Review

Key concepts in immunology

Muriel Moser *, Oberdan Leo

Laboratoire de Physiologie Animale, Université Libre de Bruxelles, Rue Profs Jeener et Brachet, 12, B-6041 Gosselies, Belgium

ARTICLE INFO

ABSTRACT

Vertebrates have developed systems of immune defence enabling them to cope with the constant threat posed by environmental pathogens. The mammalian immune system represents a multilayered defence system comprising both innate and adaptive immune responses, characterized by the increasing complexity of their antigen-recognition systems. The discovery of the intimate relationship between innate and adaptive responses has paved the way to a novel understanding of the basic mechanisms governing the regulation of an immune response. The purpose of the present review is to briefly describe the basic immunological concepts that constitute the founding principles of modern vaccinology in humans.
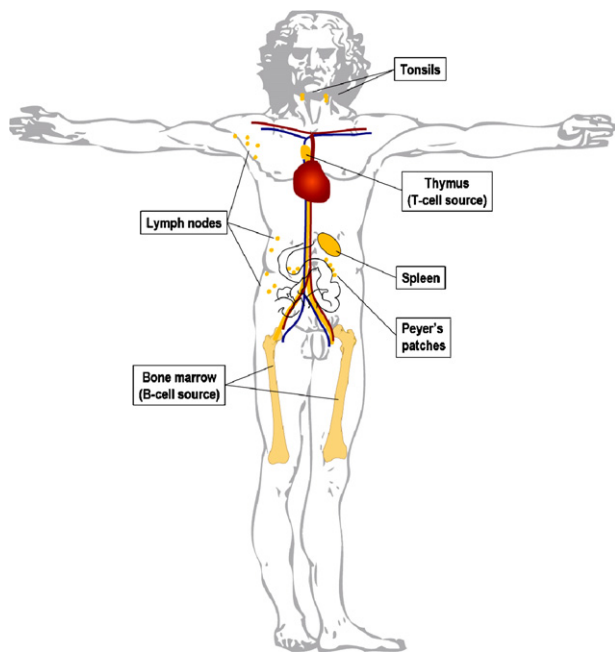
Contents

* Corresponding author. Tel.: +32 2 650 98 77x63.
  E-mail addresses: mmoser@ulb.ac.be (M. Moser), oleo@ulb.ac.be (O. Leo).

Fig. 1. Lymphoid organs. Lymphoid organs are divided into two classes: primary and secondary lymphoid organs. Primary lymphoid organs are the bone marrow and the thymus which are the sources for B-cells and T-cells, respectively. B-cells and T-cells migrate to the secondary lymphoid organs or peripheral lymphoid organs and initiate there the adaptive immune response.

1. Introduction

Protection against pathogens relies on complex interactions between organs, tissues, cells and molecules that make up the body's immune system. The immune system can be considered as a multilayered system, comprising three major defence mechanisms: (i) external barriers including physical (such as skin, ciliated epithelia, mucous membranes) and chemical (such as destructive enzymes in secretions, stomach acids) barriers; (ii) innate and (iii) adaptive immune responses.

Innate immunity represents the first line of host defence against pathogenic micro-organisms that have entered the body. This innate defence mechanism lacks memory and is mostly focused on a limited set of microbial determinants shared by a large number of pathogens. Innate responses are characterized by a lack of learning process and rapid kinetic, providing almost immediate protection against invading pathogens. Adaptive immunity provides a second line of defence, often at a later stage of infection. This immune response is activated upon pathogen encounter and is relatively slow. Adaptive responses are characterized by a very large set of effector molecules and cells, able to efficiently recognize and eliminate virtually any known pathogen. After elimination of the pathogen, the adaptive immune response establishes a state of "memory" characterized by the ability to efficiently protect the body from re-infection with the same agent. Memory is the hallmark of the adaptive immune response and can be induced by both natural infection and vaccination.

The organs of the immune system, the lymphoid organs, are distributed throughout the body (Fig. 1). They can be divided into primary lymphoid organs, where the lymphocytes—the central actors of the immune system—are generated, and secondary lymphoid organs, where the adaptive immune responses are initiated. The primary organs are the bone marrow and the thymus, whereas the secondary organs (also called the peripheral lymphoid organs) are the lymph nodes, spleen and the mucosal- and gut-associated lymphoid tissues (MALT and GALT, respectively), i.e. tonsils, ade-

noids, the appendix and the Peyer's patches of the small intestine [1].

The purpose of this review is to briefly discuss our current knowledge of the basic immunological mechanisms in humans. These constitute the founding principles of modern vaccinology, the evolution of which is outlined in an accompanying paper [2].

2. Innate immunity

2.1. Cells of the innate immune system

Cells of the innate immune system represent a very diverse set of cells of haematopoietic origin, comprising both tissue-residing cells (such as macrophages and dendritic cells) and "moving" cells (such as neutrophils, eosinophils and monocytes) that patrol throughout the body via the blood and lymph circulation. These cells can be rapidly recruited at the site of infection, thus providing an immediate line of defence against invading pathogens.

2.2. Pathogen recognition by the innate immune system

Cells of the innate immune system are able to detect an invading pathogen through a limited set of germ-line encoded receptors. These innate immune receptors (often referred to as pattern-recognition receptors, PRRs) recognize a series of conserved molecular structures expressed by pathogens of a given class. These pathogen-derived molecules (or pathogen-associated molecular patterns, PAMPs) [3] generally represent complex molecular structures that are distinctive for a set of pathogens (such as Gram-negative bacteria). Among PRRs, Toll-like receptors (TLRs) have recently emerged as pivotal components in innate immunity. These molecules are capable of sensing a wide spectrum of organisms ranging from viruses to parasites. The founding member of the TLR family, Toll, initially implicated in the development of polarity in the *Drosophila* embryo, was shown to be responsible for anti-fungal responses in the adult fly [4]. This discovery led to the identification of 10 human equivalents involved in pathogen recognition [5]. TLRs can be classified into different groups based on their localization and the type of PAMPs they recognize (see Table 1). TLRs 1, 2, 4, 5 and 6 are principally expressed on the cell surface, where they recognize mostly bacterial products, while TLRs 3, 7, 8, 9 are localized to intracellular compartments and recognize mostly viral products and nucleic acids. By specifically recognizing pathogen-derived products, TLRs represent a set of immune PRRs able to alert the immune system as soon as an infection occurs [3].

Recently, another family of "pathogen-sensing molecules", mostly expressed in the cytoplasm, has been identified. This NOD-related family of cytoplasmic molecules comprises over 20 members able to react to intracellular pathogen-derived structures, thus expanding the sensing capacity of the innate immune system to virtually all cellular compartments [6]. The most remarkable property of these molecules is probably their ability to also sense cellular damage, even in the absence of a microbial trigger. Extracellular nucleotides, alteration in cellular ion content, or lysosomal damage all seem to activate components of this intracellular sensing machinery, ultimately leading to the processing and release of inflammatory cytokines [7]. These observations have led to the concept of an innate immune system well equipped to detect both infectious events (through direct pathogen recognition) and the consequences of an infectious event (through the recognition of stress signals released by dying cells). These natural ligands, also referred to as DAMPs, for "danger associated molecular patterns", often represent normal intracellular constituents (such as ATP and uric acids), that are released upon cell lysis caused by infection or trauma [8]. It is noteworthy that expression of PRRs is not limited to cells of the innate immune response, since lymphocytes and

Table 1
Toll-like receptors (TLRs) and their microbial and endogenous ligands.

TLR	Microbial ligand	Endogenous ligand
TLR1	Peptidoglycans; lipopeptides	–
TLR2	Lipopeptides; lipoteichoic acid; glycolipids, zymosan	–
TLR3	dsRNA; siRNA	mRNA
TLR4	Lipopolysaccharide; RSV fusion protein; mouse mammary tumor; virus envelope protein; phosphorylcholine	HSP; defensin 2; fibrinogen; hyaluronic acid, HMGB-1
TLR5	Flagellin	–
TLR6	Lipopeptides	–
TLR7/TLR8	ssRNA; imidazoquinoline; resiquimod; imiquimod	U1snRNP; autoantigens-containing immune complexes
TLR9	CpG DNA	Chromatin complex
TLR10	Unknown	Unknown

non-lymphoid cells such as endothelial cells and fibroblasts have been found to express selected TLRs constitutively or in response to pathogens, stress or cytokines [9].

