Ariel Savina
Sebastian Amigorena

Phagocytosis and antigen presentation in dendritic cells

Authors' address

Ariel Savina, Sebastian Amigorena
Institut Curie, INSERM U653, Immunité et
Cancer, Paris, France.

Correspondence to:
Sebastian Amigorena
Institut Curie
INSERM U653
Immunité et Cancer
26 rue d'Ulm
75248
Paris
Cedex 05, France
Tel.: 33 1 4234 6711
Fax: 33 1 4407 0785
E-mail: sebastian.amigorena@curie.fr

Summary: Like macrophages and neutrophils, dendritic cells (DCs) are considered professional phagocytes. Even if the three cell types phagocytose parasites, bacteria, cell debris, or even intact cells very efficiently, the functional outcomes of the phagocytic event are quite different. Macrophages and neutrophils scavenge and destroy phagocytosed particles, a critical step in innate immunity. DCs, in contrast, have developed means to 'preserve' useful information from the ingested particles that serve to initiate adaptive immune responses. Thus, both phagosomal degradation and acidification are much lower in DCs than in macrophages or neutrophils. Reduced degradation results in the conservation of antigenic peptides and in their increased presentation on major histocompatibility complex class I and II molecules. In this article, we review the mechanisms that control this delicate equilibrium between phagosomal degradation/cytotoxicity and antigen presentation in the different families of phagocytes.

Keywords: *dendritic cells, antigen presentation, phagosomal pH, phagosomal degradation, cross-presentation*

Introduction

Phagocytes represent a heterogeneous family of cells that includes neutrophils, macrophages, and dendritic cells (DCs). The first two cell types are critical effectors of innate immunity. They are both involved in the immediate clearance of pathogens through local inflammatory responses. DCs entered the exclusive club of phagocytes more recently. Unlike other phagocytes, DCs are not directly involved in immediate pathogen clearance. From this perspective, they cannot be considered 'conventional' effectors of innate immunity. Like macrophages, DCs are present in all peripheral tissues and accumulate at the sites of pathogen entry. DCs express a large array of phagocytic receptors and efficiently phagocytose pathogens. DCs also express a variety of Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs). Various PRRs are selectively expressed in particular DC subpopulations, where they initiate different developmental programs (often

referred to as 'maturation'). During maturation, DCs produce cytokines and chemokines. They also undergo a series of phenotypic and functional modifications, depending on the type of PRRs they encounter (1).

Contrary to other phagocytes, however, DCs are potent antigen-presenting cells, the only ones capable of activating resting T cells and of initiating primary and memory immune responses. After taking up pathogens, infected cells, or apoptosing cells, DCs process antigens derived from these particles into peptides and load these peptides on major histocompatibility complex (MHC) class I or MHC class II molecules. Although other phagocytes are also capable of presenting antigens on MHC class I and II molecules, they do so quite inefficiently and are not capable of initiating primary immune responses. Therefore, DCs take up pathogens in peripheral tissues, undergo particular maturation programs selectively in response to different pathogens, migrate to lymphoid organs, and present antigen to T lymphocytes to initiate antigen-specific immune responses. The type of immune response initiated by DCs ultimately depends on the type of maturation signal and thereby of pathogen or of tissue environment encountered. The specificity of pathogen recognition by the adaptive arms of the immune system depends on the way DCs present pathogen-derived antigens on their surface. Thus, DCs are specialized in linking innate and adaptive immune responses, rather than directly eliminating pathogens.

The primary function of most phagocytes is to destroy pathogens. Neutrophils rely primarily on their potent oxidative burst, which generates a variety of anti-microbial weapons including toxic oxygen radicals and chloride derivatives, as well as the proper environment for proteolytic enzymatic activity. Macrophages rely on their potent lysosomal proteolytic activity to eliminate pathogens. After engulfment, they rapidly acidify their phagosomes. The concomitant recruitment of abundant lysosomal proteases results in a highly degradative environment that kills pathogens efficiently.

Neutrophils and macrophages rely on different phagocytic strategies for pathogen killing. Their respective phagocytic pathways are organized accordingly, and precise transcriptional programs determine the expression of the molecular players involved. From this point of view and considering that DC phagocytic function responds to different purposes, as compared with neutrophils or macrophages, one could predict a quite different organization of the phagocytic pathway. This review summarizes some of the recent advances in the understanding of the DC phagocytic pathway, as compared with macrophages and neutrophils. We underline how DCs have developed strategies to adapt their phagocytic pathway to their main function, i.e. antigen processing for presentation to T lymphocytes.

Phagosomal degradation

'Proteolysis' is the molecular mechanism by which proteins are consecutively cleaved in shorter fragments by hydrolysis reactions until totally broken into their constituent amino acids. Although the hydrolysis of peptide bonds is thermodynamically favored, such hydrolysis reactions are extremely slow. Without additional help, the half-life for the hydrolysis of a typical peptide at neutral pH is estimated to be between 10 and 1000 years (2). In this context, the digestion of a microbe or an apoptotic cell, in these conditions inside of phagosomes, could take a time totally incongruous for the biological purposes. Indeed, peptide unions are hydrolyzed within milliseconds in some biochemical processes. Proteases facilitate and promote very fast peptide bond cleavage, acting as highly efficient catalysts (2). Although it is most likely that it initially developed for nutrition purposes, phagocytic proteolysis also serves other functions in mammals. Macrophages and neutrophils use proteolysis to clear damaged, apoptotic, and senescent cells, as well as foreign microorganisms or other potentially dangerous particles. After phagocytosis, the ingested particles encounter a hostile environment that will ultimately result, in most cases, in their total degradation. Different phagocytes, however, use different strategies to achieve this fatal program.

Neutrophils

Neutrophils show a potent arsenal of anti-microbial compounds that include toxic reactive oxidant species (ROS) and molecules such as proteases and bioactive peptides. The neutrophil response to microbes is often referred to as 'oxidative burst' (3). During the oxidative burst, 'specific' granules containing the cytochrome b558 [the transmembrane component of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2 (NOX2), composed by two membrane subunits gp91*phox* and p22*phox*], fuse with the forming phagosomes (4). This fusion event is accompanied by the activation of the oxidase complex, through the recruitment of the cytosolic subunits (p40*phox*, p47*phox*, and p67*phox*) to the central transmembrane core (5, 6). Other cytosolic proteins, the low-molecular-weight guanosine triphosphatases (GTPases) Rac1/2, are required for the optimal activation of NOX2 (7). Once fully activated at the phagosomal membrane, NOX2 transfers electrons from the cytosol to the phagosomal lumen, where they are used to originate superoxide through a series of molecular reactions that consume oxygen (3). Superoxides

constitute the first wave of toxic molecules formed in neutrophil phagosomes. Myeloperoxidase (MPO), another enzyme released into the phagosome by the fusion of azurophil granules, catalyzes the transformation of superoxide into a variety of toxic molecules for microorganisms, such as hypochlorous acid, chlorines, chloramines, hydroxyl radicals, and single oxygen (8).

Killing of microorganisms, however, is not only because of the direct toxicity of NOX2 products. The proteolytic activity of neutrophil phagosomes is very high. During the oxidative burst, proteases and anti-microbial peptides (including defensins) and bactericidal/permeability-increasing proteins stocked in azurophil and gelatinase granules are rapidly recruited to phagosomes (9, 10). The activation of the phagosome's proteolytic content is dependent on the electrogenic activity of NOX2, which provokes an important influx of K^+ from the cytosol into phagosomal lumen (11) and generates a hypertonic intralumenal environment (12). The increase of the phagosomal K^+ concentration disaggregates the anionic proteoglycan matrix in which proteases are entrapped in azurophil granules, thus facilitating their release into the phagosome and consequently the activation of the recruited proteases. These proteases directly alter microorganism integrity and also process or activate other anti-microbial proteins or proteases in the phagosome lumen, thus enhancing further microbe damage (11, 13).

The physiological relevance of proteolysis in pathogen killing in neutrophil phagosomes has been shown using different protease-deficient mice. In these mice, pathogens such as *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Candida albicans* show much more virulence than in normal mice because of the inefficient bacteria clearing by neutrophils (11, 14, 15). The crucial importance of NOX2 in pathogen killing has been largely evidenced in patients bearing mutations in some of the subunits of the oxidase complex, causing partial or total inactivation. These patients, whose neutrophils fail to eliminate pathogens, suffer from chronic granulomatous disease, a disease characterized by severe widespread infections affecting principally childhood and often resulting in early death (16, 17).

Macrophages

Macrophages, in cooperation with neutrophils, fight against microbes providing an innate, antigen non-specific, first line of defense. Macrophages also participate actively in the removal of dead cells. After recognition, macrophages engulf their 'prey' into phagosomes, which mature to digestive organelles (phagolysosomes) by interchanging membrane and soluble material with different intracellular compartments. Macrophages lack azurophil, specific granules, and secretory vesicles

found in neutrophils (18, 19). Consequently, phagosome maturation in macrophages differs in quality and kinetics from that in neutrophils. Even if macrophages bear intracellular vesicles containing the cytochrome b558 as well as the cytosolic components of NADPH oxidase, the potency of the oxidative burst is much lower than in neutrophils. Surprisingly from this perspective, the microbicidal activity of macrophages is extremely high.

