*TRAP*ping the cellular mechanisms of lupus

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Keywords: interferon; NETosis; NETs; neutrophil extracellular traps; systemic lupus erythematosus
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Systemic lupus erythematosus (SLE) is characterized by functional changes in B and T cells, plasmacytoid dendritic cells (pDCs), and monocytes, as well as autoantibody deposition at inflammation sites and raised levels of circulating cytokines and chemokines (Bosch et al, 2011). In SLE, the autoimmune activity is mediated by chronically activated pDCs, resulting in high levels of type I interferon (IFN) and B-cell-mediated autoantibody production, especially against nuclear components such as self-DNA (Obermoser et al, 2011). However, the precise role of neutrophils in SLE pathogenesis remains unclear. Two recent studies shed light on this issue and have demonstrated neutrophil activation by IFN-α in SLE, with neutrophil extracellular traps (NETs) containing unwound deoxyribonucleic acid (DNA) and antimicrobial peptides being released. Specifically, Garcia-Romo et al (2011) demonstrated that, in SLE, NETs activate IFN-producing pDCs, while Lande et al (2011) found that neutrophils release peptide/self-DNA complexes that trigger pDC activation and autoantibody formation.

Composed of decondensed chromatin material and some granular and cytoplasmic proteins, NETs bind to both Gram-negative and -positive bacteria and regulate, extracellularly, the severity of infection. NETs are released during NETosis, a form of pathogen-induced cell death in which the nuclei swell and the chromatin melts. In particular, the cell extrudes large strands of decondensed DNA carrying cytosol proteins, granules and chromatin. Up to 24 neutrophil proteins associated with NETs have been identified, principally the cationic (DNA-binding) bactericidal proteins such as histones, defensins, proteinase 3 and myeloperoxidase (MPO) (Urban et al, 2009).

The mechanism of NET formation remains unclear. Chromatin decondensation is associated with histone H3 citrullination, in which histone arginine is transformed into citrulline remainders by peptidylarginine deiminase 4 (PAD4), an enzyme expressed in mature neutrophils. This seems to be central to NET formation since neutrophils from PAD4-deficient mice fail to form NETs. Further, elastase freed from azurophil granules degrades histones and, together with MPO, drives the chromatin decondensation necessary for NET formation, which is reliant on hydrogen peroxide produced by nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase and additionally metabolized by MPO (Papayannopoulos et al, 2010). Recent findings that nitric oxide induces NETs in an MPO-dependent fashion and that extracellular DNA strands may be released from the neutrophils' mitochondria denotes an alternative to NET creation as a neutrophil death mechanism (Borregaard, 2010).

Neutrophil extracellular traps are cleared by DNAses, and SLE patients not only have auto-antibodies against DNA-associated proteins such as NET components, but a subset of patients display decreased activity of the plasmatic NET-degrading protein, DNAse-1 (Hakkim et al, 2010). Likewise, NETs have been shown to induce *in vitro* thrombosis by stimulating platelets and to damage activated human endothelial cells, suggesting a potential pro-inflammatory role (Fuchs et al, 2010).

Evidence that NETs are released by anti-neutrophil cytoplasmic antibodies (ANCA)-stimulated neutrophils and contain the targeted autoantigens proteinase 3 and MPO highlights the pathogenic potential of NETs in autoimmune disease (Kessenbrock et al, 2009). ANCA have been shown to be pathogenic in a subgroup of patients with autoimmune small-vessel vasculitides with common renal involvement in the form of glomerulonephritis. The finding of both circulating MPO–DNA complexes and NETs in damaged kidneys suggests that NET formation promotes the autoimmune response against neutrophil components, eventually resulting in vasculitis. Importantly, TNF-α-activated neutrophils have been shown to undergo NETosis upon incubation with anti-MPO antibodies from patients with ANCA-related vasculitis and NET-related material was detected in kidney sections.

