What’s wrong with neutrophils in lupus?

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ABSTRACT

Polymorphonuclear neutrophils (PMNs) act by promoting phagocytosis, and are regarded as the first line of defense against pathogen invasion. However, recent investigations have revealed that they have many previously unknown functions. These functions include mitogen-induced cell-mediated cytotoxicity, production of cytokines/chemokines/growth factors, and release of neutrophil extracellular traps (NETs) and ectosomes/exosomes. Membrane exchange (trococytosis) is also noted following direct cell-cell contact with other immune cells for modulating innate and adaptive immune responses. These observations strongly suggest that neutrophils may play an important role in the immune network. Systemic lupus erythematosus (SLE) is a prototype of systemic autoimmune diseases characterised by the production of diverse antigen-driven autoantibodies against intra- and extracellular molecules. Multiple immune dysfunctions have been reported in SLE patients. These include excessive interferon alpha (IFN-α) expression, aberrant cytokine/chemokine/growth factor production, skewing of T cell immune responses toward Th2 and Th17 pathways, polyclonal B cell activation, increased apoptosis and NET formation, defective clearance of cell debris and NET-related molecules, and abnormal ectosome/exosome release in the plasma. We have demonstrated that SLE-PMNs per se exhibit aberrant cytokine/chemokine expression, defective glucose metabolism, and increased mitochondrial DNA D310 heteroplasmy with reduced redox capacity. Our data also indicate that autoantibodies purified from SLE sera disrupt PMN functions. In the present review, we discuss these abnormalities in detail and attempt to elucidate the potential roles of disrupted PMN functions in lupus pathogenesis.

Introduction

The pathogenesis and development of SLE is multifactorial (1), implicating genetic (2, 3), epigenetic (4-7), environmental (8) and hormonal (9) factors, and mitochondrial dysfunctions (10). The intriguing interactions among these factors ultimately elicit a perpetual vicious cycle of chronic inflammation, tissue damage, and autoimmunity in lupus patients. The disease is usually regarded as an adaptive immunity-mediated systemic autoimmune disorder. However, defects in innate immunity such as defective NK cell activity (11), disrupted IL-18 expression (12), and hereditary C2/C4 hypocomplementae mia (13) have been reported. Moreover, Hachbarth et al. (14) and Bennett et al. (15) have demonstrated that presence of low-density granulocytes (LDGs) with excessive IFN-α expression is a signature of SLE. Polymorphonuclear leukocytes (PMNs) or neutrophils are traditionally regarded as first-line defense cells carrying out phagocytosis against microbial invasion. Nevertheless, Yue et al. (16) and Dallegri et al. (17) have conducted in vitro studies, demonstrating that PMNs can mediate cytotoxicity in the presence of a mitogen (mitogen-induced cell-mediated cytotoxicity). Upon stimulation with interleukin 4 (IL-4), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon gamma (IFN-γ), PMNs can express major histocompatibility complex II (MHC-II) and the co-stimulatory molecules, cluster of differentiation (CD)80 and CD86 (18-20). These unusual PMNs can present various antigens to T cells and act like antigen-presenting cells. Oehler et al. (21) further reported that a 9-day culture of PMNs in the presence of GM-CSF, IL-4, and IFN-α resulted in elevated allogenic stimulatory activity to present specific tetanus toxoid antigen to autologous memory T cells.

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**Table I. In vitro and in vivo cytokine expression in normal polymorphonuclear neutrophils (PMNs).**

