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Mini review

Fundamental mechanisms of host immune responses to infection

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Chronic inflammatory periodontal disease is caused by host immune responses to periodontal microorganisms. Although periodontal disease seems to progress locally in the oral cavity, the actual immune responses proceed at two sites, one being the local area peripheral to the periodontium, and the other being in secondary lymphoid tissues, such as lymph nodes and the spleen. To investigate the pathogenesis of periodontal diseases, it is essential to understand exactly how the immune system works against microbial infections. The past decade has produced remarkable advances in our understanding of host immune responses. This review highlights two of these developments: cross-talk between innate and adaptive immunity mediated by dendritic cells via toll-like receptors; and antigen-specific immune regulation by dendritic cells and T cells.

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Overview

The immune system is organized in terms of cells and molecules that have specialized roles for defending against infection. Innate (natural) immunity and adaptive (acquired) immunity are two fundamental aspects of the immune system response to invading microbes (Figs 1 and 2). Innate immune responses are mediated by the release of inflammatory cytokines and chemokines, and by phagocytic or killer cells. Adaptive immune responses are mediated by the generation of antigen-specific T and B cells. Antigen-primed T cells induce clonal expansion and differentiate into effector T cells that produce various cytokines or elicit cytolysis to eliminate target cells. B cells secrete immunoglobulins, which are responsible for eliminating extracellular microorganisms. Innate responses are generated at the periphery of sites of microbial penetration, whereas adaptive immune responses are generated at secondary lymphoid tissues, such as lymph nodes and the spleen (Table 1).

It has recently been shown that dendritic cells are crucial for the initiation and regulation of both innate and adaptive immunity, and form a bridge between the two immune systems by trafficking through lymphatic vessels (Fig. 1). Innate immunity was formerly thought to comprise nonspecific immune responses, characterized by phagocytosis and digestion of microorganisms and foreign substances by macrophages and neutrophils. However, innate immunity has considerable specificity against microorganisms, and is able to discriminate pathogens from self (1–3) (Table 1). Innate immune recognition relies on a limited number of germline-encoded receptors, which are called pathogen recognition receptors. These receptors recognize conserved molecular patterns [pathogen-associated molecular patterns (PAMPs)] and produce inflammatory cytokines via intracellular signaling. The toll-like receptor (TLR) family is the best characterized class of pathogen recognition receptors and detects multiple PAMPs (4). TLRs play an essential role in the recognition of microbial components. Dendritic cells within the epithelium express TLRs and play a sentinel role at the front line of defense (5–7). Interestingly, different dendritic cell subsets express distinct sets of TLRs, and this leads to them having particular functions in innate responses and the generation of distinct T-cell subsets.

Antigen-captured dendritic cells alter their functional properties, from those of endocytic cells, which are highly responsive to pathogens, to those of nonendocytic cells that have lost their susceptibility to the peripheral infectious environment and migrate to regional lymph nodes to present antigens to naïve T cells. During migration,

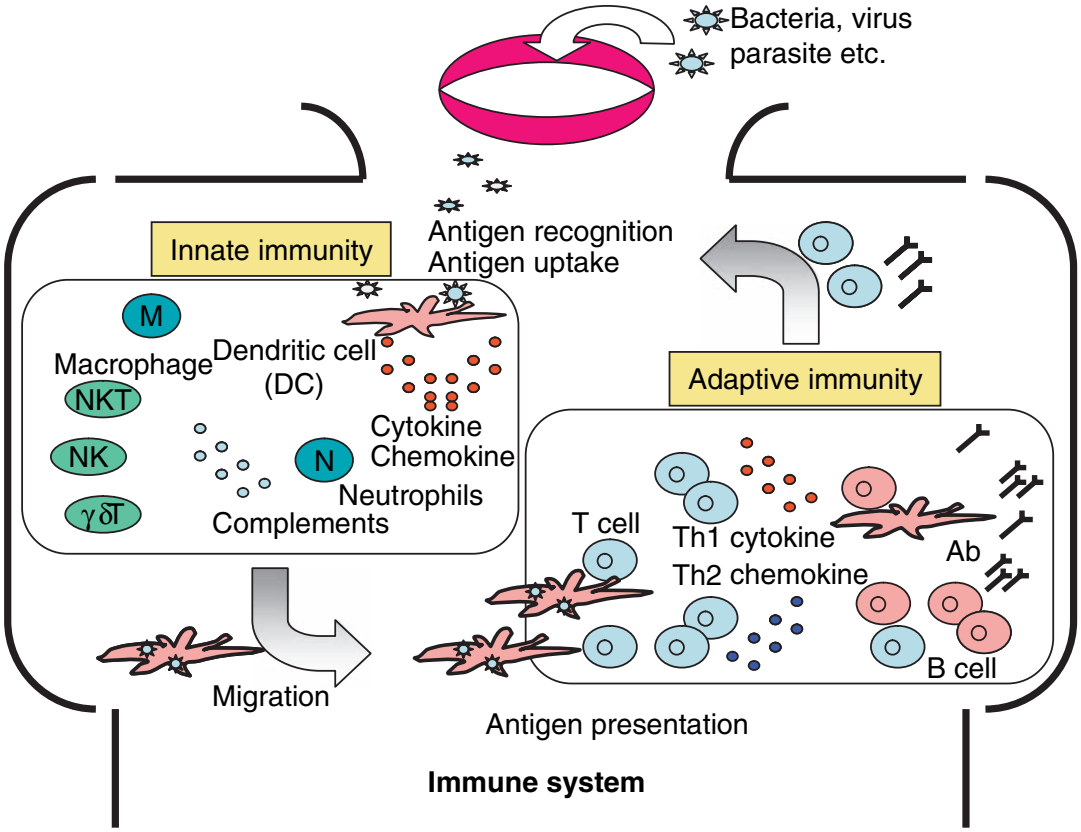

Fig. 1. Immune system. The immune system has two fundamental systems in response to invading microbes. Innate immunity is mediated by the release of inflammatory cytokines and chemokines, and by phagocytotic cells and killer cells. Adaptive immunity is mediated by the expansion of antigen-specific T and B cells. Both immune systems are well organized and interact with each other. Ab, antibody; NK, natural killer; NKT, natural killer T cells; Th, T helper.

dendritic cells display large amounts of peptide–major histocompatibility complex (MHC) and an array of costimulatory molecules, such as CD86 and CD40, and transform themselves into mature dendritic cells with high antigen-presenting capacity (Fig. 2). The migrating dendritic cells in the T-cell zone of lymph nodes interact with naïve or memory T cells and efficiently induce the activation of antigen-specific T cells.

$CD4^+$ T cells are important in determining the outcome of the T-cell immune responses against pathogens (Fig. 2). Effector $CD4^+$ T cells are classified into T helper (Th)1 and Th2 subsets (8). Th1 cells secrete interferon (IFN)-γ, tumor necrosis factor (TNF)-α and TNF-β [lymphotoxin (LT)], which are critical for the eradication of intracellular pathogens, while Th2 cells produce interleukin-4, -5, -6 and -13, which are essential for optimal antibody production and elimination of extracellular microorganisms, including helminths and nematodes. Importantly, these two T-cell subsets mediate pathologic immune responses. Th1-mediated immune responses have been associated with the tissue destruction found in inflammation and some organ-specific autoimmune diseases, whereas Th2-mediated immune responses have been implicated in allergic and systemic autoimmune diseases. Recent reports have indicated that multiple factors, including cytokines, costimulatory molecules, receptor-mediated signal transduction pathways and transcription factors, control the commitment to the Th subsets. Interleukin-12 is a critical cytokine that promotes Th1-mediated immune responses and regulates Th2 responses (9). The use of costimulatory molecules in the cognate interactions between dendritic cells and T cells may greatly affect the differentiation of Th1 and Th2 cells (10). The distinct subsets of dendritic cells in response to various TLRs direct different types of T-cell responses (11).

