Review

What's wrong with neutrophils in lupus?

C.-Y. Tsai[1], K.-J. Li[2], S.-C. Hsieh[2], H.-T. Liao[1], C.-L. Yu[2]

[1]*Division of Allergy, Immunology and Rheumatology, Taipei Veterans General Hospital, and National Yang-Ming University School of Medicine, Taipei, Taiwan;* [2]*Department of Internal Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan.*

*Chang-Youh Tsai, MD, PhD**
*Ko-Jen Li, MD, PhD**
Song-Chou Hsieh, MD, PhD
Hsien-Tzung Liao, MD, PhD
Chia-Li Yu, MD, PhD

**These authors contributed equally to this work.*

Please address correspondence to:
Dr Chia-Li Yu,
Department of Internal Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine,
7 Chung-Shan South Road, Taipei 10002, Taiwan.
E-mail: chialiyu@ntu.edu.tw

Received on May 12, 2018; accepted in revised form on September 10, 2018.

Clin Exp Rheumatol 2019; 37: 684-693.

Key words: systemic lupus erythematosus, trogocytosis, neutrophil extracellular trap, ectosome, exosome, bioenergetics

Funding: this work was partly supported by grants from Taipei Veterans General Hospital and National Taiwan University Hospital (V107D37-002-MY3-1, VN-106-01), and from the Ministry of Science & Technology (MOST 106-2634-F-075-001, MOST 107-2314-B-075-051-MY3).

Competing interests: none declared.

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 (trogocytosis) 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 hypocomplementaemia (13) have been reported. Moreover, Hacbarth *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.

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

In vitro	*In vivo*
IL-1α/IL-1β	IL-1α
IL-1ra	IL-1β
IL-8	IL-1ra
IL-12	IL-6
TNF-α	IL-8
IFN-α	IL-10
CD30L	IL-12
GROα, GROβ	MIP-2
CINC-1, 2α, 3	KC/GROα
IP-10	CINC
MIG	MIP-1α
MIP-1α/-1β	MIP-1β
TGF-α, TGFβ1	MCP-1
IL-3, G-CSF, M-CSF	TNF-α
GM-CSF (?), IL-6 (?), MCP-1 (?)	TGFβ1
SCF (?)	

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

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

Classical functions	Previously unknown functions
• Phagocytosis and killing of microbial pathogens • Vascular endothelial cell adhesion and extravasation • Release of granular proteins • Production of oxygen and superoxide radicals	• Immune complex and cell debris clearance [54,56,145] • Mitogen-induced cell-mediated cytotoxicity (MICC)[16,17] • Expression of MHC-II antigens and co-stimulatory molecules (CD80 and CD86), following the stimulation of IL-4, GM-CSF, and IFN-γ[18-21] • Production of cytokines/chemokines/growth factors[35-37] • Immunomodulation of innate and adaptive immunity *in vitro*[22-27,48] and *in vivo*[49-51] via cell–cell contact at the site of the immunological synapse[29-31] and subsequent trogocytosis[31,148,149] • Cross-talk with different cells via release of ectosomes[34,39-44] and multiple vesicle bodies (exosomes, microvesicles)[32,44-47] • NETosis following stimulation with extrusion of intracellular molecules to form NET[93,94,98-105]

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.

Takashima and Yao (20) reviewed those data and found that the authors used >99.5% ultrapure Gr-1^{high}/CD48^{-} 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 potently 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 P.151-229, 1999) lists *in vitro* and *in vivo* cytokine gene expression in normal PMNs. Moreover, PMNs can release different microvesicles such as ectosomes (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^{+}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.

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^{+}-K^{+}-ATPase activity are crucial predisposing factors for SLE (54). Na^{+}-K^{+}-ATPase is critical because it generates the ion gradients required by cells, and defective expression of this membrane enzyme renders PMNs

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

PMN functions	Anti-CL[57]	Anti-dsDNA[58]	Anti-SSB/La[59]	Anti-MP[60]	Anti-PR3[60]
Phagocytosis		↓	↓	↑	↑
IL-8 production	ND	↑	↑	↑	↑
AICD	ND	↑	↑	↑	↑
Glucose uptake	ND	ND	ND	↑	↑

Anti-CL: anti-cardiolipin antibodies; Anti-MP: anti-myeloperoxidase antibodies; Anti-PR3: anti-proteinase 3 antibodies; AICD: activation-induced cell death (apoptosis). ND: not determined.

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.

Aberrant 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 active SLE-PMNs. The molecular basis of this hyporesponsiveness to IL-8 stimulation is attributable to the defective expression of IL-8-specific receptor CXCR2 and cationic ion transporters including Na^+-K^+-ATPase, renal epithelial Na^+ channel, and outer renal medullary epithelial K^+-channel (62). Furthermore, Tsai *et al*. (63) demonstrated that defective IL-12 expression by SLE-PMN occurs by potentially deviating T cell responses towards Th2 predominance. In addition to the aforementioned abnormal neutrophil functions, SLE-PMNs have been found to exhibit enhanced cytidine deaminase expression, which may facilitate B cell maturation and autoantibody production (64). Hsieh *et al*. (65) further demonstrated defective spontaneous and LPS-stimulated IL-1ra 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 *TPP*1, *TIN*2, *POT*1, and *KU*80 is significantly reduced, whereas that of *TRF*2 and *MRE*11 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 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[53, 54, 137]
2. Enhanced apoptosis and decreased cell debris clearance[56, 145]
3. Increased cytidine deaminase production[64]
4. Spontaneous and bacterial LPS-induced cytokine production:
   - IL-1ra ↓[65]
   - IL-8 ↓[61] due to CXCR2 ↓[62]
   - Cationic ion transporter ↓[62]
   - IL-12 ↓ (SLE patients)[63]
   - IL-4 ↑[148]
   - IL-10 ↑[148]
   - IFN-γ ↑[148] (MRL-*lpr*/*lpr* mouse)[147]
5. Decreased surface lactoferrin expression and its transfer to autologous mononuclear cells[148, 149]
6. Increased oxidative stress due to:
   - Deranged bioenergetics[72]:
     - Glucose uptake ↓
     - Basal lactate level ↑
     - GLUT3 & GLUT6 ↓
     - ATP content ↓
   - Defective redox capacity[72-75]:
     - GSH ↓
     - GSH-Px ↓
     - GSSR ↓
     - GGT activity ↓
     - CD53 ↑
   - Mitochondria D-310 loop DNA heteroplasmy and functional impairment[73-75]
7. Effects on autologous mononuclear cytokine production[147, 148]
   - IL-2 ↓
   - IL-4 ↑
   - IL-10 ↑
   - IFN-γ ↑
8. Exaggerated NETosis[92-94], decreased NET clearance[94, 96, 112-117] and increased pro-inflammatory low-density granulocyte formation[14, 91-94]
9. Aberrant ectosome[43] and exosome release (?)
10. Premature telomere shortening[66, 67]

*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.

