An apoptosis differentiation programme in human polymorphonuclear leucocytes

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Abstract

Human PMNs (polymorphonuclear leucocytes or neutrophils) are essential to the innate immune response against bacterial pathogens and are a key part of the acute inflammatory response. Although progress has been made, the molecular basis for termination of inflammation during bacterial infection in humans is largely undefined. To that end, we used genomics strategies to gain new insight into processes that facilitate resolution of neutrophil-mediated inflammation and bacterial infection. On the basis of a series of recent studies, we propose that global changes in PMN gene expression after phagocytosis comprise an apoptosis differentiation programme, which represents the final stage of transcription-regulated PMN maturation. Our studies indicate that the apoptosis differentiation programme regulates multiple post-phagocytic processes in human neutrophils, such as cell fate and proinflammatory activity, and is modulated by PMN-derived reactive oxygen species. Collectively, these studies establish a global model of host cell-pathogen interaction, which provides fundamental insight into the resolution of infection in humans.

Introduction

Human PMN (polymorphonuclear leucocytes) are essential components of the innate immune response and function as the primary host defence against invading bacterial pathogens. PMNs are recruited to sites of infection, remove pathogens via phagocytosis, and subsequently kill ingested micro-organisms through oxygen-dependent and -independent mechanisms [1]. Initiation of the neutrophil-mediated acute-phase inflammatory response is crucial for the resolution of infection. However, PMNs are also implicated in the pathogenesis of tissue injury and trauma accompanying inflammatory diseases [2]. Therefore it follows that the timely removal of activated PMNs from affected sites is paramount to the resolution of inflammation. Neutrophil apoptosis provides a probable explanation towards the resolution of inflammation due to PMN activation [3,4].

The indiscriminate release of highly toxic microbialidal components as a consequence of neutrophil lysis can lead to host tissue damage and a persistent and acute inflammatory response [5]. It has been proposed that normal turnover of aging PMNs in tissues is accomplished by a constitutive or ‘spontaneous’ apoptotic process, which enables these cells to be recognized and cleared by macrophages, thereby preventing host tissue damage and inflammation [4]. Consistent with this idea, the overall importance of apoptosis in homoeostasis of the immune system has been suggested for many other cell types [6,7]. Inasmuch as massive influx of neutrophils to infected tissues has the potential to cause severe host injury, one would predict that regulation of neutrophil fate is critical during infection. Indeed, recent studies indicate that neutrophil apoptosis is accelerated significantly following phagocytosis of bacteria [8–13], although the molecular basis for this process is incompletely characterized. If spontaneous apoptosis is a mechanism intended to prevent spurious inflammation under normal circumstances, then activation-induced PMN apoptosis may very well represent an alternative means to resolve inflammation arising from infection. In the present paper, we describe a gene-regulated apoptosis differentiation programme in human neutrophils, which we suggest facilitates the resolution of infection.

Phagocytosis induces global changes in PMN gene expression

Neutrophils are terminally differentiated cells that have extraordinary capability to generate and receive many diverse signals. Signal transduction in neutrophils governs important cellular events, such as maturation and differentiation. Not surprisingly, these processes are facilitated by new gene transcription during growth and development [14,15]. Throughout maturation, neutrophils remain transcriptionally active and accumulate proteins necessary for phagocytosis, degranulation and NADPH oxidase-dependent killing [14]. As a result, mature neutrophils can migrate to infected tissues and respond rapidly to pathogenic insult. Paradoxically, PMN transcriptome analyses have revealed that these fully mature terminal cells also demonstrate complex patterns of gene expression in both resting and activated states [16–18].

