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Danger signals: a time and space continuum

Mojca Škoberne, Anne-Sophie Beignon and Nina Bhardwaj

NYU School of Medicine, MSB 507, 550 First Avenue, New York, NY 10016, USA

Sudden uncontrolled necrosis and the controlled, physiological process of apoptosis are considered to be distinct forms of cell death. Necrotic cell death is associated with the release of danger signals, the consequent maturation of professional antigen-presenting cells (dendritic cells) and the priming of immune responses, whereas apoptotic cell death is associated with the induction of tolerance. Recently, uric acid in necrotic cell lysates, in addition to other substances, has been identified as a danger signal for dendritic cells. We discuss the significance and principle of action of these molecules in alerting the immune system to dying cells and propose that, in sterile injury, they have a primary initiating role, whereas in the presence of pathogens, initiation is caused by pathogen-associated molecules.

Dendritic cells (DCs) are bone-marrow-derived professional antigen-presenting cells (APCs), which have a pivotal role in initiating immune responses. To prime and activate T cells efficiently, DCs must undergo a complex process of maturation. During maturation, DCs from peripheral tissues upregulate a variety of molecules, including major histocompatibility complex (MHC) and co-stimulatory molecules; they migrate to lymph nodes, secrete pro-inflammatory and immunostimulatory cytokines and, once the necessary signals have been received, they become 'licensed' to stimulate naïve T cells to differentiate into effector cells. DCs do not undergo maturation unless they receive an appropriate stimulus. In the steady state, constant exposure to, and uptake of, apoptotic cells leads to the active suppression of DC stimulatory capacity and to the induction of T-cell tolerance to self-antigens. By contrast, danger signals, such as microbial molecules, components of necrotic cells or tissue matrices and products of activated leukocytes, signal DCs to mature and activate immunity [1].

Recently, Shi *et al*. identified uric acid as a novel immunostimulatory signal within cell lysates [2]. Uric acid activates DCs following relocation from the inside to the outside of the cell, as do other endogenously derived ligands, such as the heat shock proteins (HSPs). In sterile injury (as a result of ischemia, tumor cell death, sterile trauma, transplantation or chemotherapy), such endogenous molecules are, presumably, the only activators of DCs. However, because endogenous activators (for example, uric acid) are also present during infection [3], we must consider how they interact with microbially derived DC activators; specifically, which signals initiate DC activation and how are the ensuing responses shaped and amplified? We propose that DC-activating signals are hierarchically organized by the nature of the pathological process (microbial infection versus sterile injury) based on the sequence (in time and space) in which the signals are received.

Uric acid: a novel endogenous danger signal

In 1994, Matzinger proposed a new concept, describing how endogenous factors that are released from tissues undergoing chaos (destruction and loss of architecture and integrity) would alert the immune system through the direct activation of DCs without the necessity of exposure to foreign substances [4]. This was a significant departure from previous theories (Box 1) [5–8]. Several groups subsequently showed that necrotic cell lysates were a source of endogenous factors that induced the activation of mouse and human DCs [9–12], and that endogenous HSPs were one of the major danger signals within these lysates [13,14]. Shi *et al.* identified another component of cell lysates that meets the criteria for an endogenous danger

Box 1. Milestones in understanding immune activation

- A few models have been proposed and improved upon to describe immune recognition and activation. The self–nonself (SNS) model was first proposed by MacFarlane Burnet in 1959 [5]. It does not involve antigen-presenting cells (APCs) and is based on clonal selection and deletion of self-reactive effector B lymphocytes early in life and the activation of the remaining cells upon an encounter with their specific ligands. Later, it became clear that B cells could become self-reactive by hypermutation. To be compatible with this discovery, a role played by helper cells (T cells) was added to Burnet's model by Bretscher and Cohn, in 1970 [6]: the recognition of an antigen (signal 1) in the absence of T helper (Th) cells (signal 2) would lead to deletion of the antigen-specific B cell. In 1975, the SNS model was modified further, by Lafferty and Cunningham, with the hypothesis that antigen-specific Th cells needed to be activated by a third partner (the APC) to survive [7].
- In his 1989 model, Janeway gave APCs a central role in initiating immune responses against infectious non-self antigens (called the INS model) [8]. Upon capture of infectious non-self antigens in the periphery, pathogens or pathogen-associated molecular patterns would activate APCs to initiate a maturation process, including migration to the lymph nodes, where mature APCs would stimulate antigen-specific T cells.
- In 1994, Matzinger, in her 'danger model', expanded the above concept to include self molecules, stating that 'stressed self' could initiate an immune response without the necessity for non-self components as a maturation stimulus [4].