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

Released molecules from SLE PMN	Relevant to lupus pathogenesis
• Cytokines:[63, 147, 148]	
• IL-2↓	Th1↓
• IL-12↓	Th1↓
• IL-4↑	Th2↑
• IL-10↑	Th2↑
• IFN-γ↑	Th1↑
• NET-related molecules induced autoantibodies:	
• Anti-dsDNA antibodies	Lupus nephritis & disease activity
• ANCA[130-132]	Arthritis, serositis, anaemia, relapse
• Granular protein release:	
• Lactoferrin↓[148]	Th1/Th2 ratio↓
• ROS↑ and RNI↑[10, 73-75]	Cell apoptosis↑, NETosis↑
• Potential exosomal microRNA release:[152]	
• miR-142-3p↓, miR-142-5p↓	T & B cell activation
• miR-146a↓	Type I IFN↑
• miR-224↑	Cell apoptosis↑
• miR-21↑, miR-31↓, miR-142-3p↓, miR-410↓	IL-10↑
• miR-125a↓, miR-125b↓	Th17/Treg ratio↑

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.

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/GSSG-R, γ-glutamyl-transpeptidase/GGT, and CD53) are all diminished. These abnormal immuno-metabolisms result 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 Ehrlich, 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 2×10^{10} 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 $CD15^{high}$, $CD14^{low}$, $CD10^{+}$, $CD16^{+}$, $CD31^{+}$, $CD11c^{+}$, 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.

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 plasmacytoid dendritic cells (*p*DC), and plays an important role in lupus pathogenesis (97). Lindau *et al*. (98) have demonstrated that LDGs in SLE produce IFN-α as *p*DC-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, [Ca^{2+}], 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 *p*DCs 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 immune receptors 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 DNase 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 autoreactive

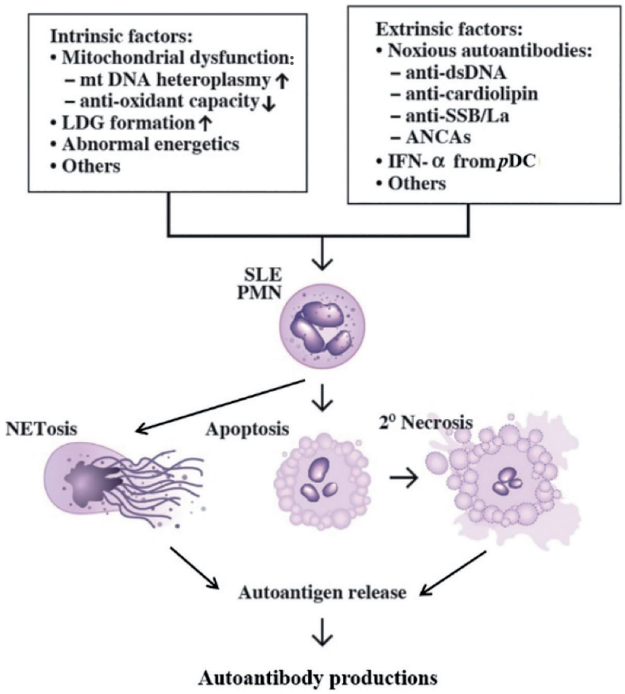

Fig. 1. The intrinsic and extrinsic factors for autoantigen release and autoantibody production in patients with SLE. LDG: low-density granulocyte; IFN: interferon; NETosis: neutrophil apoptosis to form neutrophil extracellular traps; ANCA: anti-neutrophil cytoplasmic antibody; mtDNA: mitochondrial DNA; *p*DC: plasmacytoid dendritic cell.

T cell proliferation (145). As a consequence, production of autoantibodies against C1q, CRP, DNase 1, and neutrophil granular proteins (elastase, myeloperoxidase, proteinase 3, LL-37) further delays NET remnant clearance by autologous macrophages and PMNs, and activates complements to perpetuate disease activity (146). Fig. 1 summarises the intrinsic and extrinsic pathogenic factors for PMN that may elicit autoantibody production in patients with SLE.

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 phosphatidylserine and other cell surface proteins (selectins, integrins, complement receptors, FcγRII/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 immunosuppressive signals for TGF-β1 excretion, and contribute to smooth muscle remodelling in the airways. The authors

