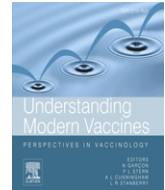




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Understanding Modern Vaccines: Perspectives in Vaccinology



Vaccine immunology

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Key concepts

- The human immune system consists of two connected compartments — the innate and adaptive — which function via the actions of secreted and cellular effectors
- The innate and adaptive immune systems work sequentially to identify invaders and formulate the most appropriate response; this interaction is crucially bridged by specialised antigen-presenting cells (APCs)
- The innate response, via the action of APCs, sets the scene for the subsequent adaptive response by providing information about the nature of the threat
- Primary exposure to a pathogen or antigen induces the production of a population of adaptive immune cells with antigen specificity that are retained for long periods and provide a rapid response upon subsequent exposure
- The vaccine concept is based on stimulating the body's defence mechanisms against a specific pathogen to establish this immunological memory
- Current vaccine strategies take advantage of immunological mechanisms, and often target the innate immune system and APCs to induce the desired specific adaptive immune response
- Future research is also set to examine ways of making the immune response more effective in generating cross protective responses against different subtypes or strains of pathogens exhibiting antigenic variation

Immunology of vaccines – a brief history

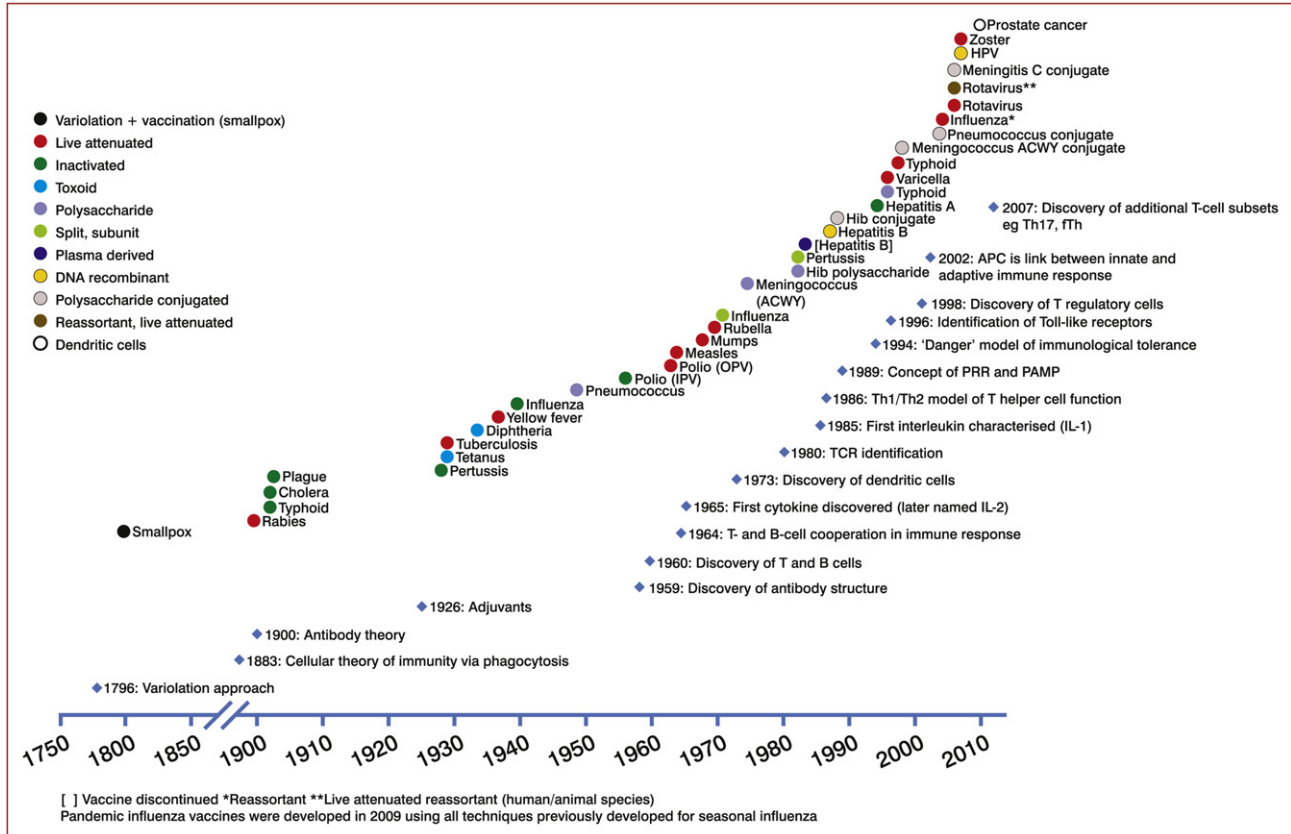
The science of immunology began in the 19th century. Louis Pasteur and Robert Koch established that microorganisms were the actual cause of infectious diseases, which greatly advanced our understanding of the specific basis of immunity. Pasteur then disproved the spontaneous generation theory of *microbes* and Koch developed his four postulates to establish the relationship between the individual agent and the cause of a disease. The discovery of antibodies in 1890 and the passive immunotherapy of diphtheria with anti-diphtheria toxin antibodies derived from horses resulted in the first Nobel Prize in Medicine being awarded to Emil von Behring. In parallel, a greater understanding of the way in which hosts and pathogens interact was unravelling some of the mysteries surrounding infection and disease. Host cells that ingested and destroyed invading microbes were identified by Élie Metchnikoff and named *phagocytes* (literally 'eating cells', from the Greek). Metchnikoff and Paul Ehrlich shared the Nobel Prize in Medicine in 1908 for their research in immunology.

The 20th century saw major advances in immunology and the related field of *vaccinology*, and recent studies continue to provide profound insights into immunological mechanisms. [Figure 2.1](#) summarises some of the important immunological milestones that are of particular relevance to the understanding of vaccinology and indicates several key parallel events in vaccine development. Our increased knowledge of the integrated immune cellular and molecular processes has facilitated, often with hindsight, an understanding of the principles underpinning effective *vaccination*. Vaccine design is now approached from a more rational, less pathogen-based perspective and, increasingly, immunology is guiding vaccine researchers towards new horizons with the potential to improve on nature. As such, the basic concepts of immunology are an essential component of the foundations of modern vaccinology.

To understand the immunology of vaccines, it is important first to examine the key players of the immune system ([Figure 2.2](#)) and to

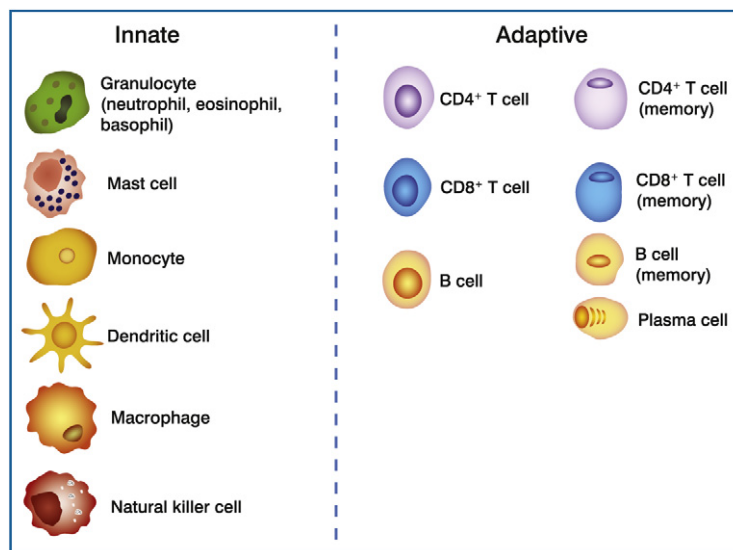
Figure 2.1 Immunological milestones of particular relevance to vaccinology. Progress in vaccine design and technology is underpinned by discoveries in immunology. This is shown by the increasing number of vaccines developed as knowledge of immunity has increased.

HPV, human papillomavirus; Hib, Haemophilus influenzae type b; OPV, oral polio vaccine; IPV, inactivated polio vaccine; Th, T helper cell; APC, antigen-presenting cell; PRR, pattern recognition receptor; PAMP, pathogen-associated molecular pattern; IL, interleukin; TCR, T-cell receptor.



understand how they are produced, activated and regulated. In the following section we will discuss the innate and adaptive phases of the immune response and how these are bridged by the actions of specialised *antigen-presenting cells* (APCs) – a key step in the successful response to vaccination.

Figure 2.2 Key players of the immune system. The innate and adaptive immune systems are populated by many different cells that vary in their roles and responsibilities. *CD*, cluster of differentiation.



Although we are continuously exposed to external antigens, foreign substances and microorganisms, under normal circumstances food and airborne antigens do not provoke the immune system. In addition, some normal commensal floras have also co-evolved with their human hosts to suppress or avoid triggering defence mechanisms. It is now known that this is partly because immune responses are usually only triggered in the context of threat or damage to the host; however, both self and non-self-antigens have the potential to trigger immune responses under conditions of acute or chronic inflammation.

