin 2008). KCS is associated with mucoid to mucopurulent discharge, blepharitis, discomfort, superficial keratitis and, in chronic severe cases, abnormal dryness (xerosis), corneal ulcers and blindness. Due to these abnormalities, the eye surface may be prone to infections; indeed, dogs with KCS allow faster growth of microorganisms (mainly coagulase-positive *Staphylococcus* species and beta haemolytic Streptococci, besides Pseudomonas species) compared to normal dogs (Davidson and Kuonen 2004; Day and Crispin 2008).

Chronic superficial keratitis/keratoconjunctivitis (CSK/CSKC), also known as corneal pannus or Überreiter's syndrome, is another immune-mediated disease of the canine cornea. CSK/CSKC is a chronic, generally bilateral, non-ulcerative, proliferative stromal keratitis, prevalent in middle-aged German Shepherd dogs. Inflammatory cell infiltration is carried out by helper (and some cytotoxic) T cells, which produce IFN-γ, macrophages and plasma cells. Hyperplasia and pigmentation of the corneal epithelium are typical consequences. Cells from corneal epithelium synthesize MHC-II molecules; often, a diffuse sedimentation of IgG in the superficial stroma of the limbal conjunctiva it is observed, and sometimes in the superficial stroma of cornea or in the basement membrane of the corneal epithelium. An immune-mediated pathology is suggested, since an autoimmune reaction to corneal antigens is involved. The clinical sign is corneal opacity caused by surface stromal inflammation, vascularisation and fibrosis (Day and Crispin 2008).

Both KCS and CSK/CSKC are characterised by expression of MHC-II and co-stimulatory signals (e.g., adhesion molecule ICAM-1 and CD40) on epithelial cells; therefore, they have the potential to present self antigens. The T lymphocytes in turn produce inflammatory cytokines, creating a vicious cycle of increased lymphocyte activation and subsequent increased inflammatory cytokines in the tissue and tear film, which leads finally to alteration of the normal cytokine milieu (Knop and Knop 2005a).

Production of inflammatory cytokines by the altered epithelial cells also occurs in ocular allergy. Allergic conjunctivitis is the most frequent form of human ocular allergy; it is a complex of hypersensitivity diseases, which affect the lid, conjunctiva and/or cornea. Symptoms include severe eczema of the eyelids and periorbital skin and chronically inflamed conjunctiva and cornea; the disease may be mild to extremely severe and may considerably impact the vision and life quality (Leonardi et al. 2008; Bielory 2010; Guglielmetti et al. 2010). Sometimes this disorder is associated with atopic dermatitis as a manifestation of an atopic state both in humans (Guglielmetti et al. 2010) and dogs (Lourenco-Martins et al. 2011). In dogs, the clinical features of allergic conjunctivitis encompass bilateral conjunctival hyperaemia (red eye), puffiness (chemosis), itch (scraping and patting the eyes) and severe discharge; in addition, marginal blepharitis and keratitis and secondary (often staphylococcal) infections may arise. Chronic patients may develop lymphoid hyperplasia, characterised by grossly observable follicles (follicular conjunctivitis). At the cytological exam, the lesion harbours a heterogeneous population of T and B cells. Probable diagnosis of canine allergic conjunctivitis is made after ruling out other potential causes of conjunctivitis and is based on anamnesis and on positivity of allergy tests (intradermal and serological tests) (Day and Crispin 2008; Lourenco-Martins et al. 2011).

Uveitis is another group of syndromes characterised by intraocular inflammation, in particular of the uvea. Clinically, the iris and ciliary body act together, while the choroid responds independently. Uveitis is generally distinguished into anterior uveitis or iridocyclitis, posterior uveitis or choroiditis and panuveitis (inflammation of the whole uveal tract) (Townsend 2008; Trbolova 2011). Uveitis

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occurs when uveal tissue or the vasculature of the blood-aqueous barrier or BRB are damaged due to a number of causes. Infectious diseases, neoplasia and immune-mediated disorders may all cause clinical signs of uveitis; therefore, the condition can be classified as non-infectious (frequently referred as autoimmune) or infectious (Townsend 2008). In many cases, uveitis is diagnosed as idiopathic/immune-mediated (Bergstrom et al. 2017). It is currently unclear whether the development of uveitis reflects a failure of immune privilege. A number of observations show the role played by the innate immune system in uveitis: for example, the ocular tissues (cornea, uvea and retina) synthesise TLRs and NOD proteins, and the uveal tract encompasses cells crucial for the innate immune response (dendritic cells and macrophages); furthermore, the innate immune response induces an adaptive autoimmune response during non-infectious uveitis (Caspi 2010; Willermain et al. 2012). Recently, it has been shown that TNF-α concentrations are increased in the aqueous humour of dogs with acute anterior uveitis compared to healthy dogs (Durieux et al. 2015). In dogs, immune-mediated uveitis generally develops with acute onset, is characterised by severe pain and is associated with blepharospasm, lacrimation and photophobia. Visual impairment or blindness were reported in some cases. The typical ocular features are ciliary injection, corneal oedema, aqueous flare and swollen iris. Uveitis has a guarded prognosis (Day and Crispin 2008).

The most common feature of canine uveitis involves local immunopathology. For example, it occurs in infectious canine hepatitis induced by canine adenovirus type 1; in this case the accumulation of viral immune complexes in the uvea induces neutrophil infiltration and secondary corneal endothelial detriment and oedema (so-called "blue eye") (Day and Crispin 2008). A similar condition was observed in dogs vaccinated with "old" modified live canine adenovirus type 1 vaccines; nowadays, this problem has been virtually eliminated by the use of adenovirus type 2 instead of type 1 in canine vaccines (Moore 2010; Day et al. 2016).