Membrane dynamics during phagosome maturation in macrophages have been studied for many years. Membrane exchange between phagosomes and endocytic compartments occur very early during phagocytosis, including during engulfment, and proceeds for several hours after the uptake (20, 21). Endocytic compartments fuse dynamically with phagosomes, following a process referred to as 'kiss and run' (22). Early phagosomes actively exchange material with early endosomes, before active membrane exchange with late endosomes and then with lysosomes. Thus, phagosomes progressively acquire the lysosomal degradative enzymes and the acidification machinery present in endocytic compartments. Among enzymes delivered to macrophage phagosomes from endosomes and lysosomes are glycosidases (such as galactosidase, mannosidase, glucuronidase, and hexosaminidase) and proteases (including cathepsins B, L, H, and S, furin and dipeptidyl peptidase II). Some of these proteases, such as cathepsins H and S, reach phagosomes early (from 20 min to 2 h after engulfment), while other cathepsins and hydrolases only show complete activity after several hours (20). Early after phagocytosis, phagosomes also exchange membrane components with the endoplasmic reticulum (ER) (23, 24), although the function of ER compounds in phagosomes is still debated (25). Phagosome maturation into phagolysomes is precisely orchestrated by small GTPases of the Rab family. Rab5 controls the early phases of phagosome maturation, while Rab7 determines later fusion events with late endosomes and lysosomes (26, 27). Phagosome maturation requires the retrieval (recycling) of certain membrane compounds, a process that involves Rab11 (28). The lipid composition of the phagosomal membrane is also critical for the interaction of phagosomes with other intracellular compartments and with the cytoskeleton (to allow phagosome intracellular movements) (29, 30).

Dendritic cells

DCs also have high phagocytic activity, both in peripheral tissues and in secondary lymphoid organs. Phagocytic DCs are referred to as immature. Upon encountering inflammatory signals or TLR ligands, immature DCs enter a developmental

program called maturation that ultimately results in the loss of the phagocytic capacity because of the changes in the activity of Rac and Cdc42 (31). Immature DCs express a large array of phagocytic receptors, including lectins, scavenger receptors, and pathogen receptors (32). Both peripheral tissues and secondary lymphoid organs contain various subpopulations of DCs. Selective expression of different phagocytic receptors on these DC subpopulations results in the selective uptake of different particles. For example, splenic $CD8^+$ DCs take up apoptotic bodies much more efficiently than other DCs in the spleen (33). $CD8^-$ DCs, in contrast, phagocytose *Leishmania* more efficiently than the $CD8^+$ subset (34).

Very little, however, is known about phagosome maturation in different DC subsets. Most of the studies were made in bone marrow-derived DCs (in mice) and monocyte-derived DCs (in humans). DC phagosomes bear various endopeptidases, exopeptidases, estearases, and reductases, such as lysosomal thiol reductase (35, 36). Nevertheless, the majority of the proteases in DCs belongs to the group of cysteine proteases, such as cathepsins S, B, H, and L; the aspartate proteases cathepsins D and E; and the asparagine endopeptidase (AEP) (37, 38). Although DCs express a variety of proteases, their potency for degradation is much lower than that of macrophages (39). The recruitment of lysosomal proteases to phagosomes is not efficient in mouse DCs (37). The low proteolytic activity of DC phagosomes is, at least in part, as a result of the reduced concentrations of lysosomal proteases (which are also low in endosomes and lysosomes). Delamarre *et al.* (39) have shown that DC lysosomes contain reduced levels of AEP and cathepsins S, D, L, and B, as compared with macrophages. Interestingly, DCs also express several members of the cystatin family of protease inhibitors that inhibit lysosomal protease activity by obstructing their active site (40, 41). Some of them are present in lysosomes and most likely contribute to restricting proteolytic activity in DC lysosomes and phagosomes. Nevertheless, the role of lysosomal proteases in DCs is not limited to antigen processing. AEP, for example, is required for the processing of cathepsins L, B, and H (42, 43). Cathepsin S and AEP as well as cystatin C control the processing of the Ii chain and, thereby, MHC class II intracellular transport and antigen presentation to $CD4^+$ T lymphocytes (44–46).

The studies summarized above suggest that phagocytic–endocytic proteolysis in DCs is aimed at degrading proteins 'partially' (processing) rather than 'totally', as is the case in macrophages and neutrophils. This is true for antigen processing, a process aimed to produce peptides of several to several tens of amino acids, and for the Ii chain, which undergoes controlled proteolysis including several discrete

intermediates (38, 47). To achieve partial or controlled proteolysis in their endocytic pathway, DCs have developed various specializations of their endosomal and phagosomal pathways, including a tight control of the pH.

pH regulation in phagosomes

Two critical rate-regulating factors for protease activity are the concentrations of the reactants and the pH of the fluid where the reaction occurs. Primarily, an acidic pH helps to disorganize and denature proteins, making them accessible to the action of the proteolytic enzymes, which themselves are unaffected by acid denaturation. The pH also determines the dissociation state of several chemical groups (acidic and basic) on proteins. In most cases, the different possible ionic forms are differentially susceptible to a particular reaction. The pH, thereby, determines the amount of the reactive species present that are sensitive to the attack by the enzyme. As a consequence, each enzyme present along the phagocytic and endocytic pathways shows a specific range of pH where its activity is optimal. Optimal pH values have been determined *in vitro* for several lysosomal enzymes using defined substrates. For example, the optimal pH for β-glucuronidase, α-manosidase, and α-galactosidase is around 4.5 and 5 for β-hexosaminidase (20). Cathepsins B and L, β-glucosidase, and AEP are most active at pH 5.5 (48), while the optimal pH for cathepsin B is around 6 (49). Other proteases exhibit their best activity at higher pH (20). Cathepsin H, furin, and cathepsin S show optimal activities at pHs 6.8, 7, and 7.5, respectively (49, 50). This variability in the optimal pH for the proteolytic activities of different proteases provides the system with an important versatility, suggesting that by regulating the phagosomal pH, the efficiency and specificity of proteolysis may be tuned. Indeed, the pH in phagosomes is regulated differently in neutrophils, macrophages, and DCs.
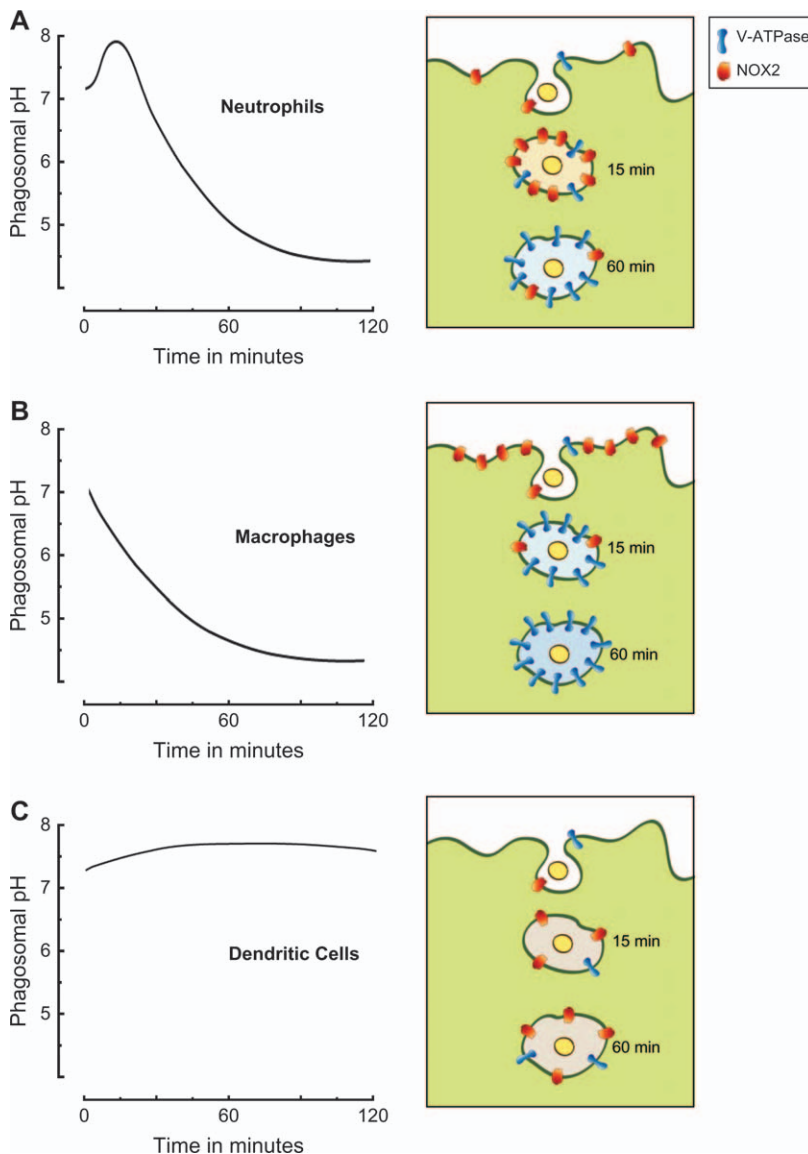
Neutrophils