2.3. Effector mechanisms of the innate immune system

Phagocytosis represents an important effector mechanism of the innate immune response. Virtually all cells of the innate immune system, whether tissue-resident or moving, are effective phagocytes. Upon contact with a phagocyte, pathogens are engulfed, trapped within an intracellular vesicle and targeted for destruction by a complex set of digestive enzymes or reactive oxygen species (such as free radicals) produced within the cell [10]. Efficient elimination of pathogens through phagocytosis requires rapid recruitment of effector cells to the infection site, a process often referred to as the inflammatory response [11]. This prototypic innate response is initiated by recognition of pathogens by innate receptors, often expressed by non-lymphoid cells (such as endothelial cells) or macrophages residing within the proximity of the infection site. Upon pathogen recognition, these cells secrete a series of chemokines (defined as small soluble proteins that function as chemotactic factors by directing cellular migration) such as CCL5/RANTES that attract phagocytes from the blood circulation to the infection site [12]. Activated resident cells and phagocytes also produce soluble mediators called cytokines (defined as proteins released by cells that affect the behaviour of other cells) such as tumour necrosis factor (TNF-α) and interleukins that further increase the phagocytic capacities of cells of the innate immune system. Elevated secretion of cytokines and chemokines leads to recruitment of cells and plasma proteins to the site of infection in tissues through increased vessel permeability, leading to the classical signs of inflammation (increased swelling, redness, pain and heat). The inflammatory response leads not only to the recruitment of cells and soluble mediators with anti-microbial activity to the site of infection but also plays an important role in the healing process of the damaged tissue [13]. It is noteworthy that this complex response is stereotyped in nature, since a subsequent infection will cause the same cascade of events, with similar kinetics and intensity.

3. Adaptive immunity

Due to the limited diversity of PRRs, pathogens displaying a high mutation rate can easily escape recognition from the innate immune system [14]. Moreover, the ability of several pathogens (such as viruses) to replicate intracellularly renders their detection and elimination particularly challenging. Adaptive immunity is a highly sophisticated biological response involving antibodies and T cell receptors as recognition systems that have evolved in response to the high mutation rate of pathogens and intracellular

replication. These antigen-specific receptors are expressed by lymphocytes, the key cell population in the adaptive immune response. Similar to cells of the innate immune response, lymphocytes originate from bone marrow-derived precursors and differentiate in the periphery into mature effector cells. These cells can be found in the blood and lymph circulation, or in secondary lymphoid organs such as lymph nodes and the spleen [1].

3.1. Antigen recognition by antibodies

Antibodies represent a set of proteins produced by a subpopulation of lymphocytes known as B lymphocytes. These molecules (also referred to as immunoglobulins) are characterized by an almost infinite diversity (in the order of 10^{12}) exceeding by far the number of known genes in the human genome. In the last decades, the mechanism by which such a highly diverse set of proteins is generated has been uncovered. Through a complex series of somatic events (including somatic recombination and mutations), a limited set of genes (in the order of 1000) has been found to generate a vast number of proteins, each expressing a distinctive binding site for an antigen (broadly defined as a molecular structure, from pathogenic origin or not, able to be recognized by an antibody) [15,16]. As a consequence of this high level of diversity, antibodies can recognize virtually all known molecular structures, whether of biological (such as proteins, lipids or nucleic acids), or synthetic (small organic compound) origin.

During B cell development in the bone marrow, each B lymphocyte expresses numerous copies of a unique antibody as a cell surface receptor (B cell receptor, BCR). As a consequence, each lymphocyte is thought to be mono-specific, i.e. able to react to a single antigenic molecule. Upon an encounter with a specific antigen (and in the presence of adequate auxiliary cells and signals), B cells expressing a given antibody are stimulated to divide and differentiate into plasma cells and memory B cells [17,18]. Most plasma cells home back to the bone marrow, where they will produce large amounts of soluble antibodies of a given specificity that will be released in the blood and other body fluids (previously referred to as "humors", hence the humoral response). In contrast to inflammatory cells, antibody producing cells do not need to be present at the site of infection, since they can fight infection "at distance" by producing soluble antibodies.

3.2. Antigen recognition by T lymphocytes and the phenomenon of MHC restriction

Although antibodies allow the immune system to react with a large variety of antigens, these large molecules cannot cross the plasma membrane and are therefore unable to bind and destroy intracellular pathogens such as viruses. T lymphocytes represent a distinct cellular subset that allows the immune system to rec-

ognize and fight intracellular pathogens. To achieve this seemingly very difficult task, T lymphocytes exploit the ability of all nucleated cells of our body to display at their cell surface peptide fragments derived from intracellular proteins. As part of a normal quality control process, intracellular proteins undergo a complex cycle of degradation and re-synthesis throughout the life of the cell [19]. Notably, rather than undergoing a complete degradation into single amino acids, a sample of intracellular proteins is subjected to limited proteolysis, giving rise to a set of small sized peptides (9–11 amino acids). These peptides are further transferred from the cytoplasm into the endoplasmic reticulum where they are bound by transmembrane "presenting molecules" encoded by the major histocompatibility complex (MHC) or human leukocyte antigen (HLA) genes in humans [20]. These molecules are composed of two chains that fold together to create a long cleft in which the peptide nests [21,22]. These peptide-binding, MHC-encoded molecules are then transferred to the plasma membrane, where they will display (or "present") these peptides (or "antigens") of intracellular origin to the cell surface.

Like B lymphocytes, T lymphocytes express an antigen-specific receptor, called T cell antigen receptor (TCR), on their cell surface. The TCR is very similar to immunoglobulin in structure, although it is encoded by a distinct set of genes. Through a similar process of somatic recombination, a limited set of TCR encoding genes will give rise to a highly diverse repertoire of antigen-specific receptors [23,24]. In marked contrast to antibodies however, TCRs are not secreted, and are unable to react with soluble antigens. TCRs represent specialized receptors adapted and able to recognize the molecular complex composed by a given peptide fragment presented by an MHC molecule. The diversity of TCRs is such that a given TCR is able to specifically react to a given peptide/MHC combination. Thus, T lymphocytes are equipped with antigen-specific receptors that are specifically designed to react to peptide fragments from intracellular origin. This complex mechanism of "antigen presentation" and "MHC restriction" allows therefore the immune system to scan and detect intracellular proteins while preserving cell integrity. T cells able to react to these protein fragments of cytoplasmic origin can be identified based on the expression of a cell surface marker known as the CD8 molecule. CD8-expressing cells react to peptide fragments presented by a subset of MHC-encoded molecules known as class I MHC molecules, expressed by virtually all nucleated cells of the organism [25].

Another T cell subset, expressing an alternative marker known as CD4, displays a similar, yet slightly distinct recognition pattern. CD4-expressing T lymphocytes react to MHC-peptide complexes that are formed in distinct cellular compartments, the endocytic vesicles. The peptides to which CD4 T lymphocytes react derive from the limited digestion of extracellular proteins that have been internalized through endocytosis or phagocytosis. Thus, CD4 T lymphocytes appear to react to protein antigens from the extracellular milieu, provided that these antigens are internalized and degraded into larger peptides (that can reach 20 residues) by a specialized set of cells, known as "antigen presenting cells" (APCs). The MHC-encoded proteins able to present peptides of endosomal origin are known as class II molecules, and are only expressed by cells of the immune system [25].

Recent observations have demonstrated that this "division of labour" (CD8-expressing cells detect peptides of cytoplasmic origin presented by MHC class I molecules, while CD4 cells react to proteins of extracellular origin whose processed peptides are loaded on MHC class II molecules) is not a strict requirement, since alternate modes of presentation have been described. In particular, "cross-presentation" refers to the ability of endocytosed material to escape the endosomal compartment and reach the cytoplasm, acquiring therefore the ability to be presented in association with MHC class I molecules [26,27]. This phenomenon, mostly restricted to a specific subset of antigen presenting cells of the dendritic cell family, can explain the ability of the immune system to activate CD8-positive MHC class I restricted cells in response to extracellular antigens. Accordingly, dendritic cells do not need to be infected by a given virus in order to express viral antigens in association with MHC class I molecules. A similar process, referred to "autophagy" has been recently invoked to explain the ability of cytoplasmic antigens to be targeted to the lysosomal compartment and to be presented in association with MHC class II proteins, although the immune consequences of this novel pathway of cross presentation remain to be firmly established [28].

In conclusion, TCRs display a distinct mode of antigen recognition when compared to antibodies, since TCRs: (i) can only react to cell surface, but not to soluble, antigens presented by MHC-encoded molecules; (ii) do not react to extracellular pathogens but only to intracellular, or previously internalized antigens; (iii) can only react to a limited set of biochemically well defined antigens (mostly proteins).

