

Basic immunology

ABDO has awarded this article 2 CET credits (GD).

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The immune system is the body's natural defence in combating organisms. Immunology has developed rapidly over the past decade owing to the refinements made in the molecular tests employed in this area of research. Therefore, the keen reader is encouraged to peruse the ophthalmic and immunological literature in order to keep abreast of the latest developments in this field.

Owing to the complex nature of this subject, it is far beyond the scope of this article to cover all aspects of immunology. Rather, the aims of the article are twofold: first to acquaint the busy practitioner with the basic concepts of the immune system; and second, to introduce the reader to the more specific topic of ocular immunology – the study of the ocular immune system.

Finally, since it is envisaged that optometrists will one day prescribe therapeutic agents, the discussion is limited to the anterior segment and anterior uvea.

Innate & adaptive immune systems

The immune system can be thought of as having two "lines of defence": the first, representing a non-specific (no memory) response to **antigen** (substance to which the body regards as foreign or potentially harmful) known as the **innate immune system**; and the second, the **adaptive immune system**, which displays a high degree of memory and specificity. The innate system represents the first line of defence to an intruding pathogen. The response evolved is therefore rapid, and is unable to "memorise" the same said pathogen should the body be exposed to it in the future. Although the cells and molecules of the adaptive system possess slower temporal dynamics, they possess a high degree of specificity and evoke a more potent response on secondary exposure to the pathogen.

The adaptive immune system frequently incorporates cells and molecules of the innate system in its fight against harmful pathogens. For example, **complement** (molecules of the innate system - see later) may be activated by **antibodies** (molecules of the adaptive system) thus providing a useful addition to the adaptive system's armamentaria.

A comparison of the two systems can be seen in **Table 1**.

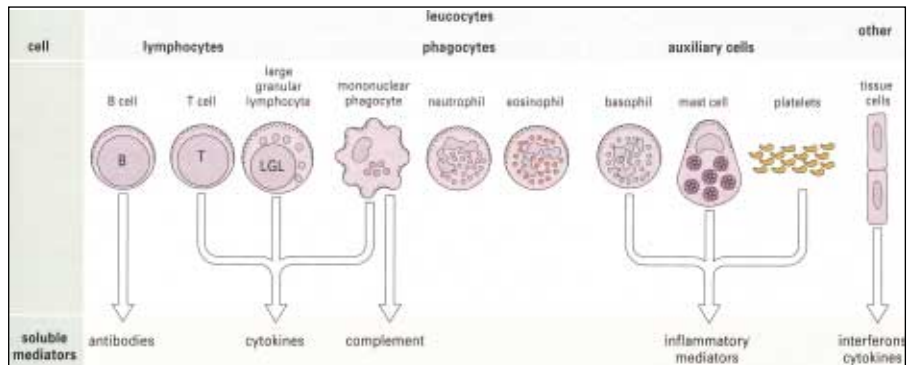


Figure 1 The principle components of the immune system are listed, indicating which cells produce which soluble mediators. Complement is made primarily by the liver, with some synthesised by mononuclear phagocytes. Note that each cell only produces a particular set of cytokines, mediators etc

Cells of the innate immune system

Phagocytes

Although sub-divided into two main types, namely neutrophils and macrophages, they both share the same function - to engulf microbes (phago - I eat, Latin).

Neutrophils

Microscopically, these cells possess a characteristic, salient feature - a multilobular nucleus (**Figure 2**). As such, these cells have been referred to as polymorphonuclear leucocytes (PMNs) and have a pivotal role to play in the development of acute inflammation. In addition to being phagocytic, neutrophils contain granules and can also be classed as one of the granulocytes. The granules contain acidic and alkaline phosphatases, defensins and peroxidase - all of which

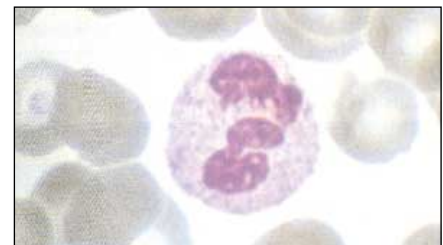


Figure 2 Morphology of the neutrophil. This shows a neutrophil with its characteristic multilobed nucleus and neutrophilic granules in the cytoplasm. Giemsa stain, x 1500

represent the requisite molecules required for successful elimination of the unwanted microbe(s).

Macrophages

Macrophages (termed monocytes when in the blood stream) have a horseshoe-shaped nucleus and are large cells. Properties of macrophages include phagocytosis and antigen presentation to T cells (see later). Unlike neutrophils (which are short-lived cells), they are seen in chronic inflammation as they are long-lived cells.

Mononuclear phagocytic system

The cells comprising the monocyte phagocytic system are tissue bound and, as a result, are further sub-divided depending on their location. A list of the cells together with their corresponding location can be found in **Table 2**.

Table 1: Cells and molecules of the innate and adaptive immune systems

Immunity	Cells	Molecules
Innate	Natural killer (NK) cells Mast cells Dendritic cells Phagocytes	Cytokines Complement Acute phase proteins
Adaptive	T and B cells	Cytokines Antibodies

Components of the immune system can be seen in **Figure 1**.

Table 2: Examples of cells of the mononuclear phagocytic system and their respective locations

Cells	Location
Monocytes	Blood stream
Alveolar macrophages	Lungs
Sinus macrophages	Lymph nodes and spleen
Kupffer cells	Liver

Phagocytosis - the process

Phagocytosis is the process by which cells engulf microorganisms and particles (Figure 3). Firstly, the phagocyte must move towards the microbe under the influence of chemotactic signals, e.g. complement (see later). For the process to continue, the phagocyte must attach to the microbe either by recognition of the microbial sugar residues (e.g. mannose) on its surface or complement/antibody, which is bound to the pathogen. Following attachment, the phagocyte's cell surface invaginates and the microbe becomes internalised into a phagosome. The resultant **phagosome** fuses with multiple vesicles containing O^2 free radicals and other toxic proteins known as lysosomes to form a phagolysosome. The microbe is subsequently destroyed.

Opsonisation ("to make tasty" - Greek)

Opsonins are molecules, which enhance the efficiency of the phagocytic process by coating the microbe and effectively marking them for their destruction. Important opsonins are the complement component C3b and antibodies.