In neutrophils, a 100-fold increase of oxygen consumption occurs following the activation of NOX2 during the respiratory burst (11, 51). The electrogenic activity of NOX2, a consequence of the movement of electrons across the phagosomal membrane, generates superoxide anions. This translocation of charges results in an important chemical gradient, which must be compensated inside phagosomes. The mechanisms proposed for charge compensation during the oxidative burst have important consequences on phagosomal pH. This compensation is, at least in part, achieved by the influx of protons pumped through the V-ATPase or other H^+ voltage-gated channels (52–54). Moreover, it has been suggested that gp91*phox* (one of

the two transmembrane subunits of NADPH oxidase) itself may function as an H^+ channel (55–57). An important consumption of protons, partially as a result of the dismutation of ROS into peroxides, causes an increase of the phagosomal pH, whereas the cytosol suffers a transient acidification because of the generation of H^+ as result of the reduction of NADPH by the oxidase (11, 58). Several studies have shown that phagosomal pH undergoes a biphasic behavior in neutrophils (*Fig. 1A*). Initially, phagosomes alkalinize, reaching values around 8 during the first 10–15 min after engulfment (11, 59, 60), which facilitates the release of proteolytic enzymes delivered by the fusion of different subset granules. The attenuation of the NOX2 activity over time, concomitantly with the continuous and increasing acquisition of V-ATPase or other proton channels, causes a rapid rise in the concentration of H^+ and a strong acidification of the phagosomal lumen (61) (*Fig. 1A*). This acidification favors the action of proteolytic enzymes to complete the killing of microbes. Supporting these views, neutrophils from patients with chronic granulomatous disease do not show the initial transient alkalinization of the phagosomal lumen (5).

Macrophages

Although macrophages can also trigger a respiratory burst, the scenario in terms of pH is significantly different to that described in neutrophils. First, the activity of NOX2 is lower than in neutrophils (62). Second, in macrophages, ROS production seems to take place mainly at the plasma membrane rather than at phagosomal membrane (19, 63). ROS production at plasma membrane in macrophages could contribute to tissue

Fig. 1. NADPH oxidase and V-ATPase replicate phagosomal pH in phagocytes. The regulation of phagosomal pH during phagocytosis in neutrophils, macrophages, and DCs is controlled through the recruitment to phagosomes of two multisubunit complexes, the NADPH oxidase (NOX2) and the V-ATPase. (A). In neutrophils, the massive recruitment of NOX2 to early phagosomes during the oxidative burst causes an important peak of proton consumption in phagosomes, resulting in a transient alkalinization (up to pH 8) of the phagosomal lumen in the first 15 min after engulfment. The subsequent recruitment of the V-ATPase to phagosomes concomitantly with the inactivation of NOX2 results in a rapid and important acidification, as the pH drops to 5–4.5 in the first 60 min. (B). In macrophages, the respiratory burst is triggered during engulfment; however, the majority of ROS production takes place at the plasma membrane instead of phagosomes, where NOX2 activity is much less important than in neutrophils. Additionally, a massive recruitment of the V-ATPase to phagosomes is observed very early during phagocytosis, resulting in a very strong acidification of the lumen. (C). In DCs, a discrete (compared with neutrophils) but sustained recruitment of NOX2 to phagosomes together with low levels of V-ATPase activity provoke a stabilization of the phagosomal pH above neutrality for several hours.

inflammation (64). The role of NOX2 in regulating the phagosomal pH is almost insignificant in macrophages. The main protagonist in pH control in macrophage phagosomes is the V-ATPase (65, 66). The kinetics of V-ATPase acquisition in macrophage phagosomes is extremely rapid. Early studies have shown that within minutes after phagocytosis of a bacteria, the pH in phagosomes decreases from 7.4 (similar to extracellular milieu) to 6.5, reaching values below 6 at 20 min after engulfment (Fig. 1B). Acidification proceeds at a decreasing rate of 0.2–0.4 units of pH/minute (65). Interestingly, in a recent study by Yates et al. (67), macrophages were shown to reduce the phagosomal acidification after activation. The pivotal role for the V-ATPase in the concentration of H^+ into phagosomes in macrophages was evidenced by pharmacological blocking of the V-ATPase. Bafilomycin A and concanamycin B, two inhibitors of the pump, neutralize the phagosomal pH (68, 69). Several microorganisms, such as *Mycobacterium tuberculosis*, survive in macrophage phagosomes by interfering with the insertion of the V-ATPase in phagosomes, thus avoiding phagosomal acidification (70). Interestingly, in none of the situations in which the V-ATPase is either absent from or inactive in the phagosomal membrane is alkalinization of the phagosomal compartment observed. These results suggest that macrophages, even if they express NOX2, are incompetent to use NOX2 activity for phagosomal alkalinization, as compared with neutrophils.

Dendritic cells

In DCs, the mechanisms of phagosomal acidification and degradation in DCs had not been analyzed until recently. Trombetta et al. (71) performed the first series of studies aimed to analyze endosomal and lysosomal acidification in this cell type. They showed that the V-ATPase cytosolic component (V1 sector) is inefficiently assembled to the membrane subunits on lysosomes in immature DCs. This incomplete assembly results in a partially inactive V-ATPase and poor lysosomal acidification *in vitro*. The V-ATPase, however, fully assembles in mature DCs, while efficient acidification is restored (71). It is most likely, although not yet documented, that the same mechanism limits acidification in immature DCs phagosomes. Indeed, we found that during at least 3 h after engulfment, no effective acidification was observed in DC phagosomes as the pH was maintained between 7 and 7.5 (72) (Fig. 1C). Our results, however, indicate that ineffective acidification is due not only to low V-ATPase activity but also to the presence of active and sustained alkalinization of the phagosomal lumen. Indeed, the V-ATPase inhibitor concanamycin B caused significant alkalinization of phagosomes in DCs, while it only produced

neutralization in macrophages. We concluded that DC phagosomes bear an active system of alkalinization, which is only evident upon inhibition of the H^+ income.

As described for phagosome alkalinization in neutrophils, we found that the NADPH oxidase plays a role in modulating the pH in DCs phagosomes. Very little is known about NADPH oxidase activity in DCs. One report from Elsen et al. (73) showed that DCs show an almost insignificant activity of NADPH oxidase compared with neutrophils (after stimulation by phorbol myristate acetate). They suggested that ROS generation in DCs is 'cryptic' because of the presence of endogenous inhibitors that prevent its activation and proposed that such inhibition would be relieved by proinflammatory cytokines. Other groups have also shown that low ROS production in DCs can be increased by TLR ligands (74) or when DCs interact with antigen-specific T cells (75). A role for NADPH oxidase in phagosomal function was also reported because NADPH oxidase activity was required to the efficient killing of intracellular *Escherichia coli* in human DCs (74).

Using bone marrow-derived DCs generated from mice lacking gp91*phox*, one of the oxidase subunits, we showed that the phagosomal alkalinization observed in normal DCs was lost. Phagosomal pH acidified over the time when NOX2 was absent, indicating that the oxidase influences significantly the regulation of the pH in DC phagosomes. Even in the absence of NOX2, however, acidification in DCs was not as effective as in macrophages, indicating that the pumping activity of the V-ATPase is limited, as proposed by Trombetta et al. (71). We also showed that gp91*phox* was recruited to DC phagosomes over time, generating an oxidative environment essential to maintain a sustained phagosomal pH between 7 and 7.5 during several hours. Taken together, these findings indicate that the phagosomal pH in DCs is controlled through an equilibrium of the activities of two multimolecular complexes present on the membrane of these compartments: the NADPH oxidase NOX2 and V-ATPase. Decreasing the activity of these complexes in DC phagosomes, either by specific inhibitors or genetically, results in strong changes in the phagosomal pH (between 1 and 2 pH units).

Therefore, DCs and neutrophils but not macrophages share the ability to alkalinize phagosomes through the activity of the NADPH oxidase. The kinetics and intensities of phagosome alkalinization in neutrophils and DCs are, however, very different. While in neutrophil phagosomes alkalinization reaches values higher than 8 within 15 min, the pH in DC phagosomes remains between 7 and 7.5 for several hours. These differences in pH regulation reflect differences in NOX2 activity and recruitment in the two cell types. In neutrophils, a massive

recruitment of NOX2 occurs through the fusion of specific granules with incoming phagosomes. Using the same protocol used for the subcellular isolation and purification of different granules in neutrophils (76), we attempted to purify the compartments bearing cytochrome b558 in DCs. We were unable to isolate a fraction corresponding to specific granules described in neutrophils (the main source of cytochrome b558 in this cell type) from DCs lysates. Additionally, MPO activity, which is a marker of azurophil granules, was not detected in any of the fractions purified (our unpublished results). Instead, we found that cytochrome b558 in DCs is stored in a population of vesicles that also bear lysosomal membrane glycoprotein 1 (Lamp1) and Lamp2 (two conventional markers of late endosomes and lysosomes) as well as Rab27a (a conventional marker of lysosome-related organelles) (77). Rab27a controls the recruitment of NOX2 to DC phagosomes. In Rab27a-deficient DCs, the acquisition of NADPH oxidase by phagosomes is delayed, resulting in a less oxidative and more acidic phagosomal lumen (the phagosomal pH is at least 1 unit less than normal cells) (77). One consequence of increased phagosomal acidification in the absence of NOX2 activity (both in gp91phox-deficient and Rab27a-deficient DCs) is an increase in protein degradation. As Rab27a is considered as a marker of lysosome-related organelles in many cell types (78), we proposed to call this novel population of NOX2-containing vesicles 'inhibitory lysosome-related organelles' because the recruitment of these vesicles to phagosomes continuously limits acidification and protein degradation (Fig. 2A,B).