<table>
<thead>
<tr>
<th>Cytokine/Chemokine</th>
<th>In vitro</th>
<th>In vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β (+/-1β)</td>
<td>IL-1α</td>
<td></td>
</tr>
<tr>
<td>IL-1α</td>
<td>IL-1β</td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td>IL-8</td>
<td></td>
</tr>
<tr>
<td>IL-12</td>
<td>IL-6</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>IL-8</td>
<td></td>
</tr>
<tr>
<td>IFN-α</td>
<td>IL-10</td>
<td></td>
</tr>
<tr>
<td>CD30L</td>
<td>IL-12</td>
<td></td>
</tr>
<tr>
<td>GR0α, GR0β</td>
<td>MIP-2</td>
<td></td>
</tr>
<tr>
<td>CINC-1, 2α, 3</td>
<td>KC/GR0α</td>
<td></td>
</tr>
<tr>
<td>IP-10</td>
<td>CINC</td>
<td></td>
</tr>
<tr>
<td>MIP</td>
<td>MIP-1α</td>
<td></td>
</tr>
<tr>
<td>MIP-1α (-/-1β)</td>
<td>MIP-1β</td>
<td></td>
</tr>
<tr>
<td>TGF-α, TGFβ1</td>
<td>MCP-1</td>
<td></td>
</tr>
<tr>
<td>IL-3, G-CSF, M-CSF</td>
<td>TNF-α</td>
<td></td>
</tr>
<tr>
<td>GM-CSF (?), IL-6 (?) , MCP-1 (?)</td>
<td>TGFβ1</td>
<td></td>
</tr>
<tr>
<td>SCF (?)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adapted from Cassatella MA et al. in “The neutrophils: new outlook for old cells”, Imperial College Press 1999, p. 151-229.
KC/GR0α: growth regulated protein alpha =CXCL-1; GR0β=CXCL-2; CINC-1: cytokine induced neutrophil chemoattractant 1; MIP: mono-kine induced by interferon gamma=CXCL-9; SCF: stem cell factor; CSF: colony stimulating factor; MIP: macrophage inflammatory protein; ra: receptor antagonist

Takashima and Yao (20) reviewed those data and found that the authors used >99.5% ultrapure Gr-1<sup>hi</sup>/CD48<sup>−</sup> cells following fluorescence-activated cell sorting (FACS), or immunomagnetic beads-sorted PMNs from bone marrow or adult C57BL/6 mice for their experiments. They concluded that neutrophil plasticity is determined by the environment to which the cells are exposed. These results suggest that PMNs can initiate and participate in coordinating immune adaptation. Recently, many investigators have demonstrated that PMNs per se, work as key players in orchestrating innate and adaptive immune responses via different mechanisms (22-27).

Intercellular communications can be achieved through many pathways including cytokine release (28), immunological synapse formation (29, 30) with subsequent membrane exchange (trogocytosis) (31), nanotubular transfer (32), and multiple extracellular vesicle release (32-34). Among these, cytokines can potentially initiate a complex network of cell-cell communications to modulate innate and adaptive immune responses. Many researchers have reported that PMNs per se can produce a number of regulatory cytokines and chemokines (35-37). As mentioned above, highly purified PMNs affect the functions of dendritic cells, monocytes/macrophages, NK cells, T cells, and B cells in addition to the defense mechanism against infections (22-27, 38). Table I (adapted from Cassatella et al. in “The neutrophils: new outlook for old cells”, Imperial College Press P151-229, 1999) lists in vitro and in vivo cytokine gene expression in normal PMNs. Moreover, PMNs can release different microvesicles such as exosomes (34, 39-44) and exosomes (32, 45-47) into body fluids to modulate the maturation and immune responses of remote dendritic and mononuclear cells. The multifaceted functions of PMNs contribute to both anti-microbial capacity and immunomodulation in innate and adaptive immunities, as evidenced by in vitro studies (48).

In animal experiments, complete depletion of neutrophils (<0.5%) in rats with monoclonal anti-granulocyte antibody, RP-3, but without depletion of lymphocytes, monocytes, NK cells, basophils, or eosinophils, altered the adaptive immune response and caused memory-type CD8<sup>+</sup>T cell exhaustion (49-51). These results have further confirmed that neutrophils play a modulatory role in innate and adaptive immunity in vivo. The classical and previously unknown functions of human PMNs are summarised in Table II. Accordingly, aberrant PMN functions may potentially derange mononuclear cell responses in some autoimmune and inflammatory diseases. In this review, we will focus in detail on the previous findings on PMNs, the molecular basis of aberrant neutrophil functions, and their relevance to lupus pathogenesis.