A peculiar example of non-infectious uveitis is the so-called uveodermatological syndrome (also known as Vogt-Koyanagi-Harada-like syndrome), an autoimmune disorder of dogs in which melanocytes are targeted by the cellular immune response (Townsend 2008). Vogt-Koyanagi-Harada syndrome is described in human as an uncommon multisystemic autoimmune disease that involves tissues containing melanin, and affects more frequently dark-skinned people (Mochizuki 2010; Greco et al. 2013). In dogs, the skin lesions seem to be mediated by T lymphocytes and macrophages (T_H1 response), whereas the ocular lesions involve more likely B cell and macrophage responses (T_H2 response) (Townsend 2008). This disease is frequently reported in the Japanese Akita (genetic predisposition), Siberian Husky, Samoyed, Chow Chow, Shetland Sheepdog, Golden Retriever, Old English Sheepdog, St. Bernard, Irish Setter and Australian Setter. Clinical symptoms are depigmentation of skin (eyelids, lips, nose, anus, scrotum, footpads) with whitening of hair (poliosis) and skin (vitiligo) and bilateral ocular lesions (anterior uveitis, chorioretinitis, severe retinal detachment and optic neuritis) (Day and Crispin 2008).

Finally, neoplasia (primary and secondary) can affect the adnexa, ocular tunics and other structures of the eye. Primary ocular neoplasia is more frequent than the secondary type. Secondary neoplasia of the intraocular components is usually of metastatic or multicentric origin (Labelle and Labelle 2013). The ocular immune privilege mechanisms may favour tumour progression by inhibiting the killing of malignant cells through direct interference with CTL functionality in the eye, or indirectly, by abrogation of the effectiveness of CTL-activated intratumoural macrophages, which are crucial for tumour rejection. Moreover, epigenetic regulation of the expression of genes coding for tumour factors can enable the generation of CTL-resistant tumour variants. Intratumoural macrophages may play a crucial role in clearing these variants, since, in contrast to CTLs, their killing mechanism is non-specific. Therefore, the impairment of macrophage activity in the eye, aimed at preserving the immune privilege by reducing ocular immunopathologies, can favour the expansion of tumour escape variants, and contribute to tumour progression in the eye (McKenna and Chen 2010). The impact of ocular neoplasms on the structure and function of the eye varies according to anatomic location, therapeutic options and prognosis; however, in any case these growths may have significant impact on vision, comfort and longevity. Ocular neoplasia, whether primary or metastatic, are relatively rare in dogs. The most frequent primary ocular neoplasia is the uveal benign melanocytoma (and to a lesser extent the uveal malignant melanoma), whereas

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the most frequent metastatic intraocular tumour is lymphosarcoma (Townsend 2008; da Conceicao et al. 2010; Labelle and Labelle 2013).

7. With an eye to the future: corneal grafting in the dog

In human medicine, an important application of ocular immune privilege is corneal transplantation (or keratoplasty). Indeed, more than 90% of corneal allografts are successful in the absence of tissue matching and without systemic immunosuppressive therapy (Zhou and Caspi 2010; Taylor 2016). For this reason, cornea is the most frequently transplanted tissue in the globe, and it is transplanted to treat a number of diseases. Penetrating keratoplasty, a type of transplantation that involves a full-thickness replacement of the cornea, has been the prevalent procedure for many decades, and it resolved many cases of corneal blindness. Recently a specialist surgeon introduced a fundamental change by developing a new procedure based on transplantation of lamellar forms, which selectively replace only the impaired layers of the cornea. Deep anterior lamellar keratoplasty is replacing penetrating keratoplasty for diseases involving the corneal stromal layers, with the advantage that there is no risk of rejection (Tan et al. 2012), which was primarily due to corneal DCs that reach regional lymph nodes and initiate the immune reaction (Knop and Knop 2005b).

Corneal perforation (ulcer) is a common clinical sign also in veterinary ophthalmology; in dogs, this condition may be caused by infection, inflammation, trauma and surgery, and the mainstay of treatment is surgery (Lacerda et al. 2017) with the goal of restoring structural integrity and preserving maximal corneal transparency (Dulaurent et al. 2014). Corneal graft surgery has proven effective, but the lack of available corneal transplants (fresh or frozen) restricts the application of this treatment in canine patients (Goulle 2012). In veterinary medicine, different surgical biomaterials have been reported to successfully resist corneal perforations: among these, the most recent ones are grafts of porcine small intestine submucosa (Goulle 2012), grafts of bovine pericardium (Dulaurent et al. 2014) and grafts of porcine urinary bladder (Balland et al. 2016). These materials can be used for corneal reconstruction, and they represent excellent alternatives to conventional conjunctival grafts, particularly for veterinary ophthalmologists (Balland et al. 2016). If graft rejection occurs, further medical or surgical treatment may be required to achieve good vision (Lacerda et al. 2017).

8. Concluding remarks and perspectives

Although several studies are under way in dogs and other species, the detailed mechanisms underlying ocular privilege are not yet completely understood. There is a substantial body of evidence to suggest that the cells and molecules of the immune system play a crucial role at this immune-privileged site. Therefore, the investigation of innate and adaptive immune responses in the healthy eye is essential for understanding the pathogenesis of ocular inflammation and diseases and for selecting the best therapeutic approach. The detailed study of ocular immunity could indeed suggest novel therapeutic strategies for blinding diseases as well as uncover more information on acute versus chronic inflammation. Moreover, the canine eye represents a valuable model to study human ocular diseases; hence, this makes it worthwhile for further investigations.

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