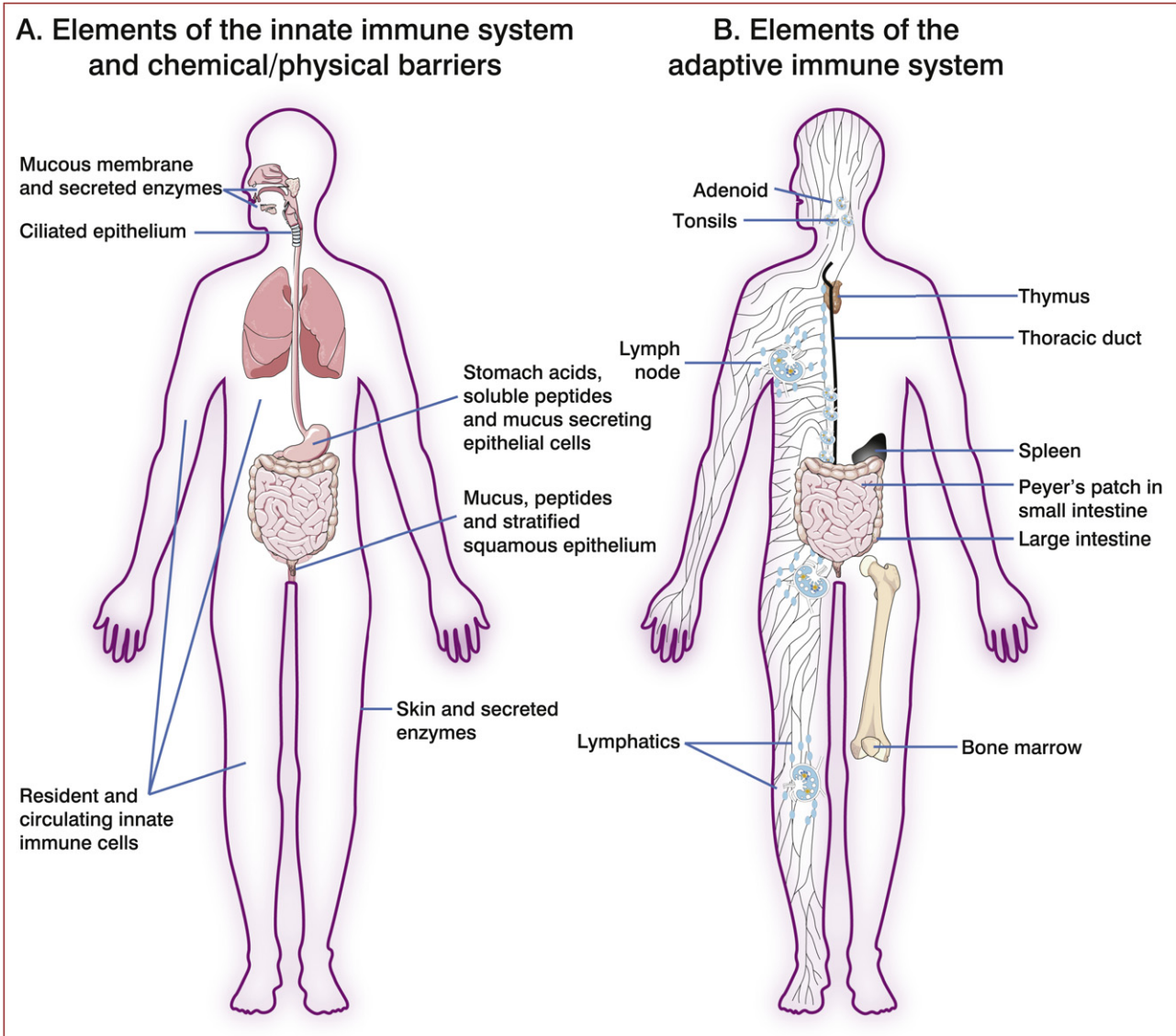
The immune system

Physical and chemical barriers comprise the body's first line of defence — including the skin, ciliated epithelia, mucous membranes, stomach acids and destructive enzymes in secretions. The immune system in vertebrates is a network of cells, tissues and organs that function in a coordinated fashion to defend the body against factors that could penetrate its physical and chemical barriers. Some of the key organs of the immune system are illustrated in [Figure 2.3](#), and include the primary lymphoid organs (bone marrow and *thymus*) where lymphocytes are generated, and the *secondary lymphoid organs* (peripheral lymph nodes, spleen, tonsils, Peyer's patches) where immune responses are initiated and regulated.

INNATE AND ADAPTIVE IMMUNITY

All organisms have some form of innate protection against the outside world, which may be as simple as a cell wall or waxy

Figure 2.3 Organs and tissues of the immune system. The innate immune system is formed from a combination of physical barriers (skin, mucus), chemical defences (acids, antimicrobial peptides) and specialised cells capable of responding to pathogens without needing to recognise specific antigens (A). The adaptive immune system consists of a network of primary and secondary organs, where immune cells are either produced or reside until they become activated (B).



coating. As higher organisms evolved, their innate defences became more sophisticated and the jawed vertebrates developed a highly specialised system of immunity – acquired (or adaptive) immunity – which may have evolved as a consequence of co-evolution with specialised parasites, increased metabolic rates due to dietary changes, and genomic instability. Jawed vertebrates thus have two interlinked systems which act sequentially to establish protective immunity – the *innate immune system* and the *adaptive immune system*. The innate immune system acts as a first line of defence which comprises both cellular and non-cellular effectors. This system provides early containment and defence during the lag time before adaptive immune effectors are available. Innate immunity comprises both soluble (eg complement, *lysozyme*) and cellular effectors (eg *natural killer* [NK] *cells*, *macrophages* and *dendritic cells* [DCs]). The innate and adaptive immune systems are principally bridged by the action of specialised APCs, which translate and transfer information from the body tissues and innate immune system to the adaptive immune system, allowing a systemic response to a localised threat. The innate immune system therefore drives and shapes the development of adaptive immune responses via chemical and molecular signals delivered by APCs to induce the most appropriate type of adaptive response. The adaptive immune system forms the second, *antigen*-specific line of defence, which is activated and expanded in response to these signals.

The innate immune system

CELLS OF THE INNATE IMMUNE SYSTEM

Cells of the innate immune system are produced in the bone marrow and then migrate to different anatomical locations. The innate immune cell repertoire includes tissue-resident cells such as macrophages and immature DCs, and cells which circulate via blood and the lymphatic system, such as *monocytes*, *neutrophils*, *eosinophils*, NK cells and *innate T cells*. Non-immune system cells

at vulnerable locations, including keratinocytes and other epithelial and mucus-producing cells, fibroblasts and endothelial cells, can also exhibit innate defensive behaviours.

DETECTION OF PATHOGENS

Invading pathogens are detected by the innate immune system through molecular-sensing surveillance mechanisms. These mechanisms include detection of pathogens via *pattern recognition receptors* (PRRs), expressed by cells of the innate immune system, which can be secreted, or expressed on the cell surface, or are present in intracellular compartments (eg *DNA/RNA sensors*). Examples of PRRs are the transmembrane *Toll-like receptors* (TLRs) and [Table 2.1](#) lists the qualities of several TLRs. The model system in [Figure 2.4](#) illustrates the location of the main human PRRs, and highlights the signalling pathways of several mammalian TLRs.

The key feature of cells of the innate immune system is their ability to directly recognise different classes of pathogens – eg viruses and bacteria – by PRRs. These receptors are able to bind to molecules (such as bacterial membrane components) that are shared by several pathogens (eg all Gram-negative bacteria express *lipopolysaccharide* [LPS]), enabling the innate immune system to sense the occurrence of an infectious event. Recently, DCs and macrophages have been shown to react to signals released by damaged cells, indicating that the innate immune system can react to both the presence of infectious microbes (via *pathogen-associated molecular patterns* [PAMPs]) and to the consequences of an infectious event.

Epithelial cells, fibroblasts and vascular endothelial cells are also able to recognise PAMPs, and signal to innate immune cells when infected, stressed or damaged. This is mediated by stress signals (such as heat-shock proteins) and active small-molecule peptides (such as *defensins*, lysozymes and *cathelicidins*) which have direct antimicrobial properties and also act to alert immune cells. Specialised chemical messengers, including *cytokines* and

TABLE 2.1. EXAMPLES OF MAMMALIAN TLRs

Receptor	Pathogen/ligand	Location
TLR1	Bacteria Parasites	Cell surface
TLR2	Bacteria Fungi Viruses Parasites	Cell surface
TLR3	Viruses	Cell compartment
TLR4	Bacteria Viruses Parasites	Cell surface
TLR5	Bacterial flagellin	Cell surface
TLR6	Mycoplasma	Cell surface
TLR7	Bacteria	Cell compartment
TLR8	Single-stranded DNA	Cell compartment
TLR9	Pathogen CpG DNA	Cell compartment

This table provides examples only and is not intended to be an exhaustive list. CpG, adjacent cytosine (C) and guanine (G) residues in a linear sequence connected by a phosphodiester bond (see *Chapter 4 – Vaccine adjuvants*, Figure 4.9).