**Table II. Classical and previously unknown functions of normal polymorphonuclear neutrophils (PMNs).**

<table>
<thead>
<tr>
<th>Classical functions</th>
<th>Previously unknown functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phagocytosis and killing of microbial pathogens</td>
<td>Immune complex and cell debris clearance [34, 36, 145]</td>
</tr>
<tr>
<td>Vascular endothelial cell adhesion and extravasation</td>
<td>Mitogen-induced cell-mediated cytotoxicity (MCC) [36-37]</td>
</tr>
<tr>
<td>Release of granular proteins</td>
<td>Expression of MHC-II antigens and co-stimulatory molecules (CD80 and CD86), following the stimulation of IL-4, GM-CSF, and IFN-γ [146-148]</td>
</tr>
<tr>
<td>Production of oxygen and superoxide radicals</td>
<td>Production of cytokines/chemokines/growth factors [32, 33]</td>
</tr>
<tr>
<td>Induction of immune inflammation in vitro [22-27, 46] and in vivo [49-51] via cell–cell contact at the site of the immunological synapse [29-31] and subsequent trogocytosis [30, 145]</td>
<td></td>
</tr>
<tr>
<td>Cross-talk with different cells via release of exosomes [34, 44-46] and multiple vesicle bodies (exosomes, microvesicles) [35, 46-47]</td>
<td>NETosis following stimulation with extrusion of intracellular molecules to form NETS [35, 94, 96-102]</td>
</tr>
</tbody>
</table>

MHC-II: major histocompatibility complex class II; CD: cluster of differentiation; IL: interleukin; GM-CSF: granulocyte macrophage-colony-stimulating factor; IFN: interferon; NET: neutrophil extracellular trap; NETosis: apoptosis of neutrophil to form neutrophil extracellular trap.

**Defective phagocytosis, aberrant cytokine/chemokine production, and hyporesponsiveness of PMNs to IL-8 predispose susceptible SLE patients to infections**

Susceptibility to infections is a major cause of morbidity and mortality in SLE patients (52). Our previous studies have revealed that defective phagocytosis, decreased TNF-α production, and lymphocyte hyporesponsiveness to stimuli make SLE patients more susceptible to infections (53). We have also demonstrated that defective C3b expression in PMNs and impaired lymphocyte Na<sup>+</sup>-K<sup>+</sup>-ATPase activity are crucial predisposing factors for SLE (54). Na<sup>+</sup>-K<sup>+</sup>-ATPase is critical because it generates the ion gradients required by cells, and defective expression of this membrane enzyme renders PMNs susceptible to infections.
and mononuclear cells sluggish to stimulation in SLE patients (55). Ren et al. (56) have reported that increased PMN and macrophage apoptosis, and impaired macrophage phagocytic clearance of apoptotic neutrophils, are responsible for leukopenia, susceptibility to infections, and autoimmunity in SLE. To determine whether circulating SLE-related autoantibodies impair PMN functions, we purified various autoantibodies from SLE sera to test their effects on immune cells and found that anti-cardiolipin (57), anti-dsDNA (58), and anti-SSB/La (59) antibodies reduced PMN phagocytosis and caused early enhancement but late suppression of IL-8 production through activation-induced cell death (AICD). In contrast, anti-neutrophil cytoplasmic antibodies, including anti-myeloperoxidase and anti-proteinase 3, conversely promoted phagocytosis, IL-8 production, and glucose uptake by neutrophils (60). Table III summarises the effects of various SLE-related autoantibodies on normal PMN functions.

