Neutrophils, apoptosis and phagocytic clearance: an innate sequence of cellular responses regulating intramacrophagic parasite infections

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SUMMARY

In complex organisms, apoptosis is a constitutive cell death process that is involved in physiological regulation of cell numbers and that can also be induced in the course of inflammatory and immune responses. Neutrophils are among the first cells recruited during inflammation. Neutrophils constitutively die by apoptosis at inflamed sites, and are ingested by macrophages. Recent studies investigated how phagocytic clearance of senescent neutrophils influences the survival of intracellular protozoan parasites that have been phagocytosed by, or have invaded phagocytes. The results indicate that neutrophil clearance plays an unexpected role in regulation of intramacrophagic protozoan parasite infection.

Key words: *Leishmania major*, neutrophils, apoptosis, macrophage, phagocytosis.

INTRODUCTION

The cell biology of phagocytic recognition and removal of dead cells has become a growing area of interest (Savill *et al*. 2002; Gregory and Devitt, 2004). Recently, a number of investigations have characterized phagocyte receptors and immune regulatory responses triggered by phagocytic disposal of apoptotic cells (Savill *et al*. 2002; Gregory and Devitt, 2004). In addition, it is now recognized that ingestion of dying cells, followed by processing and presentation of their antigens by dendritic cells (DCs), is an important source of antigenic experience for lymphocytes (Larsson, Fonteneau and Bhardwaj, 2001; Plotz, 2003).

In complex organisms, apoptosis is a constitutive cell death process that is involved in physiological regulation of cell numbers, and that can also be induced in the course of inflammatory and immune responses. Neutrophils are among the first cells recruited during inflammation. Apoptosis is central to regulation of neutrophil turnover. Senescent neutrophils constitutively die by apoptosis at inflamed sites, and are eliminated following ingestion by macrophages (Savill *et al*. 1989; Haslett, 1999) Recent studies investigated how phagocytic clearance of dying cells, including senescent apoptotic neutrophils, influences the survival of protozoan pathogens that have been phagocytosed by, or have invaded macrophages (Freire-de-Lima *et al*. 2000; Ribeiro-Gomes *et al*. 2004, 2005). Phagocytic clearance of senescent neutrophils either exacerbates the growth or induces the killing of *Leishmania major* inside macrophages, depending on the host genetic background (Ribeiro-Gomes *et al*. 2004, 2005). Fig. 1 summarizes the conclusions from these studies (discussed in detail below). Together, the results indicate that dead cell clearance plays an unexpected role in regulation of intramacrophagic protozoan infections.

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PHAGOCYTE RESPONSES TO APOPTOTIC CELL INGESTION

Cells undergoing apoptosis expose ligands for a set of conserved receptors expressed by macrophages and non-professional phagocytes, allowing adherence and engulfment (Savill *et al*. 2002; Gregory and Devitt, 2004). A central finding was that macrophages ingesting apoptotic leukocytes become deactivated, as their ability to secrete the proinflammatory cytokine TNFα is suppressed (Voll *et al*. 1997; Fadok *et al*. 1998). Suppression is mediated by autocrine and paracrine secretion of PGE_2 and TGFβ (Fadok *et al*. 1998). Macrophage activation induced by endogenous or exogenous stimuli results in distinctive phenotypes (Gordon, 2003; Mosser, 2003). Classically activated macrophages are induced by T-helper type 1 (Th1) T lymphocytes. These macrophages secrete proinflammatory cytokines and are microbicidal. On the other hand, alternatively activated macrophages are induced by Th2 T lymphocytes and by TGFβ. These macrophages secrete anti-inflammatory cytokines and are involved in tissue repair (Gordon, 2003). The anti-inflammatory effect of cells undergoing apoptosis is coupled to induction of a programme of alternative

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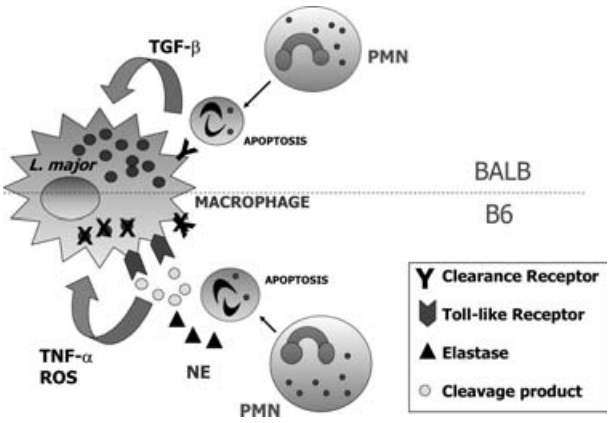