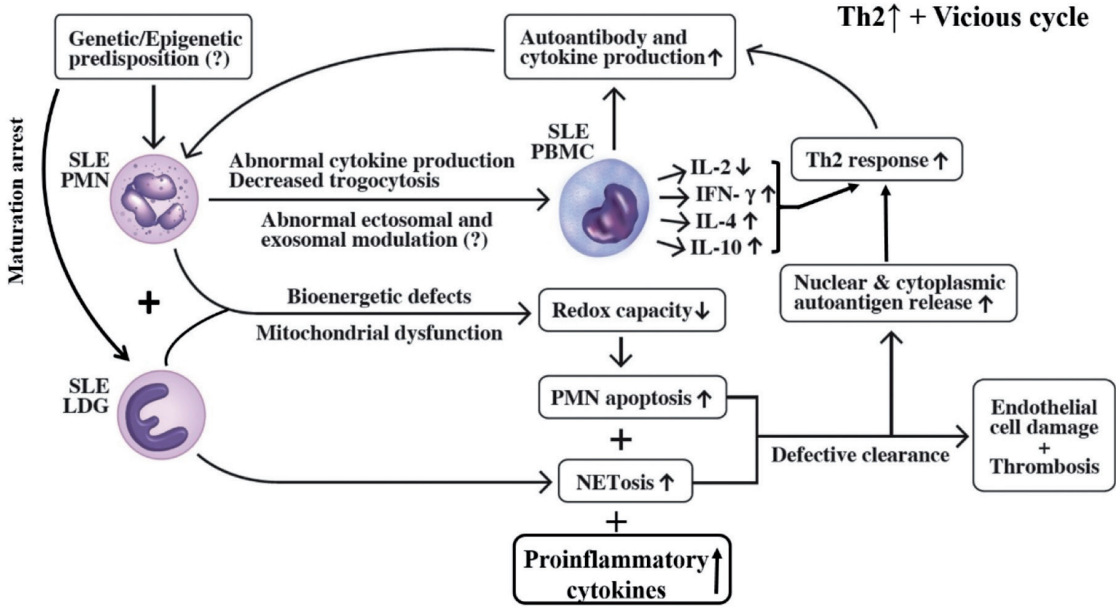

Fig. 2. Molecular basis of aberrant neutrophil functions in skewing autologous mononuclear cells toward Th2 immune responses and perpetuating vicious cycle in patients with systemic lupus erythematosus (SLE). LDG: low-density granulocyte; NET: neutrophil extracellular traps; Th: helper T cell. PMN: polymorphonuclear leukocyte; NETosis: neutrophil apoptosis to form neutrophil extracellular trap; PBMC: peripheral blood mononuclear cell.

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 ectosomes 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.

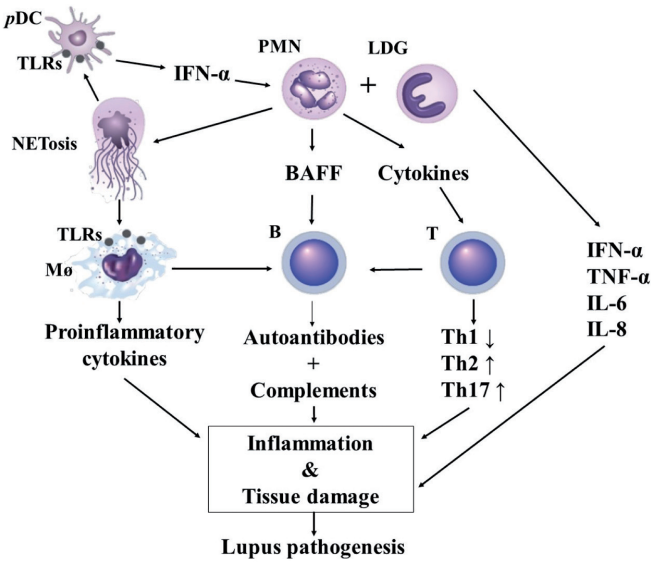

Fig. 3. Interactions among PMNs, immune factors, and different immune-related cells in SLE pathogenesis. TLR: Toll-like receptor; NETosis: apoptosis to form neutrophil extracellular trap; Mø: macrophage; Th: helper T cell; IFN: interferon; TNF: tumour necrosis factor; IL: interleukin, PMN: polymorphonuclear leukocyte; LDG: low-density granulocyte; BAFF: B cell activating factor of the tumour necrosis factor superfamily.

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 autoantibodies 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.

References

1. TSOKOS GC, LO MS, REIS PC, SULLIVAN KE: New insights into the immunopathogenesis of systemic lupus erythematosus. *Nat Rev Rheumatol* 2016; 12: 716-30.
2. MOHAN C, PUTTERMAN C: Genetics and pathogenesis of systemic lupus erythematosus and lupus nephritis. *Nat Rev Nephrol* 2015; 11: 329-41.
3. DENG Y, TSAO BP: Updates in lupus genetics. *Curr Rheumatol Rep* 2017; 19: 68.
4. LAI NS, KOO M, YU CL, LU MC: Immunopathogenesis of systemic lupus erythematosus and rheumatoid arthritis: the role of aberrant expression of non-coding RNAs in T cells. *Clin Exp Rheumatol* 2017; 187: 327-36.
5. WU H, ZHAO M, TAN L, LU Q: The key culprit in the pathogenesis of systemic lupus erythematosus: aberrant DNA methylation. *Autoimmun Rev* 2016; 15: 684-9.
6. LONG H, YIN H, WANG L, GERSHWIN ME, LU Q: The critical role of epigenetics in systemic lupus erythematosus and autoimmunity. *J Autoimmun* 2016; 74: 118-38.
7. TERUEL M, SAWALHA AH: Epigenetic variability in systemic lupus erythematosus: what we learned from genome-wide DNA methylation studies. *Curr Rheumatol Rep* 2017; 19: 32.
8. BARBHAIYA M, COSTENBADER KH: Environmental exposures and the development of systemic lupus erythematosus. *Curr Opin Rheumatol* 2016; 28: 497-505.
9. PETRI M: Sex hormones and systemic lupus erythematosus. *Lupus* 2008; 17: 412-5.
10. LEE H-T, WU T-H, LIN C-S *et al*.: The pathogenesis of systemic lupus erythematosus - From the viewpoint of oxidative stress and mitochondrial dysfunction. *Mitochondrion* 2016; 30: 1-7.
11. HENRIGUES A, TEIXEIRA L, INÊS L *et al*.:

NK cells dysfunction in systemic lupus erythematosus: relation to disease activity. *Clin Rheumatol* 2013; 32: 805-13.
12. LEE H-T, CHEN W-S, SUN K-H, CHOU C-T, TSAI C-Y: Increased spontaneous but decreased mitogen-stimulated expression and excretion of interleukin 18 by mononuclear cells in patients with active systemic lupus erythematosus. *J Rheumatol* 2009; 36: 1910-6.
13. MACEDO ACL, ISAAC L: Systemic lupus erythematosus and deficiencies of early components of the complement classical pathway. *Front Immunol* 2016; 7: 55.
14. HACBARTH E, KAJDACSY-BALLA A: Low density neutrophils in patients with systemic lupus erythematosus, rheumatoid arthritis, and acute rheumatic fever. *Arthritis Rheum* 1986; 29: 1334-42.
15. BENNETT L, PALUCKA AK, ARCE E *et al*.: Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J Exp Med* 2003; 197: 711-23.
16. YUE CL, TANIMOTO K, HORIUCHI Y: Characterization and possible mechanism of mitogen-induced cell-mediated cytotoxicity (MICC). *Scand J Immunol* 1981; 14: 397-408.
17. DALLEGRI F, FRUMENTO G, MAGGI A, PATRONE F: PHA-induced neutrophil-mediated cytotoxicity. *J Clin Lab Immunol* 1983; 11: 203-6.
18. SANDILANDS GP, MCCRAE J, HILL K, PERRY M, BAXTER D: Major histocompatibility complex class II (DR) antigen and costimulatory molecules on *in vitro* and *in vivo* activated human polymorphonuclear neutrophils. *Immunology* 2006; 119: 562-71.
19. LIN A, LORÉ K: Granulocytes: new members of the antigen-presenting cell family. *Front Immunol* 2017; 8: 1781.
20. TAKASHIMA A, YAO Y: Neutrophil plasticity: acquisition of phenotype and functionality of antigen-presenting cell. *J Leukoc Biol* 2015; 98: 489-96.
21. OEHLER L, MAJDIC O, PICKL WF *et al*.: Neutrophil granulocyte-committed cells can be driven to acquire dendritic cell characteristics. *J Exp Med* 1998; 187: 1019-28.
22. NATHAN C: Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol* 2006; 6: 173-82.
23. KUMAR V, SHARMA A: Neutrophils: Cinderella of innate immune system. *Int Immunopharmacol* 2010; 10: 1325-34.
24. BREEDVELD A, GROOT-KORMELINK T, VAN EGMOND M, DE JONG EC: Granulocytes as modulators of dendritic cell function. *J Leukoc Biol* 2017; 102: 1003-16.
25. MANTOVANI A, CASSATELLA MA, COSTANTINI C, JAILLON S: Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol* 2011; 11: 519-31.
26. MÓCSAI A: Diverse novel functions of neutrophils in immunity, inflammation, and beyond. *J Exp Med* 2013; 210: 1283-99.
27. NAUSEEF WM, BORREGAARD N: Neutrophils at work. *Nat Immunol* 2014; 15: 602-11.
28. OPPENHEIM JJ: Cytokines: past, present, and future. *Int J Hematol* 2001; 74: 3-8.
29. YOKOSUKA T, SAITO T: The immunological synapse, TCR microclusters, and T cell activation. *Curr Top Microbiol Immunol* 2010; 340: 81-107.
30. ALARCÓN B, MESTRE D, MARTÍNEZ-MARTÍN N: The immunological synapse: a cause or consequence of T-cell receptor triggering? *Immunolgy* 2011; 133: 420-5.
31. AHMED KA, MUNEGOWDA MA, XIE Y, XIANG J: Intercellular trogocytosis plays an important role in modulation of immune responses. *Cell Mol Immunol* 2008; 5: 261-9.
32. MCCOY-SIMANDLE K, HANNA SJ, COX D: Exosomes and nanotubes: control of immune cell communication. *Int J Biochem Cell Biol* 2016; 71: 44-54.
33. DHAINAUT M, MOSER M: Regulation of immune reactivity by intercellular transfer. *Front Immunol* 2014; 5: 112.
34. ROBBINS PD, DORRONSORO A, BOOKER CN: Regulation of chronic inflammatory and immune processes by extracellular vesicles. *J Clin Invest* 2016; 126: 1173-80.
35. CASSATELLA MA: The production of cytokines by polymorphonuclear neutrophils. *Immunol Today* 1995; 16: 21-6.
36. TECCHIO C, MICHELETTI A, CASSATELLA MA: Neutrophil-derived cytokines: facts beyond expression. *Front Immunol* 2014; 5: 508.
37. TECCHIO C, CASSATELLA MA: Neutrophil-derived chemokines on the road to immunity. *Semin Immunol* 2016; 28: 119-28.
38. YANG F, FENG C, ZHANG X, LU J, ZHAO Y: The diverse biological functions of neutrophils, beyond the defense against infections. *Inflammation* 2017; 40: 311-23.
39. HESS C, SADALLAH S, HEFTI A, LANDMANN R, SCHIFFERLI J-A: Ectosomes released by human neutrophils are specialized functional units. *J Immunol* 1999; 163: 4564-73.
40. EKEN C, GASSER O, ZENHAEUSERN G, QEHRI I, HESS C, SCHIFFERLI JA: Polymorphonuclear neutrophil-derived ectosomes interfere with the maturation of monocyte-derived dendritic cells. *J Immunol* 2008; 180: 817-24.
41. SADALLAH S, EKEN C, SCHIFFERLI JA: Ectosomes as modulators of inflammation and immunity. *Clin Exp Immunol* 2010; 163: 26-32.
42. EKEN C, SADALLAH S, MARTIN PJ, TREVES S, SCHIFFERLI JA: Ectosomes of polymorphonuclear neutrophils activate multiple signaling pathways in macrophages. *Immunobiology* 2013; 218: 382-92.
43. TURBICA I, GALLAIS Y, GUEGUEN C *et al*.: Ectosomes from neutrophil-like cells down-regulate nickle-induced dendritic cell maturation and promote Th2 polarization. *J Leukoc Biol* 2015; 97: 737-49.
44. MARTÍNEZ GJ, BARRACLOUGH JY, NAKHLA S *et al*.: Neutrophil-derived microparticles are released into the coronary circulation following percutaneous coronary intervention in acute coronary syndrome patients. *Biosci Rep* 2017; 37. pii: BSR 20160430.
45. JETHWANEY D, ISLAM MR, LEIDAL KG *et al*.: Proteomic analysis of plasma membrane and secretory vesicles from human neutrophils. *Proteome Sci* 2007; 5: 12.
46. VARGAS A, ROUX-DALVAI F, DROIT A, LAVOIE JP: Neutrophil-derived exosomes: a new mechanism contributing to airway smooth muscle remodeling. *Am J Respir Cell Mol Biol* 2016; 55: 450-61.
47. ZHANG J, LI S, LI L *et al*.: Exosome and exosomal microRNA: trafficking, sorting and function. *Genom Proteom Bioinform* 2015; 13: 17-24.
48. MAYADAS TN, CULLERE X, LOWELL CA: The multifaceted functions of neutrophils. *Annu Rev Pathol* 2014; 9: 181-218.
49. KUDO C, YAMASHITA T, TERASHITA M, SENDO F: Modulation of *in vivo* immune response by selective depletion of neutrophils using a monoclonal antibody, RP-3. II. Inhibition by RP-3 treatment of mononuclear leucocyte recruitment in delayed-type hypersensitivity to sheep red blood cells in rats. *J Immunol* 1993; 150: 739-46.
50. TAMURA M, SEKIYA S, TERASHITA M, SENDO F: Modulation of the *in vivo* immune response by selective depletion of neutrophils using a monoclonal antibody, RP-3. III. Enhancement by RP-3 treatment of the anti-sheep red blood cell plaque-forming cell response in rats. *J Immunol* 1994; 153: 1301-8.
51. MATSUZAKI J, TSUJI T, CHAMOTO K, TAKESHIMA T, SENDO F, NISHIMURA T: Successful elimination of memory-type $CD8^+$T cell subsets by the administration of anti-Gr-1 monoclonal antibody *in vivo*. *Cell Immunol* 2003; 224: 98-105.
52. NAVARRA SV, LEYNES MSN: Infections in systemic lupus erythematosus. *Lupus* 2010; 19: 1419-24.
53. YU CL, CHANG KL, CHIU CC, CHIANG BN, HAN SH, WANG SR: Defective phagocytosis, decreased tumour necrosis factor-alpha production, and lymphocyte hyporesponsiveness predispose patients with systemic lupus erythematosus to infections. *Scand J Rheumatol* 1989; 18: 97-105.
54. YU CL, TSAI CY, CHIU CC *et al*.: Defective expression of neutrophil C3b receptors and impaired lymphocyte Na^+-K^+-ATPase activity in patients with systemic lupus erythematosus. *Proc Natl Sci Counc ROC B* 1991; 15: 178-85.
55. LOHR JN, MEINZER F, DALLA S, ROMEY-GLUSING R, DOBLER S: The function and evolutionary significance of a triplicated Na^+-K^+-ATPase gene in a toxin-specialized insect. *BMC Evol Biol* 2017; 17: 256.
56. REN Y, TANG J, MOK MY, CHAN AW, WU A, LAU CS: Increased apoptotic neutrophils and macrophages and impaired macrophage phagocytic clearance of apoptotic neutrophils in systemic lupus erythematosus. *Arthritis Rheum* 2003; 48: 2888-97.
57. YU CL, SUN KH, TSAI CY, WANG SR: Inhibitory effects of anti-cardiolipin antibodies on lymphocyte proliferation and neutrophil phagocytosis. *Ann Rheum Dis* 1991; 50: 903-8.
58. HSIEH SC, SUN KH, TSAI CY *et al*.: Monoclonal anti-double stranded DNA antibody is a leukocyte-binding protein to up-regulate interleukin-8 gene expression and elicit apoptosis of normal human polymorphonuclear neutrophils. *Rheumatology* 2001; 40: 851-8.
59. HSIEH SC, YU HS, LIN WW *et al*.: Anti-SSB/La is one of the antineutrophil autoantibodies responsible for neutropenia and functional impairment of polymorphonuclear neutrophils in patients with systemic lupus erythematosus. *Clin Exp Immunol* 2003; 131: 506-16.
60. HSIEH SC, YU HS, CHENG SH *et al*.: Anti-myeloperoxidase antibodies enhance phago-