Natural killer (NK) cells

NK cells are also known as "large granular lymphocytes" (LGLs) and are mainly found in the circulation. They comprise between 5-11% of the total lymphocyte fraction. In addition to possessing receptors for immunoglobulin type G (IgG), they contain two unique cell surface receptors known as killer activation receptor and killer inhibition receptor. Activation of the former initiates cytokine ("communication") molecules from the cell whilst activation of the latter inhibits the aforesaid action.

NK cells serve an important role in attacking virally-infected cells in addition to certain tumour cells. Destruction of infected cells is achieved through the release of perforins and granzymes from its granules, which induce apoptosis (programmed cell death). NK cells are also able to secrete interferon- γ (IFN- γ). This interferon serves two purposes: first, to prevent healthy host cells from becoming infected by a virus; and second, to augment the T cell response to other virally infected cells (see later).

Mast cells and basophils

Morphologically, mast cells and basophils are very similar in that both contain electron dense granules in the cytoplasm. Basophils are so-called owing to the fact that their granules

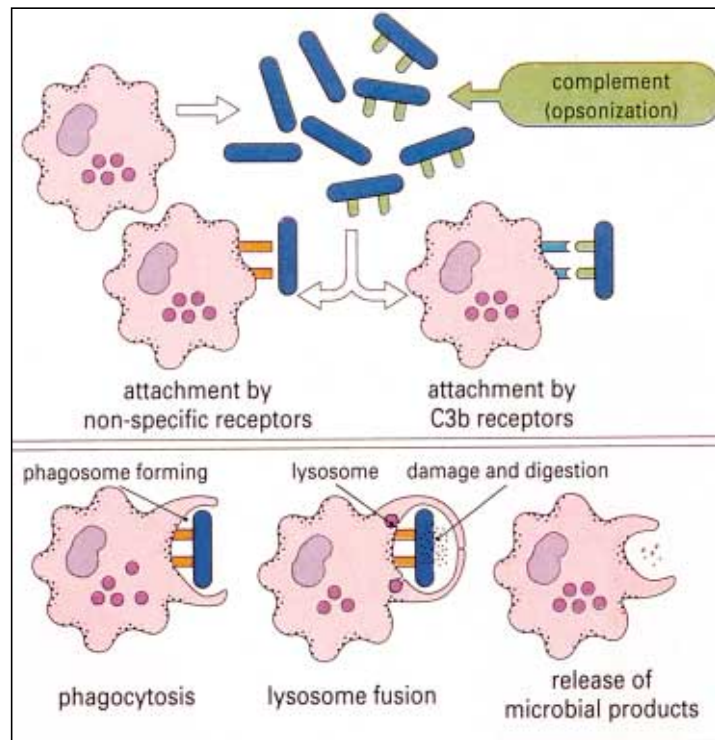


Figure 3

Phagocytes arrive at a site of inflammation by chemotaxis. They may then attach to microorganisms via their non-specific cell surface receptors. Alternatively, if the organism is opsonised with a fragment of the third complement component (C3b), attachment will be through the phagocyte's receptors for C3b. If the phagocyte membrane now becomes activated by the infectious agent, it is taken into a phagosome by pseudopodia extending around it. Once inside, lysosomes fuse with the phagosome to form a phagolysosome and the infectious agent is killed. Undigested microbial products may be released to the outside

stain with a basic dye. Unlike mast cells, which are present in close proximity to blood vessels in connective tissue, basophils reside in the circulation.

Both cell types are instrumental in initiating the acute inflammatory response. Degranulation is achieved either by binding to components of the complement system or by cross-linking of the IgE antibody which results in the release of pro-inflammatory mediators including histamine and various cytokines. The former induces vasodilation and augments vascular permeability whilst the latter are important in attracting both neutrophils and eosinophils.

Table 3: Dendritic cells and location

Cells	Location
Langerhans cell	Limbus, skin
Interdigitating cell	T cell areas in lymph nodes

Dendritic cells

Dendritic cells consist of Langerhans' and interdigitating cells and form an important bridge between innate and adaptive immunity, as the cells present the antigenic peptide to the T helper cell (adaptive immunity). Such cells are therefore known as professional antigen presenting cells (**APCs**). Table 3 illustrates the various types of dendritic cells together with an example of their location.

Eosinophils

Eosinophils (so called because their granules stain with eosin - Figure 4) are granulocytes that possess phagocytic properties. Despite the fact that they represent only 2-5% of the total leukocyte population, they are instrumental in the fight against parasites that are too big to be phagocytosed.

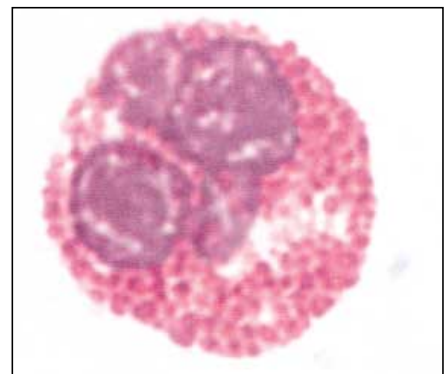


Figure 4 Morphology of the eosinophil. The multilobed nucleus is stained blue and the cytoplasmic granules are stained red. Leishman stain, x 1800

Molecules of the innate immune system

There are many molecules, which work in concert with the cells of the innate immune system and which also foster close functional links with their adaptive counterpart. The three major molecules are:

- Complement
- Acute phase proteins (APP)
- Interferons (IFNs)

Complement

The complement system represents a large group of independent proteins (denoted by the letter C and followed by a number), secreted by both hepatocytes (liver cells) and monocytes.

Although these proteins may be activated by both the adaptive immune system (**classical pathway**) or innate immune system (**alternative pathway**), the nomenclature is derived from the fact that the proteins help ("complement") the antibody response.

Activation of complement via the microbe itself is known as the alternative pathway. The classical pathway requires the interaction of antibody with specific *antigen*. The C3 component is the pivotal serum protein of the complement system. Binding of the antigen to C3 results in two possible sequelae. In either case, C3 component becomes enzymatically converted to C3b. The bacterial cell wall can either remain bound to C3b and become opsonised (since phagocytes have receptors for C3b) or act as a focus for other complement proteins (namely C5, 6, 7, 8 and 9). The latter form the membrane attack complex (MAC), which induces cellular lysis.