Corresponding author: Nina Bhardwaj (bhardn02@med.nyu.edu).

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signal. Two fractions (5 kDa and 40–100 kDa) isolated from the cytosol of necrotic cells, when delivered individually *in vivo* with particulate antigen, were shown to prime antigen-specific $CD8^+$ T cells in mice [2]. The low molecular weight compound was identified as uric acid, which exists in the extracellular environment predominantly in the form of monosodium urate (MSU). MSU treatment of DCs resulted in maturation *in vitro* and induced substantial immune responses when co-injected with antigens *in vivo*. Whether MSU is the only formulation of uric acid that is effective both *in vitro* and *in vivo* remains to be determined [2,15]. Uric acid is normally present in non-stressed tissue as a peroxisomal-degradation product of purines. Significantly, Shi *et al.* showed that, in the absence of protein synthesis, high-stress situations (such as UV irradiation or heat shock) elevate levels of uric acid in cells (probably as a result of RNA and DNA degradation) [2]. They proposed that subsequent massive cell lysis results in the release of cellular antigens as well as locally elevated MSU concentrations, which alert DCs (and other cells, such as monocytes).

Although the adjuvant effect of the uric acid that is released from lysed cells could be advantageous (for example, in tumor cell death), it could potentially incite autoimmune responses to released self-antigens, especially those to which the body is not tolerant. MSU has been recognized as an etiological agent of inflammatory gout, a condition in which elevated uric acid levels are associated with the onset of arthritis and the development of tophi (deposits of MSU). It would be interesting to determine whether other crystals, such as calcium pyrophosphate dihydrate crystals, involved in the arthropathy pseudogout also activate DCs. Whether MSU participates in the onset of true autoimmune disease is unclear, but it might amplify autoimmune responses in conditions where there is defective clearance of apoptotic cells (e.g. systemic lupus erythematosus, SLE) and consequential secondary necrosis. MSU has previously been shown to trigger inflammatory responses from monocytes. Conversely, in macrophages, exposure to MSU actually inhibits the activation that is induced by zymosan (a yeast component) [16]. The possibility of non-inflammatory removal of MSU by tissue macrophages might explain why gout-like disorders are not more common.

The stimulatory capability of MSU has not been shown for human DCs, although the fact that mice, but not humans, have the enzyme uricase (which degrades uric acid), makes the possibility of local uric acid accumulation after massive cell lysis even more likely. Uric acid levels increase markedly following stress in fibroblast and lymphoma cell lines [2], but whether this occurs in primary cells has yet to be established. However, its potential use as a clinical adjuvant might be limited by the fact that intradermal, subcutaneous or oral administration of MSU resulted not only in local inflammation, but also in acute gouty arthritis in humans [17].

Interestingly, MSU is the first primary endogenous danger signal that does not have a microbial counterpart. Because the structure of uric acid resembles that of molecules known to activate DCs through toll-like receptors (TLRs) 7 and 8, it will be interesting to identify the receptors through which MSU initiates DC maturation. Characterization of the MSU receptor would also exclude the possibility that its immunostimulatory effects are achieved indirectly through crystal-induced membrane perturbation or osmotic cell stress, which would release endogenous activators, such as HSPs [18].

Another important question is how MSU, as an endogenous danger signal, interacts with signals from microbial pathogens [19]. We propose that, upon infection with a pathogen, the primary signal will be a microbial component (e.g. a TLR agonist). Because infection is often accompanied by subsequent cell death by necrosis, the release of endogenous danger signals, including MSU, would have an augmenting effect. Interestingly, infection is associated with an increased production of uric acid, as a result of the elevated activity of the purine-degradation enzyme xanthine oxidoreductase [3]. The activity of this enzyme is greater in infected tissue than it is in ischemic tissues (sterile injury) [3,20]. This raises the question as to whether the endogenous signals would have a more pivotal role during infection. However, Christen *et al.* noted that increases in local uric acid levels were observed after a delay of more than 20 hours following the onset of infection [3]. Furthermore, substantial numbers of cells must be lysed to generate immunostimulatory levels of MSU [2], whereas only picograms of endotoxin, often present soon after bacterial exposure, are needed to activate the immune system. Therefore, we consider that MSU and other endogenous stimulators probably amplify the response to infection. Hence, the immune system becomes alerted to danger via several pathways, with MSU providing a fail-safe or complementary mechanism.

