232. INTERFERONS ALTER THE RESPONSE OF NEUTROPHILS TO INFLAMMATORY CYTOKINES IN VITRO

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Background: Neutrophils are central to the initiation, progression and resolution of inflammatory diseases such as RA. We have previously shown that neutrophils from RA patients have a gene expression signature indicating activation in vivo by interferons (IFNs), and higher expression of IFN-response genes is associated with a good response to TNFi therapy. The aim of this work was to investigate the functional effects of IFNs on neutrophils in vitro both (i) alone, and (ii) in combination with inflammatory cytokines.

Methods: Neutrophils were isolated from peripheral blood of healthy controls and incubated with Type-I IFN (IFNs) or Type-II IFN (IFNγ) at a range of concentrations in the absence or presence of GM-CSF and TNFs. Apoptosis was measured at 18h by flow cytometry using annexin V/PI; respiratory burst was measured at hourly intervals up to 5h using luminol-enhanced chemiluminescence after stimulation with fMLP or PMA; changes in protein expression and phosphorylation were measured by Western blotting; changes in gene expression were measured by RNA-Seq (Illumina).

Results: Neutrophils stimulated with IFNs in vitro underwent rapid phosphorylation of STAT proteins (5–30 min), activation of IFN-response genes (1%), and priming of the respiratory burst (3%), but only Type-II IFN delayed apoptosis, measured at 18h (unstimulated 58.6%±0.6, IFNγ 41.4%±4.1, P<0.05). Addition of IFNs to neutrophil suspensions containing GM-CSF or TNFs had a profound dose-dependent effect on the function of the inflammatory cytokines. Type-I IFNs abrogated the protective effect of GM-CSF on neutrophil apoptosis at 18h (GM-CSF 25.6%±2.7, GM-CSF+IFNγ 49.5%±1.2, P<0.01), whereas Type-II IFNs enhanced the anti-apoptotic effect of TNFs (TNFα 42.3%±3.3, TNFα+IFNγ 27.4%±1.2, P<0.05) and sustained the TNFs priming effect on the respiratory burst for up to 4h (P<0.01). Type-I and Type-II IFNs enhanced STAT3 phosphorylation by GM-CSF, and altered the activation kinetics of ERK and AKT by GM-CSF. Type-I IFN enhanced AKT phosphorylation in TNFs stimulated neutrophils.

Conclusion: IFNs profoundly alter the functional effects of inflammatory cytokines on neutrophils in vitro. This may have important consequences in vivo during therapy with biologic drugs such as TNFi. The complexity and heterogeneity of inflammatory diseases such as RA, where different cytokines dominate or act synergistically to perpetuate systemic inflammation, may explain why some patients respond better to certain biologic therapies than others. We are currently investigating the consequences of TNFi (infliximab) and JAKI (Tofacitinib) on neutrophils stimulated in vitro with IFNs, GM-CSF and TNFs.

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