On the other hands, some dendritic cells induce T-cell tolerance rather than immunity (12–14). Dendritic cells, which phagocytose the dead cell without any pathogen-related signals, are able to present antigens, but fail to express potent costimulators CD80 and CD86 and induce T-cell tolerance. Anti-inflammatory cytokines, interleukin-10 and transforming growth factor-β (TGF-β), may induce tolerogenic signals in the dendritic cells and reduce their capacity as potent antigen-presenting cells.

Immune regulation is not mediated by dendritic cells alone. Some T-cell subsets can directly inhibit the function of effector T cells. The best known of such regulatory T cells are the naturally arising $CD4^+$ $CD25^+$ regulatory T cells (natural regulatory T cells) (15). $CD4^+$ $CD25^+$ regulatory T cells are generated in the thymus, and their

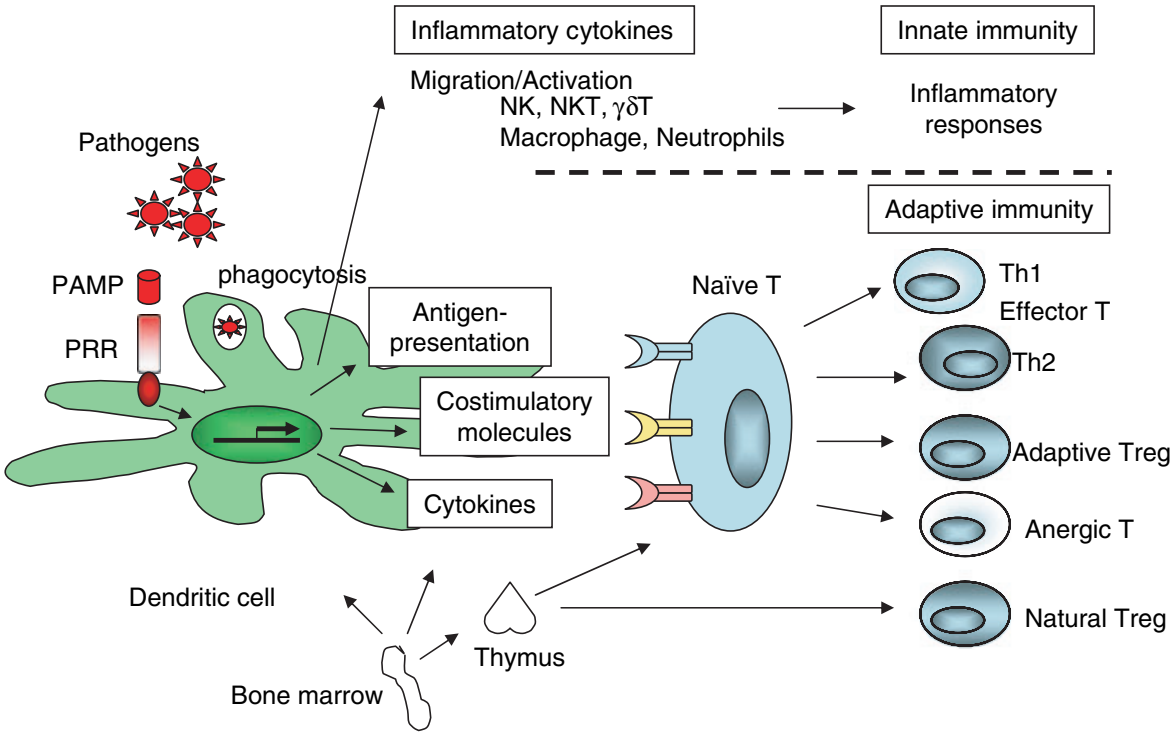

Fig. 2. Pathogen recognition by dendritic cells induces two immune systems via pathogen-recognition receptor-mediated signals. Pathogen-recognition receptor (PRR)-mediated signals induce the production of various cytokines, expression of major histocompatibility complex (MHC)/peptide complex and costimulatory molecules by dendritic cells, and trigger both innate and adaptive immune responses. See details in the text. γδT, gamma delta T cells; NK, natural killer; NKT, natural killer T cells; PAMP, pathogen-associated molecular pattern; Th, T helper; Treg, T regulatory.

expansion and activation require antigenic stimulation and high levels of interleukin-2 or CD28 costimulation (16). However, regulatory T cells exerting their suppressive effects are hypoproliferative and anergic. $CD4^+$ $CD25^+$ regulatory T cells also secrete interleukin-10 and TGF-β. It seems that their suppressive function does not directly require these cytokines and antigen-presenting cells, but their maintenance and expansion does (17). It has been suggested that the regulatory function of regulatory T cells requires cell-to-cell contact. A forkhead transcription factor, Foxp3, plays an important part in the development and function of natural regulatory T cells (17,18); however, the molecular mechanisms involved in their regulatory function are still unknown. For a long time, interest in natural regulatory T cells was focused on peripheral self-tolerance and autoimmunity, but recent observations have demonstrated that natural regulatory T cells are also capable of inducing T-cell tolerance against nonself foreign antigens, such as microbial pathogens (15). In the present study, we discuss the adaptive immune responses against pathogens regulated by particular T-cell subsets and dendritic cells.

Innate immunity

Front line of defense and mucosal surfaces

Mucosal surfaces represent a very large proportion of the body surface area and are exposed to large numbers of commensal bacteria. This commensal microflora is abundant and colonizes the large intestine, oropharynx and female genital tract without causing any harm. It is rather beneficial for the host in most cases. Although most studies of mucosal immunity have focused on the small intestine, it should be noted that the oral mucosa, including the gingiva, is clearly different from the intestinal mucosa. The intestinal mucosa has its own unique lymphoid tissues, namely the gut-associated lymphoid tissue (GALT), Peyer's patches and isolated lymphoid follicles (19). Peyer's patches are macroscopic lymphoid aggregates that are found in the submucosa of the small intestine. The isolated lymphoid follicles are smaller microscopic follicles that are distributed throughout the wall of the small and large intestines. This unique lymphoid system is involved in the induction phase of site-specific immune responses. In contrast to the intestinal mucosa, the oral mucosa does not have such a unique lymphoid system. Similarly to skin, the oral mucosa comprises a stratified squamous epithelium and underlining connective tissue. In the epithelium, many highly efficient and complementary defense mechanisms are present. The normal epithelial cells have tight intercellular junctions that impede the entry of bacteria and their metabolites. In addition, a number of proteins/peptides are involved in the eradication of invading microbes. These include

Table 1. A comparison of innate and adaptive immunity

Property	Innate immune system	Adaptive immune system
Cells involved	Phagocytes (macrophages, neutrophils, DCs), NK cells	T cells, B cells
Recognition		
Receptors	Fixed in genome Rearrangement not necessary Limited diversity	Encoded in gene segments Rearrangement necessary High diversity
Ligand/antigens	Conserved molecular patterns (components of microorganisms)	Details of molecular structure (peptides, proteins, carbohydrates)
Development	Nonclonal Selected over evolutionary time	Clonal Selected in individual
Response time	Immediate (0–4 h)	Delayed (≈ 72 h)
Site of response	Local, periphery	Secondary lympoid tissues
Response	Production of inflammatory cytokines (IL-1, IL-6, TNF-α) and chemokines (IL-8) Induction of costimulatory molecules (CD86, CD40)	Clonal expansion or anergy Production of effector cytokines (IFN-γ, IL-4)

DC, dendritic cell; IFN-γ, interferon-γ; IL, interleukin; NK, natural killer; TNF-α, tumor necrosis factor-α.

defensins, TLRs, an emerging family of proteins referred to as nucleotide oligomerization domains (NODs), and related proteins with pryin domains that can regulate apoptosis, inflammation and immune responses (20,21). Within the stratified squamous epithelial layers of the oral mucosa, Langerhans' cells are widely distributed under steady-state conditions and play a sentinel role at the front line of defense, co-operating with epithelial cells (Fig. 3) (22). In addition, interstitial dendritic cells reside in the connective tissues under the epithelium. How dendritic cells take up microbial organisms across the mucosal epithelium and distinguish pathogens from the commensal microflora are questions that remain to be answered.