Are HSPs a second danger signal in necrotic cell lysates?

Shi and colleagues mentioned, but did not describe, a second substance (of 40–100 kDa) that had similar adjuvant activity to MSU [2]. This substance remains unidentified, but HSPs are suitable candidates. There are ten HSP families, each consisting of 1–5 closely related members [21], and HSPs are found in a variety of cells and organisms (including human pathogens) and are either constitutively present or induced under stressful conditions (e.g. heat shock). Among their many functions, HSPs assist in chaperoning non-covalently bound peptide antigens from the endosome or cytosol to MHC molecules [22]. Several HSPs (gp96, HSP60, HSP70 and HSP90) are associated with the induction of immune responses in mouse and human cells, through the direct activation of monocytes and DCs and simultaneous delivery of bound antigens. The importance of several family members, especially those with high or low molecular weights, remains to be determined. HSPs bind to CD91 and Lox-1 on APCs, which internalize HSP–antigen complexes and activate T cells through cross-presentation [23]. Additional molecules on DCs that bind to HSPs include TLR4, CD40, CD36 and TLR2, most of which have been proposed to transduce stimulatory signals [21].

Matzinger's danger hypothesis brought attention to HSPs as triggers of the immune system [4] and subsequent studies have supported this. HSPs carrying tumor-specific antigens can, for example, induce effective anti-tumor

immunity in mice [21]. Furthermore, co-delivery of Hsp70 and antigen *in vivo* induces the development of autoimmune disease in a transgenic-mouse model [24]. The idea that relocation of HSPs is crucial was supported by the fact that cell-surface expression of gp96 triggers systemic autoimmune disease [25]. It will be interesting to determine the relationship between MSU and HSPs in activating DCs, considering the additional ability of HSPs to deliver antigens.

Other relocation-based danger signals

In addition to MSU and HSPs, other substances that are normally confined within cells and released following cell damage and necrosis might function as danger signals. For example, tissue injury and infection can release DNA from dying cells; genomic DNA (double stranded and methylated) exerts immunostimulatory effects on macrophages and DCs [26]. In B cells and plasmacytoid DCs (pDCs), it appears that non-CpG-containing oligodeoxynucleotides (ODNs), as well as ODNs with methylated CpG motifs, have length-dependant adjuvant capacities [27,28]. However, endogenous immunostimulatory DNA sequences are less potent and less frequent compared with unmethylated and CpG-containing microbial DNA. Similarly, it has been suggested that host chromatin, when engaged in immune complexes with immunoglobulin G2a, functions as a warning mechanism by stimulating rheumatoid-factor-producing B cells through TLR9 and B-cell receptor [29].

A chromatin-associated protein, high mobility group box−1 protein (HMGB-1), is released following primary necrosis and is associated with an inflammatory response by monocytes [30]. However, in apoptotic cells, even when they are undergoing secondary necrosis, HMGB-1 remains bound to chromatin and thus prevents induction of the inflammatory response. Interestingly, HMGB-1 also functions as a late mediator of inflammation when secreted by activated monocytes and macrophages [30]. The relationship between HMGB-1 and DCs is not known, although a recent study showed that macrophages were activated by HMGB-1 through TLR2 and TLR4 [31].

Interestingly, it has been demonstrated that synthetic, short, single-stranded RNA (ssRNA), when artificially delivered inside DCs, leads to maturation and cytokine production [tumor necrosis factor (TNF)-α and interferon (IFN)-α] through TLR7 and/or TLR8 [32,33]. However, host ssRNA is probably degraded rapidly by extracellular RNases and the physiological relevance of such an endogenous signal remains to be clarified. Nevertheless, longer sequences of mRNA released from necrotic cells were suggested to form double-stranded structures that could stimulate DCs through TLR3 [34].

Guanine derivatives might also signal to DCs. They are found at sites of inflammation and infection, and activate NF-κB, which is associated with the maturation of APCs, including DCs [35]. These naturally occurring compounds are probably the result of secondary oxidative damage to nucleic acids, including host DNA and RNA released from necrotic cells, as well as nucleic acid that is derived from invading pathogens [35,36]. The receptors involved are unknown, but the structurally related synthetic molecules loxoribine and the imidazoquinolines, which activate innate immunity, signal through TLR7 and TLR8.