Recently, we used oligonucleotide microarrays to identify changes in gene expression resulting from receptor-mediated phagocytosis in human neutrophils [16]. First, we determined...
that unactivated PMNs isolated from peripheral blood contain transcripts of at least 3000 genes, a remarkable number not appreciated previously [16]. This important finding suggested that new gene transcription probably plays a significant role in mediating post-phagocytosis sequelae in neutrophils. In all, 256 unique genes were induced or repressed 90 min after receptor-mediated phagocytosis [16]. Consistent with their role in initiation and execution of the acute inflammatory response, PMNs express numerous inflammatory mediators in a relatively short period of time after activation. For example, increased expression of genes encoding MIP (macrophage inflammatory protein)-3α (CCL20), oncostatin M, GRO-β (CXCL2), GRO-γ (CXCL3), TNFα (tumour necrosis factor α), galectin 3 (LGALS3), vascular endothelial growth factor, MIP-1α (CCL3) and MIP-1β (CCL4) occurs within 90 min after receptor-mediated phagocytosis [16,17]. These cytokines function as signalling molecules necessary for the recruitment and activation of neutrophils and other immune effector cells [1].

At least 30 genes encoding apoptosis mediators, such as the nuclear orphan receptors TR3, NOR1 and NURR1 are differentially expressed within 90 min after PMN phagocytosis [16]. Remarkably, >800 genes are up- or down-regulated between 3 and 6 h post-phagocytosis, including 127 cell fate-related genes. For example, genes encoding TNFα, TNFα receptor (TNFRSF1B), transforming growth factor-β inducible early growth response, caspase 1 and TLR2 (Toll-like receptor 2) are up-regulated [16]. The onset of apoptosis in activated PMNs correlates well with changes in expression of these and other apoptosis-related genes [16,21,22]. Importantly, actinomycin D and cyclohexamide, inhibitors of transcription and translation respectively, completely block phagocytosis-induced apoptosis (results not shown). These results indicate that PMN apoptosis is regulated partially by gene transcription, a finding that has important implications for our understanding of the pathogenesis of inflammatory diseases.

**PMN apoptosis differentiation programme**

It is becoming increasingly clear that phagocytosis of bacterial pathogens and/or interaction with bacterial products accelerates apoptosis in human PMNs. *Escherichia coli* [8], *Mycobacterium tuberculosis* [9], *Neisseria gonorrhoeae* [10], *Streptococcus pneumoniae* [11] and *Staphylococcus aureus* [12,13] have been shown to induce rapid apoptosis in neutrophils. *Anaeroplasma phagocytophilum*, the causative agent of human granulocytic ehrlichiosis, is one of the few agents identified thus far that prolongs neutrophil survival once ingested [30,31]. *A. phagocytophilum* is an obligate intracellular pathogen that survives within normal human PMNs and failure of this pathogen to promote neutrophil survival would probably result in the inability of these organisms to replicate. Taken together, a majority of studies thus far indicate that phagocytic interaction with bacteria accelerates neutrophil apoptosis. These findings are not a complete surprise as the process of PMN phagocytosis accelerates apoptosis [16,19–22,32]. Although progress has been made towards understanding these processes, the mechanisms responsible for PMN apoptosis induced by phagocytosis remain to be elucidated.

To gain insight into molecular processes that facilitate the resolution of infection, we used oligonucleotide microarrays to query the neutrophil transcriptome after ingestion of a diverse group of human bacterial pathogens [13]. PMN phagocytosis of *Borrelia hermsii*, *Burkholderia cepacia*, *Listeria monocytogenes*, *S. aureus* and *S. pyogenes* revealed a core group of genes that collectively form a common transcriptional response [13]. A total of 305 genes were up-regulated and 297 were down-regulated over a period of 6 h after phagocytosis. Of these genes, 105 encoded key factors that participate in the initiation and execution of apoptosis, including TNFα, TNFα-receptor-induced protein (GG2-1), TNFα receptor-associated factor 1, TNFSF10 (TRAIL,