The functions of the complement system may be summarised as follows:

- Opsonisation
- Lysis (destruction of cells through damage/rupture of plasma membrane)
- Chemotaxis (directed migration of immune cells)
- Initiation of active inflammation via direct activation of mast cells

It is important that complement is regulated to protect host cells from damage and/or their total destruction. This is achieved by a series of regulatory proteins, which are expressed on the host cells themselves.

Acute phase proteins

These serum proteins are synthesised by hepatocytes and are produced in high numbers in response to cytokines released from macrophages.

Interferons (IFNs)

IFNs are a group of molecules, which limit the spread of viral infections (**Figure 5**). There are two categories of IFNs, namely type I and type II. Type I IFNs may be sub-divided further into IFN- α and β . IFN- γ is the sole type II interferon. Type I IFNs are induced by viruses, pro-inflammatory cytokines and endotoxins from gram negative bacterial cell walls. Their presence remains vital

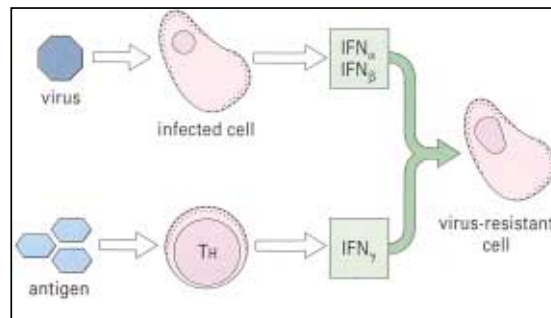


Figure 5

When host cells become infected by virus, they may produce interferon. Different cell types produce interferon- α (IFN- α) or interferon- β (IFN- β); interferon- γ (IFN- γ) is produced by some types of lymphocyte (T_H) after activation by antigen. Interferons act on other host cells to induce a state of resistance to viral infection. IFN- γ has many other effects as well

Table 4:
Primary and secondary lymphoid organs

Primary lymphoid organs	Secondary lymphoid organs
Bone marrow	Lymph nodes MALT - mucosa associated lymphoid tissue (includes bronchus, gut, nasal and conjunctival associated mucosal tissues)
	Spleen

for the successful eradication of an invading virus by the innate immune system.

Type II IFN, IFN- γ , is produced by T Helper cells and NK cells and is able to augment both the antigen presenting properties together with the phagocytic properties of the APCs (e.g. macrophages and dendritic cells).

Adaptive immunity

As mentioned previously, there is a great deal of synergy between the adaptive immune system and its innate counterpart. The adaptive immune system comprises two main types of leukocyte known as B and T lymphocytes. Before describing these important cell types, it is necessary to acquaint the reader with both the primary and secondary lymphoid organs and tissues in the body. These are summarised in **Table 4**.

The bone marrow represents the dominant site for haemopoiesis (production of blood cells and platelets). Although most of the haemopoietic cells mature in this region, T lymphocytes do so in the thymus. In the thymus, premature T cells undergo a process of positive and negative selection whereby the former are allowed to progress to maturity whilst the latter are marked for termination via apoptosis (see central tolerance).

Lymphocytes

Morphologically, there are three types of lymphocytes: T, B and NK cells. However, only T and B lymphocytes exhibit memory and specificity and, as such, are responsible for the unique quality of the adaptive immune system.

Resting B lymphocytes are able to react with free antigen directly when it binds to their cell surface immunoglobins which act as receptors. T lymphocytes do not react with free antigen and instead make use of APCs to phagocytose the antigen and then to express its component

proteins on the cell surface adjacent to special host proteins called major histocompatibility complex (MHC) class II molecules. As discussed, antigen presenting cells which express MHC class II molecules include dendritic cells and macrophages. This "afferent" phase must occur in order for the T cell to recognise the antigen. The "efferent" phase occurs when activated lymphocytes enter the tissue and meet antigen again. This results in multiplication and secretion of cytokines or immunoglobins in order to destroy the antigen.

T cells

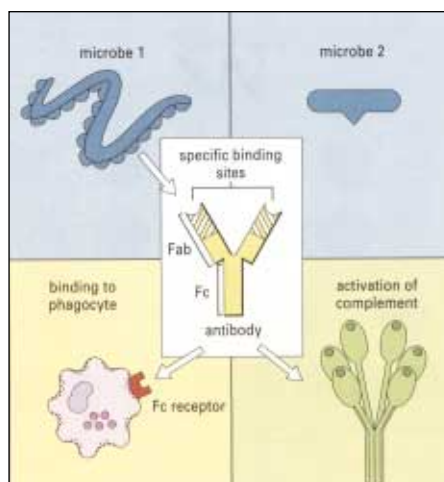
T cells can be broadly divided into both T helper (T_H) and cytotoxic T cells (T_C). Furthermore, T_H cells may be sub-divided into T_{H1} and T_{H2} . The former are pro-inflammatory T cells and stimulate macrophages whilst the latter orchestrate B cell differentiation and maturation and hence are involved in the production of humoral immunity (antibody mediated). T cells express cell surface proteins, described by cluster determination (CD) numbers. T_H cells express CD4 molecules on their cell surface, which enable the lymphocyte to bind to a MHC class II molecule. The T cell receptor is unique in that it is only able to identify antigen when it is associated with a MHC molecule on the surface of the cell.

Cytotoxic T cells are primarily involved in the destruction of infected cells, notably viruses. Unlike T_H cells, cytotoxic cells possess CD8 cell surface markers, which bind to antigenic peptides expressed on MHC class I molecules.

B cells and antibodies (immunoglobulins - Ig)

B cells are lymphocytes that produce antibodies (immunoglobulins) and can recognise free antigen directly. They are produced in the bone marrow and migrate to secondary lymphoid organs. B cells are responsible for the

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**Figure 6**

When a microorganism lacks the inherent ability to activate complement or bind to phagocytes, the body provides antibodies as flexible adaptor molecules. The body can make several million different antibodies able to recognise a wide variety of infectious agents. Thus the antibody illustrated binds microbe 1, but not microbe 2, by its 'antigen-binding protein' (Fab). The 'Fc portion' may activate complement or bind to Fc receptors on host cells, particularly phagocytes.