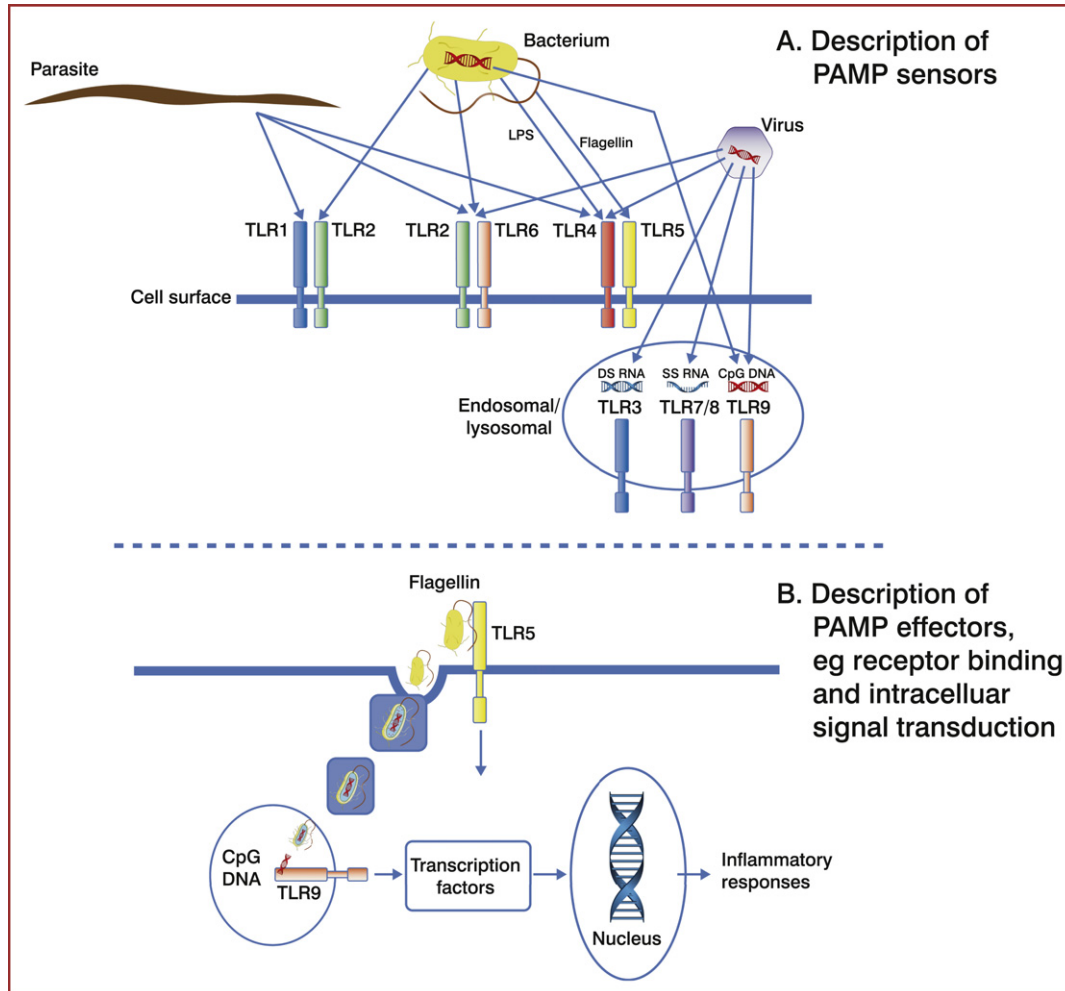
The local reactogenicity observed following vaccination probably reflects the induction of local inflammatory responses, which are important in the initiation of a successful immune response.

chemokines, are secreted by stressed/damaged cells and innate immune cells to attract other resident and circulating innate cells to the site of infection. Cells dying due to infection also release other small molecules, such as urea, which alert DCs.

Appendices, Supplementary Table 1 shows some examples of the innate biological consequences of signalling through PRRs. The downstream adaptive responses triggered by these signals are determined by the intracellular signalling pathway into which the signal feeds. Further fine-tuning of these responses to specific outcomes is believed to be achieved via the recruitment of specific intracellular *adaptor molecules*, which modify and manipulate the signal sent to the nucleus of the innate cell to tailor the profile of gene expression. Redundancy exists in pathogen detection systems, as multiple receptors may recognise the same pathogenic structure and, conversely, a single receptor may be

Figure 2.4 Cell-surface and intracellular PRRs. TLRs are a class of pathogen receptors that are known to recognise many PAMPs including surface antigens and/or a pathogen's genetic material (A). For example, TLR4 is positioned at the cell surface and recognises bacterial and viral antigens, whereas TLR9 is located intracellularly and recognises bacterial and viral DNA. Transmission of the signal is mediated by intracellular adaptor molecules (B). Activation of these messengers triggers the movement of transcription factors to the nucleus, where proinflammatory gene expression is induced or enhanced. There are many opportunities in the signalling pathways for the cell to recognise a PAMP and become responsive, maximising the chances of pathogen detection.

PAMP, pathogen-associated molecular pattern; LPS, lipopolysaccharide; TLR, Toll-like receptor; DS, double stranded; SS, single stranded; CpG, adjacent cytosine (C) and guanine (G) residues in a linear sequence connected by a phosphodiester bond.



capable of delivering more than one signal to the host cell. Overall, the integration of these signals by APCs leads to their activation. This enables them to act as messengers to precisely define the nature of the perceived danger and convey this information to the secondary lymphoid organs, where they interact with, and specifically activate, the relevant adaptive immune response.

EFFECTORS OF THE INNATE RESPONSE

Under some circumstances, pathogen clearance may be achieved by innate immune effectors without activation of an adaptive immune response. Activated innate cells act as phagocytes, engulfing and destroying the pathogen within intracellular vesicles containing digestive enzymes. To be efficient, this response requires both the recruitment and activation of phagocytes at the site of infection, a process often referred to as the inflammatory response. Cells residing in proximity to the infection site are activated upon recognition of PAMPs, and secrete a large array of soluble mediators, including chemokines and cytokines (Figure 2.5). Chemokines behave as chemoattractants (*Appendices, Supplementary Table 2*), favouring the recruitment of innate immune cells to the site of infection, while cytokines (including tumour necrosis factor and *interferons*) (*Appendices, Supplementary Table 5*) act by increasing the phagocytic activity of cells. Innate immune cells also produce a series of soluble chemical factors (such as peptides) that are able to directly target the invading microbes.

Additionally, antigens are taken up by innate cells, with immature DCs the most specialised among them. The antigen is subsequently processed and the DC differentiates into an APC. Antigen-carrying APCs then migrate to the draining lymph node and provide the link between the innate and adaptive immune responses.

Complement

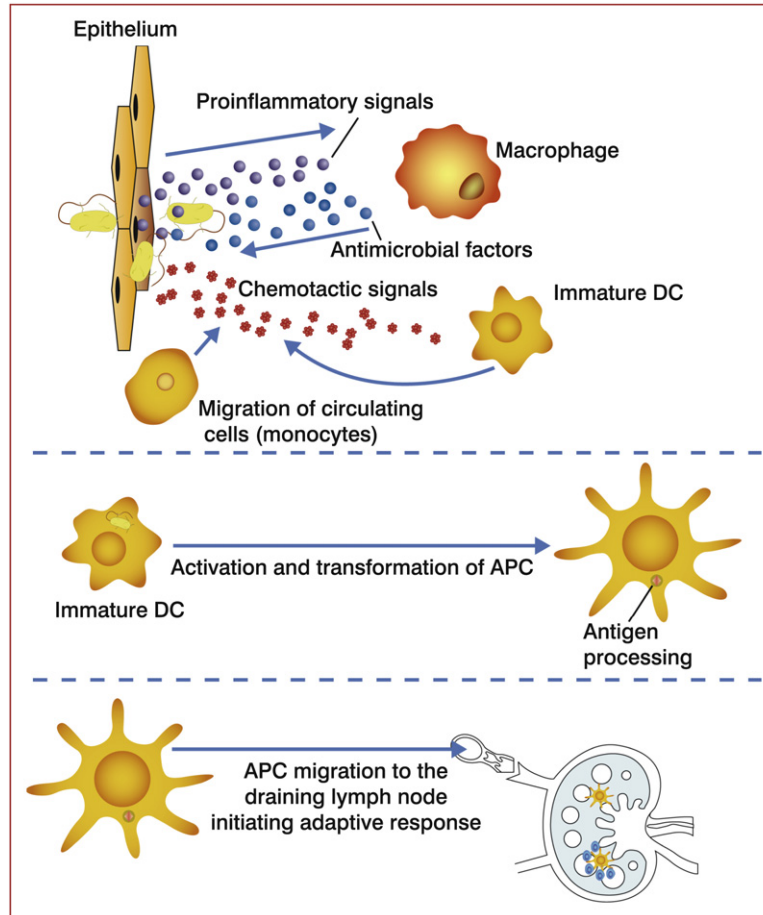
The *complement system* consists of approximately 25 proteins that work together to 'complement' the action of the adaptive immune

The inflammatory response

The local inflammatory response aims to rapidly recruit innate effector cells to an infected or damaged body site. The local, elevated secretion of cytokines and chemokines causes an increase in blood vessel permeability and the release of plasma, producing the swelling, redness, pain and heat that are typical symptoms of inflammation. Inflammation is also a protective response that helps to initiate the healing process. Soluble factors produced during an innate response can damage healthy cells; inflammation therefore needs to be a closely regulated process.

Figure 2.5 Innate effector mechanisms. The innate immune system responds to challenge by secreting factors that directly kill or inhibit the invader; or that attract more cells capable of removing the threat and, if necessary, alerting the adaptive immune system. Tissue-resident cells, such as macrophages, secrete antimicrobial factors in response to proinflammatory stimuli. Circulating cells are recruited to the site by chemotactic stimuli, released by damaged or infected epithelial and endothelial cells and other innate immune cells. If innate effectors contain and remove the invader, infection can be resolved without the intervention of the adaptive immune response. Additionally, antigens are taken up by immature DCs. The antigens are processed intracellularly and the cell transforms into an APC followed by migration to the draining lymph node.

DC, dendritic cell; APC, antigen-presenting cell.



The diversity of adaptive immune receptors

In contrast to innate cells which express a few dozen pathogen-specific receptors, lymphocytes can express an enormous diversity of antigen-specific receptors (around several thousand billion), a number that far exceeds the total number of genes present in our genome (around 25,000). Antigen receptors are in fact encoded by a set of ‘mini-genes’ that undergo complex recombination events, allowing the generation of diverse proteins from a limited number of building blocks. Additional individual changes and random insertions in the genes further increase the diversity of the receptors. The vast T- and B-cell repertoires that humans possess provide a massive potential for antigen-specific responses. This repertoire is maintained with single or very few cells expressing receptors that will recognise any given antigen, until individual clones are selectively expanded in response to a specific challenge. Notwithstanding these abilities of the adaptive immune system, rare individuals may have a genetic makeup that does not enable them to react to one or other antigen present on a pathogen.

response in destroying bacteria. Complement proteins circulate in the blood in an inactive form. Once activated, complement components serve several effector roles including the recruitment of phagocytes, the *opsonisation* of pathogens to promote *phagocytosis*, the removal of *antibody*–antigen complexes and the lysis of antibody-coated cells.

The adaptive immune system

One critical function of the innate immune system is to alert the adaptive immune response, whereby lymphocytes with antigen-specific receptors are activated and proliferate to fight the pathogenic threat. Their antigen receptors evolved in response to the selection pressure of different pathogens and therefore have very diverse characteristics.