### 3.3. Common traits of antigen recognition

3.3.1. Generation of diversity

Adaptive immunity is characterized by specificity and develops by clonal selection from a vast set of lymphocytes bearing antigen-specific receptors which are generated by a mechanism referred to as gene rearrangement. To detect, eliminate, and remember a large number of pathogens, the adaptive immune system must be able to distinguish an infinite number of different antigens, sometimes very closely related. To achieve this goal, the receptors that recognize antigens must be produced in a huge variety of configurations, essentially one receptor for each different antigen that might ever be encountered. Each T or B lymphocyte expresses one type of receptor, and the set containing the entire lymphocyte population represents what is called the repertoire of the immune system.

The vast diversity of T cell antigen receptors and antibodies is generated from a relatively small set of genes (V, D and J segments) that randomly assemble to constitute an almost infinite number of combinations during lymphocyte development [16]. This process is called gene rearrangement or V(D)J recombination and the mechanisms involved are similar in both cases. Antibody diversity is further increased with introduction of multiple mutations in the rearranged genes, which is referred to as the process of somatic hypermutation.

3.3.2. Clonal selection and immune memory

The development of a very diverse immune repertoire poses a serious threat to the host, since autoreactive receptors do arise during the process of somatic diversification. As a consequence, both T and B lymphocytes undergo an important selection process during differentiation. Cells expressing autoreactive receptors are eliminated from the repertoire through a process of "negative selection" involving the selective elimination of autoreactive cells by apoptotic cell death. Cells expressing receptors reactive to "non-self antigens" are spared by this selection procedure, and allowed to migrate to the blood and peripheral organs. Each of these mature lymphocytes will express a unique receptor out of many, and lymphocytes of a particular specificity will thus be too infrequent to mount an effective response on their own. When an antigen enters the body, it binds to cells expressing the corresponding matching receptors and induces their multiplication. This proliferative response following antigen recognition (also known as "clonal selection" [17]), leads to the overrepresentation of a subset of lymphocytes during and after an immune response that represents a unique biological "reinforcement learning process". Immune memory is indeed the consequence of this permanent alteration of the immune repertoire, whereby a fraction of previ-
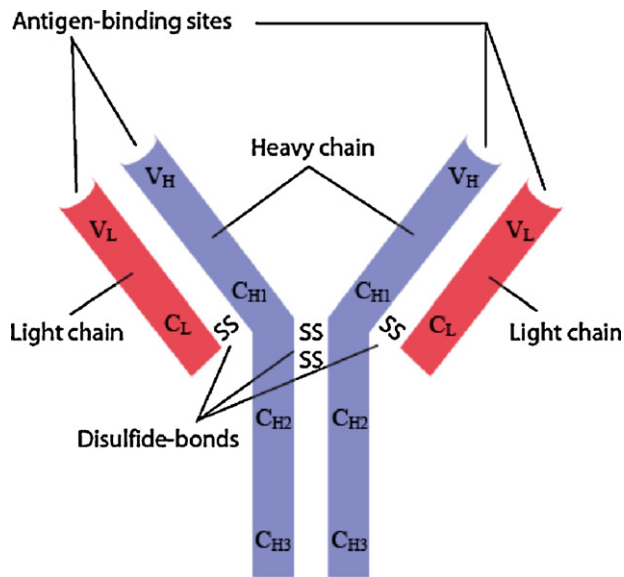
Fig. 2. Antibody structure. Antibodies are Y-shaped, flexible molecules consisting of two heavy and two light chains linked together by disulfide bonds. The light and heavy chains are composed of constant (C_L, C_{H1}, C_{H2}, C_{H3}) and variable (V_L, H_L) regions.

Table 2
Immunoglobulin (Ig) isotypes and their functions.

Immunoglobulin	Function
IgG (subclasses: IgG1, IgG2, IgG3, IgG4)	Secreted during secondary response Major form of circulating antibodies
IgA (subclasses: IgA1, IgA2)	Major form of antibodies in external secretions
IgE	Triggers immediate allergic reactions
IgM	Secreted during primary response
IgD	Exact function unknown

ously selected lymphocytes is maintained alive during the life of the host, allowing a faster and more vigorous response during a secondary encounter with the same pathogen [29]. Cells induced following a primary immune response thus represent "memory cells", able to respond again if challenged by the same pathogen. Moreover, generation of antibody variants through accumulation of somatic mutations leads to the long term survival of B lymphocytes able to secrete antibodies of very high affinity towards the invading pathogen [30]. The ability of memory cells to survive in the host for very long periods has been recently confirmed in human subjects. In particular, a study performed in aged (over 90 years old) volunteers that had been exposed to the H1N1 viral strain in 1918, demonstrated the ability of virus-specific, circulating B lymphocytes to survive in the host for over 90 years [31]. Similarly, T cells expressing immune receptors specific for smallpox have been found to subsist for long periods of times, although with a reduced half life (in the order of 10–15 years) when compared to B cells specific for the same antigen and which appear to survive for the life of the patient following vaccination [32].

3.4. Effector mechanisms of the adaptive immune response

3.4.1. Antibodies

Antibodies can be considered as bifunctional molecules, that can both recognize and eliminate a given antigen or pathogen. The structure of an antibody reflects these two functions (Fig. 2). Antibodies are roughly Y-shaped, flexible molecules made up of two heavy chains and two light chains linked together [33]. Both types of chains are composed of constant (C) and variable (V) regions, determining the functional properties of the antibody and contributing to the antigen-binding site, respectively. There are two types of light chains (κ and λ) that can associate with any of the five different heavy chains (α, γ, δ, ε and μ). The type of heavy chain determines the class, or isotype, of the antibody molecule, i.e. IgA, IgG, IgD, IgE and IgM antibodies. Immunoglobulin class is important because it determines the capacity of a given antibody to reach the site of infection and recruit the adequate effector mechanism (Table 2).

Antibodies circulate around the body in the blood and fluids. The binding of an antibody to its target is often sufficient to render the antigen harmless. Toxins produced by some bacteria can be neutralized upon recognition by a specific antibody that will block its ability to bind to specific cellular targets. Similarly, antibodies to viral particles will impede their interaction with specific cellular receptors, and therefore strongly inhibit their infectivity. More often, however, antigen–antibody complexes are able to recruit additional effector mechanisms that will lead to pathogen destruction. Binding of antibodies to surface antigens renders for example the pathogen more susceptible to phagocytosis by cells of the innate immune system, a process known as opsonization. Depending on their isotype, antibodies can also activate the complement family of proteins, leading to cell lysis and destruction of the target pathogen.

3.4.2. Effector T cells

T lymphocytes represent secretory cells, able to respond to an antigen-specific stimulation through their TCR by the production of soluble factors expressing various anti-pathogenic effects.

3.4.2.1. CD8-expressing effector T cells (CD8+ T cells).
CD8+ cytotoxic T lymphocytes (CTLs), or killer cells, were identified as cells able to induce the death of infected or otherwise damaged/dysfunctional (e.g. tumour) cells [34]. Upon recognition of a specific MHC class I/antigen complex, CD8-expressing lymphocytes secrete a pore-forming protein (perforin) that allows the intracellular delivery of a series of proteases directly into the cytoplasm of the target cell. These proteases (also known as granzymes) are able to initiate an apoptotic response leading to the rapid cell death of the antigen-expressing cell [35]. Through this complex, cell death-inducing programme, cytolytic T cells can kill infected cells expressing pathogen-derived peptides at the cell surface before the pathogen's replication programme is completed, thus stopping pathogen spread. More recently, CD8-expressing cells have also been shown to inhibit viral replication while preserving the integrity of target cells, such as neurons. Granzymes delivered into the cytoplasm of HSV-1-infected neurons by HSV-1-specific CD8+ T cells do not activate apoptosis, but rather degrade an HSV protein required for full viral expression, thus leading to inhibition of viral replication in live cells [36]. Finally, pathogen-specific T cells also secrete soluble mediators (cytokines) such as TNF or interferons (IFNs, whose name derives from their ability to *interfere* with viral replication) that bind to infected cells and inhibit intracellular pathogen replication [37].

Collectively these observations demonstrate the ability of CD8-expressing cells to inhibit intracellular pathogen replication through the secretion of soluble mediators able to interfere with pathogen replication and/or to induce the death of infected cells.

Table 3
Cytokines and their effects.