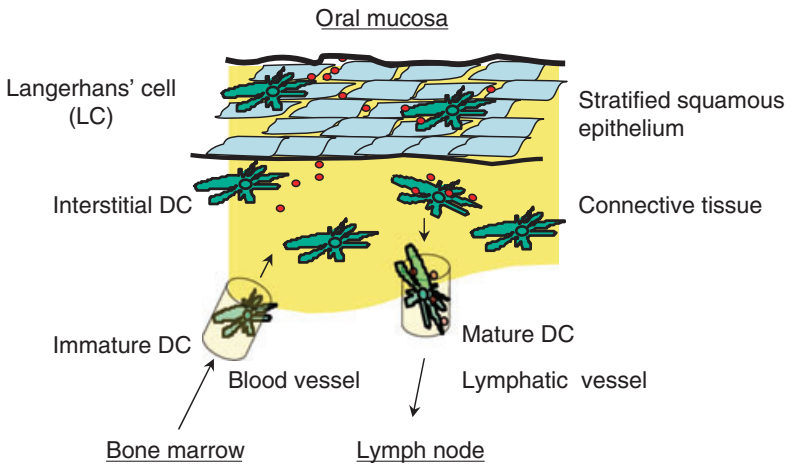

Fig. 3. Dendritic cells (DC) in the oral mucosa. In the oral mucosa, Langerhans' cells (LC) and interstitial dendritic cells play a sentinel role at the front line of defense, co-operating with epithelial cells. Antigen-captured Langerhans' cells and dendritic cells migrate to the regional lymph nodes through lymphatic vessels to present antigens to T cells.

Innate immune recognition

The innate immune system is an evolutionarily conserved form of host defense found in most multicellular organisms. Induction of innate immunity is triggered by pathogen interaction with germline-encoded pathogen recognition receptors. These receptors recognize conserved PAMPs, which represent molecular structures that are produced by microorganisms and not by the host. For example, peptidoglycan and lipopolysaccharide are produced by bacteria, but not by eukaryotic hosts. Recognition of PAMPs by pathogen recognition receptors allows the innate immune system to unerringly discriminate self-molecules from pathogen-associated nonself structures. This property of PAMPs also accounts for self/nonself recognition by the adaptive immune system. PAMPs are invariant and represent conserved molecular patterns that are essential for microbial survival. For example, the lipid A portion of lipopolysaccharide represents an invariant pattern found in all gram-negative bacteria. Lipopolysaccharide, lipoproteins, peptidoglycan and lipoteichoic acids are all synthesized by bacteria, and mutation or loss of these molecules is either lethal or reduces adaptation.

TLRs

TLRs and their ligands – Many PAMPs have been recognized by pathogen recognition receptors. TLRs function as pathogen recognition receptors, which have an essential function in innate immunity. TLRs comprise a family of type I transmembrane proteins that have been evolutionarily conserved between insects and humans (20). Toll was first identified as an essential molecule for embryonic patterning in *Drosophila*, and was shown to act as a key molecule in antifungal immunity (23). The structure of TLRs is characterized by an extracellular leucin-rich repeat domain and an intracellular toll/interleukin-1 receptor (TIR) domain (Fig. 4) (4). Mammalian TLRs comprise a large family consisting of at least 11

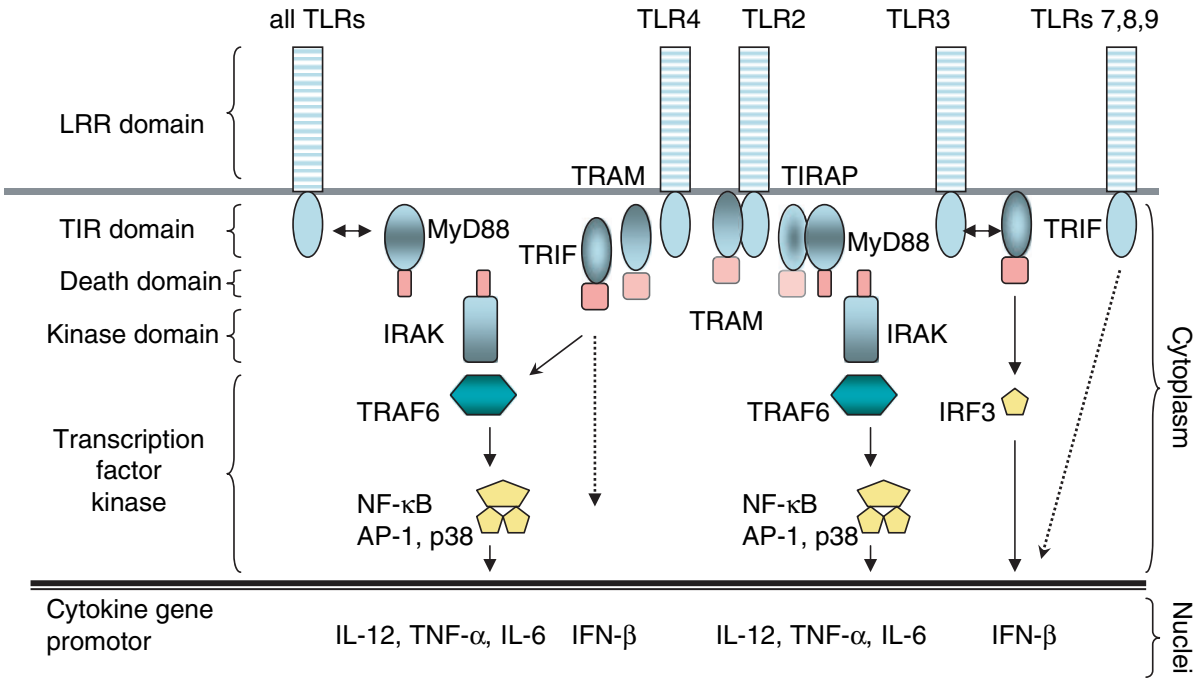

Fig. 4. Structure of toll-like receptors (TLRs) and their signaling pathway. TLRs consist of an extracellular leucin-rich repeat (LRR) domain and an intracellular toll/interleukin-1 receptor (TIR) domain. MyD88 is a TIR domain-containing adaptor molecule that associates with the cytoplasmic TIR domain of TLRs, and recruits interleukin (IL)-1 receptor-associated kinase (IRAK) to the receptor upon ligand binding. IRAK activates tumour necrosis factor (TNF) receptor-activated factor 6 (TRAF6), leading to the activation of the IκB kinase complex, resulting in nuclear translocation of NF-κB, which induces expression of inflammatory cytokines, such as IL-12, TNF and IL-6. All TLRs transduce signal through a MyD88–IRAK–TRAF6 pathway. TIR domain-containing adaptor protein (TIRAP), a second TIR domain-containing adaptor, is involved in the MyD88-dependent signaling pathway via TLR4 and TLR2. TIR-domain-containing adaptor inducing interferon-β (IFN-β) (TRIF), a third TIR domain-containing adaptor, is involved in the activation of IFN regulatory factor 3 (IRF-3), which induces IFN-β in the MyD88-independent pathway. TRIF-related adaptor molecule (TRAM) is involved in the TRIF-dependent and MyD88-independent pathway in response to the TLR4 ligand.

members. TLRs 1–9 are conserved between humans and mice, and each of these recognizes unique molecular patterns associated with different classes of pathogens (Fig. 5) (24).

