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Dendritic cells: microbial clearance via autophagy and potential immunobiological consequences for periodontal disease

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Craniofacial mucosal immune system, tissue and blood dendritic cells

The oral cavity, oropharynx and nasopharynx comprise the most proximal extent of the gastrointestinal and respiratory tracts (36). They are also part of the intricate network of the craniofacial mucosal immune system. This system shares many properties with other mucosa-associated lymphoid tissues and secondary lymphoid tissues, but is also quite distinct in terms of cellular requirements for organogenesis and mucosal imprinting molecules [reviewed in Ref. (136)]. Oral mucosa-associated lymphoid tissue must deal with the continuous onslaught of bacteria, in which the number of colonizing bacteria far exceeds the number of host cells per surface area (48). Because of this bacterial load, humans have evolved different biological mechanisms to tolerate commensal bacteria whilst preventing invasion with pathogenic bacteria. However, in some instances, the human immune response is not up to the task, being unable to maintain the delicate balance needed between tolerance and protection. Consequently, the host becomes more susceptible to the long-term effects of disruption of immune homeostasis that is manifest by several autoimmune and chronic inflammatory disorders, including periodontal disease (162).

Dendritic cells are the peripheral sentinels of the human mucosal immune system and are key regulators of tolerance and protection. Dendritic cells capture and process antigens, and express the costimulatory molecules and cytokines needed for antigen presentation to B- and T-lymphocytes. Dendritic cells also play an essential role in ‘tolerizing’ T-cells to self-antigens, thereby minimizing autoimmune reactions. As such, dendritic cells play a seminal role in deciding whether to mount a vigorous immune response against pathogenic bacteria and to tolerate commensal microbes (or self-antigens). When dendritic cell-mediated immune homeostasis is disrupted, dendritic cells can contribute to the pathogenesis of different inflammatory destructive conditions (11, 37).

Dendritic cells are commonly distinguished by their location in peripheral tissues, secondary lymphoid organs or in the blood circulatory system. Tissue ‘resident’ dendritic cells, namely Langerhans cells or interstitial dendritic cells, have relatively long lifespans and play an active role in immune surveillance, promoting host tolerance or immunity. However, nearly 50% of the dendritic cells found in these tissues are migratory dendritic cell subsets, rather than typical resident dendritic cells. Circulating blood dendritic cells are distinguished from tissue dendritic cells in that they neither show dendrite formation nor express maturation features (such as CD83) (185). Because blood dendritic cells lack lineage-specific markers, such as CD3, CD14, CD19, CD56 and glycophorin A, they are generally isolated by negative selection (156, 170, 172). Blood dendritic cells can be

divided into three general dendritic cell types – plasmacytoid dendritic cells and two types of conventional or myeloid dendritic cells ($CD1c^+$ or $CD141^+$) – based on function and phenotype (56, 84, 185). Plasmacytoid dendritic cells are derived from lymphoid progenitors and resemble plasma cells; however, plasmacytoid dendritic cells share more commonalities with myeloid dendritic cells. Plasmacytoid dendritic cells are commonly identified by expression of CD123, CD303 and CD304, and they also strongly express toll-like receptors 7 and 9 and can produce high amounts of interferon-alpha in response to C-phosphate-G bacterial DNA motifs (but not to bacterial lipopolysaccharide) (168). Therefore, plasmacytoid dendritic cells are thought to recognize predominantly viral antigens (30, 68). Myeloid dendritic cells, on the other hand, are highly phagocytic, antigen-processing dendritic cells that recognize both bacterial and viral antigens (116, 155). Myeloid dendritic cells can be characterized by their expression of $CD1c^+$ (BDCA-1^+) or $CD141^+$. $CD1c^+$ myeloid dendritic cells express all toll-like receptors (except toll-like receptor-9), whereas $CD141^+$ myeloid dendritic cells express a more restricted pattern of toll-like receptors, limited to toll-like receptor-3 and toll-like receptor-10, suggesting a more specific role in antiviral immunity (84). Recent studies have revealed an important role for blood myeloid dendritic cells in responding to periodontal infection (Tables 1 and 2).

Myeloid dendritic cells are widely distributed in the body and can regulate immune-activation or immune-tolerance functions, depending on their activation/maturation state and cytokine profile. Myeloid dendritic cells show such a degree of plasticity that some subsets can even serve as osteoclast precursors and have a role in osteoclastogenesis (2, 144). Myeloid dendritic cells are normally present at very low abundance in the blood (below ~1% of all circulating leukocytes). A rise (or fall) in the number of blood myeloid dendritic cells is often a hallmark of underlying chronic inflammatory/infectious disease; as such, blood myeloid dendritic cells have been called the 'canary in the coal mine' of these diseases (123). Most myeloid dendritic cells predominantly function within the confines of the tissues, mucosa-associated lymphoid tissue, lymphatics and secondary lymphoid organs. Myeloid dendritic cells typically exhibit short lifespans (especially after activation), as well as the need for constant replenishing. Myeloid dendritic cells can rapidly mobilize, when needed, in response to hematopoietic factors (such as fms-related tyrosine kinase 3 ligand) (92, 98). In a normal physiologic state of immune homeostasis, myeloid dendritic cells constantly undergo influx to and efflux from mucosal tissues; however, in pathophysiologic states of infection, the total number of blood myeloid dendritic cells can significantly increase (23, 123). Whether this is a consequence of active mobilization of epidermal Langerhans cells and lamina propria myeloid dendritic cells from, for example, periodontally infected tissues to the blood, increased dendritic cell differentiation from progenitors, decreased apoptosis (121) or reduced homing to secondary lymphoid organs (122), is speculative. From a histopathological context, the number of Langerhans cells decreases in the gingival epithelium in both gingivitis and periodontitis, apparently by efflux through the basement membrane. In contrast, the number of myeloid dendritic cells increases in the lamina propria in periodontitis, where they form immune conjugates with $CD4^+$ T-cells, called oral lymphoid foci *in situ* (Table 1).

Table 1. Effect of *Porphyromonas gingivalis* on myeloid dendritic cells in patients with chronic periodontitis

Tissue/cell type	Observation
Local histopathology	↓Langerhans cells in epithelium (95) ↑Maturing dendritic cells (94) and DC-SIGN$^+$ myeloid dendritic cells in lamina propria (97) ↑Dendritic cells/T-cell conjugates (94)
Blood myeloid dendritic cells	↑Noncanonical myeloid dendritic cell expansion ↑*P. gingivalis* content of blood dendritic cells ↑Dissemination of minor fimbria-1$^+$ *P. gingivalis* to atherosclerotic plaques (23)

DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin.

Canonical vs. noncanonical differentiation of dendritic cells and inflammation

Blood monocytes have the ability to differentiate into various cell lineage types, including myeloid dendritic cells and Langerhans cells. *In vitro*, this is dependent on the presence of recombinant granulocyte–macrophage colony-stimulating factor and interleukin-4 for differentiation into monocyte-derived dendritic cells (8, 99, 137, 182) and on the presence of granulocyte–macrophage colony-stimulating factor, interleukin-4

Table 2. Response of human predendritic cells, monocytes, monocyte-derived dendritic cells and $CD4^+$ T-cells to *Porphyromonas gingivalis in vitro*

Response	*P. gingivalis* strain (fimbriae; and pattern recognition receptors)				References
	Pg381 ($mfa1^+$/$fimA^+$; DC-SIGN/toll-like receptor-2)	DPG3 ($mfa1^+$; DC-SIGN)	MFI ($fimA^+$; toll-like receptor-2)	MFB (none; weak toll-like receptor-2)	(180–182)
Dendritic cell uptake	+++	++++	+++	None	(23, 94, 97, 180)
Survival in dendritic cells	+	+++	+	None	(23, 57, 180)
Dendritic cell differentiation	+++	++++	++	–	(122)
Dendritic cell apoptosis	↓	↓↓	↑	–	(122)
Maturation of dendritic cells	+/–	–	+	–	(180)
T-cell effector responses	T-helper 1	T-regulatory?	T-helper 1/ T-helper 17?	T-helper 2	(97, 180)
Allogeneic mixed leukocyte reaction	++	–	+++	–	(97, 180)
Secondary lymphoid organ homing	+	–	++	–	(123)
Autophagy/pyroptosis	+	–	++	Not internalized	(57)

DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; fimA, major fimbria; mfa1, minor fimbria.

and transforming growth factor-β for differentiation into Langerhans cells (65). These 'canonical' monocyte-derived dendritic cells and Langerhans cells are similar, in many ways, to typical myeloid dendritic cells (25, 65, 108), thus facilitating the study of their functions despite a low abundance in physiological conditions (107). When the host encounters microbial/proinflammatory signals in an infected site, $CCR2^+$ blood monocytes can rapidly migrate and then differentiate into distinct populations of myeloid dendritic cells (27, 66). Toll-like receptor recognition and stimulation seems to be particularly influential in 'noncanonical' differentiation of monocytes into $CD209^+$ $CD16^+$ or $CD1b/c^+$ monocyte-derived dendritic cells (102). However, such increases in the population of myeloid dendritic cells do not necessarily lead to efficient immune responses; in fact, the resultant pool of myeloid dendritic cells can be 'immunoincompetent' in some cases. Such a phenomenon is found particularly during chronic, low-grade infections and it is also associated with faulty pathogen-elimination and antigen-presentation processes; this may further exacerbate the immune dysregulation observed in chronic inflammatory diseases (123).

In periodontal diseases, oral mucosal infection is initiated by colonization of the tooth and oral mucosa with biofilm. The biofilm composition is eventually transformed by an increase in the numbers of certain pathogens. The host response to this biofilm ultimately leads to destruction of soft and hard tissue (24, 40). This involves the release, by local neutrophils and mast cells, of vasoactive peptides (via tumor necrosis factor-α), resulting in the secretion of inflammatory mediators by resident cells of the gingival tissue (fibroblasts); moreover, invading immune cells 'amplify' the local immune response, particularly polymorphonuclear leukocytes and other myeloid inflammatory cells that are increasingly recruited into tissues via diapedesis, in a process facilitated by vasodilation. Also, certain microbial species, notably the anaerobic gram-negative bacterium *Porphyromonas gingivalis*, have the unique ability to invade tissues and produce destructive proteolytic enzymes as well as to induce strong local inflammatory responses (21, 77).

Myeloid dendritic cells are among the leukocytes that promote proinflammatory responses (35, 37). The myeloid dendritic cells in the gingival lamina propria typically express the C-type lectin and the pattern recognition receptor, dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN or CD209), which is used as an invasin by a broad range of pathogenic organisms (45, 115, 119, 159). In the case of *P. gingivalis*, the 67-kDa minor fimbrial adhesin (denoted mfa-1) is expressed, which is glycosylated and targets the dendritic cell-specific C-type lectin, DC-SIGN, for entry into

myeloid dendritic cells (181). This interaction has also been shown to drive myeloid dendritic cell function away from maturation, leading to low expression of accessory molecules and a more immunosuppressive cytokine-production profile (179) (Table 1). Owing to their pronounced migratory function, immature status and weak bactericidal mechanisms, myeloid dendritic cells in blood may be particularly susceptible to exploitation by invasive pathogens. For example, In 2014 *in vitro* findings of our laboratory show that *P. gingivalis*, through its manipulation of DC-SIGN–toll-like receptor-2 signaling, rapidly induces differentiation of dendritic cells from monocyte progenitors. These dendritic cells, which we termed 'pathogen-differentiated' dendritic cells, are $CD1c^{+}$ $CD209^{+}$ $CD14^{-}$, but immature in phenotype and function unless forced to mature with an inflammatory cocktail (121). In addition, the *P. gingivalis*–dendritic cell interaction further dysregulates homeostasis by driving cells toward an aberrant chemokine receptor profile. These pathogen-differentiated dendritic cells display poor secondary lymphoid-organ homing capabilities (122) that prevent efficient effector cell responses. Hence, the generation of noncanonical pathogen-differentiated dendritic cells may have implications for pathogen dissemination and immune subversion in the low-grade transient bacteremia and chronic inflammation scenarios observed in periodontal diseases.

Dendritic cells and viral interactions

Targeting host cells is a fundamental step in viral infection by which viruses translocate their genome into host cells. Accordingly, two main factors are crucial in understanding such a process: (i) how viruses translocate their genomes into the target cell cytoplasm; and (ii) how the virus reaches the 'remote' targeted cells after initial entry. Studying the interactions of dendritic cells with viruses could provide clues on these two aspects of viral disease pathogenesis. As the main immune sentinels, many viruses target dendritic cells for direct propagation or systemic dissemination. Several phenotypic and functional subsets of dendritic cells are found in the peripheral tissues (185). The distinction of these subtypes is based mainly on the expression of pattern-recognition receptors, such as toll-like receptors and specific lectins for detecting high-mannose glycan motifs, at their surface. Differential receptor expression define the maturation state of dendritic cells, and subsequently their function, in immune homeostasis. In addition, the early acquaintance with each receptor determines the subsequent differentiation of dendritic cells, as well as the fate of the microorganisms. For example, in the case of dendritic cells, the dendritic cell-specific C-type lectin, DC-SIGN, can be used as a receptor by many viruses, such as HIV-1 (38), measles virus (43), hepatitis C virus (138), influenza A viruses (114), cytomegaloviruses (78), herpes simplex virus type 1 (42), Ebola virus (3), West Nile virus (41), SARS coronavirus (177) and human T-cell lymphotropic virus type 1 (90). Such early-recognition and subsequent intracellular pathways have been heavily investigated, revealing key pathogenic aspects in several viral diseases. The dendritic cell-specific C-type lectin, DC-SIGN, interacts directly with HIV through its gp120 envelope glycoprotein, and this interaction has been established as the route of HIV transmission into T-cells (63). In general, C-type lectin receptors have shown repetitive patterns of use in virus uptake, survival and even systemic dissemination (10). For instance, CD46 and CD150 have been identified as the classic measles virus receptors of epithelial and immune cells (52, 160); however, the identification of measles virus infection of alveolar macrophages and dendritic cells, which do not express CD150, suggest the involvement of other receptors (62, 105, 109). Consequently, it has been demonstrated that interaction of the measles virus with the dendritic cell-specific C-type lectin, DC-SIGN, allows CD150-mediated entry into dendritic cells (9). Hence, DC-SIGN seems to provide a means for transport of measles virus across the epithelial barrier, suggesting that dendritic cell infection facilitates measles virus to reach the lymphatic organs and replicate robustly (10). Similarly, DC-SIGN efficiently captures the glycan structures on viral envelope glycoproteins, including those of herpes simplex virus-1. Herpes simplex virus-1 infection can decrease the immunostimulatory phenotype of dendritic cells, and DC-SIGN is specifically involved in the binding and uptake of herpes simplex virus into dendritic cells (120, 134). Another example of herpesvirus infections and dendritic cell interactions is illustrated with herpes simplex virus-2, in which the persistent localized inflammatory response in the dermis below herpetic lesions contains chemokine (C-C motif) receptor 5-positive or chemokine (C-C motif) receptor 4-positive CD4 cells with mixed dendritic cell populations that express DC-SIGN (184). The latter suggests that the wide expanse of inamed surface allows more access to target cells, especially with the anatomic proximity of DC-SIGN-rich dendritic cells (184).