cytosis, IL-8 production, and glucose uptake of polymorphonuclear neutrophils rather than anti-proteinase 3 antibodies leading to activation-induced cell death of the neutrophils. *Clin Rheumatol* 2007; 26: 216-24.
61. HSIEH SC, TSAI CY, SUN KH *et al*.: Decreased spontaneous and lipopolysaccharide-stimulated production of interleukin 8 by polymorphonuclear neutrophils of patients with active systemic lupus erythematosus. *Clin Exp Rheumatol* 1994; 12: 627-33.
62. HSIEH SC, WU TH, TSAI CY *et al*.: Abnormal in vitro CXCR2 modulation and defective cationic ion transporter expression on polymorphonuclear neutrophils responsible for hyporesponsiveness to IL-8 stimulation in patients with active systemic lupus erythematosus. *Rheumatology* 2008; 47: 150-7.
63. TSAI CY, WU TH, YU CL, TSAI YY, CHOU CT: Decreased IL-12 production by polymorphonuclear leukocytes in patients with active systemic lupus erythematosus. *Immunol Invest* 2002; 31: 177-89.
64. YU CL, TSAI CY, SUN KH *et al*.: Increased spontaneous release of cytidine deaminase by polymorphonuclear neutrophils of patients with active systemic lupus erythematosus. *Br J Rheumatol* 1992; 31: 675-8.
65. HSIEH SC, TSAI CY, SUN KH *et al*.: Defective spontaneous and bacterial lipopolysaccharide-stimulated production of interleukin-1 receptor antagonist by polymorphonuclear neutrophils of patients with active systemic lupus erythematosus. *Br J Rheumatol* 1995; 34: 107-12.
66. WU CH, HSIEH SC, LI KJ, LU MC, YU CL: Premature telomere shortening in polymorphonuclear neutrophils from patients with systemic lupus erythematosus is related to the lupus disease activity. *Lupus* 2007; 16: 265-72.
67. ZHOU JG, QING YF, YANG QB, XIE WG, ZHAO MC: Changes in the expression of telomere maintenance genes might play a role in the pathogenesis of systemic lupus erythematosus. *Lupus* 2011; 20: 820-8.
68. KOMINSKY DJ, CAMPBELL EL, COLGAN SP: Metabolic shifts in immunity and inflammation. *J Immunol* 2010; 184: 4062-8.
69. PEARCE EL, PEARCE EJ: Metabolic pathways in immune cell activation and quiescence. *Immunity* 2013; 38: 633-43.
70. THIELE K, DIAO L, ARCK PC: Immunometabolism, pregnancy, and nutrition. *Semin Immunopathol* 2018; 40: 157-74.
71. VAN RAAM BJ, VERHOEVEN AJ, KUIJPERS TW: Mitochondria in neutrophil apoptosis. *Int J Hematol* 2006; 84: 199-204.
72. LI KJ, WU CH, HSIEH SC, LU MC, TSAI CY, YU CL: Deranged bioenergetics and defective redox capacity in T lymphocytes and neutrophils are related to cellular dysfunction and increased oxidative stress in patients with active systemic lupus erythematosus. *Clin Devel Immunol* 2012; 2012: 548516.
73. LEE HT, LIN CS, LEE CS, TSAI CY, WEI YH: Increased 8-hydroxy-2'-deoxyguanosine in plasma and decreased mRNA expression of human 8-oxaguanosine DNA glycosylase 1, anti-oxidant enzymes, mitochondrial biogenesis-related proteins and glycolytic enzymes in leukocyte in patients with systemic lupus erythematosus. *Clin Exp Immunol* 2014; 176: 66-77.
74. LEE HT, LIN CS, CHEN WS, LIAO HT, TSAI CY, WEI YH: Leukocyte mitochondrial DNA alternation in systemic lupus erythematosus and its relevance to the susceptibility to lupus nephritis. *Int J Mol Sci* 2012; 13: 8853-68.
75. LEE HT, WU TH, LIN CS *et al*.: Oxidative DNA and mitochondrial DNA change in patients with SLE. *Front Biosci* (Landmark Ed) 2017; 22: 493-503.
76. DE CANDIA P, DE ROSA V, GIGANTINO V, BOTTI G, CERIELLO A, MATARESE G: Immunometabolism of human autoimmune diseases: from metabolites to extracellular vesicles. *FEBS Lett* 2017; 591: 3119-34.
77. SINGH N, TRAISAK P, MARTIN KA, KAPLAN MJ, COHEN PL, DENNY MF: Genomic alterations in abnormal neutrophils isolated from adult patients with systemic lupus erythematosus. *Arthritis Res Ther* 2014; 16: R165.
78. DAI C, DENG Y, QUINLAN A, GASKIN F, TSAO BP, FU SM: Genetics of systemic lupus erythematosus: immune responses and organ resistance to damage. *Curr Opin Immunol* 2014; 31: 87-96.
79. KAUFMANN SH: Immunology's foundation: the 100-year anniversary of the Nobel Prize to Paul Ehrlich and Elie Metchnikoff. *Nat Immunol* 2008; 9: 705-12.
80. CARTWRIGHT GE, ATHENS JW, WINTROBE MM: The kinetics of granulopoiesis in normal man. *Blood* 1964; 24: 780-803.