TLR4 recognizes lipopolysaccharide, which is a major cell wall component of gram-negative bacteria, whereas TLR2 recognizes a variety of microbial components, including peptidoglycan and lipoteichoic acid from gram-positive bacteria, lipoarabinomannan from mycobacteria and zymosan from fungi (24). Initial reports have suggested that TLR2 may recognize several atypical types of lipopolysaccharide from *Leptospira interrogans* (25) and *Porphyromonas gingivalis* (26). However, a recent report indicated that the TLR2-mediated reaction is mediated by the contaminated lipoproteins in the lipopolysaccharide preparation from *P. gingivalis* (27). Therefore, careful reassessment is required to determine their specificity. It is thought that TLR1 or TLR6 is associated with TLR2 and forms heterodimers, which are involved in the discrimination between diacyl and triacyl lipopeptides. Thus, TLR2 and TLR4 recognize bacterial components that are mainly present in the bacterial cell membrane.

TLR5 recognizes flagellin, which is a protein component of the flagella extending out from the outer membrane of gram-negative bacteria. TLR9 recognizes unmethylated CpG motifs that are found in bacterial genomic DNA and also in viral DNA (28). Bacterial DNA shows high immunostimulatory effects, whereas mammalian DNA, which has few, mostly methylated, CpG motifs, is not stimulatory. TLR3 is involved in the recognition of double-stranded RNA, which is the most representative viral component. Double-stranded RNA activates immune cells and induces type I interferons (IFN-α/β), which elicit potent antiviral activity (29). TLR7 and TLR8 are highly homologous to TLR9 and are involved in viral recognition. TLR7 is a receptor for the synthetic antiviral imidazoquinolines, one of which is now used clinically for the treatment of human papillomavirus infection. Thus, TLR3, TLR7, TLR8 and TLR9 are used for viral recognition.

TLR1, TLR2, TLR4, TLR5 and TLR6 are transported to the cell surface for engagement with extracellular pathogens, while TLR3, TLR7, TLR8 and TLR9 are expressed in intracellular compartments, such as endosomes (Table 2). How do intracellular TLRs capture their ligands? In the case of bacteria that are unable to enter cells, macrophages or dendritic cells engulf bacteria by phagocytosis, and CpG DNA is exposed by degradation of

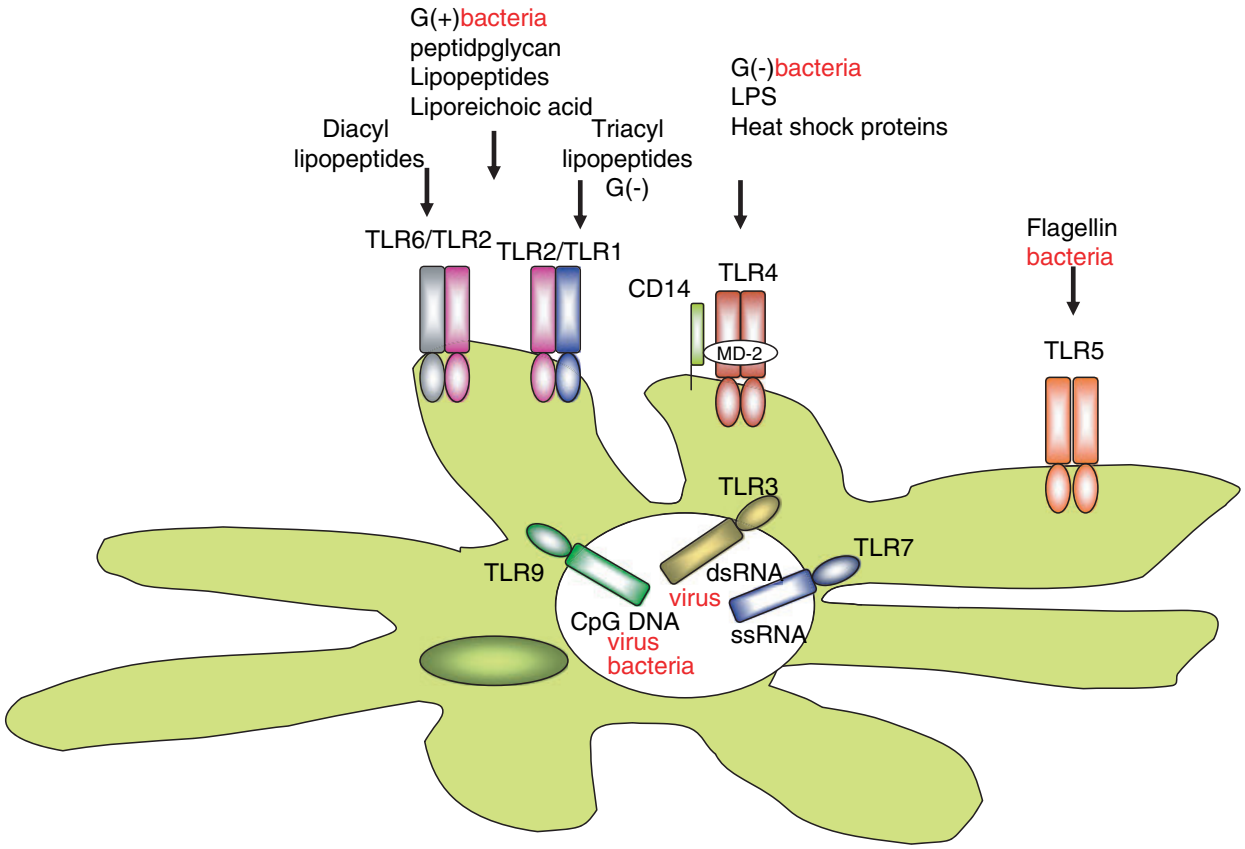

Fig. 5. Toll-like receptors (TLRs) and their ligands. TLR2 is essential in the recognition of microbial lipopeptides. TLR1 and TLR6 co-operate with TLR2 to discriminate subtle differences between triacyl and diacyl lipopeptides, respectively. TLR4 recognizes lipopolysaccharide (LPS) from gram-negative bacteria and endogenous host-derived products, such as heat shock protein 60 (hsp60). TLR5 recognizes flagellin. TLR9 is essential in CpG DNA recognition, whereas TLR3 and TLR7 are implicated in the recognition of viral double-stranded (ds)RNA and single-stranded (ss)RNA, respectively.

bacteria in phagosomes/lysosomes or endosomes/lysosomes, where TLR9 is expressed. In the case of viral infection, viruses penetrate cells by receptor-mediated endocytosis, and the viral contents are exposed to the cytoplasm by fusion of the viral and endosomal membranes. In other cases, the viral particles are degraded in the endosomal compartment, and double-stranded RNA, single-stranded RNA and CpG DNA are exposed to TLRs. Thus, ligand recognition by TLRs occurs both at the cell surface and within the phagosome/lysosome or endosome/lysosome compartments.

Table 2. Location and expression of Toll-like receptors (TLRs)

TLR	Location	Cells
TLR1	Cell membrane	mDCs, monocytes (ubiquitous)
TLR2	Cell membrane	Monocytes, NK cells, mDCs, mast cells T cells, epithelial cells
TLR3	Intracellular	mDCs, NK cells, epithelial cells
TLR4	Cell membrane	Monocytes, mast cells, neutrophils, T cells epithelial cells, endothelial cells
TLR5	Cell membrane	Monocytes, NK cells, mDCs epithelial cells
TLR6	Cell membrane	Myeloid cells, mast cells, B cells, mDCs
TLR7	Intracellular	pDCs, B cells, eosinophils
TLR8	Intracellular	NK cells, T cells, myeloid cells, mDCs
TLR9	Intracellular	pDCs, B cells, NK cells
TLR10	Cell membrane	B cells, pDCs, mDCs
TLR11		Uroepithelium (mouse)

mDCs, myeloid dendritic cells; NK, natural killer; pDCs, plasmacytoid dendritic cells.