Chronic periodontitis: microbial-induced expansion of blood myeloid dendritic cell pool and potential implications for other systemic inflammatory diseases

A recent clinical study by our group (23) showed that the pool of CD1c+ (BDCA-1) CD209+ myeloid dendritic cells is expanded in the peripheral blood of subjects with chronic periodontitis relative to that of healthy controls. This expansion of the myeloid dendritic cell pool was further increased upon scaling and root planing as a result of the induced bacteremia. Moreover, in patients with chronic periodontitis and existing acute coronary syndrome, the dendritic cell pool was further expanded relative to that of patients with chronic periodontitis alone and healthy controls. We have previously reviewed other diseases reported to influence the blood myeloid dendritic cell pool (123). The response of myeloid dendritic cells in chronic periodontitis was not caused by an overall increase in total peripheral blood mononuclear cells or an increase in canonical factors, the DC-poietins (e.g. CD135 ligand, granulocyte-macrophage colony-stimulating factor, interleukin-4- and tumor necrosis factor receptor ligands I/II) that would otherwise account for the increased numbers of myeloid dendritic cells in the circulation. It appears that the expansion occurs as a result of the microbial carriage state of the dendritic cells and their progenitors, because of the bacteremia. The circulating myeloid dendritic cells were demonstrated to carry *P. gingivalis*, along with a diverse microbiome. Moreover, DC-SIGN$^+$ myeloid dendritic cells, shown to contain *P. gingivalis*, were identified within the coronary artery plaques *in situ*. As *P. gingivalis* targets CD209 for entry into myeloid dendritic cells via its glycoprotein fimbriae, minor fimbrial adhesin (179), this phenomenon was proposed to have particular significance to the pathophysiology of both chronic periodontitis and cardiovascular disease. Chronic periodontitis and cardiovascular disease have been linked through epidemiologic studies, but the mechanisms involved in the association are still unclear (50). The results of our studies indicate that the microbial carriage state of myeloid dendritic cells activates dendritic cell differentiation and promotes trafficking of these infected myeloid dendritic cells to sites of neovascularization, such as diseased coronary arteries, thereby increasing the cardiovascular risk associated with chronic periodontitis (121).

Antigen uptake, intracellular trafficking and autophagy in dendritic cells

An important early aspect of dendritic cell function is microbial clearance, wherein the pathogens are taken up by receptor-mediated phagocytosis and subsequently processed for presentation to other immune cells. This process is a key mechanism in activation of the innate immune response and significantly affects the subsequent antigen-presenting function of dendritic cells (150, 167). The process of phagocytosis itself provides important information about the nature of the engulfed targets so that the pathogen can be routed to appropriate intracellular compartments for tailoring of the immune/inflammatory response (167). After phagocytosis, there are at least four pathways for lysosomal degradation in mammalian cells (125): (i) the endocytic/phagocytic pathway (166); (ii) macroautophagy, involving isolation in an enclosed membrane in the cytoplasm (i.e. the autophagosome), followed by fusion of the autophagosome with lysosomes for degradation (19, 55, 151); (iii) microautophagy, in which a small portion of cytoplasm is engulfed by a lysosome membrane itself (125); and (iv) chaperone-mediated autophagy (49). These different mechanisms depend on the size and the nature of the insult, early recognition by pattern recognition receptors and the type of the phagocytic cell involved.

Macroautophagy (or autophagy) is an intracellular bulk degradation system in which cytoplasmic components are directed to the lysosome in a membrane-mediated process (151, 178). The autophagic pathway proceeds through several phases: the initiation and formation of a pre-autophagosomal structure; the development of a phagophore (isolated membrane), followed by vesicle elongation and maturation (lysosomal fusion); and degradation of autophagosomal contents by lysosomal acid hydrolases (28). Autophagy is regulated by homologs of products of the autophagy-related genes, originally identified in yeast (126, 141). Starvation is a potent environmental inducer of autophagy through inhibition of the mammalian target of rapamycin pathway (103). Conversely, growth factors, along with other nutrients, signal activation of the mammalian target of rapamycin pathway, which subsequently results in autophagy inhibition. Another important pathway for autophagy is the Beclin 1-interacting complex, which consists of: (i) Beclin-1 and B-cell lymphoma 2 family proteins (that inhibit autophagy); and (ii) class III

phosphatidylinositol 3-kinase and the autophagy-related gene, *ATG14L*, which is required for autophagy (82). Autophagosomal elongation requires two ubiquitin-like conjugation systems: the autophagy-related protein 12 and the microtubule-associated protein light chain 3–protein 8 conjugation systems. After elongation, the cytoplasmic microtubule-associated proteins 1A/1B light chain 3 is the active form that associates with autophagosome formation until cargo degradation (127). The presence of light chain 3-II discrete puncta on immunofluorescence analysis indicates autophagosome formation. Impaired autophagosome–lysosome fusion may result in increases in the number of autophagosomes, as observed in several diseases (28, 141).

Autophagy was initially recognized as a mechanism for cellular homeostasis and protein turnover, especially in starvation responses. However, autophagy plays an important role in protein quality control (81, 100), degradation of toxic proteins (140) and defense against bacterial pathogens (110, 132, 135). For metabolic and antibacterial mechanisms, autophagy involves the formation of membrane vesicle autophagosomes, which then fuse with lysosomes to degrade the contents in a process similar to phagosomal maturation. Recently, autophagy has been shown to function in defense against microbes. For example, *Shigella,* an invasive bacterium, is reported to evade autophagy in hamster kidney cells by secreting the *Shigella flexneri* virulent factor, IscB (135). This study also reported that mutant bacteria, which lack the *S. flexneri* virulent factor gene, showed lower intracellular growth rates, which returned to normal in fibroblast cells with defective autophagy (autophagy protein 5 double-knockout) (135). Another study reported that autophagy eliminates *Streptococcus* after it escapes endosomes in nonphagocytic cells (131).

Autophagy is particularly active in macrophages and dendritic cells (6, 104, 110, 147). Autophagy proteins in murine macrophages are required for the fusion of phagosomes (which contain toll-like receptor enveloped particles) with lysosomes (147). Toll-like receptor engagement during phagocytosis rapidly stimulates recruitment of microtubule-associated proteins 1A/1B light chain 3 and Beclin 1 (autophagy factors) to the phagosomes, promoting rapid acidification and enhanced intracellular microbial killing in these macrophages (147). Another study, using human and murine macrophages, identified toll-like receptor-4 as a sensor for autophagy (176). These studies also showed that the signaling was dependent on toll/interleukin-1 receptor-domain-containing adapter-inducing interferon-β and toll-like receptor-4 and did not affect cell viability, indicating that it is distinct from the autophagy cell-death pathway. In addition, toll-like receptor-dependent autophagy overcomes phagosomal arrest and induces co-localization of *Mycobacterium* with autophagosomal structures (176). In addition to optimal fusion of phagosomes or autophagosomes with lysosomes, the autophagic machinery in these cells is involved in antigen presentation through the major histocompatibility complex. Autophagy proteins in dendritic cells are critical for the fusion of lysosomes with toll-like receptor apoptotic cell antigens during major histocompatibility complex class II presentation (104).

As mentioned above, dendritic cells are professional phagocytes but are more versatile than neutrophils and macrophages. When in an immature state, dendritic cells specialize in immediate pathogen clearance for which they express a wide spectrum of pattern-recognition receptors (45, 91, 150). Dendritic cells are also inclined to preserve epitopes required for antigen presentation to initiate the adaptive immune response (150). They also possess a unique phagocytic machinery for controlled antigen processing and efficient presentation (15, 83, 145). As dendritic cells mature, they up-regulate the antigen-presenting apparatus, which includes accessory molecules that prime and initiate a suitable adaptive immune response (15, 33, 83, 87, 143, 150). Dendritic cells presumably need to be efficient in controlling and inhibiting the growth of intracellular pathogens, otherwise dendritic cells would be a significant niche for pathogen dissemination through the blood to distant organs, as reported for *P. gingivalis* (23). Autophagy is recognized as an antibacterial lysosomal mechanism for dendritic cells, but also has an immunoregulatory component (13, 47, 93). Although the mechanism of autophagy apparently serves dendritic cells well in most situations, very little is known about its role in eliminating specific intracellular pathogens, the pattern-recognition receptors involved and their positive or negative regulatory roles.