81. PILLAY J, DEN BRABER I, VRISEKOOP N *et al*.: *In vivo* labeling with ^2H_2O reveals a human neutrophil lifespan of 5.4 days. *Blood* 2010; 116: 625-7.
82. SILVESTRE-ROIG C, HIDALGO A, SOEHNLEIN O: Neutrophil heterogeneity: implications for homeostasis and pathogenesis. *Blood* 2016; 127: 2173-81.
83. HSIEH SC, HUANG MH, TSAI CY *et al*.: The expression of genes modulating programmed cell death in normal human polymorphonuclear neutrophils. *Biochem Biophys Res Commun* 1997; 233: 700-6.
84. AMANULLAH A, LIEBERMANN DA, HOFFMAN B: Deregulated *c-myc* prematurely recruits both type 1 and type 2 CD95/Fas apoptotic pathways associated with terminal myeloid differentiation. *Oncogene* 2002; 21: 1600-10.
85. FRIDLENDER ZG, SUN J, KIM S *et al*.: Polarization of tumor-associated neutrophil phenotype by TGF-ß: "N1" versus "N2" TAN. *Cancer Cell* 2009; 16: 183-94.
86. GREGORY AD, HOUGHTON AM: Tumor-associated neutrophils: new targets for cancer therapy. *Cancer Res* 2011; 71: 2411-6.
87. FRIDLENDER ZG, ALBELDA SM: Tumor-associated neutrophils: friend or foe? *Carcinogenesis* 2012; 33: 949-55.
88. URIBE-QUEROL E, ROSALES C: Neutrophils in cancer: two sides of the same coin. *J Immunol Res* 2015; 2015: 1-21.
89. SMYTH MJ, NGIOW SF, RIBAS A, TENG MWL: Combination cancer immunotherapies tailored to the tumour microenvironment. *Nat Rev Clin Oncol* 2016; 13: 143-58.
90. RAN T, GENG S, LI L: Neutrophil programming dynamics and its disease relevance. *Sci China Life Sci* 2017; 60: 1168-77.
91. CARMONA-RIVERA C, KAPLAN MJ: Low-density granulocytes: a distinct class of neutrophils in systemic autoimmunity. *Semin Immunopathol* 2013; 35: 455-63.
92. DENNY MF, YALAVARTHI S, ZHAO W *et al*.: A distinct subset of proinflammatory neutrophils isolated from patients with systemic lupus erythematosus induces vascular damage and synthesizes type 1 interferons. *J Immunol* 2010; 184: 3284-97.
93. VILLANUEVA E, YALAVARTHI S, BERTHIER CC *et al*.: Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. *J Immunol* 2011; 187: 538-52.
94. KNIGHT JS, KAPLAN MJ: Lupus neutrophils: "NET" gain in understanding lupus pathogenesis. *Curr Opin Rheumatol* 2012; 24: 441-50.
95. ELKON KB, SANTER DM: Complement, interferon and lupus. *Curr Opin Immunol* 2012; 24: 665-70.
96. MITSIOS A, ARAMPATZIOGLOU A, ARELAKI S, MITROULIS I, RITIS K: NETopathies? Unraveling the dark side of old diseases through neutrophils. *Front Immunol* 2016; 7: 678
97. RÖNNBLOM L, ELORANTA ML, ALM GV: Role of natural interferon-α producing cells (plasmacytoid dendritic cells) in autoimmunity. *Autoimmunity* 2003; 36: 463-72.
98. LINDAU D, MUSSARD J, RABSTEYN A *et al*.: TLR9 independent interferon-α production by neutrophils on NETosis in responses to circulatory chromatin, a key lupus autoantigen. *Ann Rheum Dis* 2014; 73: 2199-207.
99. BARRIENTOS L, BIGNON A, GUEGUEN C *et al*.: Neutrophil extracellular traps downregulates lipopolysaccaride-induced activation of monocyte-derived dendritic cells. *J Immunol* 2014; 193: 5689-98.
100. KESHARI RS, JOYTI A, DUBEY M *et al*.: Cytokines induced neutrophil extracellular traps formation: implication for inflammatory disease condition. *PLoS One 2012*; 7: e48111.
101. PARKER H, WINTERBOURN CC: Reactive oxidants and myeloperoxidase and their involvement in neutrophil extracellular traps. *Front Immunol* 2013; 3: 424.
102. GUPTA AK, HASLER P, HOLZGREVE W, GEBHARDT S, HAHN S: Induction of neutrophil extracellular DNA lattices by placental microparticles and IL-8 and their presence in preeclampsia. *Hum Immnol* 2005; 66: 1146-54.
103. HAKKIM A, FUCHS TA, MARTINEZ NE *et al*.: Activation of the Ref-MEK-ERK pathway is required for neutrophil extracellular trap formation. *Nat Chem Biol* 2011; 7: 75-77.
104. HOPPENBROUWERS T, AUTAR ASA, SULTAN AR *et al*.: *In vitro* induction of NETosis: comprehensive live imaging comparison and systemic review. *PLoS One* 2017; 12: e0176472.
105. RADA B: Neutrophil extracellular traps and microcrystals. *J Immunol Res* 2017; 2017: 2896380. E-pub 2017 Mar 7.
106. WANG Y, LI M, STADLER S *et al*.: Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. *J Cell Biol* 2009; 184: 205-13.
107. PAPAYANNOPOULOS W, METZLER KD, HAKKIM A, ZYCHLINSKY A: Neutrophil elastase and myeloperoxidase regulate the