TLR signaling pathways – The cytoplasmic domain of TLRs is highly homologous to that of the interleukin-1R family and is known as the TIR domain. Both TLRs and interleukin-1R signals are mediated by a common adaptor protein MyD88. MyD88 has both a TIR domain and a death domain, and the recruitment of MyD88 to a TLR occurs via a TIR–TIR homotypic interaction (Fig. 4) (4,24). Upon stimulation, MyD88 recruits a death domain-containing serine/threonine kinase, interleukin-1R-associated kinase (IRAK). IRAK is activated by phosphorylation and then associates

with TNF receptor-activated factor 6 (TRAF6), leading to activation of nuclear factor-kappa B (NF-κB) and mitogen-activated protein (MAP) kinases, and transcription of immunologically relevant genes. Studies of MyD88-deficient mice revealed that MyD88-dependent NF-κB activation is critical for the production of inflammatory cytokines, such as interleukin-12, TNF-α and interleukin-6 (4).

Although MyD88-dependent NF-κB activation is essential for the production of inflammatory cytokines in response to TLR ligand stimulation, several other TIR domain-containing adaptor molecules are involved in the TLR signaling pathway. A TIR domain-containing adaptor protein (TIRAP) is implicated in the TLR2- and TLR4-mediated MyD88-dependent pathway (30). TIR-domain-containing adaptor-inducing IFN-β (TRIF) mediates the induction of IFN-β through the activation of IFN regulatory factor 3 (IRF3) in a MyD88-independent manner (31). A TRIF-related adaptor molecule (TRAM) is involved in the TLR4-mediated, TRIF-dependent pathway (32). Thus, TIR domain-containing adaptors play a pivotal role in the TLR signaling pathway, which alters subsequent immune responses.

Expression of TLRs – TLRs are expressed on a variety of cells, including both lymphoid and nonlymphoid cells, and on various epithelial surfaces. TLR2, TLR3, TLR4 and TLR5 are differentially expressed on oral, bronchial and gastrointestinal epithelia (33,34). Pathogen recognition by the TLRs expressed on epithelial cells leads to the production of cytokines, chemokines and antimicrobial peptides. These induce the expansion and activation of epithelial cells and recruit inflammatory cells to the infected sites. The constitutive interactions of TLRs with commensal microorganisms are also required to maintain epithelial homeostasis, especially in the gut (35).

The differential expression of TLRs on myeloid and lymphoid cells control various immune responses. All immune cells, including neutrophils, eosinophils, mast cells, T-, B- and natural killer (NK) cells, monocytes/macrophages and dendritic cells, express unique sets of TLRs (Table 2). Interestingly, recent studies have shown that TLR expression discriminates between dendritic cell subsets, which defines their differentiation and activation states, as well as their function in the development of subsequent innate and adaptive immune responses (Fig. 6).

TLRs and dendritic cell subsets – Human dendritic cell subsets circulating in the blood have been classified into myeloid dendritic cells and plasmacytoid dendritic cells. Freshly isolated $CD11c^{high}$ myeloid dendritic cells are immature dendritic cells, but are able to differentiate into potent antigen-presenting cells, whereas $CD11c^{low}$ plasmacytoid dendritic cells are capable of producing large amounts of type I IFNs (36). Although it has been accepted that myeloid dendritic cells are generated in the bone marrow, the ontogeny of plasmacytoid dendritic cells is still controversial. Human plasmacytoid dendritic cells express TLR7 and TLR9, whereas myeloid dendritic cells express TLRs 1 2, 3, 5, 6 and 8 (Table 2 and Fig. 6). Human blood monocytes express TLRs 1, 2, 4 and 5, but lose these receptors and acquire TLR3 as they mature in the presence of granulocyte–macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (37). Human Langerhans' cells never express TLR3, which is a receptor for double-stranded viral RNA. Plasmacytoid dendritic cells are efficient detectors for viral components, recognizing CpG DNA via TLR9 and single-stranded RNA via TLR7, but they lack TLR3.

Immature dendritic cells and TLR signaling

Dendritic cells that are present in peripheral tissues and the circulation, are not in a mature state. Freshly isolated Langerhans' cells lack sufficient expression of MHC molecules and potent costimulatory molecules CD80 and CD86, but express abundant receptors for antigen capture and phagocytosis, including TLRs, Fcγ and Fcε receptors, and c-type lectin receptors such as DEC205/CD205 and Langerin. Therefore, immature dendritic cells possess high potency for antigen capture. Different dendritic cell subsets express different sets of TLRs. Myeloid dendritic cells express a variety of cell-surface TLRs and can

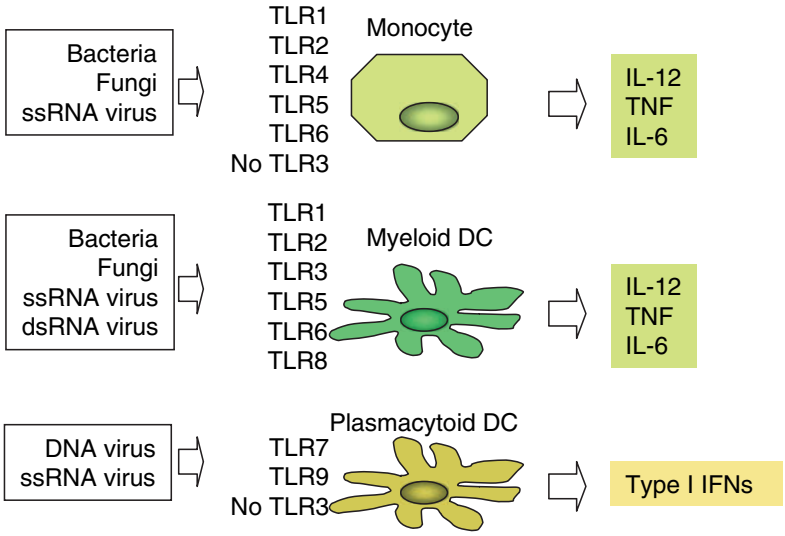

Fig. 6. Dendritic cell subsets and cytokine secretion via Toll-like receptor (TLR) signaling. Human peripheral blood monocytes, myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs) express distinct sets of TLRs. Monocytes and mDCs induce inflammatory cytokines such as interleukin (IL)-12, tumour necrosis factor (TNF) and IL-6, whereas pDCs induce type I interferons (IFNs).

recognize bacterial, fungal and viral pathogens, and secrete the inflammatory cytokines interleukin-12, TNF and interleukin-6. In contrast, plasmacytoid dendritic cells express intracellular TLR7 and TLR9, which respond to viral components and secrete type I IFNs (α, β, ω). Thus, different TLR ligands induce activation of different dendritic cell subsets and secrete distinct cytokines (Fig. 6). In other cases, the same TLR ligand on each dendritic cell subset induces different cytokines. For example, stimulation of human plasmacytoid dendritic cells with TLR7 ligand induces the secretion of IFN-α, whereas the same stimulation of myeloid dendritic cells induces interleukin-12 (11). Similar results were obtained for TLR9 ligand stimulation in mice (38). This indicates that the combinations between TLR signaling and dendritic cell subsets determine which cytokines are secreted from dendritic cells. These cytokines greatly affect sequential innate and adaptive immune responses. However, once immature dendritic cells are exposed to microbial pathogens and receive TLR signaling, they quickly induce costimulatory molecules, such as CD40, CD80 and CD86, alter homing receptors, and migrate via lymphatic vessels to regional lymph nodes where they present antigens to naïve T cells. This migration is mediated by TLR-induced down-regulation of tissue homing chemokine receptors (CCR2 and CCR5) and up-regulation of a lymph node-homing chemokine receptor, CCR7 (39). TLR-mediated signaling may function as the switch that transforms the dendritic cells from phagocytic to potent antigen-presenting cells. The cytokines secreted via TLR signals may greatly affect the sequential adaptive T-cell responses mediated by dendritic cells.