Table 5:

Antibody isotypes and corresponding functions

Antibody	Characteristics
IgG	Crosses placenta thus providing newborn with useful humoral immunity High affinity Predominant antibody in blood and tissue fluid
IgM	Large pentameric structure in circulation Present in monomeric form on B cell surface Secreted form is predominant antibody in early immune response against antigen Reaches 75% of adult levels at 12 months of age
IgA	Exists in both a monomeric and dimeric form Secretory IgA (dimeric form) represents 1st line of defence against microbes invading the mucosal surface, e.g. tears
IgE	Low levels in circulation Increased levels in worm infections Fc region has high affinity for mast cell thus involved in allergy
IgD	Antigen receptor on B cells Absent from memory cells

Table 6:

Various cytokines, their sources and functions

Cytokine	Source	Function
IL-1	Macrophages	1. T, B cell activation 2. Mobilisation of PMNs 3. Induction of acute phase proteins
IL-2	T cells	Proliferation of T and NK cells
IL-4	Th2 cells Mast cells	B cell activation IgE response
IL-8	Macrophages T cells Fibroblasts Keratinocytes	Chemotaxis of PMNs
IL-10	TH2 cells Macrophages	B cell activation Suppress macrophages
IL-12	B cells	Stimulate T _{H1} Inhibit T _{H2}
TGF β (transforming growth factor)	T cells	Inhibits other cytokines
TNF α (tumour necrosis factor)	Macrophages	Inflammation

development of antibody mediated immunity known as **humoral mediated immunity**.

When activated by foreign antigen, B cells undergo proliferation and mature into antibody secreting **plasma cells**. The latter are rich in organelles such as rough endoplasmic reticulum and mitochondria, which confer their ability to secrete soluble proteins (antibodies). Not all proliferating B cells develop into plasma cells. Indeed, a significant proportion remain as memory B cells through a process known as clonal selection. This process is vital in eliminating the antigen should the body become re-exposed to it in the future. T cells are also clonally selected and this confers to the production of T memory cells.

Although T and B cells behave differently, both are able to recirculate around the body migrating from blood to tissue and vice versa. The ability to recirculate obviously increases the efficiency with which cells of the immune system can home onto the invading antigen.

Antibodies

Antibodies have two roles to play - the first is to bind antigen and the second is to interact with host tissues and effector systems in order to ensure removal of the antigen (**Figure 6**).

There are five different types (known as **isotypes**) of antibody in the human immune system - namely IgM, IgG, IgA, IgE and IgD. In addition, there are four sub classes of IgG (IgG1-4). The basic antibody unit consists of a glycosylated protein consisting of two heavy and two light, polypeptide chains. The region which binds to the antigen is known as the **Fab** region, while the constant region, **Fc**, not only determines the isotype but is the region responsible for evoking effector systems, e.g. mast cell activation. The term immune complex refers to the combination of antigen and antibody and will be discussed later in the article (see type III hypersensitivity).

The antibody isotypes together with their corresponding function are illustrated in **Table 5**.

MHC

Major histocompatibility complex (MHC) are cell surface proteins classified as class I (also termed human leucocyte antigen [HLA] A, B and C), found on all nucleated cells and class II (termed HLA, DP, DQ and DR), found on all antigen presenting cells (APCs). MHC molecules are the *sine qua non* of T cell induced immunity.

Clinically, there is a strong association between HLA and certain systemic and ocular diseases (see later).

Cytokines

Cytokines (also termed interleukins [IL] meaning "between white blood cells") are small molecules that act as a signal between cells and have a variety of roles including chemotaxis, cellular growth and cytotoxicity. Owing to their ability to control immune activity, they have been described as the "hormones" of the immune system.

Table 6 summarises some of the cytokines pertinent to ocular immunology, their functions and their progenitors. Since interferons have been discussed earlier in the article, they have been omitted from the table.

Central and peripheral tolerance: nature's way of containing the immune response

Since various cells of the immune system are capable of reacting with self-antigens, it is therefore essential that the human body has mechanisms to suppress/eliminate autoreactive cells. Failure to do so, can, in some cases, lead to the development of autoimmune diseases (see later).

Central tolerance

Central tolerance refers to the process whereby both immature B and T cell lymphocytes, which react against normal, healthy cells (self-antigens), are eliminated via apoptosis.

Peripheral tolerance

This involves the removal of mature lymphocytes, which are not tolerant to healthy cells.

Ocular immune privilege

There are numerous sites in the body whereby tissue may be grafted with minimal risk of rejection. Such regions include, *inter alia*, the testis, thyroid lens, anterior chamber, cornea, iris and ciliary body^{1,2}.

It is important that immune privilege is not simple interpreted as the host's inability to initiate an immune response to a transplanted tissue. Rather, it is an area of the body in which there exists a paucity of various elements of the human immune system in response to an antigen.

Factors

The factors purported by investigators that contribute to the phenomenon of ocular immune privilege include:

- Isolation from a vascular supply
- Isolation from a lymphatic supply
- Presence of a vascular barrier
- Ability to suppress the immune response
- Anterior chamber associated immune deviation (ACAID)

Vascular supply

The healthy cornea is a good example of an ocular site devoid of a vascular network. The evidence to support the role a vascular network has to play in the mechanism of graft rejection is unequivocal since the risk of failure correlates positively with the degree of host vascularisation³.

Vascular barrier

There is a plethora of evidence in the ophthalmic literature to support the existence of a blood-ocular barrier. Furthermore, the same said barrier encompasses different elements including tight junctions between retinal

endothelial cells and the presence of junctional complexes linking retinal pigment epithelial cells⁴.

Lymphatic role

The fact that skin allografts were not rejected following lymph node removal⁵ led investigators to hypothesise that immune privilege was solely due to the absence of the same said system at a particular anatomical site. However, although certain immune privileged sites do indeed lack lymphatic drainage, others such as the testes⁶ and eye⁷ do possess such a system. It appears that a proportion of the aqueous humour drains via the uveoscleral pathway into the lymphatic vessels in the head and neck.

The eye, APCs & MHCs

As mentioned previously, APCs, through their ability to express MHC class II molecules, are potent progenitors of the immune response. Moreover, such cells are capable of activating T cells within the tissue itself. It is therefore not unreasonable to assume that a paucity of APCs may play an important role in immune privilege. In addition, failure to express MHC class I molecule would make a tissue immune against the lytic action of the cytotoxic T cells.

Although the aforementioned mechanisms are theoretically plausible, cells expressing both MHC class I and II molecules have been detected in the eye. **Table 7** illustrates the relationship between histocompatibility class and ocular cell type.