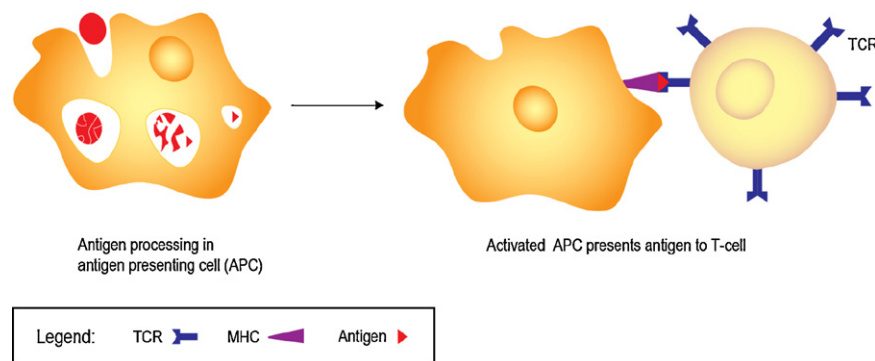| Cytokine	Secretion	Effects
Innate immunity		
Interleukin 1 (IL-1)	Myeloid cells[*]; endothelial cells; epithelial cells	Inflammation
		Fever
		Cell activation
Tumor necrosis factor-α (TNF-α)	Myeloid cells	Inflammation fever
		Neutrophil activation apoptosis
Interleukin 12 (IL-12)	Macrophages; dendritic cells	Promotion of Th1 subset
		Activation of NK cells
Interleukin 6 (IL-6)	Myeloid cells and stromal cells[a]	Proliferation and antibody secretion of B cells
		inflammation
Interferon-α (IFN-α)	Plasmacytoid DCs, fibroblasts	Promotes MHC class 1 expression
		Activation of NK cells
		Promotes CD8 T cell response
Interferon-β (IFN-β)	Fibroblasts	Promotes MHC class 1 expression
		Activation of NK cells
Adaptive immunity		
Interleukin 2 (IL-2)	T cells	Proliferation of T cells
		Promotion of AICD
		Activation and proliferation of NK cells
		Proliferation of B cells
Interleukin 4 (IL-4)	Th2 cells; mast cells	Promotion of Th2 subset
		Isotype switch to IgE
Interleukin 5 (IL-5)	Th2 cells	Activation and generation of eosinophils
Transforming growth factor β (TGF β)	T cells; macrophages	Inhibition of T cell proliferation and effector
		functions
		Inhibition of B cell proliferation
		Isotype switch to IgA
		Inhibition of macrophages
Interferon γ (IFN-γ)	Th1 cells; CD8^{+} cells; NK cells	Activation of macrophages
		Promotes expression of MHC
		Promotes antigen presentation

[*] Myeloid cells include macrophages, monocytes and dendritic cells.

[a] Stromal cells include epithelial cells, endothelial cells and fibroblasts.

3.4.2.2. CD4-expressing effector T cells (CD4^{+} T cells). CD4^{+} T cells interact with antigen MHC class II complexes that are mostly expressed by immune cells. This lymphocyte subset plays a dual role during an immune response through the secretion of a wide collection of cytokines and displays both effector and regulatory properties. As previously discussed for CD8^{+} cells, cytokines produced by CD4^{+} T cells at the site of infection can affect pathogen survival (such as shown for TNF, IFNs [37]). Moreover, several cytokines have been shown to display profound effects (both enhancing or inhibitory) on the activity of other immune effectors such as innate immune cells, B lymphocytes or cytotoxic T cells (see Table 3). The complex regulatory role of CD4-expressing lymphocytes during an immune response will be analyzed in more details in the following paragraphs.

4. Mounting and regulating an immune response

As previously described, the immune system is characterized by a complex array of effector mechanisms including phagocytes, antibody-producing cells and T lymphocytes. Selection and activation of the adequate effector mechanism is under the control of complex regulatory processes that require cooperation between different cell types of the immune system. Activation of CD4^{+} T cells represents an early and important step in the initiation of an immune response. Indeed, although helper-independent responses have been described (both antibody secretion and generation of cytotoxic CD8-expressing cells can be observed in the absence of CD4^{+} helper T cells [38–40]), optimal memory responses, displaying an increased efficiency upon secondary stim-

Antigen processing in
antigen presenting cell (APC)

Activated APC presents antigen to T-cell

Legend: TCR   MHC   Antigen

Fig. 3. Activation of helper T cells and the role of antigen-presenting cells. T cell antigen receptors (TCR) on T cells are able to recognize only processed antigen which is presented by an MHC/antigen complex.

ulation, are strictly dependent on previous activation of helper T cells [40–43].

4.1. The activation of helper T cells and the role of antigen-presenting cells

As for any T cell, helper lymphocytes can only be activated upon recognition of an adequate ligand, i.e. a peptide-MHC class II complex. Moreover, although several immune cells expressing MHC class II molecules are potentially able to generate the required peptide–MHC complex, the ability to activate naïve helper T cells appears as a specific property of a rare subset of APCs (Fig. 3) known as dendritic cells (DCs) [44]. This exclusive property of DCs is best explained by the recently developed "three signal" theory. According to this concept, developing lymphocytes exist as both immature (or naïve) and mature (expressing fully functional helper and/or effector function) cells. Transition from naïve to mature cells requires both antigen recognition (i.e. a peptide/MHC complex, signal 1) and a co-stimulatory signal (signal 2) delivered by a set of membrane bound receptors expressed by DCs (including proteins of the B7 family). Finally, by producing a distinctive set of secreted factors (cytokines, representing the third signal), DCs influence the differentiation fate of activated helper T cells toward a determined functional subset (see Section 4.3).

The role of DCs in activating naïve T cells appears to proceed in a stepwise fashion comprising three distinct steps, namely (i) antigen processing, (ii) migration to lymphoid organs and finally (iii) activation of naïve T cells through provision of a combination of antigenic, costimulatory and cytokine-borne signals [45].

(i) *DCs and the antigen capture mode*. In peripheral tissues where they reside, DCs exhibit potent endocytic activity. Through the expression of various receptors mediating endocytosis and phagocytosis of antigens, pathogens and dying cells, DCs are able to internalize and degrade a wide range of protein antigens present in their environment. This continuous process of "antigen presentation" generates a series of MHC–peptide complexes that are expressed at the cell surface of tissue resident DCs.

(ii) *DCs maturation and migration*. Upon an infectious event, DCs appear to shift from an antigen-capturing mode to a T cell-sensitizing mode during a process called maturation. DCs maturation induces multiple alterations in the function and intracellular transport of MHC class II molecules, leading to the accumulation of high numbers of antigen-loaded, MHC class II molecules to their plasma membrane. DCs maturation is also associated with a loss of adherence of these cells with the surrounding tissues, and their migration to the lymphoid organs where naïve lymphocytes reside.

(iii) *Expression of costimulatory molecules, cytokine secretion and activation of naïve T lymphocytes*. Mature DCs express high amounts of MHC–antigenic peptide complexes, as well as the costimulatory molecules required for optimal activation of T lymphocytes. Upon their migration to a lymphoid organ, these cells can deliver both antigen and costimulatory signals, thereby inducing the differentiation of naïve T lymphocytes into efficient helper cells.

Based on their location and functional properties, DCs are therefore considered as key elements in the initiation of an immune response. DCs are present in blood and in tissues, such as the skin, representing the potential entry sites for pathogens. These cells have the unique capacity to leave the infection site and migrate to the lymphoid organs where they present antigenic fragments to lymphocytes in a stimulatory mode, thus providing T cells with signals promoting their amplification, survival and differentiation.

Induction of DCs maturation represents therefore a prerequisite for an efficient immune response, and the nature and quality of signals inducing DCs maturation are of utmost importance in the initiation of immune responses.

4.2. Dendritic cell maturation and the recognition of danger signals

As members of the innate immune response, DCs express receptors, such as members of the TLR family, able to recognize pathogen-derived molecules or endogenous signals released by damaged or dying cells [46]. DCs also express receptors to several cytokines (such as TNFs or IFNs), allowing these cells to react to an occurring innate response in their environment [47]. This collection of receptors enables DCs to directly recognize a wide spectrum of organisms ranging from viruses to parasites, or to sense the consequences of a local immune response. Noteworthy, signalling through these receptors causes DCs maturation thereby functionally linking DCs response to a local infectious event. DCs maturation and the consequent migration to lymphoid organs and expression of costimulatory signals represent a "confirmation" signal, linking the development of an adaptive immune response to the previous recognition of an infectious event mediated by innate receptors. Delivery of confirmation signals can therefore be considered as both a fail-safe strategy against accidental reaction to self-components, and a mechanism to identify dangerous invaders.