Adaptive immunity

Costimulation controls T-cell immunity and tolerance

Antigen-captured dendritic cells migrate to the regional lymph nodes via lymphatic vessels, reach the T-cell areas, and present antigens to naïve and memory T cells. An efficient antigen presentation by dendritic cells requires two signals. The first signal is antigen-specific and provided by the interaction of the MHC–peptide complex with the T-cell receptor. The second signal is provided by the binding of costimulatory molecules on dendritic cells to their receptors on T cells (Fig. 7). The activation states of T cells and dendritic cells modulate the requirements of the strength and the balance of the two signals.

Costimulatory molecules can be divided into costimulators and co-inhibitors, which promote or suppress T-cell receptor-mediated responses, respectively. Most costimulatory molecules are members of the CD28-B7 immunoglobulin and TNF–TNF receptor superfamilies (Figs 7 and 8). The CD28-CD80/CD86 costimulatory pathways are the most extensively characterized, and are essential for priming naïve T cells. CD80 (B7-2) and CD86 (B7-1) are induced on antigen-presenting cells after antigenic stimulation, and CD28 is constitutively and stably expressed on all states of T cells. CD28 costimulation enhances interleukin-2 production and interleukin-2 receptor expression, induces clonal expansion of antigen-specific T cells and differentiation of effector T cells, and elicits T-cell effector functions, such as cytokine production and cytotoxicity (Fig. 7). The presence of positive costimulatory molecules prevents the induction of tolerance and develops optimal T-cell immunity. A CD28 homolog, cytotoxic T lymphocyte antigen-4 (CTLA-4), which shares its two ligands CD80 and CD86 with CD28, induces negative signals to antigen-stimulated T cells. CTLA-4-mediated negative costimulation limits the initial T-cell responses and attenuates or terminates ongoing T-cell responses. Negative costimulatory pathways may play an essential role in the induction of tolerance and maintenance of homeostasis. Thus, positive and negative costimulatory molecules are crucial for the control of T-cell immunity and tolerance (Fig. 7).

Additional novel costimulatory pathways, such as programmed death-1 (PD-1):B7-H1/B7-DC, inducible

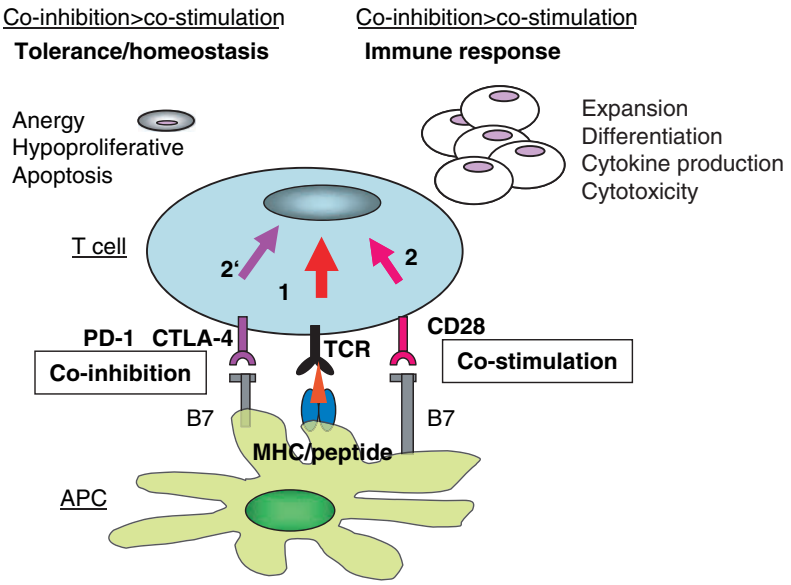

Fig. 7. Two-signal model of T-cell activation. Signal one is derived from the T-cell receptor (TCR) after being triggered by the peptide/major histocompatibility complex (MHC) on antigen-presenting cells (APCs), whereas signal two is mediated by interaction between co-stimulatory receptors and their ligands. Costimulatory receptors can be divided into costimulators and co-inhibitors. The overall balance between costimulatory and co-inhibitory signals determines the outcome of the T-cell response after antigen stimulation. CTLA-4, cytotoxic T lymphocyte antigen-4.

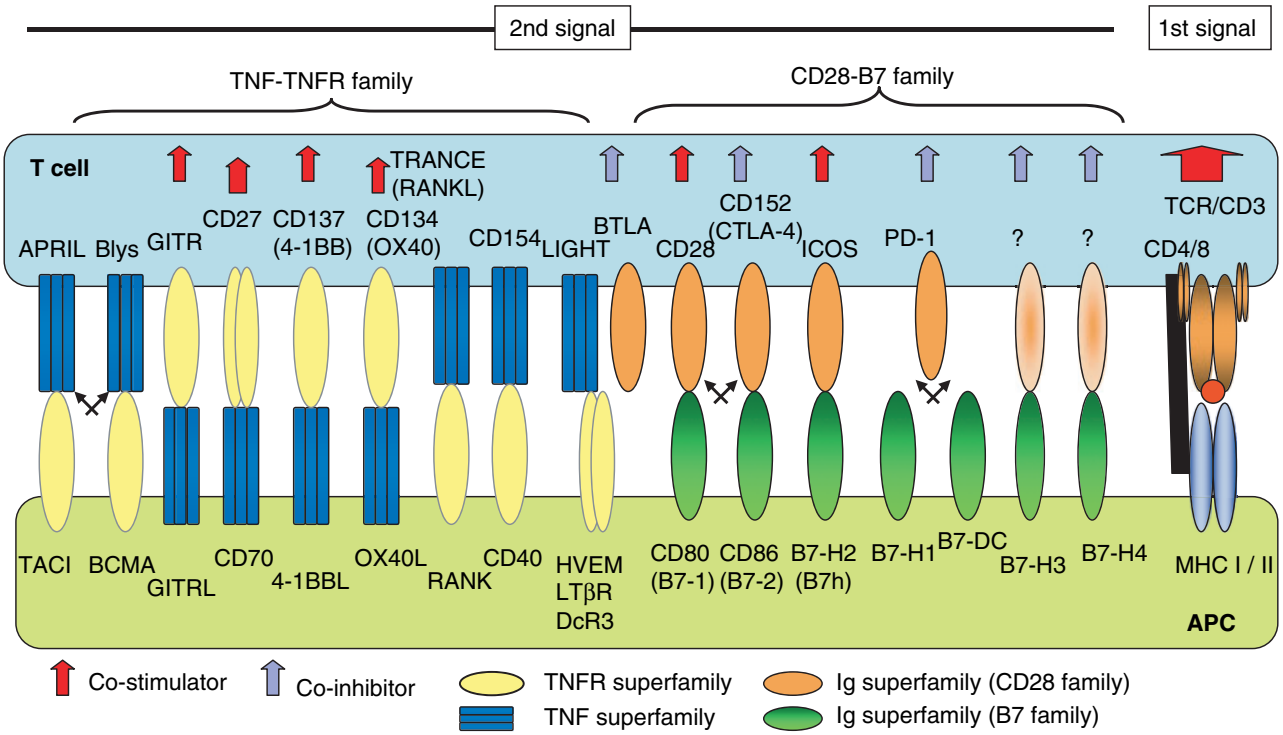

Fig. 8. Costimulatory molecules on T cells and their ligands. Two major families of costimulatory molecules are the immunoglobulin- and tumour necrosis factor superfamilies. CD28, inducible costimulator (ICOS), CD137(4-1BB) and CD134 (OX40) induce potent costimulatory signals for T-cell activation, whereas cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1) induce negative signals for T-cell inactivation or anergy.