formation of neutrophil extracellular traps. *J Cell Biol* 2010; 191: 677-91.
108. NEELI I, DWIVEDI N, KHAN S, RADIC M: Regulation of extracellular chromatin release from neutrophils. *J Innate Immun* 2009; 1: 194-201.
109. LI P, LI M, LINDBERG MR, KENNETT MJ, XIONG N, WANG Y: PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. *J Exp Med* 2010; 207: 1855-62.
110. REMIJSEN Q, VAN DEN BERGHE T, WIRAWAN E *et al*.: Neutrophil extracellular trap cell death requires both autophagy and superoxide generation. *Cell Res* 2011; 21: 290-304.
111. FUCHS TA, BRILL A, DUERSCHMIED D *et al*.: Extracellular traps promote thrombosis. *Proc Natl Acad Sci USA* 2010; 107: 15880-5.
112. PINEGIN B, VOROBJEVA N, PINEGIN V: Neutrophil extracellular traps and their role in the development of chronic inflammation and autoimmunity. *Autoimmun Rev* 2015; 14: 633-40.
113. YU Y, SU K: Neutrophil extracellular traps and systemic lupus erythematosus. *J Clin Cell Immunol* 2013; 4: 139.
114. LIGHTFOOT YL, KAPLAN MJ: Disentangling the role of neutrophil extracellular traps in rheumatic diseases. *Curr Opin Rheumatol* 2017; 29: 65-70.
115. JORCH SK, KUBES P: An emerging role for neutrophil extracellular traps in noninfectious disease. *Nat Med* 2017; 23: 279-87.
116. DELGADO-RIZO V, MARTINEZ-GUZMÁN MA, IÑIGUEZ-GUTIERREZ L, GARCÍA-OROZCO A, ALVARADO-NAVARRO A, FAFUTIS-MORRIS M: Neutrophil extracellular traps and its implications in inflammation: an overview. *Front Immunol* 2017; 8: 81.
117. SØRENSEN OE, BORREGAARD N: Neutrophil extracellular traps - the dark side of neutrophils. *J Clin Invest* 2016; 126: 1612-20.
118. GYLLENHAMMAR H, HAFSTROM I, RINGERTZ B, UDEN AM, PALMBALD J: Recombinant human leukocyte interferon modulates neutrophil function *in vitro*. *J Interferon Res* 1988; 8: 441-9.
119. AAS V, LAPPEGARD KT, SIEBKE, BENESTAD HB: Modulation by interferons of human neutrophilic granulocyte. *J Interferon Cytokine Res* 1996; 16: 929-35.
120. KOIE T, SUZUKI K, SHIMOYAMA T, UMEDA T, NAKAJI S, SUGAWARA K: Effect of interferon-alpha on production of reactive oxygen species by human neutrophils. *Luminescence* 2001; 16: 39-43.
121. BRINKMANN V, REICHARD U, GOOSMANN C *et al*.: Neutrophil extracellular traps kill bacteria. *Science* 2004; 303: 1532-5.
122. TIMÁR CI, LORINCZ AM, CSÉPÁNYI-KÖMI R *et al*.: Antibacterial effect of microvesicles released from human neutrophilic granulocytes. *Blood* 2013; 121: 510-8.
123. BRANZK N, LUBOJEMSKA A, HARDISON SE *et al*.: Neutrophils sense microbe size and selectively release neutrophil extracellular traps in resonse to large pathogens. *Nat Immunol* 2014; 15: 1017-25.
124. PATEL S, KUMAR S, JYOTI A *et al*.: Nitric oxide donors release extracellular traps from human neutrophils by augmenting free radical generation. *Nitric Oxide* 2010; 22: 226-34.
125. HAHN J, KNOPH J, MAUERODER D, LEPPKES M, HM: Neutrophils and neutrophil extracellular traps orchestrate initiation and resolution of inflammation. *Clin Exp Rheumatol* 2016; 34 (Suppl. 98): S6-8.
126. MERTELLI-PALOMINO G, PAOLIELLO-PASCHOALATO AB, CRISPIM JC *et al*.: DNA damage increase in peripheral neutrophils from patients with rheumatoid arthritis is associated with the disease activity and the presence of shared epitope. *Clin Exp Rheumatol* 2007; 35: 247-54.
127. PAPAYANNOPOULOS V: Neutrophil extracellular traps in immunity and disease. *Nat Rev Immunol* 2018; 18: 134-47.
128. LANGE C, CSERNOK E, MOOSIG F, HOLLE JU: Immune effects of neutrophil extracellular traps in granulomatosis with polyangiitis. *Clin Exp Rheumatol* 2017 (Suppl. 103); S33-39.
129. NATORSKA J, ZABCZYK M, SIUDUT J, KRAWIEC P, MASTALERZ L, UNDAS A: Neutrophil extracelluar traps formation in patients with eosinophilic granulomatosis with polyangiitis: association with eosinophilic inflammation. *Clin Exp Rheumatol* 2017 (35 Suppl.); 103: 27-32.
130. SPRONK PE, BOOTSMA H, HORST G *et al*.: Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. *Br J Rheumatol* 1966; 35: 625-31.
131. BORODIN AG, BARANOV AA, SHILKINA NP, NASONOV EL, BAZHINA OV: Clinical significance of myeloperoxydase and proteinase 3 antibodies in patients with systemic lupus erythematosus. *Klin Med* (Mosk) 1999; 77: 24-8.