Fig. 1. Opposite effects of neutrophil clearance on *L. major* infection in susceptible and resistant hosts. Upper: susceptible BALB/c mice. Inflammatory neutrophils (PMN) undergo apoptosis before infected macrophages become activated. Engagement of clearance receptors by apoptotic PMN inactivates macrophages and increases parasite replication through TGFβ secretion. Lower: resistant B6 mice. PMN secrete large amounts of Neutrophil Elastase (NE) before apoptotic PMN engage clearance receptors. NE interacts with the cell surface or the extracellular matrix, generating a cleavage product. The product is an endogenous ligand for a Toll-like Receptor (TLR). TLR signaling induces TNFα secretion and reactive oxygen species (ROS). TNFα or a downstream product disables antiinflammatory signalling originating from clearance receptors. Intracellular parasite killing is effected by ROS and TNFα.

macrophage differentiation. Ingestion of apoptotic cells induces protracted ornithine decarboxylase (ODC) activity and polyamine production, while inhibiting nitric oxide (NO) production (Freire-de-Lima *et al*. 2000). Apoptotic cell removal could trigger an ancient biochemical pathway involved in tissue repair. Recent studies using DNA microarray analysis support this notion. Both hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF), key cytokines for tissue repair, were identified as two of the most strongly induced gene products following phagocytosis of apoptotic cells (Golpon *et al*. 2004). In agreement with this notion, phagocytosis of apoptotic cells triggers angiogenesis (Golpon *et al*. 2004).

Macrophages recognize cells undergoing apoptosis through a number of conserved receptors, including integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$, scavenger receptor CD36, CD91/calreticulin, and CD14 (Savill *et al*. 2002; Gregory and Devitt, 2004). Due to loss of asymmetrical organization of the cell membrane and to proteolytic and oxidative attack, new molecular patterns are exposed by dying cells and are recognized by receptors or by opsonins that bridge apoptotic cells to phagocyte receptors. Opsonins include thrombospondin-1, that binds to $\alpha_V\beta_3$ and CD36; MFG-E8 (lactadherin), that bridges $\alpha_V\beta_3$ to exposed phosphatidylserine (PS) sites; C-reactive protein, C1q/Mannose binding lectin (MBL), that bind to CD91-calreticulin; iC3b, among others (Savill *et al*. 2002; Gregory and Devitt, 2004). Few ligands expressed by the dying cell have been characterized so far. These include exposed PS (Savill *et al*. 2002; Gregory and Devitt, 2004), and capped CD43 on early apoptotic lymphocytes (Eda, Yamanaka and Beppu, 2004). Recognition of exposed PS is important for adhesion and engulfment of apoptotic cells (Krieser and White, 2002), but is not sufficient for optimal removal of cell corpses. In addition, PS can be recognized by distinct cell surface receptors, either directly or through opsonins, such as MFG-E8 (Savill *et al*. 2002; Gregory and Devitt, 2004). The large number of receptors involved could represent multiple and co-operative interactions required for engulfment. Alternatively, each of these receptors could play a dominant role depending on anatomical site, phagocyte differentiation and stage of the apoptotic sequence expressed by the dying cell. Recent evidence favours the latter hypothesis (Gregory and Devitt, 2004). An important issue is whether engagement of these receptors mimics the anti-inflammatory effects of apoptotic cells. So far, secretion of anti-inflammatory cytokines by macrophages has been demonstrated following engagement of CD36 (Voll *et al*. 1997) and $\alpha_V\beta_3$ (Freire-de-Lima *et al*. 2000) by antibodies, in the absence of dead cells. In agreement, anti-CD36 and anti-CD51 (α_V) antibodies inhibited secretion of IL-12, and induced secretion of the anti-inflammatory cytokine IL-10 by DCs stimulated with LPS (Urban, Willcox and Roberts, 2001).