costimulator (ICOS)-B7 h, B- and T-lymphocyte attenuator (BTLA), B7-H3 and B7-H4 have been identified (Fig. 8) (40). It has been suggested that PD-1, BTLA and as yet undefined B7-H3- and B7-H4 receptors may function as negative co-inhibitory molecules. PD-1 and ICOS are induced on T cells after activation, and B7 ligands are inducible on nonlymphoid tissues at sites of inflammation (40,41). In particular, the PD-1:B7-H1 pathway seems to be critically involved in peripheral tolerance at sites of inflammation (40,41). B7-H1 is frequently induced on nonlymphoid tissue cells, such as epithelial and endothelial cells. A pro-inflammatory cytokine, IFN-γ, is a potent inducer for B7-H1 expression (40,42) PD-1:B7-H1 interactions may be important for controlling antiviral $CD8^+$ T-cell responses, and PD-1-deficient mice with adenovirus infection exhibit increased proliferation of effector T cells in the liver and enhanced clearance of the virus (43). The enhanced $CD8^+$ T-cell expansion implies that the PD-1:B7-H1 pathway regulates antiviral immunity. Thus, while the CD28-B7 pathway is essential for priming naïve T cells, these new pathways may regulate effector T-cell responses at sites of inflammation (Fig. 9).

Dendritic cells control T-cell responses

Myeloid dendritic cells and T-cell responses – Antigen-captured migrating dendritic cells in the regional lymph nodes express an array of costimulatory molecules, in addition to cytokines. Whether naïve T cells differentiate into either Th1 or Th2 is greatly dependent upon the strength of T-cell-receptor-mediated signals (i.e. the avidity between the peptides and the T cell receptor) and the types of costimulatory molecules expressed, and on the cytokines secreted by the myeloid dendritic cells (Fig. 9) (10,44). The TLR1, 2, 5 and 6-induced cytokine, interleukin-12, is particularly involved in the induction of Th1 cells (Fig. 6). Immunization of mice with adjuvants containing various TLR ligands, including lipopolysaccharide, CpG DNA and complete Freund's adjuvant, facilitates Th1 cell induction. Th1 responses in MyD88-deficient mice are severely impaired and they generate, instead, a Th2 response. These results suggest that TLR signaling induced by bacteria promotes the induction of Th1 cells via the action of myeloid dendritic cells.

Plasmacytoid dendritic cells and T-cell responses – Plasmacytoid dendritic cells produce high levels of type I IFNs in response to TLR7- and TLR9-mediated signaling, which is induced by viral components (Fig. 6) (45). Although type I IFNs are also produced by all nucleated cells, including myeloid dendritic cells, the production of type I IFNs by plasmacytoid dendritic cells upon virus stimulation is rapid and huge. Plasmacytoid dendritic cells have a limited ability to produce interleukin-12, whereas myeloid dendritic cells have a high capacity for interleukin-12 production (46). Plasmacytoid

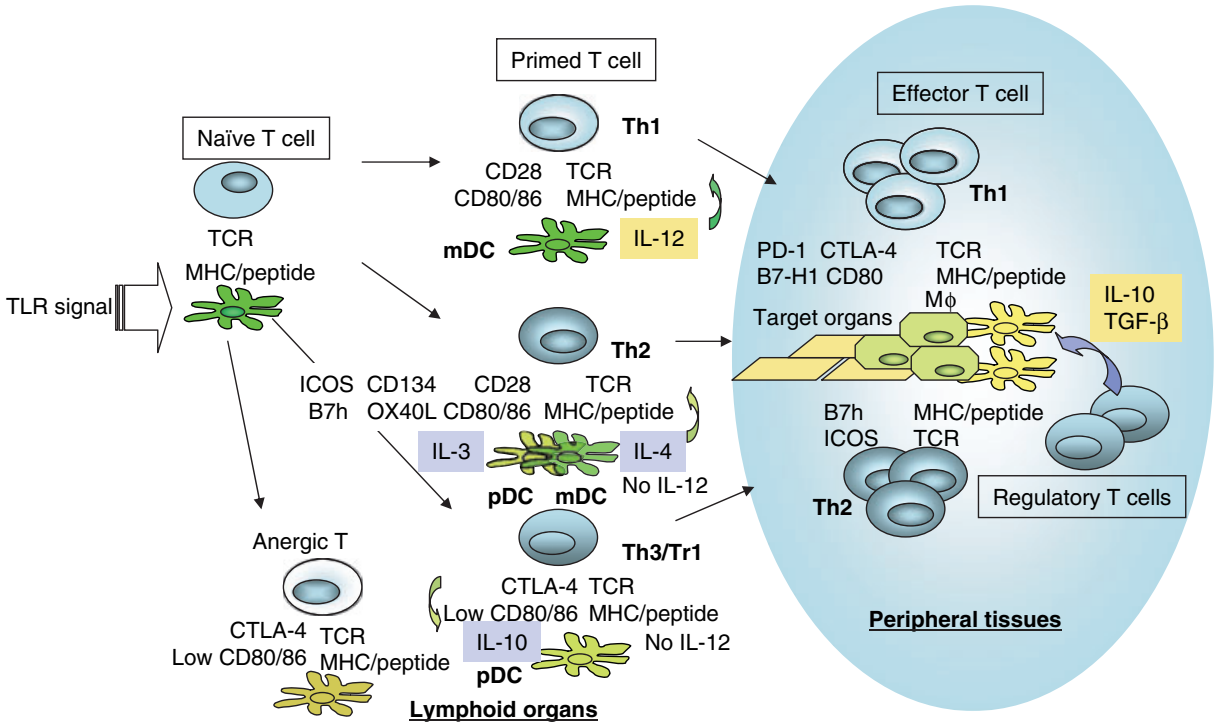

Fig. 9. Dendritic cells and T-cell responses. Cytokines and costimulatory molecules expressed on distinct subsets of dendritic cells (DCs) control the generation of effector and regulatory T cells in the lymphoid organs. The expanded various T cells migrate to the peripheral tissues and elicit their effector function. Expression of cytokines and co-stimulatory molecules in both T cells and dendritic cells further modulate local responses against target organs. See details in the text. CTLA-4, cytotoxic T lymphocyte antigen-4; ICOS, inducible costimulator; IL, interleukin; MHC, major histocompatibility complex; PD-1, programmed death-1; TCR, T-cell receptor; TGF-β, transforming growth factor-β; Th, T helper; TLR, Toll-like receptor.

dendritic cells express low levels of MHC class II and costimulatory molecules CD80 and CD86, and are unable to stimulate antigen-specific T-cell proliferation (Fig. 9). However, plasmacytoid dendritic cells acquire the capacity to differentiate into mature dendritic cells, which efficiently induce T-cell immunity. Upon viral infection, plasmacytoid dendritic cells differentiate into mature dendritic cells, mediated by autocrine IFN-α and TNF-α, which prime naïve $CD4^+$ T cells to produce IFN-γ and interleukin-10 (47). Upon parasite infection, plasmacytoid dendritic cells differentiate into mature dendritic cells, mediated by paracrine interleukin-3 release by mast cells, eosinophils and basophils, which prime naïve $CD4^+$ T cells to produce the Th2 cytokines interleukin-4, -5, -10 and -13 (48). In this review, we have not discussed mouse plasmacytoid dendritic cells. It should be noted that mouse and human plasmacytoid dendritic cells have some significant differences in surface phenotype, expression of TLRs and induction of T-cell responses.