The regulation of the pH and superoxide production in neutrophils, macrophages, and DCs follow distinct schemes. In neutrophils, the massive recruitment of NOX2 to phagosomes during the oxidative burst causes an important peak of proton consumption in phagosomes, resulting in a transient (around

15 min) but potent alkalinization (up to pH 8) of the phagosomal lumen (Fig. 1A). The subsequent recruitment of the V-ATPase to phagosomes, however, rapidly results in a strong acidification as the pH drops to 5–4.5 in the first 60 min (Fig. 1B). In macrophages, in spite of an effective respiratory burst, but less important than in neutrophils, no alkalinization of the phagosomal lumen was reported. This outcome is probably not exclusively as a result of a massive recruitment of the V-ATPase because only neutralization and no
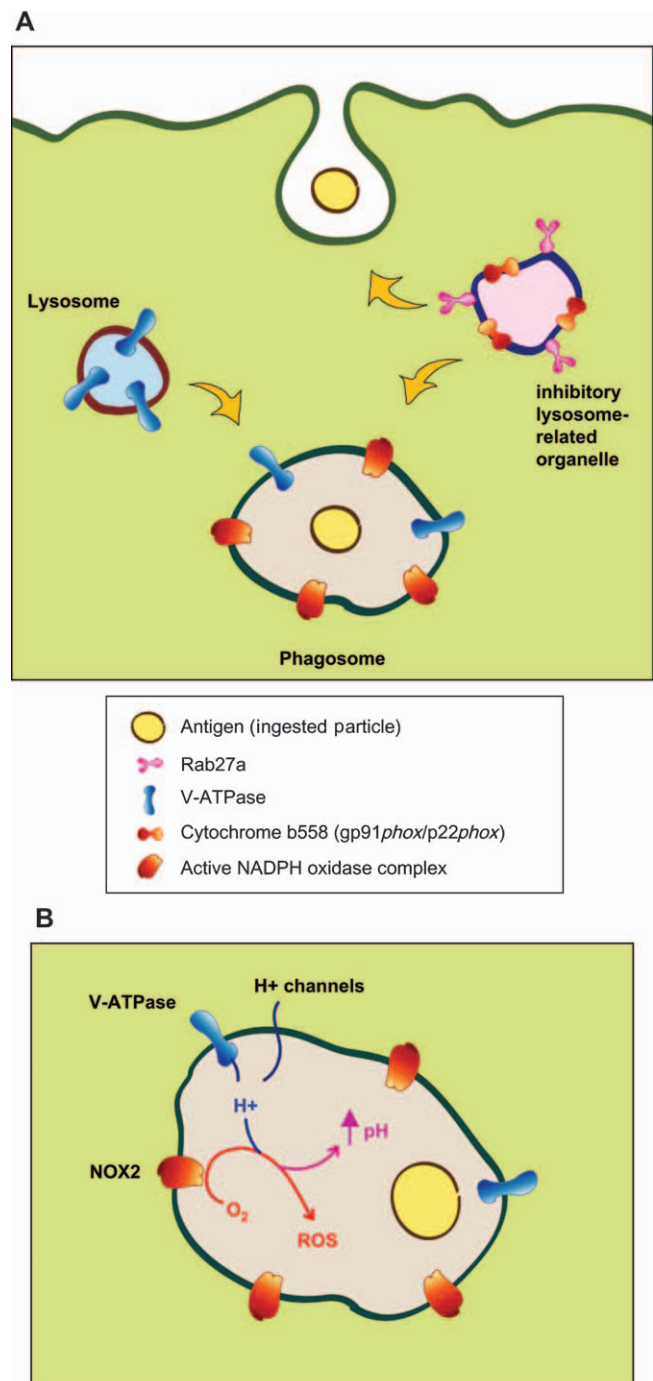
Fig. 2. NOX2 is efficiently recruited and assembled at phagosomal membranes during phagosome maturation in DCs. (A). Contrary to neutrophils, the transmembrane component of NOX2 (cytochrome b558) in DCs resides in a population of vesicles bearing both lysosomal markers (Lamp1/2) and the small GTPase Rab27a (and possibly V-ATPase), suggesting that these vesicles belong to the family of lysosome-related organelles. The acquisition of the cytochrome b558 by phagosomes is regulated by Rab27a. (B). The fusion of cytochrome b558/Rab27a-containing vesicles with phagosomes leads to the recruitment of the cytosolic subunits of NOX2, assembling the active complex. Upon activation, NOX2 consumes oxygen (O_2) and H^+ (pumped by V-ATPase and probably also contributed by other channels) to produce ROS in the phagosome lumen. Consequently, a rise of the pH is maintained for several hours. Because NOX2 limits acidification and degradation in DC phagosomes, we propose to call this population of cytochrome b558/Rab27a-containing compartments in DCs as 'inhibitory lysosome-related organelles'.

alkalinization is observed, even in the presence of a V-ATPase inhibitor. In macrophages, NOX2 is mainly assembled at the plasma membrane and not in phagosomes, most likely limiting the impact of ROS production on the phagosomal pH. In DCs, low levels of NOX2 and V-ATPase are simultaneously recruited to phagosomes. The simultaneous activity of the two membrane transporters at the phagosomal membrane maintains the pH above neutrality for several hours (*Fig. 1C*). As expected, sustained neutral pH affects phagosomal function in DCs, including proteolysis. What, then, are the consequences of this sustained neutral pH on antigen presentation?

Antigen presentation and phagocytosis in DCs

The initiation of adaptive immune responses requires CD4^{+} and CD8^{+} lymphocytes to recognize short peptides associated with MHC class II or class I molecules on the cell surface of DCs. Only a few peptides from any complex protein are effectively recognized by T cells. It is therefore critical that these 'immunogenic' peptides are not destroyed in phagosomes before association with MHC molecules. MHC class-II-restricted antigen presentation is mainly restricted to internalized proteins (47, 79). The processing of exogenous antigens occurs in the phagosomal/endosomal compartments as a result of the action of proteases that partially digest antigens along the internalization route. The same compartments contain MHC class II molecules. Relatively large antigen fragments (several tens of amino acids) are loaded on MHC class II molecules after degradation of the associated Ii chain. These large fragments are then further processed on the MHC class II molecules into 10–20 amino acid peptides. The peptide-loaded MHC class II molecules are translocated to the cell surface where they are recognized by CD4^{+} T cells. MHC class I presentation is classically restricted to antigens synthesized by the antigen-presenting cells (endogenous antigens). Defective ribosomal products are digested by the proteasome into small peptides (eight to nine amino acids) (80) that are translocated into the ER through ER transporters associated with antigen presentation (TAP1/2). Once in the ER, the peptides are loaded on MHC class I molecules with the help of the MHC class-I-loading complex and are transported to the cell surface.

There is, however, one major exception to this unifying scheme. In DCs, and under certain circumstances in other cell types, exogenous antigens can be presented on MHC class I molecules, a process called 'cross-presentation' (81, 82). Cross-presentation is required for the initiation of cytotoxic immune responses against bacteria, tumors, and certain viruses, as well as for the maintenance of tolerance to self-antigens. Phagocy-

tosis and macropinocytosis are the major routes for antigen uptake for MHC class I cross-presentation, but receptor-mediated endocytosis also results in efficient cross-presentation (1). After internalization, protein antigens are partially degraded into large fragments that are exported into the cytosol for processing by the proteasome, before translocation by TAP1/2 into the ER and loading on MHC class I molecules (83–85). The recent, although still debated, observation that phagosomes in macrophages and DCs recruit ER-resident proteins early after engulfment suggested a model for antigen export to the cytosol, involving the retro-translocation activity of sec61. Sec61 mediates both the translocation of newly synthesized leader-peptide-bearing proteins into the ER and the dislocation of improperly folded proteins from the ER into the cytosol for degradation by the proteasome. Like retro-translocation from the ER, retro-translocation from mixed ER–phagosome compartments requires p97 ATPases (86).