Abramson production of and skewed responsiveness to cytokines/chemokines in SLE-PMNs are also key factors for increased infection rates in lupus patients. Hsieh et al. (61) have demonstrated reduced spontaneous and bacterial lipopolysaccharide (LPS)-stimulated IL-8 production by SLE-PMNs, resulting in an inability to counteract IL-1-mediated inflammatory reactions and subsequent tissue damage in SLE patients. To understand the molecular basis of aberrant PMN functions in SLE, Wu et al. (66) measured genomic telomere DNA lengths in both PMNs and peripheral blood mononuclear cells (PBMCs) in SLE patients. They concluded that premature and accelerated telomere shortening is an independent feature of aberrant neutrophil biology in active SLE. In addition, Zhou et al. (67) confirmed that expression of telomere maintenance genes TP53, TIN2, POT1, and KU80 is significantly reduced, whereas that of TRF2 and MRE11 is enhanced in SLE patients. These results indicate that both innate and adaptive immune cells prematurely enter the senile stage and exhibit abnormal cell functions. It is worth noting that the purity of PMNs used in these experiments was approximately 95–98%. However, we cannot exclude the possibility that the levels of contamination by monocytes, T cells, or B cells, despite being low, might be sufficient to produce small amounts of cytokines/chemokines/growth factors and mediate immunomodulation. Recently developed purification techniques such as micro-beads for positive or negative selection have enabled investigators to achieve a PMN purity of more than 99.5%. Quite consistent results can be obtained using such ultrapure PMN populations. In summary, defective PMN functions make SLE susceptible to infections and the subsequent inflammation-induced tissue damage may release excessive intracellular autoantigens to stimulate autoantibody production in patients with SLE.

### Table III. Effects of various systemic lupus erythematosus (SLE)-related autoantibodies on polymorphonuclear neutrophil (PMN) functions

<table>
<thead>
<tr>
<th>PMN functions</th>
<th>Anti-CL</th>
<th>Anti-dsDNA</th>
<th>Anti-SSB/La</th>
<th>Anti-MIP</th>
<th>Anti-PR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phagocytosis</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>IL-8 production</td>
<td>ND</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>AICD</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Glucose uptake</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>


### Table IV. Abnormal systemic lupus erythematosus-polymorphonuclear neutrophil (SLE-PMN) functions and their effects on autologous mononuclear cell cytokine expression

**Abnormal SLE-PMN functions**

1. Defective phagocytosis and microbial killing capacity; 147, 148
2. Enhanced apoptosis and decreased cell debris clearance; 53, 54, 137
3. Increased cytidine deaminase production; 68
4. Spontaneous and bacterial LPS-induced cytokine production: IL-1ra; 147
5. Decreased surface lactoferrin expression and its transfer to autologous mononuclear cells; 108, 149
6. Increased oxidative stress due to: Deranged bioenergetics; 60
7. Effects on autologous mononuclear cytokine production: IL-1ra; 147
8. Exaggerated NETosis; 52–54, decreased NET clearance; 56, 92, 112–117, and increased pro-inflammatory low-density granulocyte formation; 147, 148
9. Aberrant ecotosome and exosome release (?)
10. Premature telomere shortening; 68, 69

*LPS: lipopolysaccharide; IL-1ra: interleukin 1 receptor antagonist; CXCR: CXC chemokine receptor; ATP: adenosine triphosphate; GLUT: glucose transporter, GSH-Px: glutathione peroxidase; GSSG-R: glutathione reductase; GGT: γ-glutamyl-transpeptidase; IL: interleukin; CD: cluster of differentiation; IFN: interferon; NET: neutrophil extracellular traps.*
Abnormal immunometabolism increases oxidative stress and apoptosis in SLE-PMNs