4.3. The diversity of helper T cell responses

As previously stated, CD4+ T lymphocytes activated by mature DCs differentiate into antigen-specific and efficient helper cells. These cells play a central role in the immune response by helping other cells to perform their effector tasks. Helper T cells regulate the activity of other immune cells through the secretion of a selected population of soluble factors known as cytokines [48]. Recently, by analyzing the panel of cytokines produced by activated T cells, at least four different subsets of helper cells have been defined (Fig. 4).

(i) Th1 cells appear to secrete mainly IFN-γ, a cytokine known to increase expression of MHC molecules and to exert potent anti-viral effects. This cytokine is also able to promote the differentiation and activity of CD8-expressing cells and phagocytes, indicating that it plays an important role against viruses and other intracellular pathogens. The available evidence suggests therefore that Th1 helper cells are able to promote an immune response particularly efficient against intracellular pathogens [49,50].

(ii) Production of cytokines such as IL-4, IL-5 and IL-13 is mostly associated with a distinct subset of helper cells known as Th2 cells. These cells appear to be particularly apt at activating cells such as eosinophils and mastocytes often involved in the immune response to large extracellular parasites [51]. Notably, supra-optimal activation of these cells is responsible for the secretion of high levels of IgE antibodies causing allergic reactions such as asthma.

(iii) A subset of cells that is often found in close association with B lymphocytes in selected structures (follicles) of lymphoid organs has been recently identified. These follicular helper T cells (fTh) are able to promote high levels of antibody secretion from antigen-specific B cells, and are therefore thought to play an important role in regulating humoral responses *in vivo* following vaccination [52,53]. The fTh cells are characterized by the production of IL-21, a cytokine known to positively affect humoral responses *in vivo*. Although originally thought to belong to the Th2 subset, the helper cell population able to promote B cell activation has been shown to express a dis-
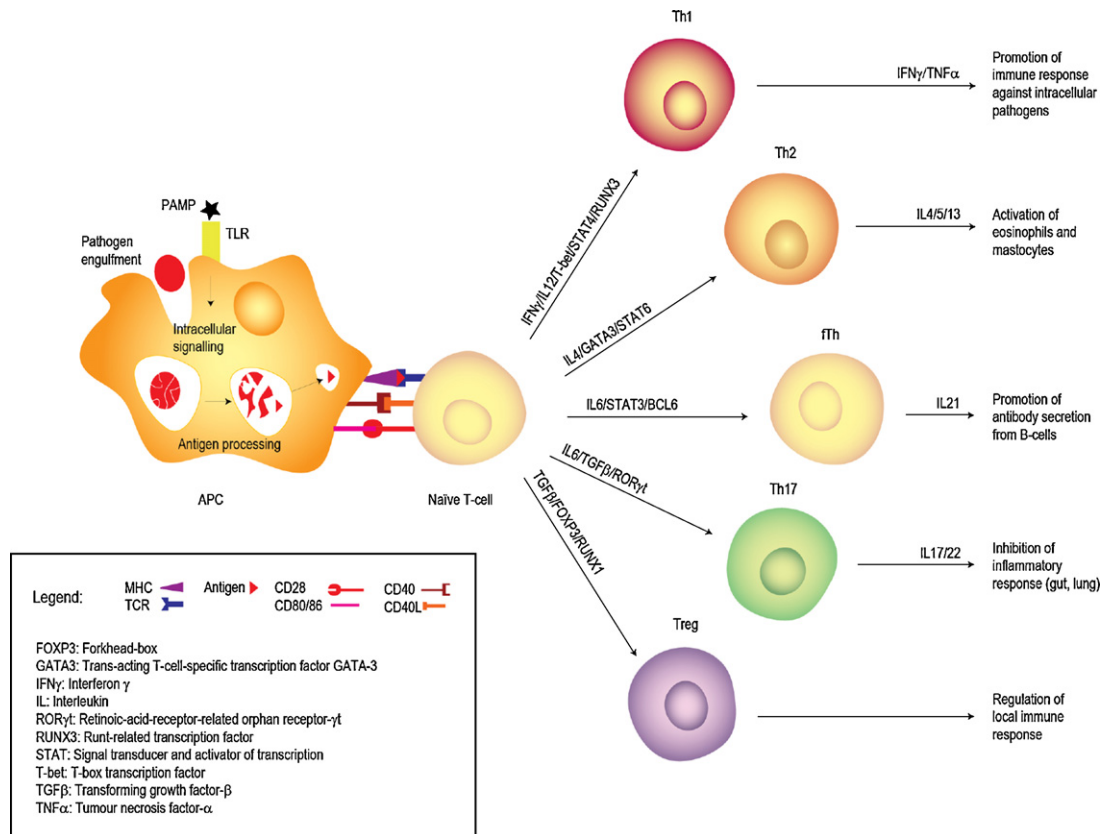
Fig. 4. Helper T cells (subsets) and regulatory T cells. The dendritic cell (DC) is the key element to T cell differentiation. DCs present antigen to naive T cells and depending on the nature of co-stimulating signals (CD86, CD40) and secreted cytokines, the transition of naive T cell to different maturated T cells is initiated. Th1 cells secrete mainly IFNγ and TNFα. Th1 cells promote an immune response against intracellular pathogens. Th2 cells secrete IL-4, IL-5 and IL-13 and they are involved in immune response to large extracellular pathogens as parasites. Follicular or fTh cells are found in the lymph nodes in close association to B cells and are characterized by the secretion of IL-21. The fTh cells stimulate antigen specific B cells to secrete high antibody levels. Th17 cells secrete IL-17 and IL-22, which regulate local immune response to gut and lung pathogens and are involved in autoimmune diseases. Regulatory or Treg cells inhibit immune response and inflammation by blocking the activity of effector, helper and/or antigen presenting cells.

tinct set of genes (notably, these cells often fail to produce high levels of the prototypic Th2 cytokines IL-4 and IL-13), and are presently believed to belong to a separate cell subset, distinct from the typical Th2 "effector" cell.

(iv) Finally, a fourth and recently identified subset has been defined based on its ability to secrete IL-17 and IL-22, cytokines that appear to play a role in response to selected pathogens including several bacterial and fungal strains [54,55]. Th17 cells appear to regulate the local immune response to gut and lung pathogens but they also represent the major pathogenic population in several models of autoimmune inflammation.

Differentiation of naïve CD4$^+$ T cells into selected helper/effector cells (Th1, Th2, fTh or Th17) is under the control of soluble mediators (mostly cytokines) produced during the early steps of antigen-specific stimulation. Several of these cytokines are produced by DCs themselves (previously referred to as the "third" signal), stressing the important role that this cell subpopulation plays in the choice of effector cells. In particular, DCs can direct the development of naïve CD4$^+$ cells into Th1 regulatory/effector cells through the production of IL-12, a well described IFN-γ-promoting cytokine [56,57]. Similarly, IL-6 appears to play an important role in both fTh and Th17 differentiation [58], while the precise nature of signals and cytokines able to promote Th2 responses remain to be firmly established [59]. An interesting, but yet not completely elucidated, feature of these responses is their ability to antagonize each other's function. In particular, Th1 and Th2 subsets appear to both crossblock each other and to inhibit Th17 development,

although the biological significance of these observations remains to be established [60].

4.4. The humoral response, a typically helper-regulated immune response

Repetitive antigens or antigens able to directly activate B cell proliferation, such as bacterial polysaccharides or TLR ligands, induce B cells to differentiate into antibody secreting cells in a T cell-independent fashion (Fig. 5a). These responses, characterized by the secretion of low-affinity antibodies (mainly IgM), display a stereotyped "innate response" behaviour, since repetitive encounters with the same antigen fail to induce a secondary, memory-like response. Overall, this type of response is poorly efficient, highlighting the important role of T cells in promoting protective humoral immune responses [61,62].