It is noteworthy that the epithelial cells of the crystalline lens are devoid of class I expression¹⁴ and that the Langerhans' cells (class II expression) are absent from the central cornea¹⁵.

It is interesting that not all cells, which express MHC class II act as professional APCs in the eye. Indeed, it has been shown that such cells reside in the iris and ciliary body and not only fail to present alloantigens to T cells, but have the ability to suppress mixed lymphocyte reactions¹⁶.

The failure to incite the inflammatory

response has attracted a great deal of interest amongst ophthalmologists and immunologists alike. It appears such suppression is achieved by various factors present in the aqueous humour (e.g. transforming growth factor - β).

Anterior chamber associated immune deviation (ACAID)

As a result of experiments with rats, investigators discovered that antigens placed in the anterior chamber resulted in systemic inhibition of delayed type hypersensitivity (DTH or type IV hypersensitivity) reactions to the same said antigens¹⁷. This phenomenon has been coined anterior chamber associated immune deviation (ACAID). The anterior chamber is thus able to suppress delayed type hypersensitivity reactions and inhibit the production of complement fixing antibodies^{18,19}. However, it has no inhibitory effect on cytotoxic T cell activity and has a minimal influence on the production of non-complement fixing antibodies.

With respect to the endothelium, two adaptations prevent it from immunological injury: first, avoidance of cytotoxic T lymphocyte (CTL)-mediated lysis; and second, inhibition of DTH responses in the anterior chamber²⁰. It achieves the former through the cells inability to express MHC class I molecules²¹. The corollary of this, however, is that virally infected cells may persist in this region. The *râison d'être* of ACAID is to protect the eye from the DTH response to pernicious antigens. As a result of its location in relation to the anterior chamber, the corneal endothelium seems well placed to reap the benefits of ACAID.

ACAID is beneficial in reducing the incidence of stromal keratitis in herpes simplex virus infection. It therefore seems reasonable to assume that such an unwanted corneal side-effect occurs as a result of a DTH response rather than the toxic effect of the virus *per se*²².

Corneal graft rejection may be due, in part, to failure to invoke ACAID. Streilein at al²³ not only discovered that the immunosuppressive effects of the cornea were abolished in corneas

Table 7:
MHC and ocular cell type⁸⁻¹³

Ocular cell type	MHC class I	MHC class II
Corneal epithelium	•	
Corneal stroma	•	
Corneal endothelium	•	
Trabecular meshwork	•	
Pigmented and non pigmented cells of ciliary body	•	
Anterior iris	•	
RPE cells	•	
Corneal limbus		•
Iris and ciliary body		•
Uveoscleral pathway		•
Ora serrata		•

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that had ACAID removed via cauterisation or keratoplasty but also abolished in corneas that had been denervated.

Ocular immunology

Anterior segment immunology may be sub-divided into the following aspects:

- Tear film
- Corneal immunology
- Conjunctival immunology
- Scleral immunology
- Uveal immunology

Tear film

a) Mucous layer

This layer is produced both by the conjunctival goblet and epithelial cells. The glycocalyx synthesised by the corneal epithelial cells serves to attach the mucous layer and in doing so binds to immunoglobulin in the aqueous²⁴. It has been suggested that the latter immunological sign may have antiviral effects. The evidence to support this is that certain intestinal mucosa, which have similar binding properties to those seen in the eye, are able to exert an inhibitory action against viral replication²⁵.

b) Aqueous layer

The aqueous layer contains the following antimicrobial factors:

1. Lactoferrin
2. Lysozyme
3. IgA
4. Miscellaneous proteins

1. Lactoferrin

Lactoferrin is produced via the acinar cells of the lacrimal gland. Its main function is to bind iron, which is required for bacterial growth. It therefore possesses both bacteriostatic and bacteriocidal properties. Furthermore, it is able to enhance the effects of certain immunoglobulins.

2. Lysozyme

Produced by type A cells in the lacrimal gland, lysozyme constitutes up to 25% of the total tear protein. It is surprising somewhat that despite being effective at lysing gram positive bacterial cell walls, staphylococcus aureus appears recalcitrant to its action²⁶. However, it is instrumental in enhancing IgA against gram negative bacteria.

3. IgA

This is the predominant isotype found in the human tears in the non-inflamed eye. Very little is present in serum with the majority secreted through epithelial cells of structures such as the lacrimal gland and the lactating breast. The IgA present in the tears is produced by plasma cells situated underneath the secretory cells lining the acini in the lacrimal gland.

IgA is very effective at binding microbes and, as such, prevents the microbe from adhering to the mucosal surface. The antibody achieves this either by interfering with the binding site directly or by agglutination. Successful binding of

the microorganism enables the tears to wash them away. IgA possesses other functions including opsonisation and inactivation of various bacterial enzymes and toxins. In addition, it may be involved in antibody dependent cell-mediated cytotoxicity. It must be emphasised, however, that IgA is unable to bind to complement and, as such, is not involved in the classical pathway²⁷.

4. Miscellaneous proteins

β -Lysin, a bacteriocidal cationic protein, is present both in the tears and aqueous humour²⁸. Its bacteriocidal properties are conferred through their ability to disrupt the microorganisms' walls. Various components of complement are present in varying degrees depending on whether the eye is closed or not.

Closed eye vs open eye

Open eye

In addition to the components of complement mentioned above, the tears are rich in lysozyme, lactoferrin and lipocalin (tear pre albumin) together with low levels of IgA^{29,30}.

Closed eye

In the closed eye environment, levels of IgA and complement components are increased. In addition, neutrophils are also recruited. The eye can be interpreted as being in a state of subclinical inflammation. However, the eye is protected from damage induced by either complement or neutrophils by DAF (Decayed Accelerating Factor [an inhibitory component of the complement system])³¹ and α^1 -antitrypsin³².

Corneal immunology

Paradoxically, the cornea, an immune privileged site, is capable of evoking an immunological response evidenced by the presence of subepithelial infiltrates, keratic precipitates, epithelial and stromal keratitis under certain clinical conditions.