Pattern-recognition receptors: crosstalk and autophagy

Pattern-recognition receptors on innate immune cells recognize microbes through their pathogen-associated molecular patterns (45, 91). Pathogen-associated molecular patterns are conserved groups of molecules, such as bacterial cell-wall and nucleic acids,

which are expressed by microbes and are essential for microbial survival. The classes of pattern-recognition receptors in dendritic cells that sense pathogen-associated molecular patterns include toll-like receptors, nucleotide-binding oligomerization domain-like family receptors, caspase recruitment domain helicases and C-type lectin receptors (e.g. the dendritic cell-specific C-type lectin, DC-SIGN). Activation of pattern-recognition receptors stimulates receptor-specific intracellular signaling pathways that regulate transcription of response genes, such as those encoding costimulatory molecules and cytokines through transcription factors such as nuclear factor-kappaB (45). Although endocytic and signaling receptors were considered separate mechanisms, emerging evidence actually shows tight links between endocytosis and signaling (157, 166, 167). These connections were presumably made early in receptor evolution, along with links to autophagy in antigen-presenting cells.

The role of toll-like receptors in autophagy bears many similarities to their role in phagosomal maturation. Toll-like receptor engagement is the characteristic difference between immune responses in apoptotic and phagocytic cellular machineries in phagocytes (17, 166). For example, the activation of toll-like receptors by bacteria has been reported to regulate phagosomal maturation and lysosomal activities in murine macrophages (17) and efficient antimicrobial autophagy (88, 104, 176). Experiments in toll-like receptor-$2^{-/-}$ or toll-like receptor-$4^{-/-}$ double knockout mice have demonstrated lack of co-localization of engulfed bacteria with lysosomal markers within the cytoplasm (17). These studies showed that the receptor recognition by the phagocytic cells of bacterial components is a decisive factor for the intracellular bacterial routing and intracellular degradative systems. Furthermore, a recent study on dendritic cells showed that lipopolysaccharide (a toll-like receptor-4 ligand) and PAM3CYS4 (a toll-like receptor-1/2 ligand) can induce autophagy in dendritic cells. Furthermore, autophagy is up-regulated in peripheral blood mononuclear cells and human gingival fibroblasts from patients with periodontitis in the presence of *P. gingivalis* lipopolysaccharide (20). Interestingly, dendritic cells obtained from patients with Crohn's disease demonstrated defective autophagy responses. In addition, the induction of nucleotide-binding oligomerization domain-2-mediated autophagy in these cells was required for bacterial routing and generation of major histocompatibility complex class II specific $CD4^{+}$ T-cells in dendritic cells (31). A mechanism through which toll-like receptors induce autophagy has been suggested as the interaction of toll/interleukin-1 receptor-domain-containing adapter-inducing interferon-β and MyD88 with the autophagy protein, Beclin 1, resulting in disruption of its interaction with B-cell lymphoma 2 (152).

Two members of the nucleotide-binding oligomerization domain-like receptor family, nucleotide-binding oligomerization domain-1 and nucleotide-binding oligomerization domain-2, have been reported to induce autophagy upon sensing bacteria. Nucleotide-binding oligomerization domain-1 agonists induce autophagy in epithelial cells, whereas nucleotide-binding oligomerization domain-2 agonists induce autophagy in myeloid cells (163). Peptidoglycan (a nucleotide-binding oligomerization domain-2 ligand) induces autophagy in human dendritic cells and enhances expression of surface major histocompatibility complex class II antigen. In addition, nucleotide-binding oligomerization domain-2 or autophagy factor (ATG16L1) knock down reduces antigen-specific CD4 T-cell proliferative responses to dendritic cells infected with a *Salmonella* vector. Moreover, dendritic cells with nucleotide-binding oligomerization domain-2 or ATG16L1 risk alleles, from patients with Crohn's disease, have impaired induction of autophagy in response to nucleotide-binding oligomerization domain-2 ligand, but not to the toll-like receptor-2 ligand PAM3CYS4. Dendritic cells with Crohn's disease-associated nucleotide-binding oligomerization domain-2 mutations have decreased co-localization of *Salmonella typhimurium* and adherent invasive *Escherichia coli* with lysosomes, and increased survival of intracellular adherent invasive *E. coli*. Hence, nucleotide-binding oligomerization domain-2 stimulation induces autophagy in dendritic cells that subsequently augments major histocompatibility complex class II antigen presentation and bacterial targeting for lysosomal degradation (31).

As mentioned earlier, the dendritic cell-specific C-type lectin, DC-SIGN (CD209), is a C-type lectin receptor involved in pathogen uptake, signaling and antigen presentation in dendritic cells (64, 158, 179). For uptake, CD209 contains internalizing motifs in its cytoplasmic tail (183). Interestingly, CD209 has been implicated in immune suppression and regulation in certain contexts (59, 60, 183). Most notably, CD209 is targeted for immune escape by several pathogens, such as HIV, hepatitis C virus, human herpesvirus-8, *Mycobacterium tuberculosis*, *Helicobacter pylori* and *Streptococcus pneumoniae* (78, 101, 139, 183). Recently, we reported that engagement of the dendritic cell-specific C-type lectin, DC-SIGN, by the

minor fimbriae (mfa-1) of *P. gingivalis* yields weak dendritic cell maturation and an immunosuppressive cytokine profile. In the absence of DC-SIGN targeting by mfa1, the same pathogen yields a very different dendritic cell response, with high levels of interleukin-23 and interleukin-6, as well as induction of a T-helper cell-1/T-helper cell-17-type response (179, 180). Furthermore, our study showed that the anaerobe *P. gingivalis* survives within dendritic cells in an aerobic atmosphere, whereas it dies rapidly in the absence of dendritic cells (179). More recent investigations from our laboratory (Ref 57) revealed higher survival rates of *P. gingivalis* after DC-SIGN-mediated uptake. This survival was associated with inhibition of expression of light chain 3-II, Rab family protein-5 and lysosomal-associated membrane protein 1 within human dendritic cells. Interestingly, the survival of this strain was inhibited within dendritic cells by autophagy induction. Alternatively, mfa1^{-} *P. gingivalis* targets a non-CD209 route, mostly through toll-like receptor-2, induced high expression of LC3-II and co-localization with Rab5/lysosomal-associated membrane protein-1-rich vesicles. Furthermore, *P. gingivalis* was enclosed in the characteristic double-membrane phagophores, whilst escaping vesicle fusion after uptake via DC-SIGN (57) (Fig. 1). The engagement of CD209 and toll-like receptors activates distinct signaling pathways (45, 70), which results in differential intracellular routing and processing of the microbe within dendritic cells. Toll-like receptors promote phagosomal and autophagosomal maturation (176) and subsequent bacterial clearance (17, 166), whereas DC-SIGN seems to serve a counter-regulatory function. The latter observation can also be exemplified in interactions of HIV with dendritic cells, in which not only does HIV take advantage of DC-SIGN receptor permissibility to facilitate engulfment, but recent evidence suggests that the interaction can shut down autophagy mechanisms on dendritic cells (16). Although HIV generally ends up in lysosomes after autophagocytosis, the process activates the mammalian target of rapamycin pathway, which results in increased HIV-1 levels in dendritic cells and increased transmission to T-cells. In fact, the pharmacological induction of autophagy by rapamycin increases HIV degradation and presentation to T-cells (16). Collectively, experimental evidence supports the concept that, because the immunoregulatory machinery enclosed in dendritic cells could be exploited by certain pathogens, augmenting the innate immunity elements (e.g. toll-like receptors/autophagy crosstalk) could be a useful tool in tipping back the immune response toward efficient microbial clearance.