132. MANOLOVA I, DANCHEVA M, HALACHEVA K: Antineutrophil cytoplasmic antibodies in patients with systemic lupus erythematosus: prevalence, antigen specificity, and clinical associations. *Rheumatol Int* 2001; 20: 197-204.
133. NAUSEEF WM, KUBES P: Pondering neutrophil extracellular traps with healthy skepticism. *Cell Microbiol* 2016; 18: 1349-57.
134. DRYER AK, HOFFMANN D, LACHMANN N *et al*.: TALEN-mediated functional correction of X-linked chronic granulomatous disease in patient-derived induced pluripotent stem cells. *Biomaterials* 2015; 69: 191-200.
135. PICKERING MC, BOTTO M: Are anti-C1q antibodies different from other SLE autoantibodies? *Nat Rev Rheumatol* 2010; 6: 490-3.
136. VAN DEN BERG RH, SIEGERT CEH, FABER-KROL MC, HUIZINGA TWJ, VAN ES LA, DAHA MR: Anti-C1q receptor/calreticulin autoantibodies in patients with systemic lupus erythematosus (SLE). *Clin Exp Immunol* 1998; 111: 359-64.
137. WILSON JG, RATNOFF WD, SCHUR PH, FEARON DT: Decreased expression of the C3b/C4b receptor (CR1) and the C3d receptor (CR2) on B lymphocytes and of CR1 on neutrophils of patients with systemic lupus erythematosus. *Arthritis Rheum* 1986; 29: 739-47.
138. EDBERG JC, WU J, LANGEFELD CD *et al*.: Genetic variation in the CRP promoter: association with systemic lupus erythematosus. *Hum Mol Genet* 2008; 17: 1147-55.
139. DELONGUI F, LOZOVOY MAB, IRIYODA TMV *et al*.: C-reactive protein +1444CT (rs1130864) genetic polymorphism is associated with the susceptibility to systemic lupus erythematosus and C-reactive protein levels. *Clin Rheumatol* 2017; 36: 1779-88.
140. PESICKOVA SS, RYSAVA R, LENICEK M *et al*.: Prognostic value of anti-CRP antibodies in lupus nephritis in long-term follow-up. *Arthritis Res Ther* 2015; 17: 371-7.
141. CHITRABAMRUNG S, RUBIN RL, TAN EM: Serum deoxyribonuclease 1 and clinical activity in systemic lupus erythematosus. *Rheumatol Int* 1981; 1: 55-60.
142. LACHMANN PJ: Lupus and deoxyribonuclease. *Lupus* 2003; 12: 202-6.
143. YEH TM, CHANG HC, LIANG CC, WU JJ, LIU MF: Deoxyribonuclease-inhibitory antibodies in systemic lupus erythematosus. *J Biomed Sci* 2003; 10: 544-51.
144. VAN DER WOUDE FJ, VAN DER GIESSEN M, KALLENBERG CG *et al*.: Reticuloendothelial Fc receptor function in SLE patients. I. Primary HLA linked defect or acquired dysfunction secondary to disease activity? *Clin Exp Immunol* 1984; 55: 473-80.
145. GAIPL US, VOLL RE, SHERIFF A, FRANZ S, KALDEN JR, HERRMANN M: Impaired clearance of dying cells in systemic lupus erythematosus. *Autoimmun Rev* 2005; 4: 189-94.
146. LEFFLER J, MARTIN M, GULLSTRAND B *et al*.: Neutrophil extracellular traps that are not degraded in systemic lupus erythematosus activate complement exacerbating the disease. *J Immunol* 2012; 188: 3522-31.
147. YU CL, SUN KH, TSAI CY *et al*.: Expression of Th1/Th2 cytokine mRNA in peritoneal exudative polymorphonuclear neutrophils and their effects on mononuclear cell Th1/Th2 cytokine production in MRL-*lpr/lpr* mice. *Immunology* 1998; 95: 480-7.
148. LI KJ, LU MC, HSIEH SC *et al*.: Release of surface-expressed lactoferrin from polymorphonuclear neutrophils after contact with $CD4^+$T cells and its modulation on Th1/Th2 cytokine production. *J Leukoc Biol* 2006; 80: 350-8.
149. LI KJ, WU CH, SHEN CY, KUO YM, YU CL, HSIEH SC: Membrane transfer from mononuclear cells to polymorphonuclear neutrophils transduces cell survival and activation signals in the recipient cells via anti-extrinsic apoptotic and MAP kinase signaling pathways. *PLoS One* 2016; 11: e156262.
150. GASSER O, HESS C, MIOT S, DEON C, SANCHEZ J-C, SCHIFFERLI JA: Characterisation and properties of ectosomes released by human polymorphonuclear neutrophils. *Exp Cell Res* 2003; 285: 243-57.
151. SADALLAH S, EKEN C, SCHIFFERLI JA: Ectosomes as immunomodulators. *Semin Immunopathol* 2011; 33: 487-95.
152. TSAI CY, HSIEH SC, LU MC, YU CL: Aberrant non-coding RNA expression profiles as biomarker/biosignature in autoimmune and inflammatory rheumatic diseases. *J Lab Preci Med* 2018; doi:10.21037/jlpm 2018. 05.02.
153. KAPLAN MJ: Role of neutrophils in systemic autoimmune diseases. *Arthritis Res Ther* 2013; 15: 219.
154. SMITH CK, KAPLAN MJ: The role of neutrophils in the pathogenesis of systemic lupus erythematosus. *Curr Opin Rheumatol* 2015; 27: 448-53.
155. NÉMETH T, MÓCSAI A, LOWELL CA: Neutrophils in animal models of autoimmune disease. *Semin Immunol* 2016; 28: 174-86.