

COMMENTARY

# Neutrophils and interferon- $\alpha$ -producing cells: who produces interferon in lupus?

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## Abstract

Interferon- $\alpha$  plays a crucial role in the pathogenesis of systemic lupus erythematosus. Nevertheless, the different human cell types producing this cytokine as well as the stimuli inducing its production have not been completely characterized. So far, a subpopulation of dendritic cells activated by immune complexes has been identified as major producers of interferon- $\alpha$  in patients with lupus. However, those cells represent a minor population and some studies have reported the secretion of interferon- $\alpha$  by other cells. On the other hand, more than 50% of blood leukocytes are neutrophils and their functions are still not fully understood. Recent data suggest that neutrophils, though usually not considered interferon- $\alpha$ -producing cells, may represent an unexpected source of this cytokine in response to some lupus stimuli.

Serum interferon (IFN)- $\alpha$  concentrations are elevated in patients with systemic lupus erythematosus (SLE) [1] and contribute to the break of peripheral tolerance by sustaining the differentiation of myeloid dendritic cells [2]. IFN- $\alpha$  actually increases the differentiation of monocytes into dendritic cells that present antigens from dying cells to CD4<sup>+</sup> lymphocytes, resulting in their proliferation. The involvement of IFN- $\alpha$  in SLE development is further supported by the IFN-inducible gene expression signature that characterizes a clinical subset of patients with SLE and more severe disease (for example, damage of the kidneys or the central nervous system) [3]. Plasmacytoid dendritic cells (PDCs) have been suggested to be the main cells producing IFN- $\alpha$  [4], and an inducer of IFN- $\alpha$  has been identified in the circulation of patients with SLE [5]. Currently, PDCs activated by DNA- and RNA-containing immune complexes are believed to be the main source of IFN- $\alpha$  in SLE upon triggering of Toll-like

receptor (TLR) 9 and TLR7, respectively, in cooperation with CD32 [6-8]. Nevertheless, PDC depletion experiments suggest that other cell types produce IFN- $\alpha$  in SLE [2] and type I IFN production occurs independently of Fc $\gamma$  receptors in a lupus mouse model [9], indicating that uptake of immune complexes is not required. In a different lupus mouse model, the double-stranded RNA analog poly I:C was shown to induce IFN- $\alpha$  production in spleen monocytes [10], confirming that – depending on the stimulus – cells other than PDCs may produce IFN- $\alpha$  independently of the presence of immune complexes.

Interestingly, Fairhurst and colleagues [11] recently showed that type I IFNs are produced by resident renal cells, suggesting that locally produced IFN- $\alpha$  may promote glomerulonephritis development. Moreover, this mechanism seems to involve the recruitment of neutrophils (polymorphonuclear leukocytes, or PMNs). In agreement with those results, granulocytes are activated in active SLE as suggested by the overexpression of granulopoiesis-related genes, known as the granulopoiesis signature [12]. This microarray analysis performed on blood cells suggests that granulocytes are involved in SLE pathogenesis. Moreover, PMNs are about 200 times more abundant than PDCs in the blood and are the first cells recruited at sites of inflammation. PMNs play a central role in host defense and, once activated, produce proinflammatory cytokines and reactive species. However, upon inappropriate activation, PMN-derived products may induce tissue damage. PMNs express TLRs 1 to 10 (except TLR3) and link innate and adaptive immunity by influencing, for example, dendritic cells. Importantly, PMNs are capable of producing IFN- $\alpha$  in response to some stimuli [13]. Normal and lupus PMNs, however, were not compared and only granulocyte colony-stimulating factor (G-CSF), in contrast to lipopolysaccharide (LPS) or N-formyl-methionine-leucine-phenylalanine (fMLP), was shown to induce IFN- $\alpha$  mRNA transcripts. A recent study confirmed the latter observation by using PMNs isolated from patients with SLE and described the induction of type I IFN upon activation with G-CSF, phorbol 12-myristate 13-acetate (PMA), or transfected poly I:C, although the natural lupus stimulus and the mechanism involved were not identified [14]. The