Cytokine release

Numerous cytokines are synthesised in the cornea including IL-1, IL-6, IL-8, TNF- α , IFN- γ , C5a and prostaglandins³³. Phagocytosis of certain antigens via corneal epithelial cells initiate the release of IL-1 which, in turn, gives rise to the recruitment of Langerhans' cells from the limbus to the central cornea. It is worthy of note that the same said APC may be recruited centrally in response to chemical or localised damage^{34,35}. Investigators have discovered that stromal fibroblasts synthesise

IL-8 in response to infection by the herpes simplex virus³⁶. Furthermore, it seems plausible that the latter cytokine release may be responsible for the neutrophilic infiltration observed in cases of herpetic keratitis.

Immunoglobulins

The three isotypes detected in the cornea are IgM, G and A. IgG predominates in the central cornea³⁷. By contrast, IgM predominates in the

limbal region. The latter antibody is restricted from the central cornea owing to its large size. Antibodies in the cornea are found in the stroma since they are cationically charged. Due to their positive ionic charge, they bind to proteoglycans and the anionic glycosaminoglycans³⁸.

Antigen/antibody complexes may sometimes be observed in the corneal stroma in a variety of pathological conditions (e.g. herpes simplex keratitis), and because of their ringed shaped appearance are known as "Wessley rings".

Complement

The elements of complement found in the cornea include C1-7³⁹ in addition to the regulatory proteins H and I and C1 inhibitor⁴⁰. Interestingly, C1 is present in high concentrations in the limbus and is restricted from the central cornea. This finding is significant since the limbal region is susceptible to ulceration following complement activation and immune complex deposition. It has been suggested that C1 is produced by corneal fibroblasts whereas the remaining components detected in the cornea appear to be derived from the plasma via linked vessels.

Conjunctival immunology

The conjunctival associated lymphoid tissue (CALT) is part of the more general mucosa associated lymphoid tissue (MALT). Langerhans' cells and lymphocytes exist within the conjunctival epithelial layer. In the substantia propria, neutrophils, lymphocytes, IgA and IgG, dendritic cells and mast cells all reside. It is noteworthy that eosinophils and basophils are not present in the healthy conjunctiva.

Langerhans' cells and dendritic cells present the antigenic peptide to the conjunctival T helper cells. Following antigenic presentation, the T cells secrete the cytokine IFN- γ which serves to promote antigen elimination by macrophages. This is the delayed-type hypersensitivity (DTH) response (see later) and is characteristic of conjunctival pathology such as phlyctenulosis.

Scleral immunology

There exists only a small number of immune cells in the sclera compared to the conjunctiva since it is relatively avascular. In its resting state, IgG appears to be present in large amounts⁴¹. However, a sclera under stress, may become immunologically active as a result of migrating cells from the overlying episcleral and underlying choroidal vasculature^{42,43}.

Uveal immunology

The uveal tract is an important site from an immunological perspective for two reasons: first, it is highly vascular in nature; and second, the majority of vessels are highly fenestrated which facilitates the recruitment of leukocytes during the inflammatory cascade. The uvea contains numerous cellular components of the immune system including macrophages, mast cells, lymphocytes and plasma cells in addition

Table 8:
Hypersensitivity reactions

Hypersensitivity type	Appearance time	Mediators	Mechanism
I. Immediate	2-30 mins	IgE	Mast cell response (enhance acute inflammation)
II. Cytotoxic	5-8 hours	IgM and IgG	Antibody and complement
III. Immune complex	2-8 hours	IgM and IgG immune complexes	Antibody/antigen complexes
IV. Delayed type	24-72 hours	CD4 and CD8 T cells, APCs	T cell mediated Macrophages activated Include granulomatous reactions
V. Stimulatory		Autoantibodies	Autoantibodies against hormone

to an appreciable number of immune factors.

Although IgG and IgM have been detected in both the choroid and iris, they exist in greater numbers in the former structure. The reason for such disparate levels is the dearth of anionic antibody binding sites on the iris surface⁴⁴. By contrast, the ciliary body harbours tremendous amounts of IgG by virtue of its anionic tissue sites.

Immunopathology and the eye

Immunopathology encompasses both pathology as a result of an over-active immune system (**hypersensitivity** and **autoimmunity**) together with that acquired through an individual's inability to fight off infection – namely immunodeficiency. Unfortunately, it is far beyond the scope of this article to describe the latter and its ophthalmic correlates.

In this section, the classification system pertaining to hypersensitivity reactions will be described and each subtype with an anterior segment manifestation will be discussed. The association between HLA and systemic and ophthalmic disease will be illustrated together with a brief overview of the concepts pertaining to autoimmunity.

Hypersensitivity

The term hypersensitivity refers to the process whereby the adaptive immune response over-reacts to a variety of infectious and inert antigens resulting in damage to the host tissue. Five types of hypersensitivity reactions exist – all of which vary in their timing following contact with the antigen (**Table 8**).

• Type I hypersensitivity: allergy

Allergies may affect approximately 17% of the population⁴⁵. The term atopic is used to describe those individuals who possess a genetic predisposition to allergy. Allergies may occur to otherwise innocuous antigens (known as **allergens**) and infectious agents, e.g. worms. Type I hypersensitivity exists in two phases, the sensitisation and effector phases.

Firstly, a harmless allergen causes production of IgE antibody on first exposure. This IgE

diffuses throughout the body until it comes into contact with mast cells and basophils. Both these cell types have receptors for IgE antibody. Although the patient experiences no symptoms after the initial binding, reintroduction of the antigen/allergen induces the production of more IgE and, furthermore, increase the likelihood of cross-linking with existing antibodies on the mast cell surface. Such cross-linking induces the mast cell to degranulate and release a host of inflammatory mediators such as histamine, prostaglandins and bradykinin.

Histamine characteristically causes the itchy symptoms experienced by patients and as a result of binding to H1 receptors in the eye, induces vasodilation and enhances mucous secretion by the goblet cells. Bradykinin augments vascular permeability, decreases blood pressure and contracts smooth muscle. Prostaglandins are also powerful inflammatory mediators.

Ocular correlates:

The following anterior segment conditions are due, if not only in part, to type I hypersensitivity:

- Seasonal allergic conjunctivitis (type I)
- Giant papillary conjunctivitis (types I and IV)
- Vernal keratoconjunctivitis (VKC) (type I and IV)
- Atopic keratoconjunctivitis (AKC) (types I and IV) (**Figure 7**)

Seasonal allergic conjunctivitis can be easily recognised by chemosis and hyperaemia of the conjunctiva, lid swelling and excessive lacrimation. The cornea is not affected.