The typical secondary antibody response observed upon multiple exposures to the same antigen is only observed when B cells are stimulated by antigen in a T cell-dependent fashion [63]. T cell-dependent, humoral responses require the concurrent activation of both B and T lymphocytes (Fig. 5b). Although B cell receptors (BCRs) can react to a wide spectrum of antigens, T cells can only be activated in response to protein antigens (see Section 3.2). The response elicited following a primary injection of a protein-containing antigen is slow and is characterized by the low affinity IgM antibodies. If the same antigen is encountered again, the secondary response develops more rapidly and is mostly composed of IgG antibodies of higher affinity [64]. Antigen-specific helper T cells play an instru-
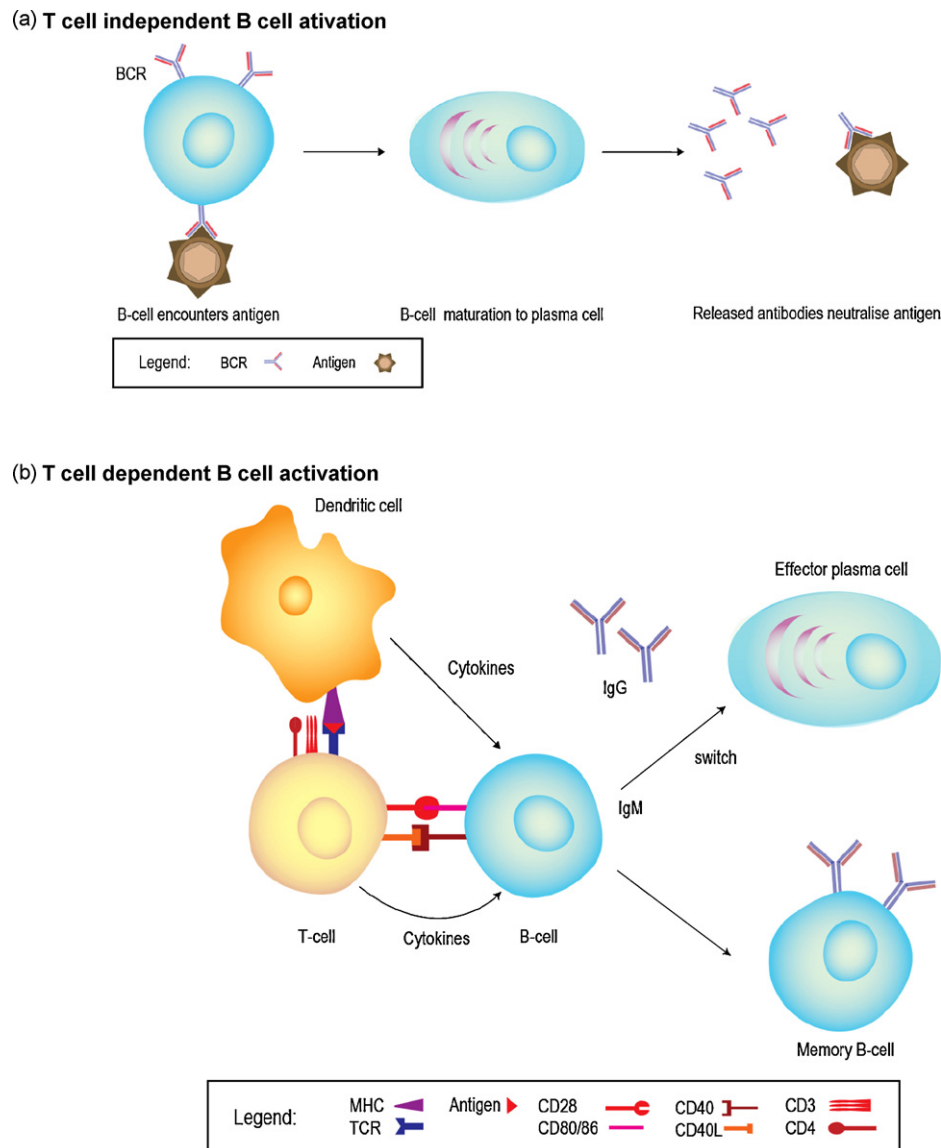
Fig. 5. (a) T cell independent B cell activation. Repetitive antigens such as bacterial polysaccharides are able to stimulate directly B cell proliferation through the B cell receptor (BCR). The interaction between antigen and BCR induces maturation to a plasma cell, which produces antigen-specific antibodies. (b) T cell dependent B cell activation. T cells are stimulated by antigen presenting cells to express CD28, CD40L and cytokines which activate B cells. Depending on the nature of the stimulating signals, the activated B cells can mature to effector-plasma cells or memory B cells.

mental role in providing to B cells the required signals (both soluble and membrane borne) enabling these cells to acquire the capacity to produce increased levels of IgG antibodies of high affinity. A secondary response is characterized by both quantitative (higher and more sustained antibody titres) and qualitative (class switch and affinity maturation) traits that are under the control of helper T cells.

4.5. Regulatory T cells

Although the existence of cells able to suppress an immune response has been long postulated, their identification and characterization have only been recently firmly established [65]. The major function of these lymphocytes (belonging to the CD4^{+} subset and constitutively expressing the CD25 marker and the Foxp3 transcription factor) is to inhibit an immune or inflammatory response by blocking the activity of effector, helper and APC cells [66]. The importance of these regulatory T cells (Fig. 4), or Treg, is best

illustrated by the severe autoimmune syndrome resulting from a genetic deficiency in Treg cells [67]. An autoimmune response can lead to tissues damage, or deregulated hormonal responses. Treg cells thus play an important role in immune tolerance, by blocking unsuitable immune reactions directed to self-antigens [68]. Although counterintuitive, it has been recently demonstrated that Treg cells can also inhibit the development of protective immune responses against "non-self antigens" [69]. It is presently assumed that by limiting these immune responses, Tregs help resolve chronic inflammatory responses that, although directed against "non-self antigens", cause extensive tissue damage if uncontrolled. Treg cells appear thus as an evolutionary tool to reduce the debilitating inflammatory responses elicited by several parasites present in the environment and that cannot be easily avoided [70].

In mice, natural, constitutively present and antigen-induced Treg cells have been described. Both these subsets appear to be under the control of TGF-β, a well known immunosuppressive cytokine secreted by numerous cell populations [71].

5. The immune system at work: basic principles of modern vaccination

The ability of the immune system to respond to virtually any pathogen, even if of recent evolutionary origin, rests on the generation of a very large set of stochastically generated antigen receptors. The major consequences of this strategy are that (i) self-recognition cannot be avoided and (ii) that the adequate effector mechanism must be selected among a large repertoire of mediators (such as antibodies and cytokines) and cells (such as T lymphocytes, macrophages and neutrophils). Moreover, an inadequate (directed to self-constituent or chronic in nature) immune response represents a potential threat for the organism, explaining therefore why the immune system appears to be in a state of "non-response". Indeed, (i) lymphocytes are sequestered within endothelia and are normally not found in tissues; (ii) soluble antigens are not able to directly activate a lymphocyte; and (iii) naturally occurring Treg cells maintain effector cells largely in a non-responsive state. DCs maturation appears therefore as the critical regulatory step enabling the initiation of an immune response. These bone-marrow derived cells leave the blood circulation and spontaneously home to virtually all tissues (mucosal surfaces, skin, etc) that represent natural entry sites for pathogens. Through a process of innate-like pathogen recognition, cell migration and delivery of both antigenic fragments and a confirmation signals to naïve T lymphocytes, DCs act as a "filter", only alerting the immune system in the presence of pathogens, and as a "lens", highlighting certain pathogenic characteristics (such as the presence of lipopolysaccharide or viral RNA) that will influence the choice of effectors (antibodies versus cytokine-producing T cells or cytotoxic effectors). Non-dangerous antigens are therefore "filtered out" by the immune system and considered as "negligible noise".

How can modern vaccinology be envisioned in such a context? As described in a companion paper [2], vaccination rests on the principle of immune memory, whereby a secondary challenge induces an enhanced immune response against a previously encountered pathogen. An ideal vaccine should therefore represent a non-virulent, innocuous form of a given pathogen, able to elicit a strong and adequate immune response in vivo. Although classically represented by attenuated or killed microorganisms, modern vaccines more often comprise pathogen-derived subcellular components or recombinant proteins [2]. In addition to representing safer and economically relevant antigenic formulations, recombinant proteins have also led to the development of therapeutic vaccines against self-antigens, such as in cancer immunotherapy.

The challenge for modern vaccinology is therefore to be able to elicit in vivo all the required steps leading to immune activation. Antigen-presentation and the maturation of DCs are presently thought to represent the limiting step in the development of efficient vaccines. A series of clinical and experimental observations have clearly illustrated the reduced immunogenicity of subcellular or subunit-based vaccines when compared with inactivated/killed whole organisms [72]. The weak immunogenicity of soluble proteins appears to be related to their inability to induce DCs maturation both in vivo and in vitro. In other words, soluble proteins appear to be considered as "negligible noise" by the immune system, lacking the inherent danger-signature often associated with a pathogen [73]. In support of this contention, addition of microbial compounds able to bind TLRs expressed by DCs strongly enhances the immune response to otherwise weakly immunogenic, recombinant proteins [74,75].