Endogenously released ATP can also stimulate immune responses. Usually, nucleotides are present at high concentrations in the cytoplasm (5–10 mM), whereas their concentration is low extracellularly (nanomolar), except when they passively leak from damaged cells or are exocytosed by activated cells. They can then be recognized by DCs that express certain P2 purinergic receptors [37]. ATP attracts DCs and induces them to mature and stimulate T cells. However, no consensus on the phenotype of the T cells induced has been reached [38,39]. Whether other nucleotides that are involved in energy metabolism (including ADP, UTP and UDP) behave similarly to ATP, and whether nucleosides, such as adenosine or sugar metabolites (e.g. uridine 5-diphosphoglucose, which is highly expressed in tumor cells), are also immunostimulatory remains controversial (Table 1) [40–43]. Nucleotides, nucleosides and guanine derivatives are unstable molecules and, therefore, we suggest that they might act only transiently in the local environment.

It is not surprising that the immune system can be alerted by various endogenously derived factors. However, further studies will be necessary to define and confirm their precise roles in DC activation and antigen presentation, and to identify the receptors involved. Furthermore, because certain studies did not rigidly exclude endotoxin and other microbial products from their preparations, we must be wary of some of the data. Finally, we do not know whether these signals function synergistically or in a hierarchical fashion, although we speculate that in sterile tissue injury, HSPs and, possibly, MSU will be among the first, and probably the most potent stimulators of DC activation [2,21].

Disruption of tissue architecture and activation of the immune system

A less appreciated feature of necrotic cell death, of any cause, is that it is often accompanied by the disruption of tissue architecture, which can yield a distinct set of signals that activate the immune system (reviewed in [44]). In some conditions, these signals might either materialize as a direct result of physical damage or be induced by factors released from stressed cells. For example, during tissue necrosis, the rupture of blood vessels and inflammation causes the extravascular relocation of fibrinogen, which has been shown to activate macrophages through TLR4 [44]. A rapid influx of blood cells, including platelets, that express the CD40 ligand (CD40L) might lead to the activation of tissue DCs through the ligation of the co-stimulatory molecule, CD40 [45]. Furthermore, components of the extracellular matrix (ECM) can initiate warning signals. Soluble fragments of heparan-sulfate proteoglycans, shed from cell surfaces and basement membranes as the result of cell injury, inflammation or tumor-cell migration, activate DCs to mature via a TLR4-dependent pathway [44]. Similarly, oligosaccharides of hyaluronan, released following enzymatic breakdown, activate murine and human DCs through TLR4 [44]. Finally, the neosynthesis of molecules after tissue injury can be interpreted as a danger signal; for example, the

Table 1. Proposed primary signals that stimulate DC maturation[a]

Signal	Receptor	Refs
Endogenous signals		
Relocation/degradation on cellular level		
MSU	?	[2]
HSP70, HSP60, HSP90, Gp96	CD91, TLR4, TLR4/TLR2, LOX-1	[21]
Self DNA	TLR9?	[26–29,58]
Chromatin binding HMGB-1 protein	RAGE, TLR2 and TLR4	[30,31]
ssRNA	TLR3, TLR7 and TLR8	[32–34]
Oxidized self guanine?	TLR7 and TLR8	[35,36]
Nucleotides (ATP)	P2 purinergic receptors (P2Y11?)	[37–40]
Nucleosides (adenosine)	Adenosine receptor A1, A2a and A3	[42,63,64]
Sugar metabolites (uridine 5-diphosphoglucose)	P2 purinergic receptor P2Y14	[43]
Granulocyte elastases	?	[65]
Relocation/destruction on tissue level		
Influx of platelets with CD40L	CD40	[45]
Fibrinogen	TLR4	[44]
Heparan sulfate	TLR4	[44]
Oligosaccharides of hyaluronan (but not the ECM polysaccharides of hyaluronan)	TLR4	[44]
Cellular fibronectin containing an extra domain A	TLR4	[44]
Pathogen derived signals (examples)		
Pathogen derived TLR ligands		
Proteoglycan	TLR2	[46]
Measles virus HA	TLR2	[46]
Zymosan (yeast)	TLR2	[46]
ds RNA (virus), synthetic poly I:C and ssRNA (probably through secondary structure)	TLR3	[34]
Some LPS (*Escherichia coli* but not *Porphyromonas gingivalis*)	TLR4	[46]
Some microbial HSPs	TLR4	[46]
F protein from RSV (but not F protein from Sendai virus)	TLR4	[46]
Flagellin	TLR5	[46]
Viral ssRNA (HIV, influenza virus)	TLR7 and TLR8	[32,33]
Oxidized microbial guanine and cytosine	TLR7 and TLR8	[46]
Unmethylated CpG-containing DNA from bacteria and some viruses (HSV-1 and HSV-2) and parasites	TLR9	[66]
Indirect DC stimulatory signals related to pathogens		
β-defensins	TLR4	[54]
Type I IFNs	IFNα/β receptor	[56]