In the cytosol, exported antigens are degraded by the proteasome into eight to nine amino acid peptides. The site of peptide loading onto MHC class I molecules during cross-presentation is still unclear. Because cross-presentation is in most cases TAP dependent and because TAP was initially thought to be present exclusively in the ER, the peptides generated in the cytosol during cross-presentation were naturally assumed to be translocated into the ER for loading onto MHC class I molecules. The reported recruitment of TAP (along with other ER residents) to phagosomes (83) suggested that the peptides generated by the proteasome during cross-presentation could be retro-translocated back into the lumen of ER–phagosome-mix compartments rather than to the ER (*Fig. 3*). There is no direct experimental evidence for MHC class I loading of peptides in the ER or in phagosomes. It is therefore very difficult to estimate the respective contribution of ER versus phagosomal MHC class I loading during cross-presentation. In any case, it is clear that while antigens are in the lumen of phagosomes, they are exposed to degradation by lysosomal proteases. Destruction of potential MHC class-I-binding peptides (which are rather scarce in most proteins) can only be detrimental to the efficiency of cross-presentation. In contrast, proteins from dying cells or from microorganisms have to be dislocated and sometimes partially degraded to be exported into the cytosol. It is therefore most likely that antigen degradation is tightly regulated to avoid the potential destruction of CD8^{+} T-cell epitopes.

Likewise, if peptide loading during cross-presentation indeed occurs in phagosomes, once the proteasome-derived peptides are transported back from cytosol into phagosomal lumen, the phagosomal milieu has to be compatible with peptide loading on MHC class I molecules. Certainly, an acidic
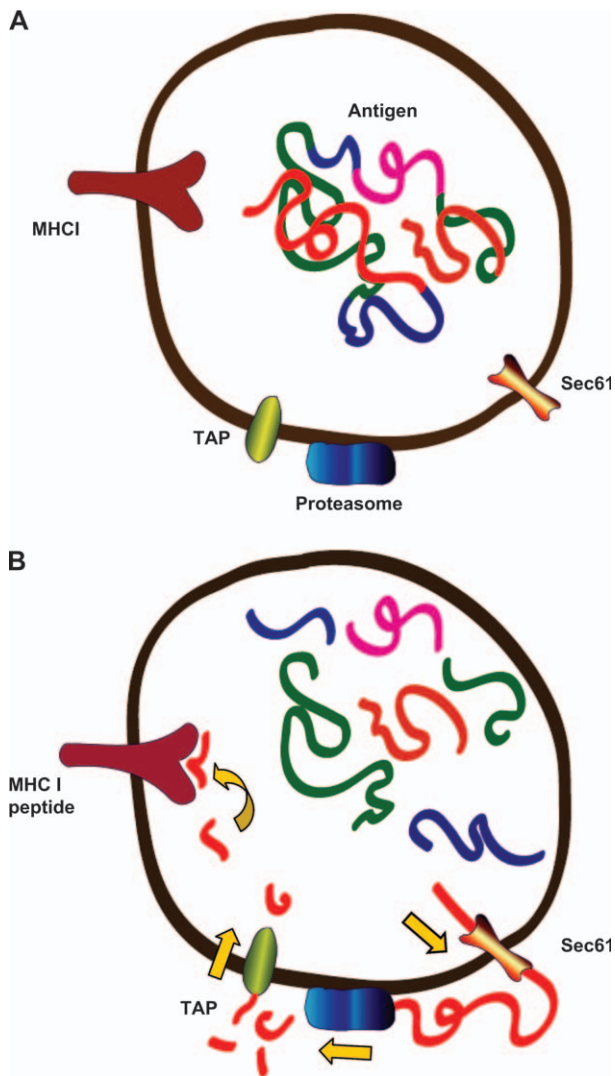
Fig. 3. Phagosomes have been proposed to behave as autonomous compartments for antigen cross-presentation in DC-phagosomes. Internalized antigens find an environment propitious for an initial degradative processing to generate the fragments that will be exported to the cytosol (probably through sec61). This initial enzyme attack must be tightly regulated to avoid destroying potentially important sequences for T-cell recognition. Once in the cytosol, exported peptide fragments are further digested by the proteasomes. The final products of this cytosolic proteasomal degradation are transported back into the phagosomes through TAP. The eight to nine amino acid peptides are then loaded onto MHC class I molecules and transported to the plasma membrane.

pH does not favor loading of peptides on MHC class I. From this point of view, the neutral pH found in DC phagosomes during the first few hours after phagocytosis and the low proteolytic activity should favor loading on MHC class I molecules in DCs, as compared with macrophages (87).

In DCs lacking or with reduced NOX2 activity (like in gp91$phox^{-/-}$ mice or Rab27a$^{-/-}$ mice), the pH in phagosomes is more acidic by 0.5–1.5 pH units (72, 77). In both mice, this acidification of the phagosomal lumen reduced the efficiency of the cross-presentation of two antigens, ovalbumin and HY, both *in vitro* and *in vivo*. The reasons for decreased cross-presentation in these mice are probably complex. Both the neutralization of the phagosomal pH (by NH_4Cl or ConB) and the partial inhibition of proteolysis (by a mix of protease inhibitors) restored cross-presentation in the mutant mice. These results suggest that decreased pH and enhanced degradation account, at least in part, for reduced cross-presentation in the absence of NOX2 activity in phagosomes. The effects of these drugs, however, are diverse. These results, therefore, do not exclude that other steps, such as transport to the cytosol or peptide loading in phagosomes, are not affected as well.

It is surprising, however, that a 1–1.5 pH unit difference in the phagosomal pH can decrease the overall efficiency of the cross-presentation process by over 60%. Why is cross-presentation reduced, rather than delayed? These observations are consistent with a model illustrated in *Fig. 3*. Proteins released from phagocytosed particles (latex beads in our case) are exposed to various proteases in the phagosomal lumen. Cross-presentation requires these proteins or large peptides derived from these proteins to be released into the cytosol. The kinetics of degradation of these proteins will determine their chance to be exported to the cytosol. If degradation is too efficient, then the class I epitopes may be destroyed and cross-presentation becomes ineffective. From this perspective, degradation and transport to the cytosol would 'compete': export from the phagosomal lumen rescues certain peptides from degradation, thus allowing cross-presentation. If proteins or large protein fragments are degraded too rapidly, they 'miss' their chance to be exported and there is no second chance, i.e. degradation destroys the potential class I epitopes. Supporting this notion, Accapezzato *et al.* (88) have shown that limiting antigen degradation improves human CD8$^+$ T-cell responses against soluble antigens both *in vitro* and *in vivo*. An additional level of complexity arises from the suggestion that antigen export to the cytosol is selective for 'early' phagosomes (as suggested by the likely role of ER-derived molecules in this process) (83–85). If such was the case, then it is essential that export to the cytosol occurs before the capacity of the phagosomes to export proteins to the cytosol is lost.

We have shown that the efficiency and the speed of transport to the cytosol are lower for high-molecular-weight molecules. Molecules below 50 kDa are transported to the cytosol more efficiently and quicker than 500-kDa molecules (86). Therefore, the partial proteolysis of large proteins should favor their transport to the cytosol and thereby cross-presentation. Which proteases, then, could perform this partial degradation during cross-presentation? The sustained high pH encountered in DC

phagosomes during the first hours after engulfment suggests that only certain proteases with optimal pH around or above 7 (such as cathepsin S) contribute importantly to cross-presentation. Proteases with optimal activity at lower pH would most likely be detrimental to cross-presentation if they were fully active (as they would be in neutrophil or macrophage acidic phagosomes). Even if the overall effect of high phagosomal pH reduces protein degradation, the activity of certain proteases (such as cathepsin S) is enhanced. The selective control of the proteolytic activity in DC phagosomes probably contributes to the efficiency of cross-presentation.

The processing of protein antigens for presentation on MHC class II should follow different rules. MHC class II molecules bind relatively large protein fragment (up to several tens of amino acids), suggesting that MHC class II molecules 'protect' the relevant epitopes from degradation. Delamarre et al. (39) showed recently that antigens resistant to degradation are more immunogenic, suggesting that they are better presented on MHC class II molecules. The same group showed that the proteolytic capacity of DCs is limited both by a low level of expression of lysosomal proteases and by low levels of V-ATPase activity in immature DCs (71). These results suggest that too much degradation is also detrimental for MHC class-II-restricted presentation. Here too, MHC class II molecules and lysosomal proteases would compete for proteins or large peptides. The outcome of this competition would determine presentation or degradation. Because MHC class II molecules can themselves protect peptides from degradation, the prediction is that presentation to CD4$^+$ T cells is not as sensitive to phagosomal pH or to the efficiency of degradation as cross-presentation to CD8$^+$ T cells. In other words, if large peptides are generated later during phagocytosis, when the phagosomal pH is already acidic, MHC class II may still bind to the peptides and protect them from degradation. At this point, it is most likely that phagosomes have lost their capacity for export to the cytosol.

This model for MHC class-I- versus MHC class-II-restricted processing in phagosomes allows for certain predictions. The first one is that processing for MHC class I and MHC class II presentation occurs, at least in part, in separate compartments. Early phagosomes, in which proteins are only partially degraded and that contain ER-resident proteins, would be competent for export to the cytosol (cross-presentation). Although the large peptides generated in early phagosomes could potentially bind MHC class II molecules (89–91), the nearly neutral pH encountered at this point and the low levels of H2-M in early phagosomes most likely does not favor loading on MHC class II molecules. Processing for MHC class-II-restricted presentation may occur in more mature phagosomes, in which the pH is lower

and that contain higher levels of H2-M. The second prediction relates to the origin or subcellular location of the antigens presented on MHC class I and MHC class II molecules. If transport to the cytosol is indeed more effective from early phagosomes (that contain ER-derived proteins), then proteins available for export at this stage of phagosome maturation should be better cross-presented. Secreted or external proteins from microorganisms, for example, should be available for transport to the cytosol in early phagosomes. Internal, non-secreted microbial proteins that required more extensive degradation of the microorganism to be released, in contrast, may not be available for export to the cytosol in time. The prediction would therefore be that external or secreted proteins should be better cross-presented than other proteins from the same microorganisms. Proteins requiring more extensive degradation of the microorganisms to be released should be better presented on MHC class II molecules.