Signal transduction resulting from recognition of apoptotic cells is incompletely understood. It has been suggested that binding of apoptotic cells is sufficient to transmit early signals that disable pro-inflammatory cytokine transcription in the absence of soluble mediators (Cvetanovic and Ucker, 2004). However, TGFβ plays an important role at later steps of macrophage inactivation (Fadok *et al*. 1998; Freire-de-Lima *et al*. 2000). The tyrosine kinase receptor MerTK is required for engulfment of the dying cell, presumably through activation of PLCγ-2 and PKC (Todt, Hu and Curtis, 2004). Phagocytes ingesting apoptotic cells activate Akt/PKB, resulting in increased cytokine-independent survival and inhibition of proliferation (Reddy *et al*. 2002). It has been suggested that ingestion of apoptotic cells inhibit, while necrotic cells stimulate, the activity of MAP kinases ERK1/2 (Reddy *et al*. 2002). However, another study found limited macrophage activation of ERK1/2 activity induced by apoptotic cells (Hu *et al*. 2002).

Some macrophage receptors are involved both in apoptotic cell clearance and in inflammatory phagocytosis of microrganisms, e.g. CD14 (Gregory and Devitt, 2004). It is not clear how phagocytes discriminate the target and initiate antinflammatory or

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proinflammatory responses. However, it has been suggested that differential engagement of Toll-like receptors (TLRs) is required to initiate a proinflammatory response (Gregory and Devitt, 2004). A similar problem may exist for discriminating between apoptotic and necrotic cells. In the worm *Caenorhabditis elegans*, a common set of engulfment genes mediates removal of both apoptotic and necrotic cells (Chung *et al*. 2000). It is generally believed that, while apoptotic cell clearance is anti-inflammatory, ingestion of necrotic cells induces a pro-inflammatory response. However, several studies suggest a more complex scenario. Both apoptotic and necrotic Jurkat lymphocytes induce a similar anti-inflammatory response in macrophages (Hirt and Leist, 2003). On the other hand, ingestion of early apoptotic cytolytic lymphocytes of the CTLL cell line is pro-inflammatory (Odaka *et al*. 2003). Engagement of TLRs by products from necrotic cells could be required for induction of a pro-inflammatory response (Li *et al*. 2001). In agreement with this study, apoptotic cells co-operate with the TLR ligand bacterial LPS to generate a pro-inflammatory response in macrophages (Lucas *et al*. 2003). These results suggest that, more important than being necrotic or apoptotic, dying cells would activate macrophages if they express or release a ligand for TLRs. In the absence of such a ligand, dead cell removal would trigger an anti-inflammatory response in the phagocyte.

PHAGOCYTIC REMOVAL OF APOPTOTIC LYMPHOCYTES INACTIVATES MACROPHAGES AND DRIVES GROWTH OF INTRACELLULAR PATHOGENS

Parasitic infection of mammalian hosts leads to both parasite and host cell apoptosis, which could have pathogenic implications (DosReis and Barcinski, 2001). Infection of mice with *Trypanosoma cruzi* leads to induction of both T- (Lopes *et al*. 1995) and B-cell apoptosis (Zuniga *et al*. 2002). Induction of T cell apoptosis through T cell receptor or Fas death receptor exacerbates replication of *T. cruzi* in co-cultured macrophages (Nunes *et al*. 1998). Further studies confirmed that the uptake of apoptotic T lymphocytes by macrophages increased the intracellular growth of *T. cruzi* (Freire-de-Lima *et al*. 2000). Macrophages attach and ingest apoptotic T cells through a mechanism that requires the $\alpha_V\beta_3$ integrin. Moreover, engagement of $\alpha_V\beta_3$ is sufficient to promote exacerbated replication of *T. cruzi*, since anti-$\alpha_V\beta_3$ antibodies mimic the effect of apoptotic cells on intra-macrophagic parasite growth (Freire-de-Lima *et al*. 2000). Blockade of $\alpha_V\beta_3$ by anti-α_V Fab fragment decreased the adhesion of apoptotic cells and inhibited the exacerbating effect of apoptotic cells on parasite growth. The biochemical pathway initiated by apoptotic cell ingestion was identified. It consisted of PGE_2 and TGFβ production, followed by increased ODC activity, and increased production of the polyamine putrescine (Freire-de-Lima *et al*. 2000). Putrescine production was required for increased parasite replication. On the other hand, ingestion of apoptotic cells inhibited NO production by macrophages. A pathogenic role for this pathway was suggested by the findings that: (1) injection of apoptotic cells exacerbated and accelerated parasitaemia *in vivo*; and (2) parasitaemia was reduced by treatment with cyclooxygenase inhibitors aspirin and indomethacin, which block PGE_2 production (Freire-de-Lima *et al*. 2000). Polyamine synthesis is required for the replication of several pathogenic parasites (Müller, Coombs and Walter, 2001), including intra-macrophagic growth of *T. cruzi* (Majumder and Kierszenbaum, 1993) and *L. major* (Iniesta, Gomez-Nieto and Corraliza, 2001). Since ingestion of apoptotic cells influences production of TGFβ and polyamines by macrophages, it could play a deleterious role in infection by other intracellular pathogens.