[a]Abbreviations: ATP, adenosine triphosphate; CD40L, ligand of CD40; ds, double stranded; ECM, extracellular matrix; F protein, fusion protein; HA, hemagglutinin; HMGB-1, high mobility group box-1 protein; HSP, heat shock protein; HSV, herpes simplex virus; IFN, interferon; LPS, lipopolysaccharide; MSU, monosodium urate; RAGE, receptor for advanced glycosylation end productsRSV, respiratory syncytial virus; ss, single stranded; TLR, toll like receptor.

cellular fibronectin splice-variant, containing an extra domain A, activates macrophages through TLR4 [44].

Exogenous danger signals: pathogen-related activators of dendritic cells

During infection, pathogen-associated molecular patterns (PAMPs) can induce DC maturation. PAMPs are recognized by pattern-recognition receptors (PRRs), which include the TLRs, among others. PRRs can be intracellular, cell-surface expressed or secreted. In addition to the innate recognition of molecules, PRRs can enhance the uptake and phagocytosis of microbes, activate complement and induce signaling pathways. Several TLR ligands of pathogenic origin have been identified, many of which activate DCs and differentially polarize T cells (Table 1) [46]. TLRs might simultaneously trigger antigen uptake, but it is not clear whether they do so directly or indirectly [47,48]. Alternatively, members of other PRR families, such as the C-type lectins, that recognize microbes might mediate their internalization but do not signal full DC maturation, and can even negatively influence the DC maturation that is induced through other receptors, including TLRs [49].

The only TLR that is solely associated with agonists of microbial origin, and not endogenous signals, is TLR5 (which binds bacterial flagellin). It is worth noting that endogenous TLR agonists might trigger a different response than microbial agonists when binding to the same receptor. For example, the low TLR9 response to endogenous agonists might be quantitative, because CpG stimulatory motifs are more frequent in bacterial DNA, some viral DNA [50] and protozoan genomic DNA than they are in host DNA. Alternatively, there could be differences in affinity and structure or a differential involvement of co-receptors [51]. Endogenous substances and microbes might not only share the same TLRs, but activation of a TLR with a microbial agonist could also induce the production of a TLR agonist of endogenous origin. For example, CpG-containing DNA induces the release of peptides bound to the inducible form of Hsp70 from murine macrophages, which, in turn, mediates cross priming by neighboring DCs [52]. Additionally, endogenous ligands can aid TLR agonists in signaling, as shown recently for mindin (a member of the ECM proteins) and lipopolysaccharide in macrophages [53].

We are rapidly learning that microbes possess an extraordinary array of DC-activating factors, which can, in some cases, be mimicked by host cellular constituents. The time scale involved indicates that DC-activating components of microbial origin predominate early in the local environment. Endosomal localization of TLR7, TLR8 and TLR9 ensures the detection of microbes as soon as they enter the cells. These receptors recognize microbial nucleic acids, ssRNA (RNA viruses) and CpG-containing DNA (e.g. bacteria or DNA viruses), respectively. Later, during the course of microbial replication, molecules such as double-stranded RNA or mRNA are produced and sensed by DCs through TLR3. Finally, even those DC-activating microbial compounds that are only released following the death of the microbes probably accumulate and initiate DC activation before sufficient tissue damage is achieved to provide high enough concentrations of endogenous ligands.