**Down-regulation of proinflammatory capacity accompanies neutrophil apoptosis**

As mentioned previously, neutrophils play a prominent role in the initiation and execution of the inflammatory response. Consistent with the idea that apoptosis facilitates the resolution of inflammation after phagocytosis, PMNs down-regulate expression of genes encoding proinflammatory mediators [22]. For instance, genes encoding TNFα, vascular endothelial growth factor, oncostatin M, IL (interleukin)-6, CXCL2 and CXCL3, which play important roles in the early modulation of inflammation and/or recruitment of other leukocytes, are up-regulated early due to PMN activation [16,22]. However, expression of these up-regulated proinflammatory molecules decreases significantly as apoptosis progresses [22]. Consistent with that observation, genes encoding 133 key proinflammatory mediators or signal transduction molecules are down-regulated during the initial stages of apoptosis. Repression of the gene encoding IL-8Rβ (CXCR2, CXC chemokine receptor 2) correlates with down-regulation of the surface expression of this protein [22]. This finding is significant in that the IL-8 receptor plays a prominent role in the modulation of PMN inflammatory responses [23,24]. Phosphoinositide metabolism and calcium-mediated signal transduction regulate important PMN functions including IL-8 signalling [24–29]. Ligation of cell surface receptors such as CXCR2 activates phosphoinositide 3-kinases, which in turn regulate second messengers involved in signal transduction events that lead to cell activation [24–29]. Thus the finding that 19 genes encoding proteins involved in phosphoinositide 3-kinase and calcium signal transduction are down-regulated [22] is consistent with the view that there is overall down-regulation of proinflammatory capacity during PMN apoptosis. Inasmuch as IL-8 is a potent chemokine that modulates key leucocyte responses such as chemotaxis [24,29], it is probable that down-regulation of CXCR2 during apoptosis is critical for the resolution of inflammation after neutrophil activation.
TNF-related apoptosis-inducing ligand), TNFRSF10C (TRAILR3) and tumour suppressing subtransferable candidate 3 [13]. In addition, genes encoding nearly a dozen members of the TLR2 signal transduction pathway were up-regulated [13]. These findings suggest that bacteria induce an apoptosis differentiation programme in human PMNs, which facilitates rapid neutrophil turnover during infection.

Of the pathogens tested, S. pyogenes alone induces unique changes in neutrophil gene expression [13]. In all, expression of 393 neutrophil genes (168 up-regulated and 225 down-regulated) change due to phagocytosis of S. pyogenes, including 71 cell fate-related genes. For example, phagocytic interaction with S. pyogenes up-regulates activator protein (1) complex related genes FOS, FOSL1, FOSB, JUNB and TNFRSF5 (CD40). The activator protein (1) complex has been shown to participate in the induction of apoptosis [33]. Furthermore, S. pyogenes modulates the expression of at least 26 neutrophil genes involved in responses to interferon and repressed several members of the JAK-STAT cell survival pathway [13].

Our previous studies have demonstrated that differential expression of key regulators of apoptosis correlates well with the initiation of programmed cell death in human PMNs [16,21,22]. Thus we hypothesized that phagocytosis of B. cepacia, B. hensiti, L. monocytogenes, S. aureus and S. pyogenes accelerates apoptosis in human PMNs. In addition, unique neutrophil gene expression patterns induced by live S. pyogenes suggested an accompanying difference in PMN apoptosis. In fact, each of the five bacterial species tested accelerates PMN apoptosis, and notably, apoptosis caused by S. pyogenes is more rapid by comparison and leads to neutrophil necrosis [13].

There are three notable implications of these findings. First, accelerated neutrophil apoptosis after phagocytosis of bacteria is a normal phenomenon that contributes to the resolution of infection. Secondly, alteration of PMN fate is a critical for the resolution of inflammation. We propose that these transcription-regulated processes involve an apoptosis differentiation programme, which represents the final stage of PMN maturation and is critical for the resolution of inflammation.

Concluding remarks
The ability of PMNs to mobilize rapidly and mount an appropriate response to pathogenic insult is critical to the overall maintenance of health in humans. As terminal cells, neutrophils have the ability to ingest and kill microbial pathogens without new transcription. Nevertheless, recent evidence indicates that new gene transcription initiates important processes involved in the down-regulation of pro-inflammatory capacity in activated PMNs, facilitating rapid cell turnover. Phagocytosis of bacterial pathogens initiates a cascade of molecular signalling events that culminate in accelerated programmed cell death essential for the resolution of infection. We propose that these transcription-regulated

References

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