Subsequent studies demonstrated that ingestion of apoptotic lymphocytes exacerbates replication of HIV in human macrophages (Lima *et al*. 2002) and facilitates infection of *Coxiella burnetti* in mouse macrophages (Zamboni and Rabinovitch, 2004). Furthermore, amastigote forms of *L. amazonensis* expose PS sites on their surface, and PS exposure is involved in macrophage deactivation following infection (de Freitas Balanco *et al*. 2001). This mechanism of evasion was called 'apoptotic mimicry', to suggest that certain parasites mimic apoptotic cells – in this case, by exposing PS – in order to infect phagocytes silently (de Freitas Balanco *et al*. 2001). Expression of PS by amastigotes recalls an early study, where treatment of *Leishmania*-infected macrophages with liposomes containing PS deactivated macrophages and increased parasite replication (Gilbreath *et al*. 1985). Furthermore, erythrocytes infected by *Plasmodium falciparum* express the protozoal protein PfEMP-1, which binds to CD36 and to thrombospondin-1. Erythrocytes infected by *P. falciparum* mimic apoptotic cells by modulating DC maturation in response to an inflammatory stimulus (Urban *et al*. 2001).

NEUTROPHILS AS INNATE REGULATORS OF IMMUNITY AGAINST INFECTION

Neutrophils could represent an important example of immune regulation through phagocytic removal of apoptotic cells. Neutrophils are among the first cells to reach an inflammatory site. Inflammatory neutrophils secrete proteases, chemokines and soluble mediators that regulate inflammation. However, activated neutrophils have a short lifespan and undergo constitutive apoptosis, leading to their phagocytic removal by macrophages (Savill *et al*.

1989; Haslett, 1999). This removal prevents lysis of dying neutrophils and leakage of destructive cytotoxic molecules in which neutrophils are rich (Henson and Johnston, 1987). Phagocytic removal of apoptotic neutrophils functionally deactivates macrophages through secretion of PGE_2 and TGFβ (Fadok *et al*. 1998). Therefore, clearance of neutrophils has been associated with resolution of inflammation (Savill *et al*. 2002; Haslett, 1999). Furthermore, phagocytosis of apoptotic neutrophils by immature DCs inhibits their maturation by decreasing IL-12 secretion, expression of co-stimulatory molecules, and by reducing their ability to stimulate naive T cells (Urban *et al*. 2001; Stuart *et al*. 2002). Therefore, clearance of neutrophils could have additional immune regulatory consequences.

The role of neutrophils in the host defence against intracellular infections was long neglected. One reason for this was the belief that intracellular pathogens, by growing inside macrophages, were sheltered from the phagocytic activity of neutrophils. The classical view of the host defence mechanisms against intra-macrophagic infections was that the macrophage is both the host and the effector cell – with T lymphocytes playing a crucial role in activation of the macrophage antimicrobial mechanisms (Adams and Hamilton, 1984). However, advances in the knowledge of host defence mechanisms against intracellular pathogens have demonstrated that neutrophils are important partners of macrophages in those mechanisms.

Studies with experimental murine mycobacteriosis showed for the first time a chronic recruitment of neutrophils to mycobacterium-infected foci (Silva, Silva and Appelberg, 1989). Furthermore, these same studies demonstrated that: (1) in mycobacterium-infected inflammatory exudates neutrophils were phagocytosed by macrophages and the neutrophilic molecule lactoferrin was extensively transferred to macrophages; (2) such a transfer preferentially occurred to infected macrophages; and (3) the *in vitro* anti-mycobacterial activity of peritoneal macrophages was increased when macrophage cultures were supplemented with neutrophil material. These observations led to the concept that neutrophils participate in the control of intra-macrophagic infections by a mechanism of neutrophil-macrophage co-operation whereby macrophage anti-microbial ability is increased by the ingestion of neutrophils or neutrophilic molecules (Silva *et al*. 1989). At that time, the ingestion of senescent neutrophils by macrophages was considered a possible mechanism for the interaction between the two phagocytes, but association of neutrophil senescence to apoptosis was still unknown. The observation that selective neutrophil depletion by a monoclonal antibody rendered mice more susceptible to experimental mycobacteriosis (Appelberg *et al*. 1995), supported the interpretation that neutrophils were involved in the defence mechanisms against intra-macrophagic mycobacterial infections. Following the initial observations with mycobacterial murine infections (Silva *et al*. 1989), several reports described neutrophil participation in the control of intra-macrophagic infections by other intracellular pathogens including *Listeria, Salmonella, Yersinia, Francisella, Chlamydia* and *Toxoplasma* (reviewed in Pedrosa *et al*. 2000).