Dendritic cells inducing T-cell tolerance – Dendritic cells are not only responsible for priming T cells, they are also responsible for inducing tolerance. Immature dendritic cells in the peripheral tissues retain a capacity for endocytosis. Under steady-state conditions, the targeting of dendritic cells by antigen-capture receptors with a low dose of antigens leads to deletion of the corresponding T cells and maintains peripheral tolerance (49). Endocytosis alone does not induce dendritic cell maturation, and dendritic cells migrate to the regional lymph nodes in the absence of infection as part of their life cycle. Immature dendritic cells that present self-antigens express low levels of MHC and costimulatory molecules, and induce anergy in autoreactive T cells (50). Thus, under steady-state conditions, immature dendritic cells function as tolerogenic antigen-presenting cells for self-antigens.

Interleukin-10 and TGF-β are well known as immunosuppressive cytokines and are secreted from various types of cells. In addition, specialized T cells, T regulatory type 1 (Tr1) and Th3 cells, which secrete high amounts of interleukin-10 and TGF-β, respectively, have been characterized (51). The immunosuppressive properties of Tr1 and Th3 cells can probably be explained by the ability of cytokines to inhibit antigen-presenting cell function (Table 3). Interleukin-10-treated dendritic cells reduce the production of inflammatory cytokines, including interleukin-1β, interleukin-6, TNF-α and interleukin-12, and inhibit the induction of antigen-specific Th1 cells (52,53). Such dendritic cells may induce peripheral tolerance by regulating effector T-cell responses. Although these tolerogenic signals are not fully understood, one potential candidate is

indoleamine 2,3-dioxygenase (IDO). IDO is a tryptophan-catabolizing enzyme expressed on macrophages and dendritic cells, and regulates T-cell proliferation and function (54). In immature dendritic cells, which express low amounts of B7 antigens (CD80 and CD86), prominent binding of CTLA-4 to B7s may induce direct signaling and trigger IDO-mediated T-cell regulation (55,56).

Naturally arising regulatory T cells

Properties of natural regulatory T cells – Another mechanism of peripheral tolerance is achieved by special subsets of regulatory T cells. Regulatory T cells are divided into two groups. One consists of the $CD4^+$ $CD25^+$ naturally arising regulatory T cells, and the other includes Tr1 and Th3 cells, which are induced regulatory T cells (Table 3) (15,57,58). Natural regulatory T cells constitutively express high levels of CD25, CTLA-4 and the glucocorticoid-inducible tumor necrosis factor receptor (GITR). As these molecules are also induced on conventional $CD4^+$ $CD25^-$ T cells after activation, they are not specific markers of regulatory T cells. The immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome in humans (59) and the scurfy mutant mice (60) show multi-organ autoimmune diseases, allergy and inflammatory bowel disease, which are similar to the manifestations seen in mice lacking natural regulatory T cells (61). These abnormalities are caused by mutations in the Foxp3 gene (62). Foxp3 is predominantly expressed on $CD25^+$ regulatory T cells, and the gene transfer of Foxp3 converts $CD25^-$ naïve T cells into regulatory T cells, which possess phenotypes and function similar to those of natural regulatory T cells (18). It seems that Foxp3 is definitively required for the development and suppressive function of regulatory T cells (17).

Natural regulatory T cells are able to produce high amounts of interleukin-10, but it is unlikely that the interleukin-10 secreted from regulatory T cells is directly involved in immunosuppressive function. However, interleukin-10 may generate surrounding dendritic cells that are involved in the maintenance and expansion of regulatory T cells (63,64). Low, but constitutive, expression of B7 on immature dendritic cells plays an important role in the maintenance and function of $CD4^+$ $CD25^+$ regulatory T cells in the context of CTLA-4 binding (65,66). Thus, surrounding antigen-presenting cells do not directly contribute to regulatory function, but may be actively involved in the maintenance and expansion of regulatory T cells.

Regulatory T cells and infection – It has been well established that Tr1 and Th3 cells regulate host responses against various infections (67). Recent reports suggest that natural regulatory T cells also regulate T-cell responses against pathogens. The T-cell receptor repertoire of natural regulatory T cells is as broad and diverse as that of conventional $CD4^+$ $CD25^-$ T cells. Therefore, natural regulatory T cells recognize nonself, foreign antigens as well as self-antigens. The consequences, following the participation of natural regulatory T cells in responses to infection, are variable. The outcome of an infection is determined by the overall equilibrium between effector and regulatory mechanisms. In some cases, natural regulatory T cells control excessive immune responses and maintain homeostasis or protective immunity. In other cases, natural regulatory T cells inhibit effector immune responses against pathogens and cause chronic infection or disease re-activation. In such a case, microbes can stay alive in the host, and are transmitted. The functions of regulatory T cells are modulated by several factors arising between host and pathogen, such as the phase of infection, dose of pathogens and immunological status of the host.

For example, the transfer of $CD4^+$ T-cell populations, lacking regulatory T cells, into mice with severe-combined immunodeficiency induces inflammatory bowel disease. In the absence of regulatory T cells, commensal bacteria in the gut trigger harmful access inflammation and cause massive gut inflammation. In such a case, natural regulatory T cells may play an important role as a critical regulator for gastrointestinal homeostasis (68). Similarly, in the *P. carinii* infection model, the absence of regulatory T cells leads to lethal pneumonia in immunodeficient mice, while transfer of regulatory T cells prevents the disease (69). Thus, in immunocompromised hosts, regulatory T cells act protectively against infection.

Natural regulatory T cells actively suppress pathogenic T-cell responses. It has been demonstrated that regulatory T cells inhibit memory $CD4^+$ T-cell responses to *Helicobacter pylori* (70) and inhibit $CD8^+$ T-cell responses

Table 3. A comparison of natural and adaptive regulatory T cells

Feature	Natural Treg cells	Adaptive T reg cells
Site of induction	Thymus	Periphery
Phenotype	$CD25^{high}$ $GITR^{high}$ $CTLA\text{-}4^{high}$ Foxp3+	Variable
Costimulation (CD28 CD80/86)	Required (for expansion)	No
IL-2	Strongly required (for expansion)	Required
Specificity	Self-antigens (foreign antigens)	Tissue antigens Foreign antigens
Mechanism of effector cell suppression	Required T-cell to T-cell contact APC Cytokine independent	Required T-cell to T-cell contact Cytokine dependent
Cytokine production	IL-10, TGF-β	IL-10 (Tr1), TGF-β (Th3)

APC, antigen-presenting cell; CTLA-4, cytotoxic T lymphocyte antigen-4; GITR, glucocorticoid-inducible tumor necrosis factor receptor; IL, interleukin; TGF-β, transforming growth factor-β: Th3, T helper 3 cell; Tr1, T regulatory type 1 cell.

to hepatitis C virus (71) and hepatitis B virus (72). Depletion of T regulatory T cells enhances protective immunity against invading microbes, leading to their eradication from the host. In addition, natural regulatory T cells express TLR4, -5, -7 and -8, and ligand stimulation induces the proliferation and activation of regulatory T cells (73). Therefore, pathogens may alter the balance for their survival by enhancing regulatory T cells and reducing effector cells. Natural regulatory T cells play an important role in controlling the magnitude and nature of antimicrobial immune responses, and in the maintenance of concomitant immunity, in addition to maintaining immunological self-tolerance.

Conclusion

The immune system is well organized by a multitude of closely co-ordinated mechanisms. These include a multiple receptor and ligand complex for pathogen recognition, multifariously changing dendritic cells that induce immunity or tolerance, an array of costimulatory molecules and cytokines that enhance or regulate the function of various immune cells, and several subsets of effector and regulatory T cells that have pathogenic or protective immune functions. All these mechanisms contribute to the infectious process and, although they are not yet fully understood, recent studies have provided important information for estimating the pathology and healing properties of periodontal diseases and for developing possible novel therapies.

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