What is the neutrophil-NET-SLE connection? The origin of the self-antigens, which provoke the altered immune reactions in SLE, and the manner in which inflammatory responses are provoked by immune complexes remain unclear. Blood samples from SLE patients with disease bouts and increased IFN responses contain large amounts of immature neutrophils, reflecting raised bone marrow release, probably due to death of mature

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DOI 10.1002/emmm.201100167

neutrophils after activation by cytokines including IFN. Although mature dying neutrophils seem to be involved in tissue lesions in SLE, their disease-promoting role is unclear (Craft, 2011).

Garcia-Romo et al discovered that, in SLE, mature neutrophils suffer accelerated cell death compared to normal neutrophils, and undergo NETosis after exposure to anti-ribonucleoprotein (RNP) auto-antibodies in an Fc gamma receptor IIA (FcγRIIa)- and Toll-like receptor 7 (TLR7)-dependent process. They also found that anti-RNP auto-antibody-containing immune complexes bind to FcγRIIa on neutrophils, with TLR7 engagement and reactive oxygen species (ROS) generation. TLR7 expression in neutrophils is heightened by pDC-secreted IFN-α. Neutrophil death by NETosis is promoted by FcγRIIa mediated anti-RNP neutrophil binding, which generates the ROS required for neutrophil enzyme activation and transport to the cellular nucleus to begin the DNA disentangling vital for NET creation (Craft, 2011). The authors also demonstrated that NETs contained neutrophil-encoded antimicrobial peptide LL37 (cathelicidin) (Fig 1).

Lande et al found that, in SLE, immunogenic DNA-containing complexes contain LL37/self-DNA and human neutrophil peptides that stimulated Toll-like receptor 9 (TLR9) in pDCs, resulting in IFN-α production. Furthermore, NET release was induced by autoantibodies against antimicrobial peptide autoantibodies. In SLE, neutrophils release peptide/self-DNA complexes that accelerate pDC activation and autoantibody generation. The authors found that LL37 protects NET DNA against extracellular degradation while enabling DNA uptake by and activation of pDCs via TLR9 engagement, leading to IFN-α production. *In vitro* internalization of DNA complexes via FcγRIIa on pDCs was raised by anti-LL37 and anti-DNA autoantibodies, of which significant amounts were found in sera from SLE patients but not those with progressive systemic sclerosis or healthy controls. Self-DNA-containing immune complex immunogenicity has been ascribed to internalization of DNA into TLR9-containing compartments, which requires neutrophil antimicrobial peptides that shield DNA against enzymatic degradation. LL37 can protect DNA against degradation, possibly due to its exclusive α-helical configuration, which efficiently stabilizes charge interactions with DNA (Lande et al, 2011). Lande et al also propose that complexes of self-DNA and antimicrobial peptides help activate self-reactive B cells in SLE, resulting in the origination of anti-LL37 autoantibodies, a deduction founded on the relationship between these autoantibodies and those directed against DNA, an auto-antigen known to elicit autoreactive B cell activation. Further, the authors found that neutrophils exposed to IFN-α *in vitro* displayed raised levels of antimicrobial peptides on their membranes, where they underwent antimicrobial peptide antibody binding to incite NET release. These findings, like those of Garcia-Romo et al, show that IFN-α primes neutrophils for NETosis.