From this perspective, the DCs' endocytic and phagocytic pathways seem to be extraordinarily well adapted to cross-present antigens to CD8$^+$ T cells efficiently. First, the recruitment of lysosomal proteases to phagosomes is much slower in DCs than in macrophages. Second, phagosomes have a remarkably prolonged early phase. The pH remains neutral for several hours in DCs, while it acidifies very rapidly (within 30 min) in macrophages (72). Finally, the presence of ER-derived proteins last much longer in DCs (up to 2 h) (83, 84), as compared with macrophages (less than 1 h) (23, our own unpublished results). Altogether, these observations suggest that by slowing down the degradation of ingested particles and by prolonging the early phases of phagosome maturation, DCs optimize the chances for antigens to be exported to the cytosol and thereby to be cross-presented. This would not hamper MHC class-II-restricted presentation, at least when DCs also receive a maturation signal. In this case, the increased activity of the V-ATPase would allow a progressive increase of acidification, a slow degradation of the microorganism, and efficient presentation to CD4$^+$ T lymphocytes.

The working model described above was drawn from results obtained with immature bone-marrow-derived DCs and resting bone-marrow-derived macrophages. It does not take into account the complex environment that surrounds both cell types in vivo or the existence of various subpopulations of cell types. It was shown recently, for example, that lipopolysaccharide (LPS)-activated macrophages acidify their phagosome less efficiently than resting macrophages, suggesting that they may cross-present antigens more efficiently (67). If, as proposed by various groups recently, certain DC subpopulations are more efficient at cross-presenting antigens than others, then phagosomal maturation and function should also be different among them.

Although they do so less efficiently than DCs, macrophages (and very rarely, neutrophils) are competent for both MHC class-I- and class-II-restricted presentation. This lower efficiency for antigen presentation, as compared with DCs, was observed *in vitro* and *in vivo* using inert particles or apoptotic bodies (33, 92–94). The situation with infectious agents is more complex, and in general, it is very difficult to compare the efficiency of antigen presentation between different cell types. Indeed, most microbes infect or are phagocytosed differentially by different phagocytes, making any conclusion about antigen presentation (rather than targeting) difficult. In addition, most pathogens interfere with the phagosomal function as a survival strategy, resulting in the inhibition of phagosome–lysosome fusion. This inhibition has different effects on the phagocytic pathway of macrophages or neutrophils, including the inhibition of protein degradation, of acidification, or of superoxide production. In doing so, microorganisms certainly also influence antigen presentation. The analysis of the effects of these microorganisms on phagosomal function in DCs is only starting to be considered as a major issue. It will certainly be very exciting, in the next few years, to uncover how the effects of microorganisms on the phagocytic pathway of DCs influences the intracellular pathways involved in antigen presentation and cross-presentation.

Signaling through TLRs is one aspect of microorganism sensing by DCs that has started to be analyzed recently. Several groups showed that in DCs, LPS initiates antigen presentation to $CD4^+$ T cells (95–97). The engagement of TLR4 in individual phagosomes was suggested to determine the presentation of antigens contained within that individual phagosome but not from other phagosomes in the same cells (97). The mechanisms underlying these effects of TLR ligands are starting to be analyzed. LPS-treated DCs acidify their lysosomes more efficiently because of a better assembly of the cytosolic subunits of the V-ATPase on lysosomes (71). The immediate effect of LPS on acidification and phagosome maturation in macrophages and DCs, however, remains a matter of debate. LPS was shown to accelerate the recruitment of lysosomal markers and lysosome-tropic dyes to phagosomes in DCs (98). However, it has also been suggested that TLR4 engagement has an inhibitory effect on phagosome–lysosome fusion in macrophages (99). More recently, Yates and Russell (69) showed that LPS does not affect the extent or kinetics of early acidification in the macrophage phagosome. Future studies should address the effects of TLR engagement in phagosomal functions in DCs and investigate in more detail how the quality of the cargo in phagosomes determines the type of immune response that will be triggered.

Conclusions

DCs play a critical role in the initiation of $CD8^+$ T-cell-mediated cytotoxic immune responses. In the cases of immune responses against certain bacteria, against viruses that do not infect DCs directly, or against tumors, DCs must present internalized antigens on MHC class I molecules. Certain studies, however, suggest that the role of cross-presentation is critical even for viruses in principle capable of infecting DCs (100, 101). It is remarkable, in any case, to see how different the functional organization of the DC phagocytic pathway is compared with macrophages or neutrophils. The ability to export proteins from phagosomes to the cytosol (87), recruitment of certain ER-resident proteins (83), the inefficient assembly of the V-ATPase (71), and the efficient assembly of NOX2 (72) all contribute to provide DC phagosomes with a unique competence for cross-presentation. Nevertheless, all these specializations of endosomes and phagosomes, so far, have been characterized in 'artificial' DCs (derived from the bone marrow or from monocytes *in vitro*) and, for the most part, using artificial phagosomes (containing latex beads, not real microorganisms). From the very incomplete and biased picture available today, the DC phagocytic pathway could be viewed as a combination of certain traits from macrophages and others from neutrophils. From macrophages, DCs have borrowed the overall structure and morphology: the two populations of myeloid cells are only distinguished by a few surface markers. Their respective anatomical distributions, their migratory capacities, and, most importantly, their main biological functions are very different. Although there is a lot still to do before a complete picture of the DC's phagocytic pathway can be drawn precisely, the results available show important differences. The macrophage phagocytic system seems adapted for a unique main function: destroying the engulfed particle. The main effector system used appears to be proteolysis. An extremely high concentration of proteolytic lysosomal enzymes, which are very active at the very pH encountered in macrophage's phagosomes (around 4.5), make macrophage phagosomes a 'dangerous place for bugs to be'. Although the underlying mechanisms may not be exactly the same, the overall situation in neutrophil phagosomes seems to be quite similar: the main purpose of phagocytosis is destruction. Although the respective role of reactive oxygen, chloride derivatives, and proteases in the killing of microbes is still debated, the efficiency of the cytotoxic process is not in question. The initial rise in the pH observed in the first minutes of the oxidative burst is probably part of a complex series of ionic fluxes that finally allow the freeing of and the activation of proteases that participate to the killing process.
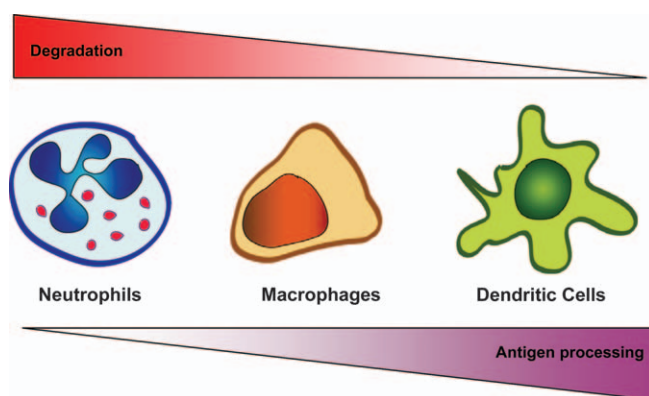
Fig. 4. Degradation-processing balance in phagocytic cells. The high destructive capacity of neutrophils is essential to eliminate pathogens. However, a consequence of such a 'destructive' power is that the conservation of peptides from microorganisms is compromised. DCs, in contrast, degrade proteins inefficiently, thus preserving the antigenic information contained in peptides. Macrophages are equipped to eliminate pathogens efficiently because of their high degradative phagosomal capacity, but under certain conditions, they can also process antigens to stimulate T cells.

The situation in DCs is quite different. What we know so far about the DC phagocytic system suggests that the main function of phagocytosis is not killing microbes. DC phagosomes rather seem to function as 'peptide factories' for adaptive immune recognition. They put microorganisms in an environment that combines nearly neutral pH, limited proteolytic activity, and low levels of oxidation. This kind of environment is probably sufficient to limit the proliferation of microbes and perhaps cause their partial degradation to liberate individual proteins that could serve as potential antigens for T-cell recognition (and maybe also for B-cell recognition). The main adaptation of the DC phagocytic pathway to this role in adaptive immunity seems to be the remarkable delay in phagosome maturation. The initial phase of phagosome maturation, neutral or alkaline pH, and low proteolytic activity, which in both macrophages and neutrophils lasts for minutes, is prolonged in DCs for hours. This important delay in phagosome maturation gives the antigen processing and presentation machinery of DCs a better chance to extract (protect from degradation) potential peptides for immune recognition. Some of the molecular mechanisms underlying this delay have been showed in the past few years. Many of them as well as their role in effective antigen recognition are still to be uncovered. Strategies for the attenuation of protein degradation seem to constitute the delicate 'bridge' that antigen-presenting cells have developed to go through from innate destructive immunity to effective adaptive immune responses (Fig. 4).