Lymphocytes can be found circulating in the blood/lymph and residing within secondary lymphoid organs, such as the lymph nodes and spleen. There are two main subsets of lymphocytes involved in adaptive immune responses, whose nomenclature reflects the site of their development – *B cells* develop in the bone marrow and *T cells* develop in the thymus.

The adaptive immune system essentially functions via the production of three key types of effector: *antibodies* (produced from B cells), cytokines and cytolytic molecules (produced by T cells) (Figure 2.6).

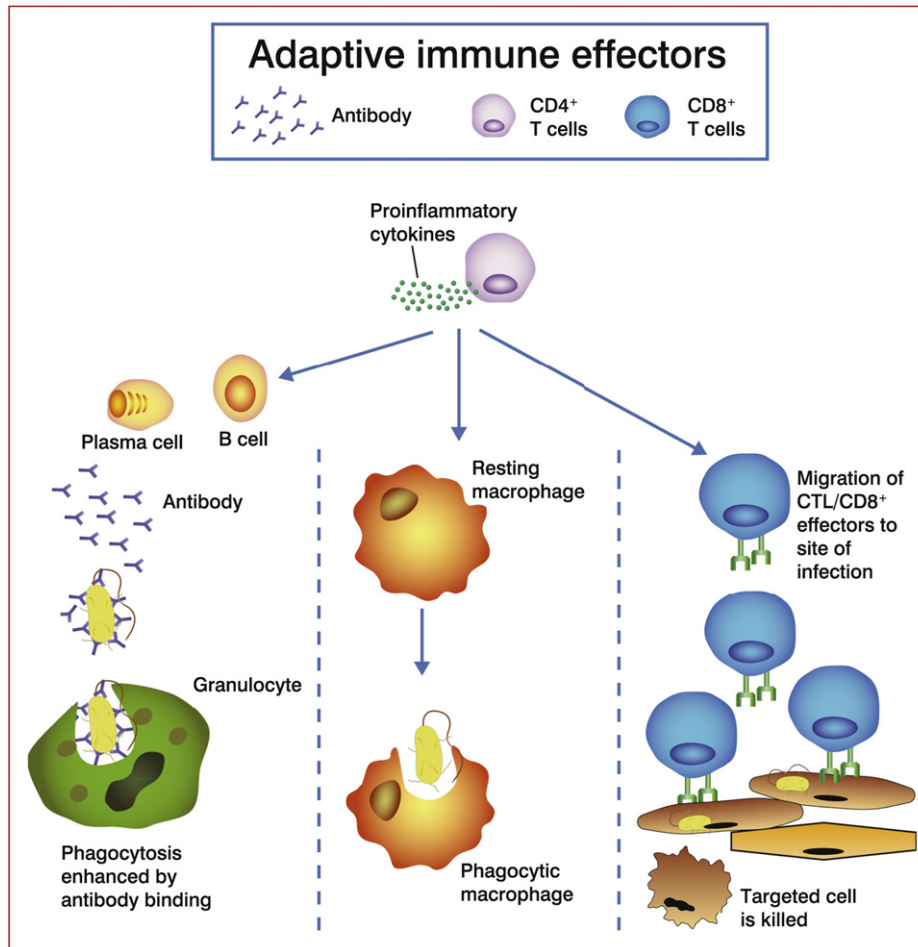
THE FACILITATORS AND EFFECTORS OF THE ADAPTIVE IMMUNE RESPONSE

Facilitators: the role of the APC

The first cells to interact with an incoming pathogen are often the phagocytes of the innate immune system, which can engulf and degrade pathogens. However, it is now clearly recognised that professional APCs, typified by DCs, can ingest pathogen-derived proteins, partially digest, process and transport the peptide products to the cell surface, rather than targeting them for complete destruction.

Figure 2.6 Adaptive immune effectors. Adaptive immune effectors are recruited to the site of challenge and also circulate systemically to deal with any disseminated pathogens/antigens. Adaptive effectors can act by interacting directly with the pathogen, or by modulating the action of innate immune effectors. $CD4^+$ cells often represent the first lymphocyte subset activated during an adaptive immune response and are able to affect the behaviour of other immune cells (thereby acting as 'helpers'). Soluble antibodies reaching the site of infection perform effector functions such as enhancing phagocytosis (greater detail on antibody effector functions is provided in Figure 2.7). $CD4^+$ T cells expressing a Th1-type phenotype activate macrophages locally via secretion of proinflammatory cytokines, increasing their phagocytic and antimicrobial activity. $CD8^+$ T cells recruited to the site of infection/inflammation express surface molecules that are directly cytotoxic to infected target cells via apoptosis but can also exert antiviral activity on these cells via cytokines.

CD, cluster of differentiation; CTL, cytotoxic T lymphocyte; Th, T helper cell.



MHC restriction and T-cell subsets

MHC molecules display antigenic peptides to T cells. MHC class I molecules receive endogenous proteins, including those derived from intracellular pathogens, and are expressed by virtually all nucleated cells of the body. MHC class I peptides are recognised by the T-cell receptor (TCR) expressed by CD8⁺ T cells. MHC class II molecules are usually expressed by a restricted set of cells such as macrophages, DCs and B cells. They present peptides derived from exogenous antigens, taken up via mechanisms such as endocytosis and phagocytosis. Antigenic peptides presented by MHC class II molecules are bound by TCRs expressed by CD4⁺ T cells.

These pathogen-derived peptide antigens are bound by a specialised set of receptors known as *human leukocyte antigens* (HLA) that act as 'antigen-presenting' molecules. These molecules are encoded by a gene family called the *major histocompatibility complex* (MHC). DCs displaying pathogen-derived antigen on the cell surface are also endowed with migratory properties that allow them to leave the infected site and migrate towards the nearest lymph node. DCs therefore represent an important cellular messenger, able to transport molecular pathogen fragments to secondary lymphoid organs. Antigen fragments displayed by DCs are destined to activate pathogen-specific T cells residing in the lymph nodes.

Facilitators: T-cell activation

T cells represent a subset of lymphocytes that differentiate within the thymus, a small bi-lobular organ situated in the anterior mediastinum. Each T cell expresses a unique antigen-specific receptor (the TCR) with a unique recognition capacity.

T cells do not directly recognise whole pathogens, but are only specifically activated by DCs transformed into APCs which present molecular fragments (mostly peptides derived from limited digestion of protein antigens) in association with MHC molecules at the cell surface. *Naïve lymphocytes* are therefore 'blind' to live microorganisms and need the help of APCs to adequately react to an invading pathogen.

Facilitators: CD4⁺ T cells

An individual naïve T cell can only be activated by a protein antigen for which it has a specific receptor, and which has been processed and presented by an APC. Cells activated by antigen-bearing DCs express the cluster of differentiation (CD)4 cell-surface protein, and are thus referred to as *CD4⁺ T cells*. These cells often represent the first lymphocyte subset activated during an adaptive immune response, and play both modulatory and effector roles during an immune response. CD4⁺ cells act primarily by secreting soluble factors (cytokines) that are able to exert direct antimicrobial

properties and affect the behaviour of other immune cells. In most cases, $CD4^+$ cells help other immune cells perform their task and are, therefore, referred to as *helper T cells* (Th). Based on the types of cytokines they secrete and differing abilities to help other subsets of immune cells, several sub-populations of Th cells have been identified (*Appendices, Supplementary Table 3*).

One subset of Th cells, the Th1 cells, appear to secrete mainly interferon-gamma ($IFN\gamma$), a cytokine known to limit pathogen survival and spreading. It is also known to promote the differentiation of cytolytic cells that are able to destroy cells infected with intracellular pathogens (see $CD8^+$ T cells). Th1 cells are, therefore, considered important for inducing immune responses involved in the clearance of pathogens.

Another subset of T helper cells, the Th2 cells, produce cytokines (*interleukins* [IL] IL-4, IL-5, IL-13) that appear particularly apt at activating innate cells (eosinophils, *mast cells*) which are often involved in the immune response to large extracellular *parasites*.

Another subset, termed *follicular T helper cells* (*Tfh*) based on their tissue localisation in follicular structures, have been defined by secretion of IL-21, a cytokine thought to favour the secretion of antibodies by antigen-specific B cells.

Activation of $CD4^+$ cells represents a key step in setting in motion an adaptive immune response. Through their ability to secrete cytokines, these helper cells will augment the capacity of other immune cells to perform their tasks. The adaptive immune response is frequently characterised by two effector cell populations, the $CD8$ -expressing cytolytic T cells and the antibody-secreting B cells.

Adaptive effectors: $CD8^+$ T cells

$CD8^+$ T cells exploit the TCR/MHC interaction around pathogen-derived peptides to detect and fight intracellular pathogens. To achieve this, $CD8^+$ T cells rely on the fact that virtually all nucleated cells (with a few notable exceptions) present fragments of intracellular proteins at their surface as part of the body's normal

Identified around 2005, Tfh cells were thought to be part of the Th2 subset based on the profile of cytokines they produced, but have subsequently been identified as a distinct subset of T cells that fulfil some of the roles originally attributed to Th2 cells.

surveillance processes. In contrast to classically defined APCs, which display antigenic fragments in association with MHC class II molecules, non-immune cells use a closely related set of molecules to display peptides derived from the cytoplasm – the MHC class I molecules. This complex mechanism of antigen presentation allows $CD8^+$ T cells to scan proteins from within the cell, while preserving the integrity of the cell membrane.

The salient feature of $CD8^+$ T cells is their ability to secrete cytotoxic factors in addition to cytokines. These factors enable $CD8^+$ T cells to kill cells displaying pathogen-derived peptides presented by MHC class I molecules, for example a virus-infected cell. By killing cells expressing high levels of virus-derived peptides at their surface, $CD8^+$ cells are able to eliminate infected cells before the completion of a viral replication cycle, thus limiting viral spread within an infected individual. In addition, $CD8^+$ T cells can inhibit viral replication without destroying the target cells by producing cytokines that are able to interfere with pathogen replication.