Besides the neutrophil-macrophage co-operation with transfer of neutrophilic anti-microbial materials to the macrophage, other modalities of neutrophil participation in the control of intra-macrophagic infections must be considered. These include: (1) transfer to macrophages of pathogens ingested by neutrophils, through the phagocytosis of infected neutrophils (Silva *et al*. 1989; Afonso *et al*. 1998; Gregory and Wing, 2002; van Zandbergen *et al*. 2004). This transfer would pass on to the macrophages the task of eliminating pathogens that the neutrophil ingests but cannot eliminate. The simultaneous transfer of neutrophil molecules would potentiate the macrophage capacity to destroy the pathogen; (2) secretion of neutrophilic granule components that can activate infected macrophages (Lima and Kierszenbaum, 1985; Lincoln *et al*. 1995); and (3) immunomodulation through production of cytokines and chemotactic factors (reviewed in Pedrosa *et al*. 2000).

The role neutrophils play can also be deleterious for the host. Studies that compared genetically susceptible and resistant mice found that neutrophils play either protective or deleterious roles in responses to infection, depending on host genetic background. In *T. cruzi* infection, neutrophils protected BALB/c mice by increasing Th1 T cell responses, but aggravated infection of B6 mice by reducing Th1 responses (Chen *et al*. 2001). On the other hand, early neutrophil recruitment induced susceptibility of BALB/c mice to *L. major* infection by instructing a Th2 T cell response (Tachini-Cottier *et al*. 2000).

FAS LIGAND REGULATES *LEISHMANIA* INFECTION BY PROMOTING NEUTROPHIL RECRUITMENT AND RAPID NEUTROPHIL CLEARANCE

Recent studies demonstrated that engagement of Fas death receptor in resident macrophages promotes neutrophil recruitment (Hohlbaum *et al*. 2001). Since Fas ligand (FasL) regulates host responses to infectious diseases (Dockrell, 2003), the role of FasL on neutrophil clearance was investigated in *L. major* infection of susceptible BALB/c mice Ribeiro-Gomes *et al*. (2005). Expression of FasL was deleterious for the host, since FasL-deficient *gld* mutant

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mice were more resistant to infection. Injection of promastigotes into the peritoneal cavity attracted neutrophils in wild-type, but not in *gld* mice (Ribeiro-Gomes *et al*. 2005). Neutrophil recruitment was concomitant with resident macrophage apoptosis and chemokine secretion. Apoptosis was mediated by Fas receptor and was absent in *gld* macrophages. These results agree with an important role of FasL in macrophage apoptosis and neutrophil recruitment (Hohlbaum *et al*. 2001). Recently, conditional ablation of macrophages also demonstrated that peritoneal macrophages are required for neutrophil recruitment to the inflamed peritoneal cavity (Cailhier *et al*. 2005). Since *gld* mice expressed increased levels of tissue resident neutrophils, interactions of neutrophils with infected macrophages were investigated. Both live and dead wild-type neutrophils exacerbated *Leishmania* replication in macrophages through a mechanism dependent on TGFβ production. Dead *gld* neutrophils also exacerbated parasite growth, but live *gld* neutrophils induced NO-dependent killing of *Leishmania* (Ribeiro-Gomes *et al*. 2005). Kinetic experiments demonstrated that *gld* neutrophils remained alive for longer periods, and that clearance by macrophages was delayed. In agreement, delaying the death and clearance of wild-type neutrophils with an anti-FasL antibody abolished exacerbation of parasite growth and allowed macrophages to control infection. Therefore, the leishmanicidal activity of *gld* neutrophils derived from their increased lifespan and co-operation with macrophages. These results were confirmed *in vivo*, showing that neutrophil depletion abolished increased susceptibility of wild-type over *gld* mice (Ribeiro-Gomes *et al*. 2005). These results suggest that FasL exacerbates *Leishmania* infection in susceptible hosts at two steps. First, *Leishmania* induces resident macrophage apoptosis through FasL, which attracts neutrophils. Second, FasL accelerates the rates of neutrophil death and clearance. Ingestion of senescent neutrophils functionally deactivates macrophages, allowing increased *Leishmania* replication. On the other hand, delaying neutrophil apoptosis plays a protective role, presumably through macrophage activation by products from live neutrophils.