References

1. Guermonprez P, Valladeau J, Zitvogel L, Amigorena S. Antigen presentation and T cell stimulation by dendritic cells. Annu Rev Immunol 2002;**20**:620–667.

2. Radzicka A, Wolfenden R. A proficient enzyme. Science 1995;**267**:90–93.

3. Segal AW, Coade SB. Kinetics of oxygen consumption by phagocytosing human neutrophils. Biochem Biophys Res Commun 1978;**84**:611–617.

4. Segal AW, Dorling J, Coade S. Kinetics of fusion of the cytoplasmic granules with phagocytic vacuoles in human polymorphonuclear leukocytes. Biochemical and morphological studies. J Cell Biol 1980;**85**:42–59.

5. Segal AW, Geisow M, Garcia R, Harper A, Miller R. The respiratory burst of phagocytic cells is associated with a rise in vacuolar pH. Nature 1981;**290**:406–409.

6. Babior BM. NADPH oxidase: an update. Blood 1999;**93**:1464–1476.

7. Bokoch GM, Diebold BA. Current molecular models for NADPH oxidase regulation by Rac GTPase. Blood 2002;**100**:2692–2696.

8. Klebanoff SJ. Myeloperoxidase: friend and foe. J Leukoc Biol 2005;**77**:598–625.

9. Borregaard N, Cowland JW. Granules of the human neutrophilic polymorphonuclear leukocyte. Blood 1997;**89**:3503–3521.

10. Ganz T, et al. Defensins. Natural peptide antibiotics of human neutrophils. J Clin Invest 1985;**76**:1427–1435.

11. Reeves EP, et al. Killing activity of neutrophils is mediated through activation of proteases by K+ flux. Nature 2002;**416**:291–297.

12. Ahluwalia J, et al. The large-conductance Ca2+-activated K+ channel is essential for innate immunity. Nature 2004;**427**:853–858.

13. Geraghty P, et al. Neutrophil elastase up-regulates cathepsin B and matrix metalloprotease-2 expression. J Immunol 2007;**178**:5871–5878.

14. Belaaouaj A. Neutrophil elastase-mediated killing of bacteria: lessons from targeted mutagenesis. Microbes Infect 2002;**4**:1259–1264.

15. Belaaouaj A, et al. Mice lacking neutrophil elastase reveal impaired host defense against gram negative bacterial sepsis. Nat Med 1998;**4**:615–618.

16. Quie PG, White JG, Holmes B, Good RA. In vitro bactericidal capacity of human polymorphonuclear leukocytes: diminished activity in chronic granulomatous disease of childhood. J Clin Invest 1967;**46**:668–679.

17. Segal BH, Leto TL, Gallin JI, Malech HL, Holland SM. Genetic, biochemical, and clinical features of chronic granulomatous disease. Medicine (Baltimore) 2000;**79**:170–200.

18. Ryter A. Relationship between ultrastructure and specific functions of macrophages. Comp Immunol Microbiol Infect Dis 1985;**8**:119–133.

19. Berton G, Bellavite P, de Nicola G, Dri P, Rossi F. Plasma membrane and phagosome localisation of the activated NADPH oxidase in elicited peritoneal macrophages of the guinea-pig. J Pathol 1982;**136**:241–252.

20. Claus V, et al. Lysosomal enzyme trafficking between phagosomes, endosomes, and lysosomes in J774 macrophages. Enrichment of cathepsin H in early endosomes. J Biol Chem 1998;**273**:9842–9851.

21. Vieira OV, Botelho RJ, Grinstein S. Phagosome maturation: aging gracefully. Biochem J 2002;**366**:689–704.

22. Duclos S, et al. Rab5 regulates the kiss and run fusion between phagosomes and endosomes and the acquisition of phagosome leishmanicidal properties in RAW 264.7 macrophages. J Cell Sci 2000;**113**:3531–3541.

23. Gagnon E, et al. Endoplasmic reticulum-mediated phagocytosis is a mechanism of entry into macrophages. Cell 2002;**110**:119–131.

24. Hatsuzawa K, et al. Involvement of syntaxin 18, an endoplasmic reticulum (ER)-localized SNARE protein, in ER-mediated phagocytosis. Mol Biol Cell 2006;**17**: 3964–3977.

25. Touret N, et al. Quantitative and dynamic assessment of the contribution of the ER to phagosome formation. Cell 2005;**123**: 157–170.

26. Roberts EA, Chua J, Kyei GB, Deretic V. Higher order Rab programming in phago-lysosome biogenesis. J Cell Biol 2006;**174**:923–929.

27. Vieira OV, et al. Modulation of Rab5 and Rab7 recruitment to phagosomes by phos-phatidylinositol 3-kinase. Mol Cell Biol 2003;**23**:2501–2514.

28. Leiva N, Pavarotti M, Colombo MI, Damiani MT. Reconstitution of recycling from the phagosomal compartment in streptolysin O-permeabilized macrophages: role of Rab11. Exp Cell Res 2006;**312**:1843–1855.

29. Griffiths G. On phagosome individuality and membrane signalling networks. Trends Cell Biol 2004;**14**:343–351.

30. Anes E, Kuhnel MP, Bos E, Moniz-Pereira J, Habermann A, Griffiths G. Selected lipids activate phagosome actin assembly and matu-ration resulting in killing of pathogenic mycobacteria. Nat Cell Biol 2003;**5**:793–802.

31. Nobes C, Marsh M. Dendritic cells: new roles for Cdc42 and Rac in antigen uptake? Curr Biol 2000;**10**:R739–R741.

32. Bancherau J, et al. Immunobiology of dendritic cells. Annu Rev Immunol 2000;**18**:767–811.

33. Iyoda T, et al. The CD8+ dendritic cell subset selectively endocytoses dying cells in culture and in vivo. J Exp Med 2002;**195**:1289–1302.

34. Ritter U, Meissner A, Scheidig C, Korner H. CD8 alpha- and Langerin-negative dendritic cells, but not Langerhans cells, act as prin-cipal antigen-presenting cells in leishmania-sis. Eur J Immunol 2004;**34**:1542–1550.

35. Riese RJ, Chapman HA. Cathepsins and com-partmentalization in antigen presentation. Curr Opin Immunol 2000;**12**:107–113.

36. Maric M, et al. Defective antigen processing in GILT-free mice. Science 2001;**294**:1361–1365.

37. Lennon-Dumenil AM, et al. Analysis of pro-tease activity in live antigen-presenting cells shows regulation of the phagosomal pro-teolytic contents during dendritic cell acti-vation. J Exp Med 2002;**196**:529–540.

38. Honey K, Rudensky AY. Lysosomal cysteine proteases regulate antigen presentation. Nat Rev Immunol 2003;**3**:472–482.

39. Delamarre L, Pack M, Chang H, Mellman I, Trombetta ES. Differential lysosomal proteolysis in antigen-presenting cells determines antigen fate. Science 2005;**307**:1630–1634.

40. El-Sukkari D, et al. The protease inhibitor cystatin C is differentially expressed among dendritic cell populations, but does not control antigen presentation. J Immunol 2003;**171**:5003–5011.

41. Hall A, Ekiel I, Mason RW, Kasprzykowski F, Grubb A, Abrahamson M. Structural basis for different inhibitory specificities of human cystatins C and D. Biochemistry 1998;**37**:4071–4079.

42. Shirahama-Noda K, et al. Biosynthetic processing of cathepsins and lysosomal degradation are abolished in asparaginyl endopeptidase-deficient mice. J Biol Chem 2003;**278**:33194–33199.

43. Maehr R, et al. Asparagine endopeptidase is not essential for class II MHC antigen pre-sentation but is required for processing of cathepsin L in mice. J Immunol 2005;**174**:7066–7074.

44. Driessen C, et al. Cathepsin S controls the trafficking and maturation of MHC class II molecules in dendritic cells. J Cell Biol 1999;**147**:775–790.

45. Manoury B, et al. Asparagine endopeptidase can initiate the removal of the MHC class II invariant chain chaperone. Immunity 2003;**18**:489–498.

46. Pierre P, Mellman I. Developmental regulation of invariant chain proteolysis controls MHC class II trafficking in mouse dendritic cells. Cell 1998;**93**:1135–1145.

47. Hsing LC, Rudensky AY. The lysosomal cysteine proteases in MHC class II antigen presentation. Immunol Rev 2005;**207**: 229–241.

48. Manoury B, Hewitt EW, Morrice N, Dando PM, Barrett AJ, Watts C. An asparaginyl endopeptidase processes a microbial antigen for class II MHC presentation. Nature 1998;**396**:695–699.

49. Barrett AJ, Kirschke H, Cathepsin B, cathep-sin H, and cathepsin L. Methods Enzymol 1981;**80**(Pt C):535–561.

50. Kirschke H, Wiederanders B. Cathepsin S and related lysosomal endopeptidases. Methods Enzymol 1994;**244**:500–511.

51. DeCoursey TE. During the respiratory burst, do phagocytes need proton channels or potassium channels, or both? Sci STKE 2004;**2004**:pe21.