$CD8^+$ cytolytic cells can also eliminate cells displaying abnormal host peptides, such as those presented by tumour cells, and therefore play an important role in the immune control of aberrant cell growth. Although $CD8^+$ T cells can directly react to cells expressing the appropriate antigen/MHC class I complexes, their optimal activation programme (proliferation and acquisition of full cytolytic potential) is best achieved in the presence of cytokines produced by type 1 $CD4^+$ T helper cells.

B cells and antibodies

Antibodies represent a highly diverse set of soluble proteins secreted by the subset of lymphocytes referred to as B cells. B cells develop in the bone marrow before undergoing a process of differentiation and maturation in the spleen. As with T lymphocytes, each B lymphocyte expresses a unique antigen receptor (B-cell receptor [BCR]) enabling the cells to react to a specific antigen. In marked contrast to TCRs, BCRs can directly bind to molecules expressed by pathogens, with no need for previous internalisation

and presentation by APCs or other innate immune cells. Upon antigen encounter, B cells expressing the cognate BCR are induced to proliferate and differentiate into *plasma cells*, which can secrete large amounts of a soluble form of the BCR that we know as an *antibody*. This soluble protein is thus released in the blood and other body fluids (referred to as the 'humors') enabling them to fight infection at distant sites.

Antibodies can be viewed as bifunctional molecules, able to both recognise and eliminate a given antigen or pathogen. Antibody molecules consist of a 'constant' fragment, a structural feature common to all antibodies of a given isotype, and a 'variable' region, which includes the portion that gives the antigen specificity (or antigen-binding characteristics) of the antibody. The variable region of the antibody can exist in a huge number of molecular configurations, and an individual's BCR repertoire is generated to maximise capability to produce antibodies that are useful against diverse potential pathogenic threats. The constant part of the molecule exists in five different forms (isotypes) (*immunoglobulin* [Ig] A, IgD, IgE, IgG and IgM, see *Appendices, Supplementary Table 4*) that determine the ability of an antibody class to localise to particular body sites and target specific types of infection, and to recruit the optimal local effector cells (see [Figure 2.7](#)).

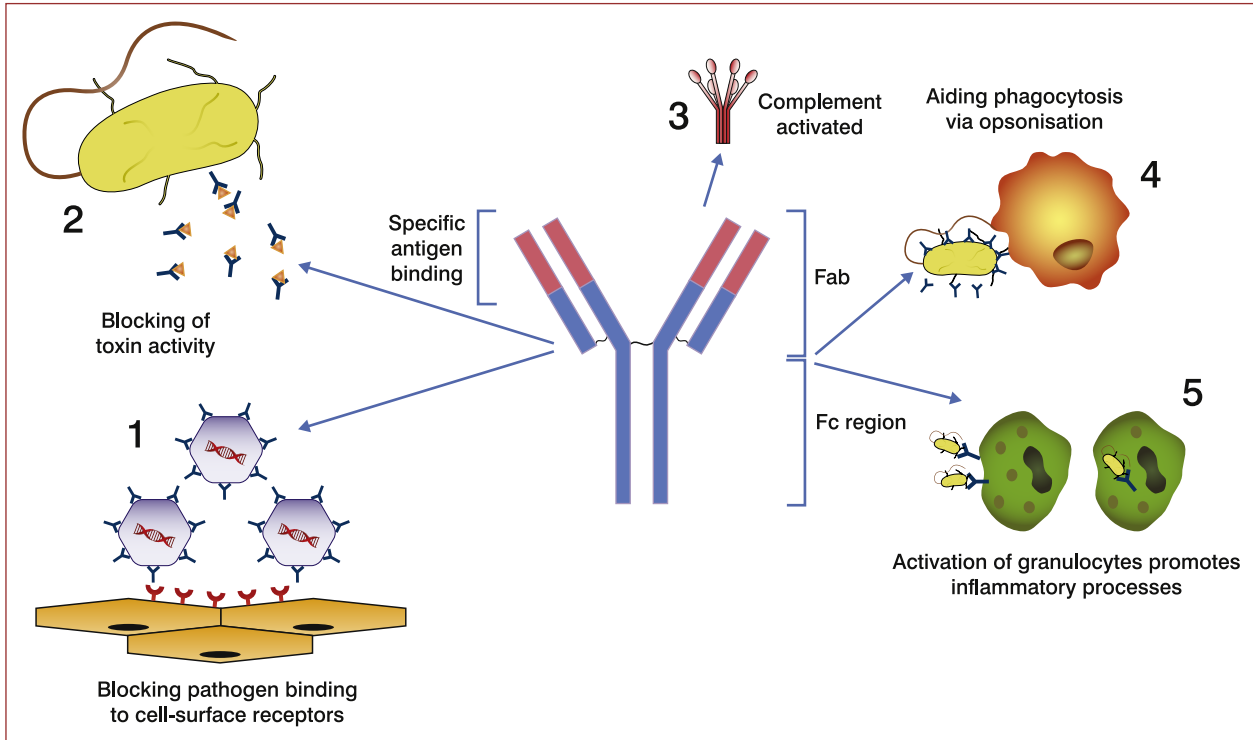
B cells can differentiate into antibody-secreting cells upon encounter with a given antigen or pathogen. In most cases, direct activation of B cells by an antigen is observed in response to repetitive antigenic structures, such as carbohydrates found in bacterial walls. These T cell-independent responses are characterised by the secretion of low-*affinity* antibodies of the IgM type. This type of response is often stereotyped in nature, lacking the typical *memory response* upon re-exposure to the same antigen (see section titled *Immunological memory*).

In most cases, optimal B-cell activation and differentiation into antibody-secreting plasma cells is only observed when both B and T cells are simultaneously activated by the same pathogen.

Antibodies play multiple roles in the control and elimination of pathogens, and in the response to vaccination. Binding of targets by antibodies is often sufficient to initiate processes that render the pathogen harmless.

Figure 2.7 Effector functions of antibodies. Antibodies play multiple roles in the control and elimination of pathogens and in the response to vaccination. Antibodies can bind directly to the surface of pathogens (1) or soluble mediators (2) preventing infection of host cells and neutralising pathogen-derived toxins. Antibodies can recruit effector molecules such as complement (3) or activate effector cells via their Fc portion (4, 5). Binding of the Fc region of an antibody to a specific Fc receptor expressed by innate cells can facilitate pathogen internalisation and destruction by phagocytes (by a process referred to as 'opsonisation') (4). Similarly, ligation of Fc receptors on granulocytes promotes their degranulation and triggers potent antimicrobial responses including the removal of antibody–antigen complexes and the lysis of antibody-coated cells (5).

Fab, fragment antigen binding; *Fc*, fragment, crystallisable.



In these instances, $CD4^{+}$ T cells differentiate into T_{fh} cells that are able to provide a helper signal to B cells. T cell-dependent B cell responses are characterised by the secretion of high-affinity antibodies and a large spectrum of isotypes (in particular IgG), and are typically associated with immunity resulting from natural exposure.

THE ROLE OF CYTOKINES

Cytokines are small proteins secreted by activated innate and adaptive immune cells (such as DCs, macrophages and T cells), which direct the activity of other cells to coordinate an appropriate immune response. Cytokines are a diverse family of molecules which include interleukins, interferons and growth factor responses (Appendices, Supplementary Table 5). Cytokines may act in an *autocrine*, *paracrine* or *endocrine* fashion, by binding cell-surface receptors and stimulating signalling pathways, ultimately affecting the gene expression of the target cell. Cytokines are referred to as either proinflammatory or anti-inflammatory, depending on their role during the establishment of immune responses. These two types then act together to control and regulate different aspects of the immune response.

REGULATORY T CELLS

Immune responses are prevented, down-regulated or terminated by multiple mechanisms. These mechanisms include *clonal deletion*, the activity of suppressor monocytes and anti-inflammatory cytokines, induction of *apoptosis*, induction of unresponsiveness by resting APCs, expression of inhibitory cell-surface co-receptors and the activity of regulatory CD4⁺ T cells.

Regulatory T cells (T_{reg} cells) belong to the CD4⁺ T-cell subset. Their role is to inhibit immune or inflammatory responses by blocking the activity of *effector T cells*, *helper T cells* and APCs. They are key to the down-regulation of immune responses at the appropriate point following a protective response, the immunological *tolerance* process (avoiding immune response towards *self-antigens* and non-threatening antigens, eg from food and commensal floras), and prevention of uncontrolled or chronic inflammatory responses.

Antibodies produced during the immune response may also down-regulate subsequent immune responses, for example by elimination or masking of antigen, hence limiting the activation of additional T cells. Antibody–antigen complexes may also bind to inhibitory receptors, initiating suppressive responses.

A genetic deficiency of T_{reg} cells results in severe autoimmune syndrome; conversely, infection may be established where responses are inappropriately suppressed by selective activation of T_{reg} cells, for example by the stomach pathogen *Helicobacter pylori*. Over-suppression of immune responses by regulatory mechanisms may also result in an inadequate response to vaccination in some individuals.