Normal neutrophils are highly dependent on glucose for ATP generation via anaerobic glycolysis (68-70). This is evidenced by the fact that PMNs contain few mitochondria and consume little oxygen (71). Li et al. (72) reported that the disrupted bioenergetics of SLE-PMNs are due to the defective expression of glucose transporters (GLUT) 3 and 6, increased basal intracellular lactate concentration, and reduced ATP production. Moreover, the redox capabilities of SLE-PMNs including the levels of intracellular glutathione (GSH) and redox-related enzymes (glutathione peroxidases/GSH-Px, glutathione reductase/GSSGR, γ-glutamyl-transpeptidase/GGT, and CD53) are all diminished. These abnormal immuno-metabolism results in increased oxidative stress and accelerated apoptosis in patients with SLE (72). Lee et al. (73) have demonstrated that the expression levels of human 8-oxoguanine DNA glycosylase 1, anti-oxidant enzymes, mitochondrial biogenesis-related proteins, and glycolytic enzymes are reduced in SLE leukocytes. Furthermore, the defective mitochondria-related redox capacity in SLE-PMNs was found to be parallel with a decrease in mitochondrial DNA copy number and an increase in D310 heteroplasmy (10, 74, 75). These metabolic defects could certainly enhance oxidative stress, apoptosis, and NETosis in SLE-PMNs. It is believed that these immunometabolic changes are not only caused by hereditary predisposition but also by environmental metabolites and extracellular vesicles released from surrounding cells, as reported by de Candia et al. (76). In brief, sophisticated genetic (2, 3, 77, 78) and epigenetic (4-7) predispositions, and defective immunometabolic status (68-72, 76) can further obfuscate the abnormal innate and adaptive immune responses in patients with SLE. Table IV summarises the aberrant PMN functions and their effects on autologous mononuclear cell immune responses in SLE. The aberrantly released molecules from PMNs relevant to abnormal adaptive immune responses and lupus pathogenesis are summarised in Table V.

Generation of low-density granulocytes (LDG) in SLE

PMNs were discovered by Paul Ehlich, while Elie Metchnikoff pioneered the fundamental works on PMN biology over 100 years ago (79). PMNs are the most abundant leukocytes in peripheral blood, accounting for over 2 × 10¹⁰ cells in blood circulation. The lifespan of circulating PMNs is usually quite short (6–8 h) (80). However, longer lifespans have been observed in human PMNs (up to 5.4 days) (81, 82). Hsieh et al. (83) demonstrated that the Fas/CD95 death pathway is activated in PMNs. These mature PMNs, interacting with neighbouring neutrophils already expressing both Fas and FasL on their cell surfaces, are one of the sources for spontaneous PMN apoptosis. A lack of proto-oncogene c-myc expression in PMNs is responsible for their non-proliferative nature. Amanullah et al. (84) demonstrated that deregulated c-myc expression in normal myeloid cells blocks the differentiation of c-myc itself owing to premature recruitment of the Fas/CD95 death pathway, which normally induces apoptosis at the end of the differentiation program. PMNs account for 50–70% of circulating white blood cells, and serve as the first line of defense against infections and tumour-associated inflammatory conditions in human diseases (85-90). Hacbarth et al. (14) firstly reported the presence of “low buoyant density-granulocytes” or LDG in the circulation of patients with active SLE. These distinct abnormal granulocytes characterised by high CD15 expression and low CD14 expression, can be distinguished from monocytes/macrophages (91). Carmona-Rivera and Kaplan (91) found that SLE-LDGs express unique surface markers such as CD14⁹⁶, CD-14low, CD-14high, CD-14low, CD10, CD16, CD31, CD11c, G-CSFR, and GM-CSFR. Singh et al. (77) investigated the genetics of LDGs and reported that control neutrophils and normal-density SLE neutrophils had similar levels of copy number variations, whereas LDGs isolated from the peripheral blood of lupus subjects and those derived from myeloid progenitors had an over twofold greater number of copy number variations per genome. The additional copy number alterations found in LDGs occurred preferentially on chromosomes 19, 17, 8, and X. The presence of LDGs may thus indicate genomic instability in SLE subjects.

Table V. Molecules released from PMNs relevant to abnormal mononuclear cell responses and lupus pathogenesis.