Phagosome heterogeneity and maturation in dendritic cells

Phagocytosis relies on a network of endocytic vesicles to deliver antigen load from early phagosomes to

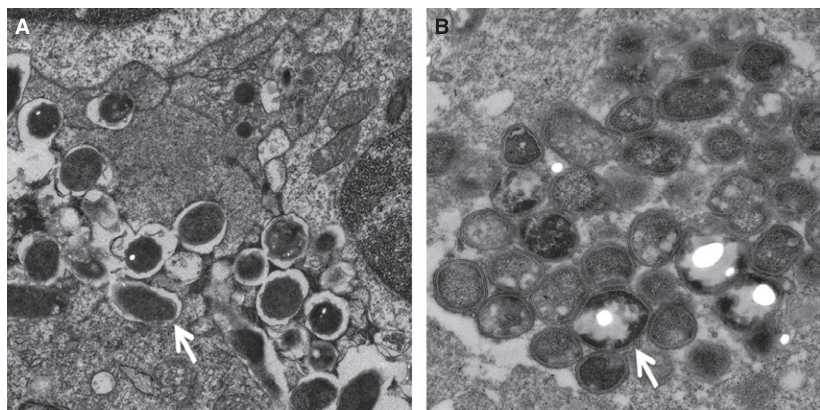

Fig. 1. *Porphyromonas gingivalis* is differentially routed within myeloid dendritic cells based on its expression of minor/major fimbriae. Electron microscopy images of intracellular vesicles containing *P. gingivalis* within human myeloid dendritic cells (white arrows). (A) Myeloid dendritic cells infected with *P. gingivalis* strain DPG3 expressing minor fimbriae (mfa-1) only. *Porphyromonas gingivalis* can be detected within single-membrane structures in the cytoplasm of monocyte-derived dendritic cells. Although some *P. gingivalis* are occasionally detected within double-membrane vesicles, a significant number of intracellular bacteria were detected in single-membrane phagosomes, resulting in increased intracellular survival. (B) Section of myeloid dendritic cells infected with a *P. gingivalis* strain expressing major fimbriae (fimA). The double-membrane vesicles exhibit selective autophagy characteristics, which target noncytoplasmic structures (e.g. bacteria). The vesicle membranes are visible as a parallel bilayer membrane separated by an electron-lucent cleft and contain noncytoplasmic structures. This phenomenon could be responsible for the efficient clearance of intracellular *P. gingivalis*

lysosomes for degradation. The bilayer phagosomes formed at the plasma membrane and the composition of these membranes gains a unique character depending on the type of pattern-recognition receptors engaged during phagocytosis (18). The maturation of the phagosome will not necessarily proceed through similar steps in different phagocytic cells. Moreover, different phagosomal maturation pathways have been reported in the same cell type. These different pathways are dictated primarily by the cargo contained in the phagosomes, as well as by external signals and the activation and/or differentiation state of phagocytes (69). In other words, 'all phagosomes are not created equal and there is a significant degree of phagosome heterogeneity and individuality' (18). In this regard, dendritic cells seem very well equipped for such heterogeneity in phagosome pathways. The functional organization of the dendritic cell phagocytic pathway is unique in several aspects, such as: (i) the ability of dendritic cells to export proteins from the phagosome to the cytosol (145); (ii) the characteristic cross-presentation and subsequent cross-priming by dendritic cells to defeat viral immune-evasion strategies and promote self-antigen tolerance (15, 83); and (iii) evidence of a phagosomal active system of alkalinization in dendritic cells that suppresses acidification within the phagosomes, especially in the first few hours after pathogen engulfment (149, 150). The complex phagosomal system in dendritic cells is therefore designed to suit its crucial role as a link between innate and adaptive immune responses, as well as a link between systemic and peripheral immunity.

Dendritic cell phagophores contain a wide range of esterases, reductases as well as endopeptidase. The majority of the proteases in dendritic cells belong to the group of cysteine proteases, such as cathepsins S, B, H and L; the aspartate proteases cathepsins D and E; or the asparagine endopeptidase (86, 106). Characteristically, dendritic cell proteases have a lower degradative potency than those within macrophages (44). These low lysosomal activities in dendritic cells could be caused by a reduced concentration of proteases (44) or the expression of protease inhibitors (cystatin family) within intracellular vesicles (79, 153). Dendritic cells express several members of the cystatin family of protease inhibitors that inhibit lysosomal protease activity by obstructing their active site. Some are present in lysosomes and probably contribute to restricting proteolytic activity in dendritic cell lysosomes and phagosomes (153). It seems that the phagosomal machinery within dendritic cells is set to partially degrade the target, rather than to eliminate it completely.

pH is a critical rate-regulating factor for protease activity in phagocytes. Basically, the acidic pH enhances the accessibility of proteases to target proteins by denaturation. However, each class of enzyme requires a specific range of pH for optimal activity (150). The optimal pH of proteolytic enzymes provides a system with a crucial adaptability function that suits the role of each phagocyte. For instance, studying acidification in immature dendritic cells showed no change in pH at 3 h after engulfment, in contrast to the immediate decrease of pH in macrophages. This was not only caused by the presence of a low vacuolar-type H^+-ATPase pump but also because of active alkalinization of the phagosomes in dendritic cells (149). The active alkalinization process in dendritic cells was assumed to resemble the NADPH oxidase process in neutrophils. However, the NAPDPH oxidase activity in dendritic cells is negligible compared with that in neutrophils (58). It has been proposed that this weak generation of reactive oxygen species in dendritic cells is caused by endogenous inhibitors, which could be abolished by proinflammatory signals. Reactive oxygen species production was also improved in dendritic cells by toll-like receptor ligation and T-cell interactions (117, 169). Although dendritic cells and neutrophils (but not macrophages) show active alkalinization processes, the kinetics and intensities are different. Whereas neutrophil phagosomes 'actively' raise the pH to 8 for 15 min, dendritic cell phagosomes maintain a high pH for several hours. As suggested by others, this extended alkalinization process could be caused by a microbicidal oxidase system contained in the vesicles or by 'inhibitory lysosome-related organelles' within dendritic cells that consume O_2 and H^+ to produce reactive oxygen species within the phagosomal lumen (149). This highly controlled machinery allows dendritic cells to preserve the useful epitope information of the antigen for ultimate presentation. However, it could also be exploited by certain pathogens to evade intracellular degradation, thereby using dendritic cells as a protective niche for persistence and systemic dissemination.

A significant factor in the regulation of phagosomes in dendritic cells is their maturity state. In immature dendritic cells, the H^+-ATPase cytosolic component is inefficiently assembled to the membrane subunits on lysosomes. This results in poor lysosomal acidification. In contrast, in mature dendritic cells, vacuolar-type H^+-ATPase was fully assembled and efficient acidification was restored (164). Interestingly, we recently reported that dendritic cells infected through the dendritic cell-specific C-type lectin, DC-SIGN,

maintain the immature form and display a reduced apoptotic rate (121). In addition, bacteria targeting DC-SIGN were able to survive at a higher rate for a longer time within these immature dendritic cells (57). Typically, immature dendritic cells have a short life as a result of the initiation of active apoptosis shortly after maturation, presumably to avoid immune overstimulation (26, 32). CD209 engagement seems to alter this homeostatic balance. It also appears to have a negative influence on the ability of dendritic cells to expel intracellular cargo by pyroptosis (121). The antibacterial autophagy pathway aims to route the cargo for fusion with lysosomes to facilitate final degradation. Hence, several intracellular pathogens are suggested to block autophagosomal maturation by hindering lysosome fusion or inhibiting autophagy protein-dependent fusion with the lysosome. Interestingly, some reports suggest that certain pathogens use autophagosomes for replication by blocking autophagosomal maturation (5, 13, 53). One of the earliest studies describing such a mechanism was with *P. gingivalis*, in which it trafficked to autophagosomes to evade conventional endocytic trafficking to lysosomes in endothelial cells (14). However, in our recent results, *P. gingivalis* seems to use an alternative tactic in myeloid dendritic cells, involving evasion of autophagic capture through early engagement with CD209 (57).