IMMUNOLOGICAL MEMORY

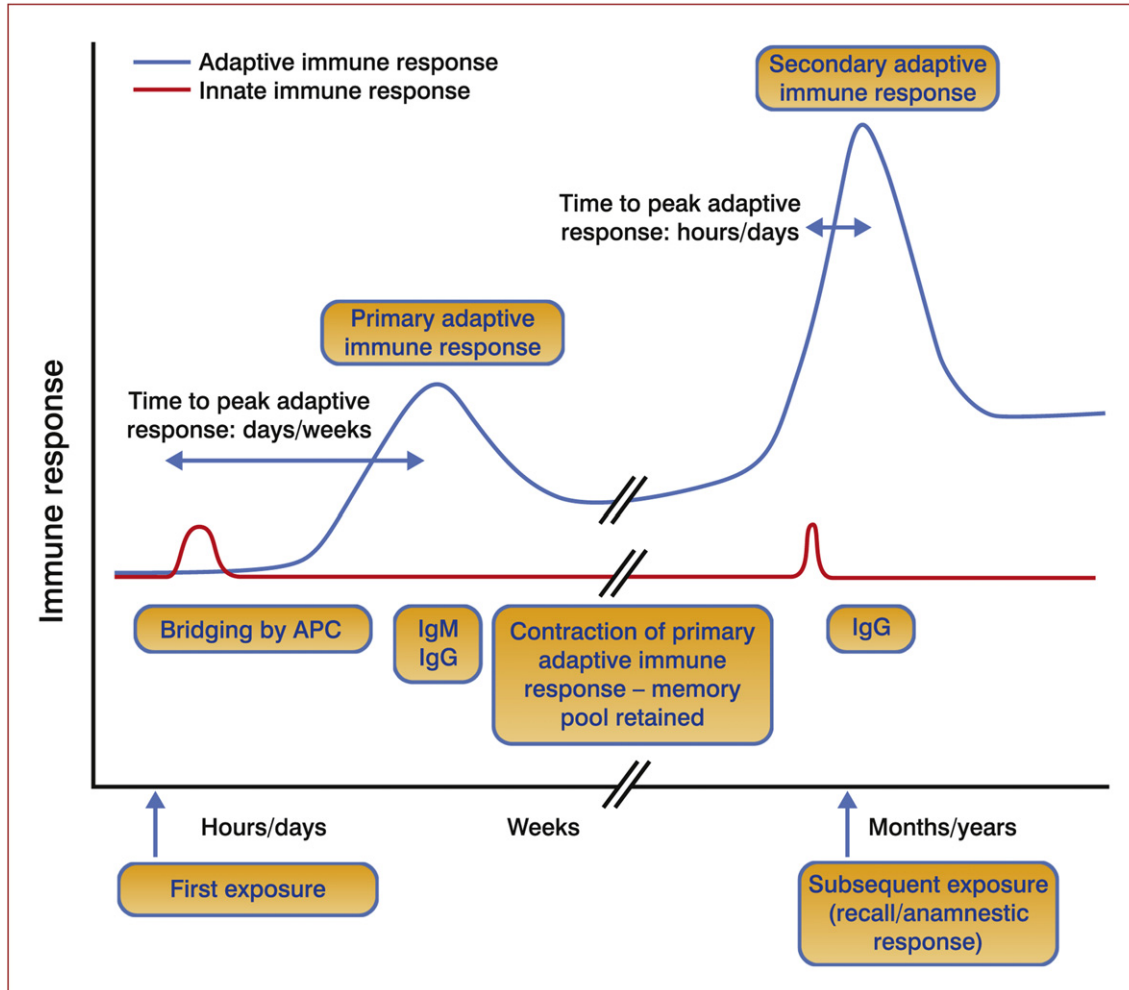
Upon differentiation, naïve T and B cells, each expressing a unique TCR and BCR, migrate to the blood and *peripheral lymphoid organs*. Due to the large number of possible immune receptors, lymphocytes expressing a given antigen specificity will be too infrequent to mount an effective immune response on their own. Thus, upon antigen encounter, T and B lymphocytes must undergo rapid proliferation, leading to the accumulation of an increased number of cells expressing receptors for the incoming antigen. Some of these cells will differentiate into effector cells (such as cytokine-producing T cells or antibody-secreting plasma cells), while others will become 'memory cells', able to survive for a long period of time within the host.

Exposure to an antigen (pathogen or vaccine) therefore leads to a long-term (and sometimes permanent) modification of the cellular repertoire, such that the relative frequency of T and B cells specific for an individual antigen is increased in antigen-exposed individuals compared with naïve individuals (Figure 2.8). In addition to their increased frequency, memory T and B lymphocytes also display novel functional properties, enabling them to develop secondary (recall) responses on re-encounter with their specific antigen, or a closely related antigen. The adaptive response on secondary exposure leads to a rapid expansion and differentiation of memory T and B cells into effector cells, and the production of high levels of antibodies. A higher proportion of IgG and other isotypes of antibodies compared with the level of IgM characterises memory antibody responses.

By definition, all effective vaccines lead to the development of *immune memory*, by mimicking the threat of an infection and providing antigens derived from the specific pathogen. The ability to generate immune memory is the key attribute of the adaptive immune system, which is crucial for the long-term protection of individuals and populations. Generating immune memory depends on a high degree of interaction among many

Figure 2.8 The kinetics of primary and recall (memory) immune responses. On first exposure to a pathogen or antigen (referred to as 'priming' in vaccination), the innate immune system must detect, process and translate the threat into a form that can be understood by the adaptive immune system. This occurs via the bridging actions of APCs and takes days/weeks. Following resolution of the challenge, a specialised 'memory' cell population remains. The cells within this population are maintained for a long time (months/years) and may remain within the host for the rest of their host's life. On subsequent exposure to the same antigen (referred to as 'boosting' in vaccination), the innate immune response is triggered as before but now the memory cell populations are able to mount a greater and more rapid response as they do not need to undergo the same activation process as naïve cells.

APC, antigen-presenting cell; IgM, immunoglobulin M; IgG, immunoglobulin G.



different cell types, which maintains higher numbers of T and B cells that were selected as the most useful in the *primary immune response*. However, while the relative contribution of clonal memory cells to protection can be inferred from the molecules they express and their functional behaviour, the presence of memory cells *per se* is not indicative of absolute protection against disease.

There are several theories as to how new effector cells are generated from memory cells when needed, and it is likely that all of these mechanisms play a role in immunological protection. One possibility is that short-lived effector cells are produced continuously by memory cell division, replacing the older cells of the same specificity — this process would be driven by the persistence of antigen. Long-lived effector cells may also be generated from memory cells in a process driven by cytokines and engagement of PRRs in response to a new encounter with the pathogen. A third possibility is that effector cells remain for long periods in specialised survival niches — there is some evidence that this is an important mechanism in *B-cell memory*, since depletion of memory B cells does not significantly impact the level of circulating antibodies, probably due to the presence of long-lived plasma cells.

Summary of differences between the innate and adaptive immune systems

As we have seen, the innate and adaptive immune systems occupy distinct evolutionary and functional niches. The innate immune system, along with physical and chemical barriers, provides a first line of defence against invasion or damage. A system of cellular and soluble mediators then transmits the nature of the threat to the adaptive immune system, which selectively expands the appropriate repertoire in order to deal with the threat. The key differences between these two systems are summarised in [Table 2.2](#). In the next section, the mechanisms linking innate and adaptive immunity will be discussed.

TABLE 2.2. KEY DIFFERENCES BETWEEN INNATE AND ADAPTIVE IMMUNITY

Innate immunity	Adaptive immunity
First line of defence against pathogens	Second line of defence against pathogens
Acts rapidly (hours/days), vital to triggering of adaptive responses	Relatively slow to develop (days/weeks)
Triggered by threat/damage	Triggered by exposure to specific antigen
Activated by microbial general patterns and damage	Very focused, targets a specific pathogen
Acts through pathogen class-specific mechanisms of neutralisation/control	Acts through antigen-specific and non-specific effectors
No recall response on subsequent exposure to same threat	Rapid recall response on subsequent exposure to same antigen (immunological memory)
Initiates processes of tissue repair	

Innate and adaptive immune responses are bridged by the actions of APCs

As previously discussed, the innate immune system provides an essential link between the first encounter with a pathogen at the site of infection and the eventual establishment of immune memory. Innate cells (such as macrophages and DCs) are strategically located at body sites with a high risk of infection (such as epithelia and mucosal surfaces). These types of cells act as both a first line of defence against danger and as key messengers that are able to alert the adaptive immune system. Since naïve T and B cells are not pre-positioned in most organs and tissues of the body, they rely on the innate immune system to sense an infectious event.

Among tissue-resident cells, the most efficient APCs are DCs. Immature DCs which have captured antigen become activated and mature into functional APCs, while migrating to the regional draining lymph node or submucosal lymphoid tissue. Mature DCs express high levels of antigen/MHC complexes at the cell surface and undergo morphological changes, which render them highly specialised, to activate naïve T cells. When they arrive in the lymph node, DCs present processed antigen and express *co-stimulatory signals*. The signals provided by DCs promote T-cell differentiation and proliferation, initiating the adaptive T cell-mediated immune

response. APC activation is therefore a necessary prerequisite for an efficient adaptive immune response.

DCs not only provide antigen and co-stimulation to naïve T cells, but also contribute to the initial commitment of naïve T helper cells into Th1, Th2 or other subsets. This directs the efficient induction of T helper cells with appropriate cytokine profiles early during infections, without the need for direct contact between antigen-specific T cells and pathogens.

Undigested pathogen-derived antigens are also drained by the lymph and transported to the B cell-rich area of the lymph node, where they are exposed to BCR-expressing cells. An adaptive immune response is therefore initiated in a draining lymph node by the concerted action of innate immune cells and free antigens. These activate T and B lymphocytes, respectively, to proliferate and differentiate into effector and memory cells.

The type of communication employed by the immune system represents a unique approach to multi-system signalling and communication over distances. As well as employing the soluble mediators — proinflammatory messengers, chemokines and soluble danger signals — the immune system uses migratory APCs to physically transport messages from the periphery to the induction sites of adaptive immune response, eg in lymph nodes.