<table>
<thead>
<tr>
<th>Released molecules from SLE PMN</th>
<th>Relevant to lupus pathogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokines: [55, 147, 148]</td>
<td></td>
</tr>
<tr>
<td>• IL-2↑</td>
<td></td>
</tr>
<tr>
<td>• IL-12↓</td>
<td></td>
</tr>
<tr>
<td>• IL-4↑</td>
<td></td>
</tr>
<tr>
<td>• IL-10↑</td>
<td></td>
</tr>
<tr>
<td>• IFN-γ↑</td>
<td></td>
</tr>
<tr>
<td>NET-related molecules induced autoantibodies:</td>
<td>Lupus nephritis &amp; disease activity</td>
</tr>
<tr>
<td>• Anti-dsDNA antibodies</td>
<td>Arthritis, serositis, anaemia, relapse</td>
</tr>
<tr>
<td>ANCA [130-132]</td>
<td></td>
</tr>
<tr>
<td>Granulocyte protein release:</td>
<td></td>
</tr>
<tr>
<td>• Lactoferrin [148]</td>
<td>Th1/Th2 ratio↑</td>
</tr>
<tr>
<td>• ROS↑ and RNI [65-75]</td>
<td>Cell apoptosis↑, NETosis↑</td>
</tr>
<tr>
<td>Potential exosomal microRNA release: [132]</td>
<td></td>
</tr>
<tr>
<td>• miR-142-3p↑, miR-142-5p↑</td>
<td>T &amp; B cell activation</td>
</tr>
<tr>
<td>• miR-146a↑</td>
<td>Type 1 IFN↑</td>
</tr>
<tr>
<td>• miR-224↑</td>
<td>Cell apoptosis↑</td>
</tr>
<tr>
<td>• miR-21↑, miR-31↑, miR-142-3p↑, miR-410↑</td>
<td>IL-10↑</td>
</tr>
<tr>
<td>• miR-125a↑, miR-125b↑</td>
<td>Th17/Treg ratio↑</td>
</tr>
</tbody>
</table>

PMN: polymorphonuclear leukocyte; SLE: systemic lupus erythematosus; IL: interleukin; IFN: interferon; Th: helper T cell; NET: neutrophil extracellular trap; ANCA: anti-neutrophil cytoplasmic antibody; ROS; reactive oxygen species; RNI: reactive nitrogen intermediate; miR: microRNA; Treg: regulatory T cell.
Increased percentage of circulatory LDGs with pro-inflammatory properties in patients with active SLE

SLE-LDGs exhibit a proinflammatory nature through degranulation, protease release, and production of high levels of proinflammatory cytokines including IFN-α, IL-6, IL-8, and TNF-α to induce blood vessel damage (92-94). The low phagocytic capacity and high NETosis tendency of SLE-LDGs in association with their defective NET clearance capacity may further induce endothelial damage (92-96). It is conceivable that IFN-α is mainly secreted by activated plasmacytid dendritic cells (pDC), and plays an important role in lupus pathogenesis (97). Lindau et al. (98) have demonstrated that LDGs in SLE produce IFN-α as pDC-like cells through a Toll-like receptor (TLR)-9-independent pathway and easily undertake NET formation. Interestingly, Barrientos et al. (99) demonstrated that NET could downregulate LPS-induced monocyte-derived DC activation.

In addition to infections, a number of stimuli have the potential to trigger NETosis. These include proinflammatory cytokines (IL-8 and TNF-α), phorbol myristate acetate [PMA], nitric oxide [NO], reactive oxygen species [ROS], monosodium urate crystals, bacterial LPS, fungal zymosans, agonistic PMN autoantibodies (anti-proteinase 3, anti-myeloperoxidase), and immune complexes (100-105). Activation of ROS, [Ca²⁺], peptidylarginine deiminase 4, caspase, elastase, myeloperoxidase, microtubular polymerisation, and autophagy are all involved in NETosis (106-110). Excessive NETosis may potentially cause thrombus formation (111), vascular damage (92, 93), downregulation of LPS-activated macrophages/DC (99), and exposure of neutrophilic cytoplasmic/nuclear autoantigens to the adaptive immune system to induce chronic inflammation, autoimmunity, and autoantibody production (112-117).

Autoantibodies and IFN-α can derange PMN functions in patients with SLE

As described in the previous paragraph and Table III, different autoantibodies purified from SLE sera can cause AICD. In addition, excessive production of IFN-α from pDCs and SLE-LDG further suppress PMN functions including phagocytosis, aggregation, migration, activation, degranulation, and ROS production (118-120).