GPC is well known to optometrists as an allergic response to contact lenses, prosthetic lenses, protruding corneal sutures and scleral buckles. Histological examination of eyes suffering from GPC have revealed degranulated mast cells (type I hypersensitivity)⁴⁶ and CD4+ T cells (type IV)⁴⁷, thus corroborating the theory that such a condition is mediated by both types of hypersensitivity.

Vernal conjunctivitis can present either in a palpebral form characteristically exhibiting



Figure 7:
Corneal thinning and vascularisation in AKC, excess mucous is present



Figure 8:
Epithelial macroerosion in VKC



Figure 9:
Corneal plaque in VKC

giant cobblestone papillae, or in a limbal form with gelatinous deposits known as Trantas'-dots, which represent degenerating epithelial cells and eosinophils. The first corneal change is a punctate epithelial keratitis, which if left unchecked develops into a macroerosion (**Figure 8**) and finally a corneal plaque develops (**Figure 9**). The conjunctiva itself is characteristically oedematous and contains an array of immunological cells including lymphocytes and mast cells. Evidence of type I involvement includes the histological detection of, *inter alia*, degranulated mast cells, eosinophils and increased levels of IgE in affected eyes⁴⁸. The detection of CD4+ T cells and macrophages is indicative of a delayed inflammatory component⁴⁹.

Module 4 Part 2

Type II hypersensitivity: cytotoxic

This classification of hypersensitivity involves either IgG or IgM antibodies, which may induce cellular lysis due to the involvement of the classical complement pathway (as seen in blood transfusion reactions) or recruit and activate inflammatory cells via complement. The components of complement include the C5a, which serves to attract inflammatory cells to the site of interest. The hypersensitivity reaction is a result of the excessive amount of extracellular mediators released by the inflammatory cells to antigens that are too big to be completely phagocytosed.

Antibodies to self-antigens, such as the acetylcholine receptor in myasthenia gravis, is not only another example of type II hypersensitivity but also an example of autoimmune disease.

Ocular manifestations

- Mooren's ulcer
- Cicatricial pemphigoid

Mooren's ulcer is a rare peripheral ulcerative keratitis that exists either as a unilateral, non-progressive form which has a predilection for elderly patients or as a more severe, progressive form affecting both eyes of relatively young individuals. The signs range from a small patch of grey infiltrate near the margin to frank ulceration involving the entire corneal circumference and, in some cases, the central region as well. The healing process results in a thin, vascularised, opaque cornea. Investigators have identified a significant number of lymphocytes, neutrophils and plasma cells⁵⁰ in the cornea, thus providing unequivocal evidence to support the theory that this condition has an immunopathological aetiology.

Cicatricial pemphigoid is a chronic, blistering disease, which has a predilection for both the ocular and oral mucous membranes. Unlike its self-limiting counterpart, pemphigus vulgaris, cicatricial pemphigoid rarely affects the skin. Ocular cicatricial pemphigoid is a serious, bilateral condition that represents effective shrinkage of the conjunctiva. Although the initial presentation may be subacute and non-specific, it frequently progresses to symblepharon, entropion with secondary trichiasis, dry eye, ankyloblepharon and conjunctival fornix shortening. There is evidence to support the presence of IgG antibodies directed against self-antigen in the basement membrane of both the skin and eye⁵¹. Binding of the aforementioned antibodies may activate complement with subsequent recruitment of inflammatory cells into the area. The process of cicatrization is achieved through the secretion of collagen via fibroblasts as a result of stimulation via cytokines released from the invading inflammatory cells.

Type III hypersensitivity: immune complex

Large pathogens with multiple antigenic sites have several antibodies bound to them forming

immune complexes. Normally, these complexes are removed by the mononuclear phagocytes in the liver and spleen with no adverse sequelae. However, persistence of immune complexes does occur in certain individuals leading to their deposition in tissue. As a consequence of the latter action, complement may be activated thus paving the way for inflammatory cells to enter the deposition site. Since blood vessels (which filter plasma at high pressure and exhibit a great deal of tortuosity) are more susceptible for immune complex deposition, the ciliary body is particularly vulnerable to this type of hypersensitivity reaction.

Ocular manifestations

- Uveitis (Cohn's disease)
- Peripheral corneal lesions associated with rheumatoid arthritis
- Stevens Johnson syndrome
- Sjögren's syndrome

The signs and symptoms of the above will be described in future articles in the series.

Type IV hypersensitivity: delayed-type hypersensitivity (DTH)

The term DTH has been used to describe a reaction owing to its prolonged time-scale relative to the other hypersensitivity types. Although DTH can be transferred by T cells that have been previously sensitised by an antigen, it cannot be transferred in serum.

The sequence of events leading to DTH begins with initial presentation of the antigen peptide to T cells by APCs (e.g. Langerhans' cells). The primed T cell migrates to the site of antigenic entry whereby it releases pro-inflammatory mediators such as TNF. The release of these cytokines facilitates blood flow and extravasation of plasma contents to the area. The activation of CD4 T helper and CD8 cells results in the release of IFN- γ and, as a consequence, enhances macrophage activity in that area. Resolution of DTH is dependent on the efficacy with which such phagocytes can remove the offending antigen.

Recalcitrant infectious agents result in a chronic DTH that causes the chronically activated macrophages to fuse together and form multinucleated giant cells. In an attempt to contain the infectious agent, macrophages may undergo further inter connections to resemble an epithelial layer. Owing to the similarity to this layer they are referred to as epithelioid cells. Both epithelioid and multinucleated giant cells secrete factors that induce fibrosis resulting in granuloma formation. Thus granulomata are the hallmark of chronic inflammation. Damage and loss of function of the neighbouring tissues frequently ensues until the agent is removed either chemically or surgically.

Ocular manifestations

- Ocular allergies (VKC, AKC GPC)
- Idiopathic uveitis
- Sympathetic ophthalmia
- Phlyctenulosis

Type V hypersensitivity

This relatively new category encompasses the concept of autoantibodies binding to hormone receptors that mimic the hormone itself. This results in stimulation of the target cells. Examples include thyrotoxicosis.

Autoimmunity

The ability to react against self-antigens is known as autoimmunity. However, a significant number of people exist who harbour auto-antibodies and yet remain asymptomatic. The corollary of this is that the presence of autoreactive cells *per se* is not sufficient to trigger autoimmune disease. In fact, autoimmune disease is a result of breakdown of one of the immunoregulatory mechanisms. Furthermore, autoimmune disease may be classified as either being organ specific (e.g. insulin dependent diabetes mellitus, Grave's disease) or non-organ specific (e.g. Sjögren's syndrome, ankylosing spondylitis).