OPPOSITE OUTCOMES OF NEUTROPHIL CLEARANCE IN GENETICALLY DISTINCT HOSTS

Most studies on neutrophil clearance have employed resting blood neutrophils in which apoptosis was induced by irradiation or aging. However, the physiological setting of parasitic infection involves interactions with neutrophils that have undergone trans-endothelial migration and activation. In this regard, inflammatory neutrophils differ from resting neutrophils, since they actively degranulate, releasing proteases in the extracellular medium (Rainger, Rowley and Nash, 1998). Interactions of inflammatory neutrophils with *Leishmania*-infected macrophages were investigated in susceptible and resistant hosts (Ribeiro-Gomes *et al*. 2004). Live and dead neutrophils from susceptible BALB/c mice exacerbated *Leishmania* growth in macrophages by a mechanism dependent on cell contact and TGFβ, similar to that described for *T. cruzi* growth driven by apoptotic lymphocytes (Freire-de-Lima *et al*. 2000). In agreement, neutrophil depletion *in vivo* reduced parasite loads in infected BALB/c mice. Surprisingly, neutrophil depletion exacerbated infection in resistant B6 mice, suggesting that neutrophils protect against infection in B6 mice. In fact, live or dead B6 neutrophils induced *Leishmania* killing in macrophages by a mechanism dependent on TNFα that did not require cell contact (Ribeiro-Gomes *et al*. 2004). The neutrophil serine protease Neutrophil Elastase (NE) activates human macrophages and induces TNFα secretion (Fadok *et al*. 2001). Since inflammatory neutrophils secrete NE, we investigated the role of NE in defence against *Leishmania*. The NE inhibitor peptide MeOSuc-AAPV-cmk prevented macrophage leishmanicidal activity in the presence of neutrophils. Furthermore, injection of MeOSuc-AAPV-cmk *in vivo* exacerbated *L. major* infection in resistant B6 mice (Ribeiro-Gomes *et al*. 2004). These results suggest a role for NE in the pro-inflammatory and microbicidal function of B6 neutrophils. Although functional differences between BALB/c and B6 neutrophils are not completely understood, inflammatory B6 neutrophils release more NE into supernatants than BALB/c neutrophils (Ribeiro-Gomes and DosReis, unpublished results). Following neutralization of NE or TNFα activity, clearance of B6 neutrophils becomes anti-inflammatory, like BALB/c neutrophils (Ribeiro-Gomes *et al*. 2004). This result suggests that previous contact with soluble NE disables the anti-inflammatory signalling pathway induced by contact with the neutrophil corpse.

Another study found that phagocytosis of stressed apoptotic neutrophils – generated by contact with bacteria – also activates macrophages and induces TNFα secretion. Macrophage activation required expression of heat shock proteins HSP60 and HSP70 by neutrophils (Zheng *et al*. 2004). It has been suggested that HSPs are endogenous ligands for TLRs (Binder, Vatner and Srivastava, 2004), and that NE activates TLR4 on bronchial epithelial cells (Devaney *et al*. 2003). Therefore, it is possible that under certain conditions, senescent neutrophils express TLR ligands and activate macrophages during the process of phagocytic clearance. The proposed differences in the outcome of phagocytic clearance of BALB/c and B6 neutrophils are summarized in Fig. 1. However, it should be noted that, in order to prevent inflammation, additional mechanisms

counteracting the proinflammatory clearance of B6 neutrophils must exist.

CONCLUDING REMARKS AND PROSPECTS FOR THE FUTURE

Host immune responses to *Leishmania* infection are under the control of several independent genes (Lipoldova *et al*. 2000). Our results suggest that a genetic polymorphism exists in innate macrophage activation by clearance of neutrophils. Mapping of the genes involved will be important for understanding genetic differences in innate resistance to *Leishmania* infection among human subjects. In addition, clearance of dead neutrophils affects DC maturation and costimulatory activity for T cells (Stuart *et al*. 2002). It will be important to investigate the roles of neutrophil clearance in DC interactions with T cells in the course of *Leishmania* infection.

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