Impaired clearance of necrotic cell debris and NET-associated autoantigens in SLE

Increased NET formation and decreased NET remnant clearance in SLE

Recently, it was discovered that PMN kill microbial pathogens by either phagocytosis or release of neutrophil extracellular traps (NETs) dependent on the size of the invading microbes (121-123). In addition to microbes and their product such as LPS, many stimuli such as pro-inflammatory cytokines, IL-8 and IL-1β, the released intracellular components of connective tissues, reactive oxidants, calcium ionophores, nitric oxide donors, and pharmacological agents including PMA are involved in these processes (100-105, 121, 124, 125). Among these, PMA is a potent inducer of NETosis in vitro (103). However, all these inducers exhibit different real-time confocal imaging patterns after PMN activation to result in NET formation (104).

The released NETs contain DNA, histones, and DNA-attached neutrophil granular proteins such as elastase, proteinase 3, myeloperoxidase, and LL-37 (CAP-18). The released NETs are rapidly cleaved by DNase 1 or inactivated by enzyme inhibitors, and cleared by complements and mononuclear phagocytes. Defective clearance of NET remnants would result in long-term exposure to nuclear and cytoplasmic autoantigens, which would subsequently elicit chronic inflammation (124-127), autoimmune diseases (112-116), and ANCA-associated vasculitis (128, 129). It is equally interesting that in clinical practice, these NET-related autoantibodies in SLE patients are associated with arthritis, serositis, leukopenia, and disease expression in addition to ANCA-associated vasculitis (130-132). On the other hand, there has also been contradictory evidence against these hypotheses. For instance, Nauseef and Kubes (133) suggested that identification of NET components in vivo should be a prerequisite to distinguish vital (active) NETs from necrotic cell remnants. All the NET-DNAs in tissue should be identified from PMNs. PMA, a plant-derived organic compound and a well-known activator of ubiquitous signal transducer, protein kinase C, is reported as NETosis inducer. However, it is not physiologically relevant, since it does not activate physiological processes in vivo (103, 104). However, until now, there have seemed to be no impeccable study methods to differentiate in vivo NETosis from in vitro NET variants. Nevertheless, in some clinical conditions such as X-linked chronic granulomatous disease, the host cannot effectively kill invasive pathogens due to deficiency in ROS generation, which is a crucial NET inducer. Thus, chronic granulomatous lesions occur at the site of infection (134). This scenario is quite different from impaired NET clearance in SLE.

Factors involving defective clearance of necrotic cell- and NET-derived cytoplasmic and nuclear molecules in SLE patients

Defective clearance of necrotic cell- and NET-derived cytoplasmic and nuclear molecules may originate from congenital complement C2 and C4 deficiency (13), or may be acquired from anti-C1q/C1q receptor antibody production (135, 136) in patients with active SLE. Furthermore, congenital defects in the gene expression of immunoreceptors FcγR, CR1, and CR2 in PMNs (54, 137), hereditary CRP gene variants (138), and defective CRP promoter gene expression in hepatocytes (139), acquired anti-CRP antibody production (140), deficient DNsase 1 synthesis (141, 142), anti-DNase 1 autoantibody production (143), as well as hypofunction of the reticuloendothelial system (144) are all implicated in this process. Defective clearance of necrotic cell debris and NET-associated molecules results in overexposure of adaptive immune cells to intracellular autoantigens, which then elicits autoantibody production and autoactive...
Abnormal neutrophil functions derange autologous mononuclear cell immune responses that potentially induce lupus development

In our previous studies, we found that the peritoneal exudative PMNs obtained from an autoimmune MRL-lpr/lpr mouse progressively suppressed the expression of IL-4, IL-10, and IFN-α, but promoted IL-2 production in autologous mononuclear cells, which were remarkably different from those derived from a normal BALB/c strain (147). Furthermore, Li et al. (148, 149) have demonstrated that a less pronounced release of surface-bound lactoferrin from SLE-PMNs and reduced PMN-PBMC trogocytosis inclines mononuclear cells toward the Th2 pathway.