These novel findings show that type I IFNs are central to SLE pathogenesis, and

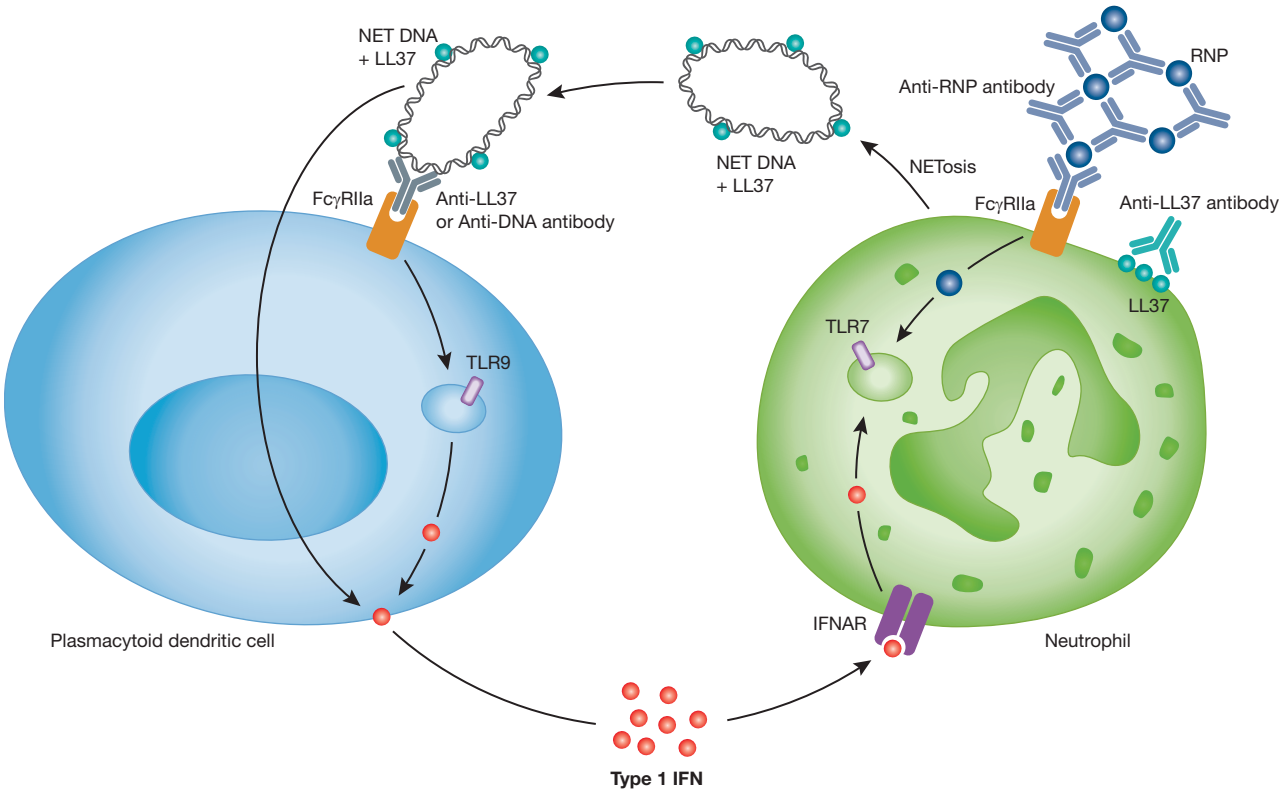

Figure 1. NETs in SLE. IFNs stimulate neutrophil activation, which triggers NET formation in autoantibody engagement, leading to pDC activation and IFN production.

that NET release by neutrophils and their uptake and activation of pDCs result in the chronic IFN-α production often seen in SLE. By directly linking autoantibodies to neutrophil NETosis, they also prove a new pathogenic role of autoantibodies in SLE (Fig 1).

The vital role of IFN-α observed in SLE supports IFN antagonism as a therapeutic approach in this disease, while TLR signalling blockade with biologics, small molecules or nucleic acid-based drugs may also offer a potential therapeutic option. Future studies will assess whether suppression of NET formation through ROS scavenging could cease chronic autoimmunity in SLE.

» . . .this new evidence suggests that neutrophils might play an important connecting role in SLE autoimmunity and pathogenesis «

In conclusion, this new evidence suggests that neutrophils might play an important connecting role in SLE autoimmunity and pathogenesis, with autoantibody activation leading to the release of DNA-containing NETs which, in turn, prompt IFN production by pDCs, one of the main drivers of inflammation and damage in SLE. More studies are required to explore the involvement of NETs in SLE and other autoimmune and inflammatory disorders with confirmed neutrophil activation.

The author declares that he has no conflict of interest.

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