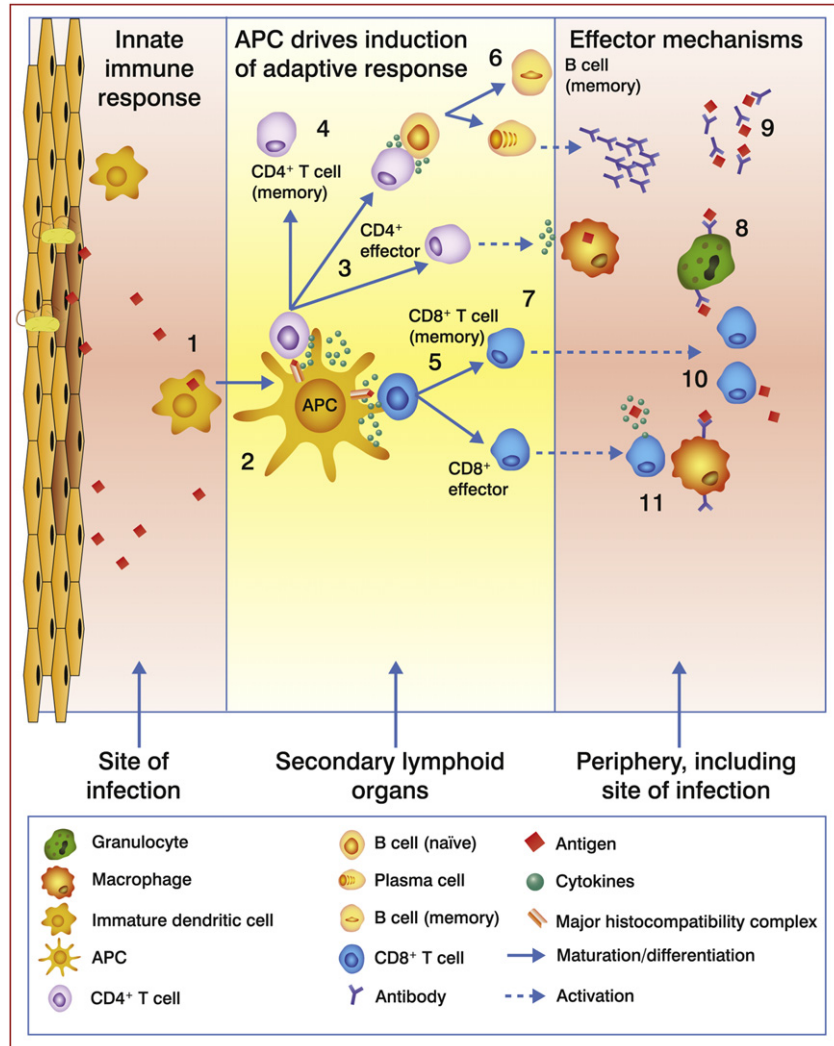
Notably, by selectively migrating in response to infectious/cell-damaging events, DCs act as filters for the adaptive immune response, helping T and B cells to ignore innocuous foreign antigens. Thus, the innate immune response plays an important role in selecting antigens that represent a real threat to the organism that requires an adequate adaptive response.

The immune response — summary

The response to pathogens in humans takes place over a large anatomical distance and in distinct phases, which are summarised in [Figure 2.9](#).

Figure 2.9 An overview of the human immune response. The innate immune response is initiated (1). Innate immune cells begin to mature and differentiate, while migrating to the lymph nodes (2). APCs induce the adaptive immune response including activation of T cells into effector cells and differentiation of T cells into memory cells (3–5). Naïve B cells differentiate into antibody-secreting plasma cells and memory B cells following activation by helper $CD4^+$ T cells (6) and activated $CD4^+$ T cells activate tissue-resident macrophages (7). Antibodies can enhance the effector functions of innate cells (8) and also neutralise pathogens directly (9). Cytotoxic T cells can directly kill infected tissue/cells via molecular and chemical signalling (10) and also induce infected cells/phagocytes to kill intracellular pathogens, or inhibit pathogen replication (11). *Not all T cell subsets are represented in this illustration.*

APC, antigen-presenting cell; CD, cluster of differentiation.



The innate immune response is initiated at the site of challenge when a foreign entity triggers a defensive response, which is mediated by chemical signals. These signals attract responding innate immune cells (monocytes, DCs etc) which travel to the site and engulf fragments of the pathogen. The monocytes and DCs then leave the site via lymphatic vessels and begin to mature and differentiate, while travelling to the local draining lymph nodes. Differentiation gives rise to APCs that interact with naïve T cells at the lymph nodes and bear receptors for the antigenic peptides expressed on the surface of the APC. Molecular, antigenic and cytokine signals combine to direct the differentiation and activation of $CD4^{+}$ T cells into distinct effector subtypes. This is the induction phase of the adaptive immune response.

A sub-population of $CD4^{+}$ T cells differentiates into memory cells, which are capable of responding rapidly on repeat exposure to the same antigen. $CD8^{+}$ T cells also receive antigenic and cytokine stimulation from APCs and undergo differentiation either into memory-type cells or armed effector cytotoxic cells. Activated helper $CD4^{+}$ T cells interact with antigen-bearing naïve B cells and, via molecular and cytokine signals, direct their activation into i) antibody-secreting plasma cells, and ii) memory populations that persist for long periods. Activated $CD4^{+}$ T cells also travel to the site of infection and, via cytokine signals, activate tissue-resident macrophages to become fully active and to destroy phagocytosed antigen/pathogens.

Antibodies can enhance the effector functions of innate cells, for example, by enhancing phagocytosis. Soluble antibodies at the site of challenge can neutralise pathogens directly by binding to their surface. *Cytotoxic T cells* can directly kill infected tissue/cells via molecular and chemical signalling. These cells can also induce infected cells/phagocytes to kill intracellular pathogens, or can inhibit pathogen replication via chemical and molecular signals.

On secondary exposure to the same antigen or pathogen, specific adaptive effectors with a memory phenotype can rapidly proliferate and produce a new wave of adaptive immunity at the site of

challenge. This pathway can occur independently of further innate immune events and is the basis of vaccination.

The coordination of all these phases ensures that a call for help from the periphery is relayed to the regional secondary lymphoid tissues and that appropriate effectors are directed to the site of infection by a series of chemical and molecular signals. This cycle of an immune response forms the basis for the principles of vaccination (Figure 2.10) and is discussed further in the next section.

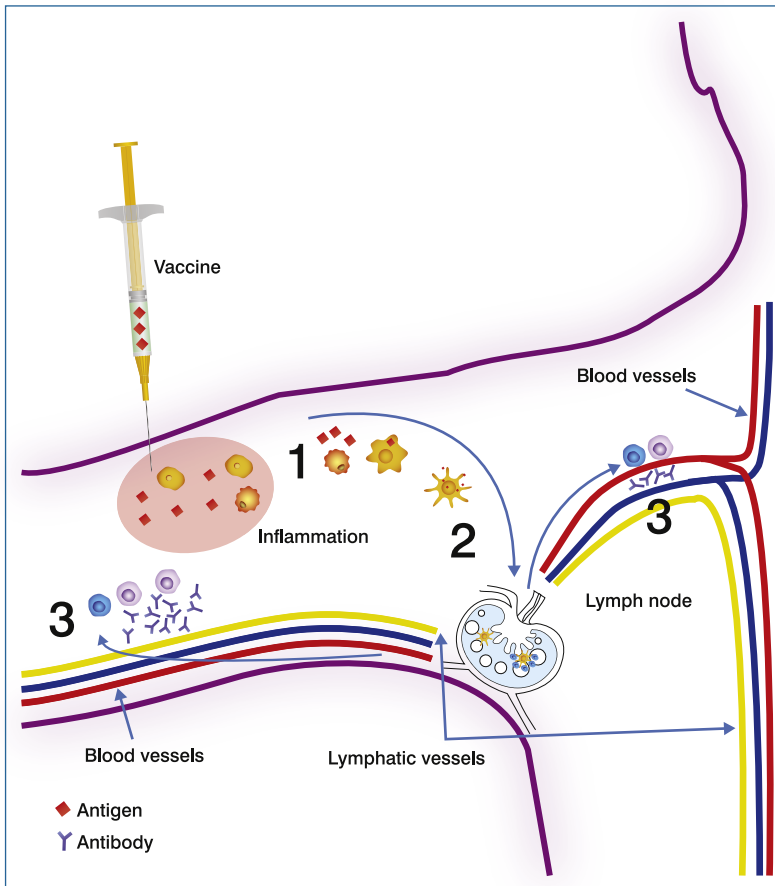


Figure 2.10 The flow of information following intramuscular vaccination. An antigen delivered by a vaccine is taken up by macrophages and immature APCs (1). APCs migrate to the lymph node draining the site of vaccination (2). The adaptive immune response is now initiated and effectors, such as CD4⁺ effector T cells, cytotoxic T cells and soluble antibodies (3), are produced which travel throughout the bloodstream and back to the site of vaccination.

APC, antigen-presenting cell.

Modern immunology applied to vaccinology

The modern immunology concepts described in this chapter are of great importance both for the design of new vaccines and to help us understand why vaccines do — or do not — work as efficiently as desired.

IMMUNOLOGICAL REQUIREMENTS OF A VACCINE

Identification and selection of the most appropriate antigen

Vaccines aim to prevent the disease symptoms that are the consequences of a pathogenic infection. In most cases, this does not occur by completely preventing infection but by limiting the consequences of the infection. As discussed earlier, an understanding of the disease pathogenesis and natural immune control is, therefore, very useful when selecting appropriate antigens upon which to base a vaccine.

Vaccines developed from pathogens can vary in the complexity of the pathogen-derived material they contain. Our understanding of fundamental immunology, as well as the selection techniques used, has resulted in new vaccines that are better characterised than ever before, and has also initiated a more rational approach to vaccine design. The different approaches to antigen selection are discussed in depth in *Chapter 3 – Vaccine antigens*.

Induction of innate immune responses

The immune system is triggered by a combination of events and stimuli, as described previously. The requirement for more than the presence of a 'foreign' antigen to elicit an immune response must therefore always be considered in vaccine design, particularly when using highly purified or refined antigens (see *Chapter 3 – Vaccine antigens*). Highly refined *subunit antigen* formulations, and some inactivated whole pathogens, do not contain many of the molecular features and defensive triggers that are required to alert the innate immune system. These types of antigen are designed to minimise

excessive inflammatory responses but, as a result, may be suboptimally *immunogenic*. Under these circumstances, the addition of *adjuvants* (see *Chapter 4 – Vaccine adjuvants*) can mimic the missing innate triggers, restoring the balance between necessary defensive responses and acceptable tolerability.