Phagosomal maturation is heterogeneous, with variation being derived predominantly from receptor–ligand interactions. When microbes (e.g. *Staphylococcus aureus* and *S. typhimurium*) engage toll-like receptors, they are delivered to lysosomes at an inducible rate manifested by increased clearance and phagolysosomal fusion (173). However, slower phagolysosome maturation has been detected in pathogen interactions involving the C-type lectin family and scavenger receptors. *Porphyromonas gingivalis*, by virtue of its fimbriae, is capable of engagement of at least two different classes of pattern recognition receptors in dendritic cells, namely the C-type lectin, DC-SIGN, and toll-like receptors, using the mfa-1 (minor) and fimA (major) fimbriae, respectively (179). In addition, the expression of these fimbriae could be altered by environmental conditions (175). This suggests that major and minor fimbriae may control the intracellular routing of *P. gingivalis* within the dendritic cells and shift the phagosomal maturation of intracellular vesicles containing the microbe. Studying the phagosomal maturation in dendritic cells after *P. gingivalis* phagocytosis could reveal an important mechanism for *P. gingivalis* survival through manipulation of CD209–toll-like receptor-2 signaling. This could profoundly impact upon not only the survival of *P. gingivalis* within dendritic cells, but also dendritic cell maturation and T-cell activation at secondary lymphoid organs and oral lymphoid foci at sites with chronic periodontitis (94–96).

Autophagy in the context of immune-inflammatory diseases

Defective autophagy has been associated with microbial survival, but also with several other chronic inflammatory conditions. Crohn's disease is a chronic inflammatory disease of the small intestine characterized by inflammatory alterations in the mucosal barrier combined with failure of microbial clearance. A genome-wide association study revealed the potential involvement of autophagy proteins in the pathogenesis of Crohn's disease. Genetic defects in nucleotide-binding oligomerization domain-2, immunity-related GTPase family M protein and the autophagy-related protein 16, ATG161L1, were suggested to be involved in the pathogenesis of Crohn's disease. Although immunity-related GTPase family M protein was reported as an autophagy inducer during the elimination of *Mycobacterium*, its role in microbial elimination in Crohn's disease has not yet been tested (12). However, dendritic cells from patients with Crohn's disease with the high-risk nucleotide-binding oligomerization domain-2 mutation showed defects in autophagy induction as well as altered expression of inflammatory signaling (31). In human dendritic cells, nucleotide-binding oligomerization domain-2 is required for induction of autophagy and subsequent efficient antigen presentation to $CD4^+$ T-cells. In this context, the role of nucleotide-binding oligomerization domain-2 in the regulation of proinflammatory mediators was crucial in the pathogenic process. Indeed, the recently established link between nucleotide-binding oligomerization domain-2 and autophagy helps to clarify the links between nucleotide-binding oligomerization domain-2 defects and the manifestations of Crohn's disease. Hence, it seems that dendritic cells with defective nucleotide-binding oligomerization domain-2 maintain the presence of opportunistic microbes and the proinflammatory signals that trigger intestinal inflammation.

Chronic periodontitis is initiated by an interaction between bacterial components of oral biofilms and host response mechanisms (161). The pathogenicity of chronic periodontitis involves excessive activation of innate and adaptive immune responses and/or

failure of sufficient T-cell polarization for its resolution. This continuous immune activation is influenced by a unique microbial complex (i.e. biofilm) at the oral mucosal interface; however, certain microbes (e.g. *P. gingivalis*) are able to infect and penetrate the epithelial barrier. In addition, this persistent immune activation induces endotoxin tolerance, which can prevent excessive immune responses but can also prevent microbial clearance (128–130). *Porphyromonas gingivalis* has been called a 'keystone' species in the oral microbial community as a result of its ability to influence the entire biofilm and contribute to innate immune-mediated pathogenesis (39). In chronic periodontitis, *P. gingivalis* exhibits several virulence factors, which include endotoxin, cysteine proteinases (gingipains), hemagglutinins and its adhesive fimbriae (72, 75). Still, the ability of *P. gingivalis* to invade cells and survive within, particularly in migratory cells, raises the specter of systemic dissemination and contribution to chronic diseases [e.g. cardiovascular disease and diabetes mellitus (67, 142)]. We have been interested, for some time, in the communication between *P. gingivalis* fimbriae and host immune elements. The two adhesins of *P. gingivalis*, termed the mfa-1 (minor) and fimA (major) fimbriae, have been shown to play roles in the pathogenesis of periodontal disease in the rat model (165). Several lines of evidence show that *P. gingivalis* fimbriae are essential in the communication with the innate immune system (and thus the adaptive immune system) through toll-like receptor-2/4, as well as through integrins and complement receptor 3 and CD14 (4, 73, 74, 131, 171).

The minor fimbriae are composed of a 67-kDa protein which is encoded by the *mfa1* gene (80) and is glycosylated with CD209 ligands fucose, mannose and *N*-acetyl galactosamine. Although little is known about the cellular receptors targeted by the minor (mfa1) fimbriae, our recent study showed that the minor fimbriae target DC-SIGN (CD209) on human monocyte-derived dendritic cells for entry. In addition, interplay between the major and minor fimbriae of *P. gingivalis* play critical and counter-regulatory roles in dendritic cell maturation and T-cell responses (179). Specifically, CD209 ligation by minor fimbriated *P. gingivalis* results in immature dendritic cells with a low proinflammatory cytokine profile and low allostimulatory response. On the contrary, fimA^{+} *P. gingivalis* strains, lacking minor mfa-1 fimbriae, induce more robust dendritic cell maturation with a strong proinflammatory cytokine response and an allostimulatory profile. In dendritic cell–CD4^{+} T-cell coculture, *P. gingivalis* strains expressing only the minor fimbriae induced dendritic cells to prime naïve T-cells into T-helper cell-2 effectors. Alternatively, *P. gingivalis* strains expressing only the major fimbriae induced dendritic cells to prime naïve T-cells into the T-helper 1 subtype (179). These and other *in vitro* findings are summarized in Table 2. This distinction in dendritic cell responses to the different strains of *P. gingivalis* can be understood in the context of intracellular trafficking of these strains within dendritic cells and antigen-presentation functions of dendritic cells. This is evidenced from our recent observations of the ability of mfa1^{+} *P. gingivalis* to evade autophagic vesicles (LC3^{+}) after uptake of the dendritic cell-specific C-type lectin, DC-SIGN, by dendritic cells (57). In the intracellular milieu of the dendritic cells, *P. gingivalis* can exploit the controlled proteolysis process. However, the fate of *P. gingivalis* is sealed early in the process by CD209–toll-like receptor-2 manipulation [reviewed in Ref. (76)] leading to differential endosomal and phagosomal routing, *P. gingivalis* survival and dendritic cell activation.

Microbial tactics: evasion of antibacterial autophagy by several pathogens

Bacterial pathogens exhibit several mechanisms though which they can avoid elimination by host cells. Certain intracellular pathogens can evade antibacterial autophagy by several mechanisms that range from evading early recognition to inhibition of protease activity within lysosomes. For instance, *Listeria monocytogenes* masks itself from recognition of autophagic machinery by the formation of a propulsive actin tail (54). An *L. monocytogenes* surface protein, ActA, recruits the host actin polymerization apparatus (Arp2/3 and Ena/VASP) to polymerize and elongate the actin tail on the bacterial surface. In addition, *L. monocytogenes* seems to harbor another 'anti'-autophagic protein (In1K). Although the mechanism of In1k is as yet unclear, the absence of both In1k and Acta1 causes more bacteria to be targeted by autophagy than the absence of Acta1 alone (54).