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aforesaid cells represent a subset of proinflammatory PMNs secreting increased levels of several cytokines, such as tumor necrosis factor (TNF) and IFN- $\gamma$  (upon activation), and may induce vascular damage. Those cells are low-density PMNs and represent an abnormal subset of neutrophils present in peripheral blood mononuclear cell (PBMC) preparations from patients with lupus. Low-density PMNs present features of both immature and activated PMNs and have a decreased phagocytic potential, but it is still unknown whether they represent a distinct cell population. It should be noted that another study has shown that human PMNs isolated from healthy donors also produce IFN- $\beta$  mRNA upon stimulation with transfected poly I:C [15], although IFN- $\alpha/\beta$  secretion was not detected. Likewise, mouse PMNs infected with encephalomyocarditis virus express IFN- $\beta$  mRNA, a mechanism partially dependent on the cytoplasmic RNA helicase MDA5. In regard to IFN- $\alpha$ , we could show that PMNs activated by chromatin, a major autoantigen in SLE, produced IFN- $\alpha$  in an immune complex-independent manner whereas non-stimulated PMNs did not (Dennis Lindau and colleagues, University of Paris 13, France, manuscript submitted). Mono-nucleosomes were particularly efficient in inducing PMN activation. In contrast to the findings of Denny and colleagues [14], IFN- $\alpha$  production was detected in steady-state PMNs and with PMNs isolated from both patients with SLE and healthy donors, suggesting that a key event is the presence of increased concentrations of circulating nucleosomes, a situation especially common *in vivo* in patients with SLE. Together with our previous results showing that free chromatin stimulates PMNs [16] independently of TLR9 [17], leading to interleukin (IL)-8, IL-6, and TNF secretion, CD11b upregulation, and increased phagocytic activity, all of those studies reveal an unsuspected role for PMNs in SLE pathogenesis. Nevertheless, further studies will be needed to identify the pathway elicited. A cellular cross-talk might be required to efficiently trigger IFN- $\alpha$  secretion by PMNs. Owing to their proinflammatory activity [18,19], platelets might be involved. Indeed, platelets activated by immune complexes were recently shown to potentiate IFN- $\alpha$  secretion by PDCs in SLE through a CD154-CD40 interaction [20]. Depending on the stimulus, activated PMNs may secrete IFN- $\alpha$  early during the lupus disease, before immune complexes are produced, bypassing the classical mechanism requiring the induction of autoantibodies and the formation of immune complexes activating PDCs. Thus, two complementary pathways might be involved in IFN- $\alpha$  induction in SLE.

## Conclusions

Because IFN- $\alpha$  secretion is a key pathogenic event in SLE, the characterization of the cellular sources and the

lupus stimuli represents a crucial step for the development of new therapeutic strategies aiming at controlling IFN- $\alpha$  secretion, especially at an early stage of the disease. Because they are abundant and recruited in inflamed tissues, activated PMNs may represent a new therapeutic target. Finally, future studies will be necessary to determine whether neutrophil extracellular traps (NETs) are involved in the induction of IFN- $\alpha$  production by PMNs and reciprocally whether circulating chromatin triggers the formation of NETs. Indeed, NETs are structures formed by activated PMNs and contain chromatin [21]. Because they form fibers that bind and kill bacteria, NETs represent a type of innate response. In particular, mature SLE PMNs have been shown very recently to release immunogenic NETs upon exposure to autoantibodies, leading to an activation-induced cell death named NETosis, and those lupus NETs trigger the production of IFN- $\alpha$  by PDCs in a TLR9-dependent manner [22,23]. Interestingly, this mechanism occurs in IFN- $\alpha$ -primed PMNs, implying that IFN- $\alpha$  had already been induced. Collectively, the data suggest that PMN-derived or PMN-induced IFN- $\alpha$  or both might have been underestimated.

## Abbreviations

G-CSF, granulocyte colony-stimulating factor; fMLP, N-formyl-methionine-leucine-phenylalanine; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; NET, neutrophil extracellular trap; PBMC, peripheral blood mononuclear cell; PDC, plasmacytoid dendritic cell; PMA, phorbol 12-myristate 13-acetate; PMN, polymorphonuclear leukocyte; SLE, systemic lupus erythematosus; TLR, Toll-like receptor; TNF, tumor necrosis factor.

## Competing interests

The author declares that he has no competing interests.

## Authors' information

Until August 2010, the author was affiliated with the Department of Immunology, Institute for Cell Biology, University of Tübingen (Tübingen, Germany). In September 2010, he moved to the University of Paris 13, France.

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