In vitro experiments have demonstrated the release of ectosomes and exosomes from human PMNs (at a purity approaching 95–98%) on stimulation. PMN-derived ectosomes are defined as vesicles that are shed directly from the cell surface, and contain phosphatidyserine and other cell surface proteins (selectectins, integrins, complement receptors, FcyRII/III, HLA-I, CD66d and CD87, and neutrophil granular proteins) (39, 150). Such ectosomes activate multiple signalling pathways in macrophages (42), interfere with immune cell maturation, and down-regulate monocyte-derived DCs by suppressing the expression of surface molecules including CD40, CD80, CD83, CD86, and HLA-class II on neutrophils (40, 43, 151). Exosomes, which are 30–100 nm-sized microvesicles that bud (so-called “exocytosis”) from neutrophils on activation, contain proteins, nucleic acids, and lipids (47). Vargas et al. (46) revealed that neutrophil-derived exosomes transduce multiple immuno-suppressive signals for TGF-β1 excretion, and contribute to smooth muscle remodelling in the airways. The authors
cultured human neutrophils (99.50% purity) for 18 h. The supernatants were harvested following centrifugation. The obtained microvesicles, or exosomes, were then isolated by serial centrifugation/ultracentrifugation, and their distribution according to shape and size was determined. Although exosomes and exosomes are frequently found in SLE plasma, specific PMN-derived ectosomes and exosomes from SLE patients, and their effects on immune modulation, remain elusive. Recently, Tsai et al. (152) reviewed the aberrant microRNAs release from SLE-PMNs that may potentially be implicated in lupus pathogenesis (Table V).

The eccentric PMN functions observed in SLE patients undoubtedly play an important role in lupus pathogenesis through disrupted cell-cell communications among PMNs and other innate and adaptive immune cells (153-155). Defective clearance of apoptotic cells, secondary necrotic cell debris, and NET-associated molecules further perpetuate autoantigen-driven autoantibody production and T cell responses. Aberrant PMN functions implicated in abnormal adaptive immunity as well as the ensuing vicious cycle resulting in lupus are illustrated in Fig. 2. Finally, the interactions among PMNs, immune factors, and immune-related cells in SLE pathogenesis are illustrated in Fig. 3.

Concluding remarks and unsolved problems

PMNs are traditionally regarded as phagocytic and inflammatory cells that form the first line of defense against exogenous and endogenous pathogenic microbial invasions. In recent years, many previously unknown PMN functions have been discovered. These include cytokine/chemokine/growth factor production, NET formation, granular proteins/ectosomes/exosomes release, and membrane exchanges among PMNs and other innate and adaptive immune cells for modulating immune responses. Abnormal PMN functions are derived from hereditary predispositions and acquired factors that exert certain adverse effects on immune responses in SLE patients. Hereditary predispositions may originate from gene mutations in immune complex processing molecules, cytokines, IFN-α, CRP, DNase 1, mitochondrial DNAs, and phosphatidylserine (2, 3, 13-15, 54, 55, 137, 141-144). Epigenetic dysregulation of microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) (4-7, 152) are all involved in lupus pathogenesis. Moreover, as listed in Table III, the deleterious effects of SLE-related autointermediaries can disrupt neutrophil functions and further perpetuate the vicious cycle during its immunopathogenesis. However, missing links remain in the detailed molecular basis of interactions among PMNs and other innate and adaptive immune cells when comparing normal individuals and SLE patients. These gaps in our understanding include the following: (a) The specific SLE-PMN-derived ectosomes and exosomes that mediate the immune dysfunctions of SLE have not yet been reported; (b) The signalling pathways responsible for membrane exchanges among PMNs and other immune-related cells or vice versa in normal and SLE patients have not been well investigated; (c) The subsequent events of post-trogocytosis among PMNs, macrophages/DCs, and lymphocytes require further investigation; (d) Most of the data regarding the interactions among PMNs and other innate/adaptive immune cells were obtained in vitro or ex vivo, but not from in vivo experiments; (e) Whether NET formation really occurs in vivo in blood circulation or in lymph nodes is still ambiguous. In conclusion, the relevance of neutrophils to immune responses, rheumatic diseases, and autoimmune diseases, especially SLE, remains open for further investigations.

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