Recognition of the important role of the innate immune response in regulating the induction of an adaptive response has led to a reappraisal of the role of adjuvants in vaccinology [78]. Adjuvants, referred to as the "immunologists' dirty little secret" [76], are generally defined as compounds, or association of compounds, that increase and/or modulate the intrinsic immunogenicity of an antigen. In some instances adjuvants also permit the use of a lower dose of antigen in vaccine preparations without compromising the resulting immune response. Although the functional properties of most adjuvants were originally thought to be related to their ability to retain antigens within tissues (thus increasing their exposure to the immune system), recent observations have clearly indicated that most efficient adjuvants (including the widely used aluminium-based salts) are able to activate an innate immune response by directly interacting with DCs, or by inducing in vivo the release of cellular constituents able to activate DCs. Aluminium salts have been recently shown to activate components of the "inflammasome" complex (a member of the NOD-like family of PRRs), leading to the processing and release of pro-inflammatory cytokines such as IL-1β and IL-18 [77].

These observations have led to the concept that the ability to activate the innate immune system may represent an obligatory property for any given adjuvant. Greater understanding of the signals regulating innate responses in vivo has thus led to a more rational design of "immune potentiators" acting as adjuvants [78]. In particular, a new generation of adjuvants has been developed based on the ability of TLR-ligands to induce DCs activation and maturation in vivo. As previously stated, DCs maturation represents a prerequisite for the delivery of antigen-MHC complexes to naïve T cells in an immunogenic fashion. However, DCs can not only activate naïve T helper cells, but also direct their differentiation into functionally distinct helper cell subsets (such as Th1, Th2 and fTh) that will ultimately affect the choice of effector cells (antibodies, cytotoxic T cells, activated macrophages, etc). Aluminium salts, for example, represent potent adjuvants in vivo, leading to the secretion of high levels of antigen-specific antibodies. Although this response appears particularly apt in fighting extracellular pathogens, it may prove less effective against viral strains that are mostly sensitive to Th1-type cytokines (such as IFN-γ) or CD8-expressing cells.

The challenge of modern vaccinology will be to devise new immunological strategies and/or antigen formulations able to counteract the natural tendency of the immune system to ignore non-dangerous antigens. These strategies will also have to selectively induce the adequate effector mechanism adapted to the pathogen envisioned. Identification of TLRs' natural ligands has led to the development of purified and synthetic ligands that can activate TLR pathways in a well defined, and safer, manner increasing immunogenicity of antigens while minimizing local and systemic inflammatory responses. Imidazoquinolines, synthetic compounds binding to TLR7 and TLR8 are presently being considered as candidate adjuvants [78]. Similarly, detoxified forms of lipopolysaccharide (LPS) such as monophosphoryl lipid A (MPL) have now been licensed as adjuvants for several anti-viral vaccines, based on their safety profile and ability to induce the appropriate Th1-like, cellular response in vivo. Based on these promising results, LPS mimetics (such as aminoalkyl glucosaminide 4-phosphate or AGP) are presently being developed for clinical [79,80]. Finally, as described in a companion paper [78], combinations of classical and newly designed adjuvants that have been shown to cooperate and trigger both humoral and cell-mediated immunity have been recently licensed for use in humans. The aim of ongoing studies is to identify the adjuvant formulations able to both enhance and direct an immune response toward a desired choice of effectors [74].

In conclusion, both our increased knowledge of the complex regulatory circuits regulating an immune response and greater understanding of the mode of action of adjuvants should enable the development of efficient vaccines against cancer and infectious diseases (such as AIDS, tuberculosis and malaria) for which no vaccines are presently available.

References

[1] Murphy, Travers, Walport. Janeway's immunobiology. 7th edition; 2008.
[2] Zepp F. Principles of vaccine design—lessons from nature. Vaccine 2010;28(Suppl 3):C14–24.
[3] Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell 2006;124(4):783–801.
[4] Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell 1996;86(6):973–83.
[5] Medzhitov R, Preston-Hurlburt P, Janeway Jr CA. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature 1997;388(6640):394–7.
[6] Meylan E, Tschopp J, Karin M. Intracellular pattern recognition receptors in the host response. Nature 2006;442(7098):39–44.
[7] Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell 2002;10(2):417–26.
[8] Shi Y, Evans JE, Rock KL. Molecular identification of a danger signal that alerts the immune system to dying cells. Nature 2003;425(6957):516–21.
[9] Alegre ML, Leemans J, Le Moine A, Florquin S, De Wilde V, Chong A, et al. The multiple facets of toll-like receptors in transplantation biology. Transplantation 2008;86(1):1–9.
[10] Stuart LM, Ezekowitz RA. Phagocytosis: elegant complexity. Immunity 2005;22(5):539–50.
[11] Luster AD, Alon R, von Andrian UH. Immune cell migration in inflammation: present and future therapeutic targets. Nat Immunol 2005;6(12):1182–90.
[12] Bachmann MF, Kopf M, Marsland BJ. Chemokines: more than just road signs. Nat Rev Immunol 2006;6(2):159–64.
[13] Li M, Carpio DF, Zheng Y, Bruzzo P, Singh V, Ouaaz F, et al. An essential role of the NF-kappa B/Toll-like receptor pathway in induction of inflammatory and tissue-repair gene expression by necrotic cells. J Immunol 2001;166(12):7128–35.
[14] Bowie AG, Unterholzner L. Viral evasion and subversion of pattern-recognition receptor signalling. Nat Rev Immunol 2008;8(12):911–22.
[15] Brack C, Hirama M, Lenhard-Schuller R, Tonegawa S. A complete immunoglobulin gene is created by somatic recombination. Cell 1978;15(1):1–14.
[16] Murre C. Epigenetics of antigen-receptor gene assembly. Curr Opin Genet Dev 2007;17(5):415–21.
[17] Burnet. A modification of Jerne's theory of antibody production using the concept of clonal selection. Aust J Sci 1957;20:67–9.
[18] Hodgkin PD, Heath WR, Baxter AG. The clonal selection theory: 50 years since the revolution. Nat Immunol 2007;8(10):1019–26.
[19] Schubert U, Anton LC, Gibbs J, Norbury CC, Yewdell JW, Bennink JR. Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. Nature 2000;404(6779):770–4.
[20] Rock KL, York IA, Saric T, Goldberg AL. Protein degradation and the generation of MHC class I-presented peptides. Adv Immunol 2002;80:1–70.
[21] Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. Structure of the human class I histocompatibility antigen HLA-A2. Nature 1987;329(6139):506–12.
[22] Fremont DH, Matsumura M, Stura EA, Peterson PA, Wilson IA. Crystal structures of two viral peptides in complex with murine MHC class I H-2Kb. Science 1992;257(5072):919–27.
[23] Hedrick SM, Cohen DI, Nielsen EA, Davis MM. Isolation of cDNA clones encoding T cell-specific membrane-associated proteins. Nature 1984;308(5955):149–53.
[24] Yanagi Y, Yoshikai Y, Leggett K, Clark SP, Aleksander I, Mak TW. A human T cell-specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. Nature 1984;308(5955):145–9.
[25] Loureiro J, Ploegh HL. Antigen presentation and the ubiquitin–proteasome system in host–pathogen interactions. Adv Immunol 2006;92:225–305.
[26] Kovacsovics-Bankowski M, Rock KL. A phagosome-to-cytosol pathway for exogenous antigens presented on MHC class I molecules. Science 1995;267(5195):243–6.
[27] Norbury CC, Hewlett LJ, Prescott AR, Shastri N, Watts C. Class I MHC presentation of exogenous soluble antigen via macropinocytosis in bone marrow macrophages. Immunity 1995;3(6):783–91.
[28] Dengjel J, Schoor O, Fischer R, Reich M, Kraus M, Muller M, et al. Autophagy promotes MHC class II presentation of peptides from intracellular source proteins. Proc Natl Acad Sci USA 2005;102(22):7922–7.
[29] Gourley TS, Wherry EJ, Masopust D, Ahmed R. Generation and maintenance of immunological memory. Semin Immunol 2004;16(5):323–33.
[30] Neuberger MS, Ehrenstein MR, Rada C, Sale J, Batista FD, Williams G, et al. Memory in the B-cell compartment: antibody affinity maturation. Philos Trans R Soc Lond B Biol Sci 2000;355(1395):357–60.
[31] Yu X, Tsibane T, McGraw PA, House FS, Keefer CJ, Hicar MD, et al. Neutralizing antibodies derived from the B cells of 1918 influenza pandemic survivors. Nature 2008;455(7212):532–6.
[32] Hammarlund E, Lewis MW, Hansen SG, Strelow LI, Nelson JA, Sexton GJ, et al. Duration of antiviral immunity after smallpox vaccination. Nat Med 2003;9(9):1131–7.
[33] Amzel LM, Poljak RJ. Three-dimensional structure of immunoglobulins. Annu Rev Biochem 1979;48:961–97.
[34] Cerottini JC, Nordin AA, Brunner KT. Specific in vitro cytotoxicity of thymus-derived lymphocytes sensitized to alloantigens. Nature 1970;228(5278):1308–9.
[35] Chowdhury D, Lieberman J. Death by a thousand cuts: granzyme pathways of programmed cell death. Annu Rev Immunol 2008;26:389–420.
[36] Knickelbein JE, Khanna KM, Yee MB, Baty CJ, Kinchington PR, Hendricks RL. Noncytotoxic lytic granule-mediated CD8+ T cell inhibition of HSV-1 reactivation from neuronal latency. Science 2008;322(5899):268–71.
[37] Bartee E, Mohamed MR, McFadden G. Tumor necrosis factor and interferon: cytokines in harmony. Curr Opin Microbiol 2008;11(4):378–83.
[38] Liu YJ, Zhang J, Lane PJ, Chan EY, MacLennan IC. Sites of specific B cell activation in primary and secondary responses to T cell-dependent and T cell-independent antigens. Eur J Immunol 1991;21(12):2951–62.
[39] Macpherson AJ, Gatto D, Sainsbury E, Harriman GR, Hengartner H, Zinkernagel RM. A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. Science 2000;288(5474):2222–6.
[40] Sun JC, Williams MA, Bevan MJ. CD4+ T cells are required for the maintenance, not programming, of memory CD8+ T cells after acute infection. Nat Immunol 2004;5(9):927–33.
[41] Janssen EM, Lemmens EE, Wolfe T, Christen U, von Herrath MG, Schoenberger SP. CD4+ T cells are required for secondary expansion and memory in CD8+ T lymphocytes. Nature 2003;421(6925):852–6.
[42] Shedlock DJ, Shen H. Requirement for CD4 T cell help in generating functional CD8 T cell memory. Science 2003;300(5617):337–9.
[43] Sun JC, Bevan MJ. Defective CD8 T cell memory following acute infection without CD4 T cell help. Science 2003;300(5617):339–42.
[44] Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. J Exp Med 1973;137(5):1142–62.
[45] Moser M. Dendritic cells. In: Paul W, editor. Fundamental Immunology. 6th edition 2008. p. 451–82.
[46] Joffre O, Nolte MA, Sporri R, Reis e Sousa C. Inflammatory signals in dendritic cell activation and the induction of adaptive immunity. Immunol Rev 2009;227(1):234–47.
[47] Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J Exp Med 1994;179(4):1109–18.
[48] Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 1986;136(7):2348–57.
[49] Heinzel FP, Sadick MD, Holaday BJ, Coffman RL, Locksley RM. Reciprocal expression of interferon gamma or interleukin 4 during the resolution or progression of murine leishmaniasis. Evidence for expansion of distinct helper T cell subsets. J Exp Med 1989;169(1):59–72.
[50] Yamamura M, Uyemura K, Deans RJ, Weinberg K, Rea TH, Bloom BR, et al. Defining protective responses to pathogens: cytokine profiles in leprosy lesions. Science 1991;254(5029):277–9.
[51] Finkelman FD, Shea-Donohue T, Morris SC, Gildea L, Strait R, Madden KB, et al. Interleukin-4- and interleukin-13-mediated host protection against intestinal nematode parasites. Immunol Rev 2004;201:139–55.
[52] Breitfeld D, Ohl L, Kremmer E, Ellwart J, Sallusto F, Lipp M, et al. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. J Exp Med 2000;192(11):1545–52.
[53] Schaerli P, Willimann K, Lang AB, Lipp M, Loetscher P, Moser B. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. J Exp Med 2000;192(11):1553–62.
[54] Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 2005;6(11):1123–32.