Another mechanism by which microbes can resist autophagy is through inhibition of critical steps of autophagosome formation or maturation. *Legionella pneumophila* shows the ability to de-conjugate LC3-II and lipid phosphatidylethanolamine (29). Conjugation of LC3 with phosphatidylethanolamine on the vacuole membrane is a crucial step in autophagosome elongation and development. *Legionella*

pneumophila RavZ protein irreversibly inhibits vesicle development, 'shielding' the microbe within these vacuoles, despite early autophagic recognition. Modulation of autophagosomal maturation is also a mechanism widely used by several intracellular pathogens (13). For instance, *Anaplasma phagocytophilum* has been reported to secrete Ats-1 protein, which induces autophagy through Beclin-1 (133); however, the vacuoles formed fail to fuse with lysosomes, providing a double-membrane vacuole for microbe survival.

Bacterial persistence can result from active evasion of the degradative machinery component of autophagy, but the mechanism underlying this evasion involves inflammatory signaling pathways. For example, *Yersinia* spp. recruit YopJ protein, which inactivates several levels of the mitogen-activated protein kinase pathway. In addition, YopJ induces apoptosis and secretion of caspase-1 by inhibiting inhibitor of nuclear factor kappa-B kinase subunit beta (nuclear factor-kappaB subunit) (118, 124). *Shigella* spp. are reported to regulate nuclear factor-kappaB through secretion of IpaH (E3 ligases), type III secreted effector proteins (7). *Escherichia coli* and *Burkholderia pseudomallei* use similar approaches by secretion of Cell cycle inhibiting factor (Cif) and Cif homologue in *Burkholderia pseudomallei* (CHBP) (type-III secreted effectors), which deactivate nuclear factor-kappaB by blocking the degradation of nuclear factor-jB inhibitor (34). Alternatively, *Shigella* spp. decrease the acute inflammatory responses by type-III secreted protein (OspF), which specifically inhibits mitogen-activated protein kinases. It seems that microbes can suppress certain pathways, and activate others, to their advantage. Certain species attempt to hinder acute responses whilst augmenting chronic immune activation. This concept could be exploited through pharmacological induction of autophagy and its potential scheme in the treatment of chronic infectious diseases (28). One of the important candidates for such a strategy is sirolimus (a mammalian target of rapamycin inhibitor). Rapamycin functions through inhibiting the mammalian target of rapamycin pathway in mammalian cells and has been widely used to augment the antimicrobial properties of immune cells, whilst serving an immunosuppressive role (28, 51). Recently, we showed that rapamycin impairs the intracellular survival of *P. gingivalis* strains that utilize the CD209-dependent route to evade autophagy (57). This could be particularly relevant in the treatment of chronic periodontitis and other chronic persistent microbial infections (121, 123, 179, 180).

Autophagy induction: targeting dendritic cells as a potential therapeutic approach

One of the earliest studies providing the first clue that autophagy up-regulation may be beneficial in the treatment of infectious diseases was in 1998. The study showed that genetic up-regulation of the autophagy gene, *Becl1*, protected mice against the lethal alphavirus encephalitis in neuronal tissues (112). Later, several studies reported that important pathogens are degraded *in vitro* by autophagy, including group A *Streptococcus pyogenes*, *M. tuberculosis*, *S. flexneri*, *Salmonella enterica*, *L. monocytogenes* and *Francisella tularensis*; viruses such as herpes simplex virus type 1 and chikungunya virus; and parasites such as *Toxoplasma gondii* (46, 97). In addition, *in vivo* data showed that the involvement of key autophagy genes could be protective against certain pathogens, such as *L. monocytogenes*, *M. tuberculosis* and *T. gondii* (71, 97). Such evidence suggests pharmacological induction of autophagy as a promising approach against certain persistent pathogens.

Besides the antimicrobial effect of autophagy induction, the immune-regulatory arm of autophagy seems also to be beneficial. Autophagy showed regulatory actions in the innate and adaptive immune elements (as described above). Of particular interest, induction of autophagy seems to augment antimicrobial innate immunity whilst preventing excessive inflammatory responses. Hence, autophagy induction has great potential, especially in targeting chronic infectious diseases such as periodontitis. For instance, autophagy can enhance production of type I interferon and innate immune responses by delivering viral nucleic acids to endosomal toll-like receptors. However, autophagy was also reported to control cellular levels of reactive oxygen species production, which prevent the response from being excessive (146). Regarding adaptive immunity, it was reported that autophagy protein-5 is required for dendritic cells to process and present extracellular microbial antigens for major histocompatibility complex class II presentation (104). In addition, autophagy also enhances the presentation of endogenous viral antigens on major histocompatibility complex class I molecules, as well as cross-presentation for tumor cell antigens in dendritic cells (61, 104, 111). The link between autophagy and antigen-presenting cells suggests that pharmacological induction of autophagy may be beneficial, not only in the treatment of acute infection but also in enhancing vaccine efficacy.