52. Murphy R, DeCoursey TE. Charge compen-sation during the phagocyte respiratory burst. Biochim Biophys Acta 2006;**1757**:996–1011.

53. Morgan D, DeCoursey TE. Diversity of volt-age gated proton channels. Front Biosci 2003;**8**:s1266–s1279.

54. Decoursey TE. Voltage-gated proton channels and other proton transfer pathways. Physiol Rev 2003;**83**:475–579.

55. DeCoursey TE, Morgan D, Cherny VV. The gp91phox component of NADPH oxidase is not a voltage-gated proton channel. J Gen Physiol 2002;**120**:773–779.

56. Henderson LM, Meech RW. Proton conduc-tion through gp91phox. J Gen Physiol 2002;**120**:759–765.

57. Henderson LM, Meech RW. Evidence that the product of the human X-linked CGD gene, gp91-phox, is a voltage-gated H(+) pathway. J Gen Physiol 1999;**114**: 771–786.

58. Jankowski A, Grinstein S. Modulation of the cytosolic and phagosomal pH by the NADPH oxidase. Antioxid Redox Signal 2002;**4**: 61–68.

59. Jiang Q, Griffin DA, Barofsky DF, Hurst JK. Intraphagosomal chlorination dynamics and yields determined using unique fluorescent bacterial mimics. Chem Res Toxicol 1997;**10**:1080–1089.

60. Cech P, Lehrer RI. Phagolysosomal pH of human neutrophils. Blood 1984;**63**:88–95.

61. Jankowski A, Scott CC, Grinstein S. Deter-minants of the phagosomal pH in neutro-phils. J Biol Chem 2002;**277**:6059–6066.

62. Forman HJ, Torres M. Reactive oxygen spe-cies and cell signaling: respiratory burst in macrophage signaling. Am J Respir Crit Care Med 2002;**166**:S4–S8.

63. Yamaguchi T, Kaneda M. Presence of cyto-chrome b-558 in guinea-pig alveolar macrophages-subcellular localization and relationship with NADPH oxidase. Biochim Biophys Acta 1988;**933**:450–459.

64. Forman HJ, Torres M. Signaling by the respiratory burst in macrophages. IUBMB Life 2001;**51**:365–371.

65. Lukacs GL, Rotstein OD, Grinstein S. Phagosomal acidification is mediated by a vacuolar-type H(+)-ATPase in murine macrophages. J Biol Chem 1990;**265**:21099–21107.

66. Lukacs GL, Rotstein OD, Grinstein S. Deter-minants of the phagosomal pH in macro-phages. In situ assessment of vacuolar H(+)-ATPase activity, counterion conductance, and H+ "leak". J Biol Chem 1991;**266**:24540–24548.

67. Yates RM, Hermetter A, Taylor GA, Russell DG. Macrophage activation downregulates the degradative capacity of the phagosome. Traffic 2007;**8**:241–250.

68. Yates RM, Hermetter A, Russell DG. The kinetics of phagosome maturation as a func-tion of phagosome/lysosome fusion and acquisition of hydrolytic activity. Traffic 2005;**6**:413–420.

69. Yates RM, Russell DG. Phagosome matura-tion proceeds independently of stimulation of toll-like receptors 2 and 4. Immunity 2005;**23**:409–417.

70. Vergne I, Chua J, Singh SB, Deretic V. Cell biology of mycobacterium tuberculosis phagosome. Annu Rev Cell Dev Biol 2004;**20**:367–394.

71. Trombetta ES, Ebersold M, Garrett W, Pypaert M, Mellman I. Activation of lysosomal function during dendritic cell maturation. Science 2003;**299**:1400–1403.

72. Savina A, et al. NOX2 controls phagosomal pH to regulate antigen processing during crosspresentation by dendritic cells. Cell 2006;**126**:205–218.

73. Elsen S, et al. Cryptic O2- -generating NADPH oxidase in dendritic cells. J Cell Sci 2004;**117**:2215–2226.

74. Vulcano M, et al. Toll receptor-mediated regulation of NADPH oxidase in human dendritic cells. J Immunol 2004;**173**: 5749–5756.

75. Matsue H, et al. Generation and function of reactive oxygen species in dendritic cells during antigen presentation. J Immunol 2003;**171**:3010–3018.

76. Kjeldsen L, Sengelov H, Lollike K, Nielsen MH, Borregaard N. Isolation and characterization of gelatinase granules from human neutrophils. Blood 1994;**83**:1640–1649.

77. Jancic C, et al. Rab27a regulates phagosomal pH and NADPH oxidase recruitment to dendritic cell phagosomes. Nat Cell Biol 2007;**9**:367–378.

78. Stinchcombe J, Bossi G, Griffiths GM. Linking albinism and immunity: the secrets of secretory lysosomes. Science 2004;**305**: 55–59.

79. Cresswell P. Antigen presentation. Getting peptides into MHC class II molecules. Curr Biol 1994;**4**:541–543.

80. Yewdell JW, Nicchitta CV. The DRiP hypothesis decennial: support, controversy, refinement and extension. Trends Immunol 2006;**27**:368–373.

81. Rock KL, Gamble S, Rothstein L. Presentation of exogenous antigen with class I major histocompatibility complex molecules. Science 1990;**249**:918–921.

82. Heath WR, et al. Cross-presentation, dendritic cell subsets, and the generation of immunity to cellular antigens. Immunol Rev 2004;**199**:9–26.

83. Guermonprez P, Saveanu L, Kleijmeer M, Davoust J, Van Endert P, Amigorena S. ER-phagosome fusion defines an MHC class I cross-presentation compartment in dendritic cells. Nature 2003;**425**:397–402.

84. Cresswell P, Ackerman AL, Giodini A, Peaper DR, Wearsch PA. Mechanisms of MHC class I-restricted antigen processing and cross-presentation. Immunol Rev 2005;**207**: 145–157.

85. Ackerman AL, Cresswell P. Cellular mechanisms governing cross-presentation of exogenous antigens. Nat Immunol 2004;**5**:678–684.

86. Ackerman AL, Giodini A, Cresswell P. A role for the endoplasmic reticulum protein retrotranslocation machinery during crosspresentation by dendritic cells. Immunity 2006;**25**:607–617.

87. Rodriguez A, Regnault A, Kleijmeer M, Ricciardi-Castagnoli P, Amigorena S. Selective transport of internalized antigens to the cytosol for MHC class I presentation in dendritic cells. Nat Cell Biol 1999;**1**:362–368.

88. Accapezzato D, et al. Chloroquine enhances human CD8+ T cell responses against soluble antigens in vivo. J Exp Med 2005;**202**:817–828.

89. Lee P, Matsueda GR, Allen PM. T cell recognition of fibrinogen. A determinant on the A alpha-chain does not require processing. J Immunol 1988;**140**:1063–1068.

90. Lindner R, Unanue ER. Distinct antigen MHC class II complexes generated by separate processing pathways. EMBO J 1996;**15**:6910–6920.

91. Villadangos JA, Driessen C, Shi GP, Chapman HA, Ploegh HL. Early endosomal maturation of MHC class II molecules independently of cysteine proteases and H-2DM. EMBO J 2000;**19**:882–891.

92. Regnault A, et al. Fcgamma receptor-mediated induction of dendritic cell maturation and major histocompatibility complex class I-restricted antigen presentation after immune complex internalization. J Exp Med 1999;**189**:371–380.

93. Albert ML, et al. Immature dendritic cells phagocytose apoptotic cells via alphavbeta5 and CD36, and cross-present antigens to cytotoxic T lymphocytes. J Exp Med 1998;**188**:1359–1368.

94. Albert ML, Sauter B, Bhardwaj N. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. Nature 1998;**392**:86–89.

95. Delamarre L, Holcombe H, Mellman I. Presentation of exogenous antigens on major histocompatibility complex (MHC) class I and MHC class II molecules is differentially regulated during dendritic cell maturation. J Exp Med 2003;**198**:111–122.

96. Manickasingham S, Reis e Sousa C. Microbial and T cell-derived stimuli regulate antigen presentation by dendritic cells in vivo. J Immunol 2000;**165**:5027–5034.

97. Blander JM, Medzhitov R. Toll-dependent selection of microbial antigens for presentation by dendritic cells. Nature 2006;**440**:808–812.

98. Blander JM, Medzhitov R. Regulation of phagosome maturation by signals from toll-like receptors. Science 2004;**304**: 1014–1018.

99. Sachs DH, Kiszkiss P, Kim KJ. Release of Ia antigens by a cultured B cell line. J Immunol 1980;**124**:2130–2136.

100. Sigal LJ, Crotty S, Andino R, Rock KL. Cytotoxic T-cell immunity to virus-infected non-haematopoietic cells requires presentation of exogenous antigen. Nature 1999;**398**:77–80.

101. Ramirez MC, Sigal LJ. Macrophages and dendritic cells use the cytosolic pathway to rapidly cross-present antigen from live, vaccinia-infected cells. J Immunol 2002;**169**:6733–6742.