It is important to realise that the causes of autoimmune diseases are multifactorial. The main predisposing factors are age, gender, infection and genetics. However, one of the most important factors of interest to immunologists and clinicians alike is the association between HLA and autoimmune disease. When determining the likelihood of contracting a disease both epidemiologists and clinicians refer to the relative risk. In the case of HLA antigen, the relative risk compares the chance of a person who has a particular HLA antigen acquiring a disease to those individuals who do not have such an antigen. The association is exemplified by the relative risk of suffering from ankylosing spondylitis (AS) in individuals who possess HLA-B27. In patients suffering from AS, the prevalence of HLA-B27 is 90% and this figure rises to 95% in patients who suffer both with the disease and acute iritis. Indeed, in the UK approximately 45% of patients who present with acute iritis will harbour HLA-B27⁵².

It is therefore important that patients who present with anterior uveitis are screened for HLA-B27 because although a positive result may not necessarily be diagnostic, its presence will certainly improve the sensitivity of further radiological tests.

Table 9 compares the HLA associations with both ocular disorders together with those systemic disorders relevant to the ophthalmic practitioner.

Conclusion

This article has only broached the fascinating subject of ocular immunology. A basic understanding of immunology is required if practitioners are to therapeutically manage their patients. Further articles in the series will help to reinforce the concepts of this challenging subject.

Acknowledgement

The author would like to thank Professor Roger Buckley for his permission to use **Figures 7 to 9**.

Figures 1 to 6 reprinted from Immunology, Third Edition, Roitt, Brostoff and Male, 1993 by permission of the publisher Mosby.

About the author

Gregory Heath is an optometrist working part-time in private practice. He was recently awarded the diploma in clinical optometry at City University and is currently reading medicine at the Royal Free and University College, London, Medical School.

References

References are available upon request. Please fax 01252-816176 or email info@optometry.co.uk.

Disease	HLA association	Relative risk ⁵⁴	Ocular manifestation
Ankylosing spondylitis	B27	90	Anterior uveitis
Reiter's disease	B27	33	Mucopurulent conjunctivitis Anterior uveitis Keratitis
Rheumatoid	DR4	7	Keratoconjunctivitis sicca Keratitis Scleritis
Primary Sjögrens' syndrome	DR3, DR5	10	Keratoconjunctivitis sicca
Sarcoidosis	DR3	Not known	Anterior uveitis (acute and chronic) Dacryoadentitis Retinal vasculitis, neovascularisation, Optic nerve granulomata
Cicatrical pemphigoid	DQw7	Not known	Shrinkage of conjunctiva
Behcet's disease	B5	3	Anterior uveitis Retinitis, periphlebitis, retinal oedema
Sympathetic ophthalmia	DR4, A11, B40	Not known	Panuveitis
Systemic lupus erythematosus	DR2, DR3	3	Punctate epithelial keratopathy Keratopathy Necrotising scleritis Retinal lesions (cotton wool spots) Autoimmune optic neuropathy

Table 9:
HLA and ophthalmic disease

Multiple choice questions - Basic immunology

Please note there is only one correct answer

- Which one of the following is not part of the innate immune system?
 - Mast cells
 - Complement
 - Phagocytes
 - T cells
- Which one of the following statements is correct regarding the innate immune system?
 - It is specific
 - It evokes a more potent response on secondary exposure
 - It represents the first line of defence
 - It is able to memorise pathogens on subsequent exposures
- Which one of the following statements is correct regarding the cells of the innate system?
 - Basophils are important phagocytes
 - During phagocytosis the pathogen becomes initially internalised as a phago-lysosome
 - Eosinophils play an important role in combating virally-infected cells
 - Langerhans' cells form a bridge between innate and adaptive immunity
- Which one of the following statements is correct regarding the adaptive immune system?
 - It consists of all types of lymphocytes
 - T cells produce antibodies
 - T cells mature in the thymus
 - B cells are produced in the spleen
- Which one of the following statements is correct regarding T cells?
 - T cells can be subdivided into TH₁ and TH₂ subtypes only
 - T cells alone can identify any type of antigen
 - T cells express cell surface proteins denoted by cluster determinant (CD) numbers
 - All T cells are involved in initiating the inflammatory response
- Which one of the following statements is incorrect?
 - B cells have antibodies as their cell surface receptor
 - There are five types of antibody
 - IgE is an important antibody in allergies
 - All B cells differentiate into plasma cells
- Which one of the following statements is correct regarding ocular immune privilege?
 - There is an absence of Langerhans' cells in the central cornea
 - Aqueous humour has no role
 - Abundant vascular supply is vital
 - All ocular cells express MHC class II
- Which one of the following statements concerning ocular immunology is incorrect?
 - Levels of IgA and complement increase when the eyes are closed
 - IgA is the predominant antibody in blood and tissue fluid
 - IgM, IgG and IgA antibody isotypes have been identified in the cornea
 - The sclera contains a smaller number of immune cells than the conjunctiva
- In a patient suffering from vernal conjunctivitis, which one of the following statements is correct?
 - Trantas'-dots represent infiltrating T cells
 - Affected eyes have increased levels of IgD
 - Histologically, mast cells, lymphocytes and macrophages have been identified
 - Is due to type III Hypersensitivity
- Which one of the following statements is incorrect?
 - HLA-B27 is a risk factor for both anterior uveitis and ankylosing spondylitis
 - Granulomas are present in type IV hypersensitivity reactions
 - Histamine is an important vasoconstrictor
 - IgE mediated hypersensitivity is of rapid onset
- What proportion of patients with acute iritis will harbour HLA-B27?
 - 15%
 - 25%
 - 45%
 - 65%
- Which one of the following statements is correct regarding a type I hypersensitivity reaction?
 - It always occurs in isolation
 - It is characterised by the presence of macrophages
 - It is associated with myasthenia gravis
 - It involves the degranulation of mast cells following the cross-linking of IgE bound to its cell surface

An answer return form is included in this issue. It should be completed and returned to: CPD Initiatives (c4082c), OT, Victoria House, 178-180 Fleet Road, Fleet, Hampshire, GU51 4DA by March 6, 2002.