Stimulatory signals produced by infected cells

Microbial invasion activates DCs by triggering the secretion of molecules from infected cells. One example is the β2-defensins, which are secreted from the mucosa and skin in response to the infection of epithelial cells and which stimulate the maturation of DCs through TLR4 [54]. The second example is type I interferons. All DCs, but especially pDCs (Box 2), secrete large amounts of IFNα upon viral infection, stimulated either through TLR-dependent or -independent pathways [55]. IFNα was recently shown to be involved in, and sufficient for, the licensing of DCs for T-cell priming [56]. Additionally, together with TNFα and, possibly, other cytokines, IFNα has been associated with the induction of bystander maturation of uninfected myeloid DCs (mDCs) [57]. This mechanism is important for the initiation of T-cell responses during viral infection. Type I interferons might contribute to T-cell priming through the autocrine activation of pDCs and/or paracrine activation of bystander mDCs and pDCs. Interestingly, in contrast to viral infections, type I interferons might play a pathogenic role in the autoimmune disease SLE, where high levels of circulating IFNα have been associated with active disease. How this contributes to disease pathology is uncertain, but a recent study has shown that immune complexes containing DNA activate pDCs through FcR and TLR9 [58]. Furthermore, sera from patients with SLE can induce DC differentiation from monocytes through a type I interferon-dependent pathway [59]. Thus, type I interferons might be induced by pDC activation and, in turn, amplify the pathogenic response through mDC activation.

Box 2. Dendritic cell subsets

There are two main dendritic cell (DC) subsets that have been identified in humans: the $CD11c^+$ DCs (mDCs), which include Langerhans cells, dermal, interstitial and blood mDCs, and the $CD11c^-CD123^+$ and $CD4^+$ plasmacytoid DCs (pDCs), which are found in the blood and after inflammation, in the periphery.

Amplification of responses mediated by danger signals

How do we distinguish the primary signals that initiate DC maturation from the numerous events that subsequently amplify and sustain the stimulatory pressure on DCs? Primary signals would emanate from dying cells (uric acid and HSPs) or microbes (by PAMPs). The ensuing inflammatory response, mediated by proinflammatory cytokines such as interleukin (IL)-1β, IL-6, TNFα and the type I interferons, as well as certain enzymes that induce further cell death, would sustain the effects of the initial signals.

The *in vivo* effects of secreted cytokines would lead to the paracrine activation of DCs, the production of chemokines and the recruitment of inflammatory cells and lymphocytes. Various other substances produced during stressful conditions can act as chemoattractants, including ATP, adenosine and β-defensins [60,61]. Recruited cells can then initiate new rounds of activation, such as the recruitment of mast cells, which release histamine and stimulate DCs [44,62]. Danger signals, therefore, initiate a complex continuum of signaling molecules and cascades that culminate in the induction of immunity (Figure 1).

How are responses that are initiated by danger signals turned off? Undoubtedly, multiple mechanisms are involved, including: (i) the induction of regulatory cells specific for both self and microbial antigens; (ii) Th2 cells that might modulate Th1 responses; (iii) natural-killer-cell-mediated removal of DCs; (iv) the loss of antigen-presenting DCs through antigen-specific mechanisms; and (v) the apoptosis of antigen-specific effectors.

Concluding remarks

A growing number of endogenous molecules share a common feature: their ability to activate DCs to become potent APCs. DC-activating signals are hierarchically organized by the nature of the pathological process (microbial infection versus sterile injury) and the sequence in which the signals are received. During infection, microbial factors will trigger DC maturation, with amplification of the response a result of the subsequent release of endogenous activators. In non-pathogenic situations, for example, ischemia or traumatic injury, endogenous activators are predominant. Because the human host has several mechanisms to avoid autoimmunity, in most cases sterile injury will be non-injurious in the long term.

The possibility of using certain activators to stimulate immunity is appealing for the development of antimicrobial vaccines and to modulate immune responses in cancers. Further studies of the relative potency and efficacy of these molecules, of their effects on cells other than DCs or macrophages, and the mechanisms controlling their activity will lead to a better understanding of their function. This will be especially important to retain tolerance to self-antigens in individuals prone to autoimmune disorders.

