Neutrophil extracellular traps and low-density granulocytes are associated with the interferon signature in systemic lupus erythematosus, but not in antiphospholipid syndrome

With great interest we read the article by Rahman *et al*¹ about the clinical associations and functional characteristics of low-density granulocytes (LDGs) in patients with systemic lupus erythematosus (SLE).

LDGs are a subset of proinflammatory neutrophils which numbers are increased in patients with SLE as well as other rheumatic diseases including antineutrophil cytoplasmic antibody-associated vasculitis, rheumatoid arthritis and antiphospholipid syndrome (APS).^{2–5} LDGs are proposed to be involved in the pathogenesis of rheumatic diseases by spontaneously releasing neutrophil extracellular traps (NETs).^{5,6} NETs consist of web-like structures of chromatin decorated with antimicrobial and other neutrophil-derived peptides. NETs induce vascular damage,⁶ thrombosis⁷ and are a source of autoantigens⁵ to trigger autoimmunity and thus could contribute to the pathogenesis of autoimmune diseases.

Arguably, the most important contribution of NET release to the pathogenesis of SLE is their effect on plasmacytoid dendritic cells (pDCs). In vitro, NETs trigger toll-like receptors on pDCs resulting in the production of interferon alpha (IFN α).^{6,8,9} In turn, IFN α primes neutrophils to release NETs^{8,9} resulting in a perpetuating pathogenic cycle of NET release and IFN α production. Elevated levels of IFN α induce a gene signature termed the IFN signature. This signature is characteristic of several autoimmune diseases, most notably SLE, and considered a therapeutic target. NETs are thus considered major contributors to the IFN signature in these diseases. In support of this hypothesis, Rahman *et al* report elevated numbers of LDGs only in patients with SLE displaying a high IFN signature.

We recently reported increased numbers of LDGs in patients with SLE and/or APS.³ Similar to the findings by Rahman *et al*

we also found elevated numbers of LDGs confined to patients with SLE displaying a high IFN signature.¹⁰ In addition to LDG numbers, we studied NET release induced by patient's plasma and found increased NET release only in patients with SLE with a high IFN signature.¹⁰ Our observations are therefore confirmed by Rahman *et al* and show that the in vitro capacity of NETs to induce IFN α secretion by pDCs translates to an in vivo association between LDG numbers and NET release with the IFN signature in patients with SLE.

However, in our analysis the association between the IFN signature and NET release or LDG numbers was confined to patients with SLE. We did not find such an association in patients with APS, neither in SLE+APS nor in primary APS (PAPS, patients with APS and without SLE),¹⁰ despite the presence of an IFN signature and increased NET release and LDGs in patients with (P)APS.^{7,11,12} On a closer look, this was caused by an increase in NET release and LDG numbers in both IFN-negative and IFN-positive patients with APS. Therefore, in APS, LDGs and NET release are increased irrespective of the IFN signature.¹⁰

Possibly, this difference between SLE and APS lies in the presence of anti- β 2 glycoprotein I (β 2GPI) antibodies in patients with APS. Anti- β 2GPI antibodies induce the release of NETs in neutrophils from healthy donors⁷ and are associated with increased NET release and higher LDG numbers in patients with primary APS.^{3,7} In APS, NET release and LDG numbers are therefore increased due to the presence of anti- β 2GPI antibodies, even in patients without the IFN signature. Consequently, the role of NETs on the presence of an IFN signature is stronger in SLE as compared with APS, in which other factors may influence the IFN signature.

In addition to their role in the maintenance of the IFN signature, Rahman *et al*¹ highlight a novel role for LDGs in the pathogenesis of SLE by showing that LDGs interfere with T cell activation. Supernatants of LDGs were found not to suppress T cell proliferation, in contrast to supernatants of normal-density granulocytes. Furthermore, LDGs induced the production of

Figure 1 Increased numbers of LDGs in association with disease activity and low complement levels in patients with SLE. Numbers of LDGs were determined by flow cytometry in peripheral blood as described¹⁰ in patients with SLE stratified by disease activity as measured by SLEDAI (A) or low complement levels (either C3 or C4 below the lower limit of normal) (B). HC, healthy control; LDGs, low-density granulocytes; ns, not significant; SLEDAI, systemic lupus erythematosus disease activity index. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

proinflammatory cytokines such as IFN γ in T cells. The critical role for LDGs in the clinical manifestations of SLE is further substantiated by Rahman *et al* by showing increased numbers of LDGs in patients with active disease (SLE Disease Activity Index ≥ 4) and patients with signs of complement activation. In our cohort, we confirm an association of higher numbers of LDGs with disease activity and low complement levels in patients with SLE (figure 1).

Whether or not LDGs have similar effects on T cells in APS and other autoimmune diseases remains to be determined. Several key immunological features are often shared among rheumatic diseases, increased NET release and LDG numbers and the IFN signature form an example for SLE and APS. Our observations, however, suggest different roles for NETs and LDGs in the pathogenesis of these diseases. Therefore, we advocate the inclusion of different patient groups when studying NETs in order to increase our understanding of the contribution of NETs and LDGs to the pathogenesis of autoimmune diseases.

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