[55] Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol 2005;6(11):1133–41.

[56] Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. Science 1993;260(5107):547–9.

[57] Maldonado-Lopez R, De Smedt T, Michel P, Godfroid J, Pajak B, Heirman C, et al. CD8alpha+ and CD8alpha− subclasses of dendritic cells direct the development of distinct T helper cells in vivo. J Exp Med 1999;189(3):587–92.

[58] Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 2006;441(7090):235–8 [Epub 2006 April 30].

[59] van Panhuys N, Tang SC, Prout M, Camberis M, Scarlett D, Roberts J, et al. In vivo studies fail to reveal a role for IL-4 or STAT6 signaling in Th2 lymphocyte differentiation. Proc Natl Acad Sci USA 2008;105(34):12423–8.

[60] O'Quinn DB, Palmer MT, Lee YK, Weaver CT. Emergence of the Th17 pathway and its role in host defense. Adv Immunol 2008;99:115–63.

[61] Maizels N, Bothwell A. The T-cell-independent immune response to the hapten NP uses a large repertoire of heavy chain genes. Cell 1985;43(3 Pt 2):715–20.

[62] Vos Q, Lees A, Wu ZQ, Snapper CM, Mond JJ. B-cell activation by T-cell-independent type 2 antigens as an integral part of the humoral immune response to pathogenic microorganisms. Immunol Rev 2000;176:154–70.

[63] Fazilleau N, McHeyzer-Williams LJ, McHeyzer-Williams MG. Local development of effector and memory T helper cells. Curr Opin Immunol 2007;19(3):259–67.

[64] Allen CD, Okada T, Cyster JG. Germinal-center organization and cellular dynamics. Immunity 2007;27(2):190–202.

[65] Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995;155(3):1151–64.

[66] Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell 2008;133(5):775–87.

[67] Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 2001;27(1):20–1.

[68] Kim JM, Rasmussen JP, Rudensky AY. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. Nat Immunol 2007;8(2):191–7.

[69] Oldenhove G, de Heusch M, Urbain-Vansanten G, Urbain J, Maliszewski C, Leo O, et al. CD4+ CD25+ regulatory T cells control T helper cell type 1 responses to foreign antigens induced by mature dendritic cells in vivo. J Exp Med 2003;198(2):259–66.

[70] Sakaguchi S, Regulatory. T cells: mediating compromises between host and parasite. Nat Immunol 2003;4(1):10–1.

[71] Horwitz DA, Zheng SG, Gray JD. Natural and TGF-beta-induced Foxp3(+)CD4(+)CD25(+) regulatory T cells are not mirror images of each other. Trends Immunol 2008;29(9):429–35.

[72] Geeraedts F, Goutagny N, Hornung V, Severa M, de Haan A, Pool J, et al. Superior immunogenicity of inactivated whole virus H5N1 influenza vaccine is primarily controlled by Toll-like receptor signalling. PLoS Pathog 2008;4(8):e1000138.

[73] Matzinger P. The danger model: a renewed sense of self. Science 2002;296(5566):301–5.

[74] Garcon N, Chomez P, Van Mechelen M. GlaxoSmithKline Adjuvant Systems in vaccines: concepts, achievements and perspectives. Expert Rev Vaccines 2007;6(5):723–39.

[75] van Duin D, Medzhitov R, Shaw AC. Triggering TLR signaling in vaccination. Trends Immunol 2006;27(1):49–55.

[76] Janeway Jr CA. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol 1989;54(Pt 1):1–13.

[77] Lambrecht BN, Kool M, Willart MA, Hammad H. Mechanism of action of clinically approved adjuvants. Curr Opin Immunol 2009;21(1):23–9.

[78] Leroux-Roels G. Unmet needs in modern vaccinology. Adjuvants to improve the immune response. Vaccine 2010;28(Suppl 3):C25–36.

[79] Dupont J, Altclas J, Lepetic A, Lombardo M, Vazquez V, Salgueira C, et al. A controlled clinical trial comparing the safety and immunogenicity of a new adjuvanted hepatitis B vaccine with a standard hepatitis B vaccine. Vaccine 2006;24(49–50):7167–74.

[80] Johnson DA. Synthetic TLR4-active glycolipids as vaccine adjuvants and stand-alone immunotherapeutics. Curr Top Med Chem 2008;8(2):64–79.