Induction of CD4⁺ T cell help

The induction of CD4⁺ T cells is essentially controlled by the nature of this initial inflammatory response. Therefore, vaccine adjuvants can play a role in guiding how CD4⁺ T cells are induced and how they further differentiate and influence the quality and quantity of the adaptive immune response.

Selection and targeting of effector cells

It is important to recognise that the dominant immune response to a given pathogen or antigen may not necessarily be the optimum response for inducing protection; indeed through evolution some pathogens have developed strategies to evade or subvert the immune response, as is the case with *Neisseria gonorrhoeae*, where the dominant antibody response actually facilitates infection by preventing complement-dependent bactericidal activity. Antibody *titres* are often considered to represent adequate indicators of immune protection but, as discussed above, may not be the actual mechanism by which optimal protection is achieved. Useful specific so-called immune *correlates of immunity/protection* may be unknown or incompletely characterised. Therefore, modern vaccine design still looks to clinical trials to provide information about clinical efficacy and, if possible, the immunological profiles of protected individuals. *Immunogenicity* is assessed by laboratory measurement of immune effectors, typically antibodies. Increasingly, however, specific T-cell activation is included in the parameters assessed, as adequate T-cell immunity may be essential for recovery from some infections and improved assay techniques have allowed these evaluations to become more standardised and offer more robust data. This can then open the door to understanding observed clinical efficacy (or lack of) and to defining at least some of the features of

vaccine-induced protection. By preferentially targeting the best immunological effectors, vaccines can then hope to mimic or improve on nature's own response to infection.

VACCINE DEVELOPMENT CHALLENGES

Immune correlates of protection – what, when and where?

Successful natural immune responses can be measured in protected individuals and assessed in terms of, for example, the production of specific types of antibody or a particular pattern of cytokine expression by T cells – this gives the correlates of protection, which can then be reproduced using a vaccine. Correlates of protection can only be determined from a clinical trial where protection from disease or infection is determined in cohorts of vaccinated versus unvaccinated individuals.

The majority of vaccines developed so far have been assessed by their ability to elicit antibody responses. One example where this is well defined is rubella, where protective antibody titres can be reliably assessed to determine whether an individual is protected post-vaccination. However, immune correlates of protection are not well defined in many diseases, including human immunodeficiency virus (HIV) where the presence of antibodies is not a correlate of immunity/protection, since infected individuals develop antibodies without being protected against disease. This is a significant barrier to HIV vaccine research and reflects the generation of variants of the virus which evade serological effectors such as antibodies. There is evidence that some highly exposed individuals can develop resistance to HIV infection, suggesting that immunity and, therefore, a vaccine are possible. However, the complex immunological profiles of these rare individuals make it difficult to define the protective effectors and their immunological triggers.

Historically, the generation of antibodies has been the main goal of vaccination; however, for future vaccines this may be insufficient or inappropriate. Thus, developments are focused on the generation of specific CD4⁺ (Th1) lymphocyte or CD8⁺ *cytotoxic T cell*

responses. These are approaches under investigation for herpes simplex virus (HSV) and tuberculosis vaccines, where selected *T-cell determinants* delivered as recombinant proteins or via live viral vectors aim to target the CD4⁺ and CD8⁺ T-cell compartments.

The need to guide the immune response towards protective mechanisms has been demonstrated in trials of respiratory syncytial virus (RSV) vaccines, where exposure of vaccinees to natural RSV infection led to severe pulmonary pathology characterised by infiltration of mononuclear cells and eosinophils, suggesting a strongly Th2-biased response. This resulted in hospitalisations and deaths of at least two young children following a study in the 1960s. Hence, insufficient knowledge of the factors affecting natural control of an infection or the inability to balance the integrated immune response induced by a vaccine can affect the ability to produce a safe, effective vaccine.

Host–pathogen interactions in vaccine immunology

Vaccine immunology is greatly affected by the complex interactions that occur between the host and the pathogen. These interactions can determine the type of immune response a vaccine needs to induce to offer protection against an actual challenge. Many pathogens have complex life cycles and sophisticated strategies which allow them to be successful in their pathological niche. This may be as simple as a waxy coating which makes opsonisation more difficult, or as complex as the ability to modulate host gene expression and manipulate or change the molecular signals displayed by infected cells. Examples of the immunological challenges posed by some pathogens are discussed below.

Bacteria

Mycobacterium tuberculosis is a good example of a bacterial pathogen with several defensive mechanisms. These include an unusual waxy coating which facilitates survival once the bacterium is taken up into APCs – this is particularly effective as it allows the bacterium to escape from degradation within macrophages,

establishing an immunologically 'silent' refuge. In addition, *M. tuberculosis* is able to down-regulate the expression of antibacterial immune effectors, such as nitric oxide, by infected macrophages. The intestinal Gram-negative pathogen *Salmonella enterica* is able to modify its LPS into a form that is less identifiable by TLR4. Impairment of the LPS/TLR4 interaction reduces early activation of the innate immune response and hence allows *Salmonella* to better survive and proliferate within the host's intestinal cells.

Viruses

Viruses such as cytomegalovirus (CMV) also have highly evolved host avoidance strategies. This member of the herpesvirus family has evolved multiple genes for the manipulation of host immunity, including those whose products prevent the display of viral proteins in association with MHC class I molecules (hence avoiding triggering or being targets of specific CD8⁺ cytotoxic T cells) by both diverting viral products out of the degradation pathway and by suppressing expression of MHC class I molecules. Ordinarily, this would attract the attention of NK cells, which are activated by nucleated cells lacking surface expression of MHC class I molecules. However, CMV possesses genes encoding MHC class I mimics, which are expressed on the surface of infected cells and are able to bind to receptors which switch off the cytotoxic activity of circulating NK cells.

Parasites

Parasites present a challenge to vaccine design because the parasite life cycle comprises distinct phases within a single host, during which it will reside in different anatomical locations and, most importantly, express different surface antigens. Thus, parasites represent an immunological 'moving target'. In addition, the immune response to parasites is very complex and may be modulated by the parasite itself, and host–parasite interactions are often poorly defined. There are currently no available vaccines for parasitic

diseases of humans, although one vaccine for malaria is currently in Phase III clinical trials (see *Chapter 6 – Vaccines of the future*).

IMMUNOLOGICAL IMPEDIMENTS TO EFFECTIVE VACCINATION – TOLERANCE

Other important considerations in vaccine immunology include the phenomena of immune tolerance and *immunological/antigenic interference*, which can suppress or prevent development of adequate immune responses following vaccination. Immune tolerance refers to the induction of immunological non-responsiveness by repeated exposure to similar antigens, such as polysaccharide antigens; this effect is dose-dependent and may be limited in time as increasing the interval between subsequent doses can partially restore responsiveness. Immunological/antigenic interference occurs when previous or concomitant exposure to another antigen prevents the development of adequate responses to the vaccine antigen, which may be due to previous or concurrent vaccinations. Another potential cause of reduced vaccine efficiency is the presence of passively induced immunity, eg that transferred from mother to foetus, where the vaccine antigen is neutralised by pre-existing maternally-derived antibody without triggering a host-derived immune response in the infant. These phenomena can be avoided, however, by taking them into account in immunisation schedules.

Modern vaccines – interactions with the immune system

Vaccines developed within the last decade have benefited from an increased knowledge of the innate and adaptive immune responses, and are better characterised in terms of their immunological mechanism of action than many of their predecessors. It has become apparent that the most successful vaccines mimic infection by actively targeting the innate phase of the immune response and modulating or enhancing the interface bridged by APCs. The immune response to a vaccine can be substantially improved

Live viral vaccines may cause immunological interference with each other if administered at the wrong intervals – for example, live varicella virus vaccines and the measles, mumps and rubella vaccine should be given at the same time or 1 month apart to avoid interference.

through the use of adjuvants, which stimulate the innate immune response by providing elements that are normally present in most pathogens but absent from a highly purified antigen. The vaccines which we know most about tend to be those that include an adjuvant, as the effects of these compounds on the innate immune system and the downstream adaptive response can be studied both in isolation and in combination with antigen. There are several points during the innate response at which adjuvanted vaccines are known or believed to influence the subsequent adaptive immune response, thereby initiating a long-lasting immune response. This includes modulating or mimicking the interaction between PAMPs and innate receptors such as TLRs; influencing or promoting intracellular signalling pathways; enhancing antigen uptake by APCs; and up-regulating or modifying cell-surface crosstalk between APCs and naïve T cells. Some examples of specific adjuvanted vaccines that exert direct effects on the innate immune response are discussed in *Chapter 4 – Vaccine adjuvants*.

We are increasingly able to understand the balance between mechanisms of immune activation and immune regulation. In parallel, the detailed assessment of the immunological mechanism of action of vaccines helps us to achieve effective immune stimulation without inducing a chronic inflammatory state. This information also helps us to reassess the role of vaccines and natural infections as potential triggers of autoimmune diseases.

Recently, much effort has been devoted to the design of vaccines that induce CD8⁺ T cell responses, as they have a central role in the host response to viral infections and cancers. However, as yet, it is not easy to measure the CD8⁺ T cell response in humans in a consistent and reliable way; this will remain the focus of research in the coming years.

Conclusion

Advancing knowledge of the immunological mechanisms of action of existing vaccines provides essential information that is vital to the

production of new, well-tolerated, effective vaccines. How immunological requirements are balanced with the complexities of the pathogen, the needs of the target vaccinees, the practicalities of antigen production, and the stability and tolerability of the eventual vaccine represents a constantly evolving challenge. The factors affecting the selection and production of different types of antigens are discussed in *Chapter 3 – Vaccine antigens*.

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