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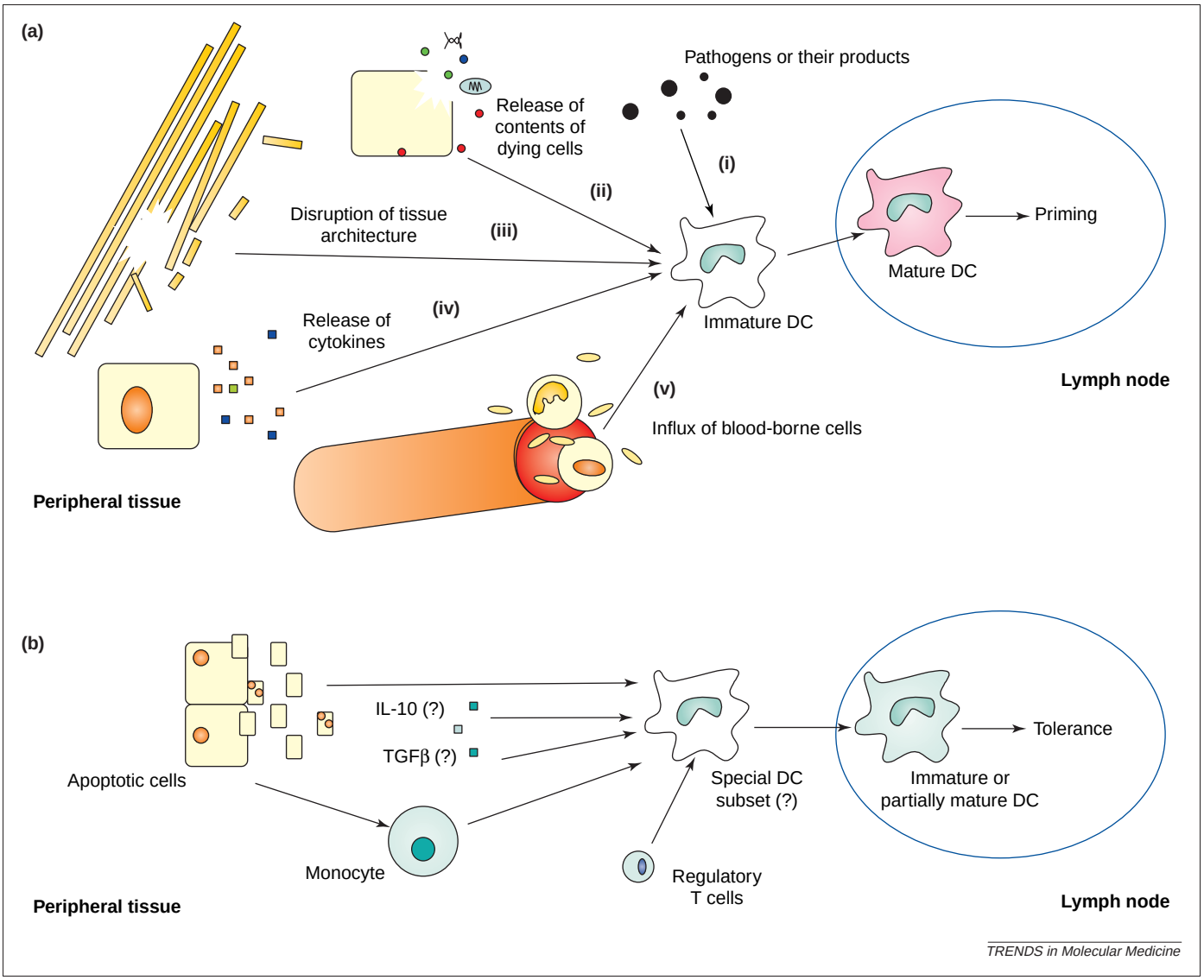

Figure 1. Understanding immune activation in the broad context of danger signals. **(a)** Potential danger signals that can activate dendritic cells (DCs) include: (i) the direct presence of pathogenic microbes or their products; (ii) the death of a stressed cell and the release of its contents upon microbial infection or sterile injury; (iii) immunostimulatory compounds, such as heparan sulfate and oligosaccharides of hyaluronan, released following the uncontrolled degradation of the extracellular matrix (ECM) in response to injury; (iv) soluble molecules produced upon injury, such as type I interferon (IFN) or tumor necrosis factor-α (TNF-α), which can amplify and sustain DC activation; and (v) injury, either by the direct rupture of vessels or by chemotaxis, causing an influx of blood-borne cells. **(b)** The potential consequences in the absence of danger signals. Here, controlled cell death by apoptosis not only fails to stimulate immune responses, but can also actively render antigen-presenting cells into an immunosuppressive state.

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