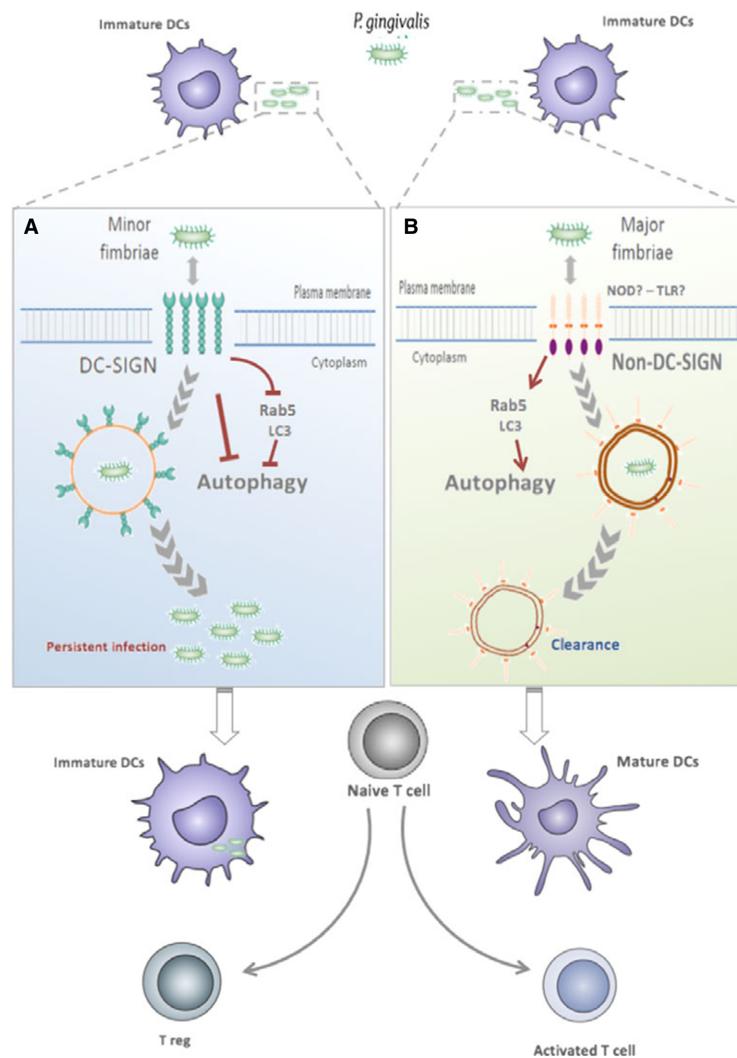

Fig. 2. Hypothetical model of the role of autophagy in the survival of *Porphyromonas gingivalis* within dendritic cells and the immunobiological consequences of the process. Upon dendritic cell recognition, immature myeloid dendritic cells take up *P. gingivalis* for intracellular trafficking and antigen processing and presentation; however, *P. gingivalis* contains two different adhesins [minor (mfa-1) and major (fimA) fimbriae], the expression of which can vary in response to environmental cues (pH and temperature). Immature myeloid dendritic cells express specific pattern recognition receptors to recognize these fimbriae. In our model, recognition is mediated by either dendritic cell-specific C-type lectin receptor DC-SIGN-dependent (A) or non-DC-SIGN dependent (B) routes. (A) DC-SIGN recognizes minor fimbriae promoting optimal pathogen intake (180). Experimental evidence shows that the DC-SIGN-dependent pathway is consistently used by several pathogens to escape intracellular killing (78, 102, 140, 184). *Porphyromonas gingivalis* exploits the DC-SIGN 'regulated' lysosomal pathway within dendritic cells. Hence, DC-SIGN-mediated uptake facilitates autophagy escape via inhibition of early endosomal GTPase (e.g. Rab5) and autophagy marker LC3 with subsequent lysosomal evasion. Autophagy evasion is most clearly evidenced by localization of *P. gingivalis* (expressing minor fimbriae) to single membrane intracellular vesicles (Fig. 1). The DC-SIGN-dependent pathway prolongs the life of the immature myeloid dendritic cell and promotes the intracellular survival of *P. gingivalis,* thus potentially facilitating persistent infection and systemic dissemination. This immature myeloid dendritic cell secretes proinflammatory cytokines and, upon interaction with naive T-cells, leads to a T-cell regulatory response typically found in chronic inflammatory conditions. (B) *Porphyromonas gingivalis*-expressing major fimbriae could potentially be recognized by nucleotide-binding oligomerization domain/toll-like receptor pathways (currently unknown). Evidence shows that the non-DC-SIGN pathway mediated by the nucleotide-binding oligomerization domain or toll-like receptor promotes efficient autophagy processes (31, 88, 105, 177). The uptake of *P. gingivalis* expressing major fimbriae is directed toward Rab5/$LC3^{+}$ phagophores fused with lysosome for efficient degradation. This leads to induction of dendritic cell maturation and efficient *P. gingivalis* clearance. The intracellular trafficking pathway ultimately determines the T-cell effectors that are induced – induced regulatory T-cells or a T-helper 1/T-helper 17 response – resulting in either a state of acquired immune privilege or resolution of infection. DC, dendritic cell; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; LC3, light chain 3 protein; NOD, nucleotide-binding oligomerization domain; Rab5; TLR, toll-like receptor; T reg, regulatory T-cell.

In mouse dendritic cells, autophagy induction with rapamycin enhanced the efficacy of the bacillus Calmette–Guérin vaccine by increasing peptide processing and presentation (89).

Drugs that modulate autophagy can be classified broadly as mammalian target of rapamycin-dependent and -independent agents. Rapamycin and its analogs (CCI-79, AP23573 and RAD001) inhibit the mammalian target of rapamycin and subsequently induce autophagy in primary cell culture, mammalian cells and *in vivo* (89, 110, 113). Sirolimus (rapamycin) is a clinically approved immunosuppressive and anti-cancer drug; hence, it exerts pleiotropic effects in which it induces autophagy and downregulates excessive inflammatory mediators (28). However, it is important to note that the mammalian target of rapamycin regulates several biological pathways and more specific targeted therapy may be required. Another similar approach was studied by targeting 5′ adenosine monophosphate-activated protein kinase activity, which induces autophagy by mammalian target of rapamycin inhibition and activation of the serine/threonine-protein kinase ULK1 (an autophagy transcriptional factor) (1). Autophagy can also be induced by inhibition of the phosphatidylinositol signaling pathway, using agents such as lithium, carbamazepine and valproic acid (148). It has been suggested that these drugs induce autophagy through reduction in mitochondrial uptake of Ca^{2+} released by the inositol(1,4,5)trisphosphate-3-kinase receptor, which causes a mild defect in mitochondrial respiration and 5′ adenosine monophosphate-activated protein kinase activation (22). However, these agents have several additional targets that could limit their application in the treatment of simple infectious conditions (85). Recently, the US Food and Drug Administration approved pharmacological agents for inducing mammalian target of rapamycin-independent autophagy. This group included clonidine and rilmenidine (an imidazoline receptor), calpain inhibitors and verapamil (l-type Ca^{2+} channels) (174). Many of these drugs are used for the treatment of hypertension, cluster headaches and angina. In addition, they seem to have favorable safety profiles. However, further investigation is needed to clarify their autophagic induction properties.

Some reports (as described earlier) did not recognize autophagy as an antibacterial mechanism but suggested that certain pathogens use autophagosomes for replication by blocking autophagosomal maturation (5, 13, 53). These observations shaped the need for agents that do not regulate the autophagic process as whole, but specifically target certain levels of autophagosomal formation and maturation (lysosomal fusion). In addition, a promising therapeutic strategy tackles the microbial properties that antagonize antibacterial autophagy. For instance, *S. flexneri* and *L. monocytogenes* (discussed earlier) are able to secrete proteins that enable them to escape recognition by the autophagic machinery (54, 135). Presumably, inhibition of these virulence factors would provide a safer and more therapeutic specific approach. This will entail specific approaches to address each type of infection individually and even the targeted host cells. One recent approach that investigated the interaction between the autophagy gene, Bec-1, and phosphoinositide 3-kinase revealed a candidate peptide (Tat-becline 1) for inducing antibacterial autophagy. Tat-becline 1 specifically induced autophagy and subsequently decreased the replication of several pathogens, including HIV, and reduced the mortality rates of mice infected with lethal West Nile viruses (154). This substantial line of evidence shows that autophagy has diverse functions in innate and adaptive immune responses and a crucial role in the chronic infectious diseases. A thorough understanding of initiation, development and regulation of the processes is therefore necessary in order to design efficient therapeutic strategies.

The pathogenesis of chronic periodontitis encompasses innate and adaptive immune responses. Both have destructive and protective potential, depending on the microbial insult and host susceptibility. Actually, chronic periodontitis illustrates the ‘double-edged sword’ of immune responses, in which the principal clinical signs of the disease are the result of activated immuno-inflammatory mechanisms, rather than direct effects of the bacteria. However, microbial elements are essential for the initiation of periodontitis, and bacterial virulence factors are decisive in progression of the disease. Substantial evidence from different studies indicates that the disease is a combination of hyper-responsive immune mechanisms, along with failure of microbial clearance. Autophagy could add a new dimension in understanding the multifactorial basis of chronic inflammatory conditions, such as periodontitis. In addition, therapeutic strategies, tackling autophagy regulation, could present a very promising approach in the treatment of chronic periodontitis.

Summary and Conclusions

In conclusion, we see promotion of autophagy as integral to restoration of immune homeostasis and

hence essential for resolution of infection in periodontitis. Figure 2 illustrates our model of the hypothetical role of autophagy in bacterial infection (*P. gingivalis*). Recognition of the oral pathogen, *P. gingivalis*, by dendritic cells, through either dendritic cell-specific C-type lectin, DC-SIGN-dependent or -independent routes (the latter probably dominated by toll-like receptor-mediated signaling), results in a very different outcome. The first scenario blocks autophagy, favoring pathogen survival and differentiation of long-lived immature immunoregulatory or suppressive dendritic cells that may favor immune dysregulation via altered inducible regulatory T-cell function. The second scenario promotes autophagy and bacterial degradation, resulting in mature immunostimulatory dendritic cells that promote effective immune responses capable of long-term resolution of infection. The use of autophagy promoters could favor the latter scenario and promote periodontitis resolution, and thus may represent a novel therapeutic approach in the search of a 'cure' for periodontal disease in humans.

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