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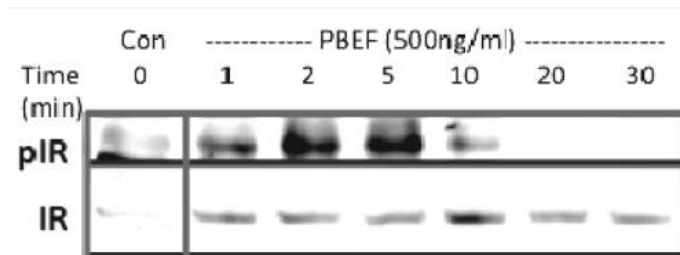
PRE-B CELL COLONY-ENHANCING FACTOR (PBEF) PRIMES NEUTROPHIL (PMN) OXIDATIVE BURST BY ACTIVATING THE INSULIN RECEPTOR AND DOWNSTREAM MAPKS

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Introduction: PBEF is a pleiotropic 52 kD protein that is upregulated in sepsis and ARDS. We have previously shown that PBEF can inhibit PMN apoptosis, and prime PMN for enhanced respiratory burst activity. PBEF exerts both extracellular cytokine-like activity, possibly through interactions with the insulin receptor (IR), and intracellular enzymatic activity as the rate-limiting step in a salvage pathway of NAD biosynthesis. We hypothesized that extracellular PBEF competes with insulin to prime for PMN oxidative burst, and sought to define the downstream pathways signaling this effect.

Methods: PMN (10^6) from healthy human donors were treated with recombinant PBEF (does), LPS (1· g/ml), NAD (1mM), or nicotinamide (1mM). In some experiments, cells were pre-incubated with: IR inhibitor – HNMPA (50uM); insulin (100nM); ERK Inhibitor-PD98059 (1uM); p38 inhibitor-SB203580 (1uM); or PBEF inhibitor-FK866 (250nM). Oxidative burst was measured by flow cytometry as fluorescence of dihydrorhodamine; priming was measured by further stimulation with fMLP (100nM) for 10mins. Co-immunoprecipitation was conducted on control and LPS-stimulated cell lysates using anti-IR or –PBEF antibodies and protein G sepharose beads followed by SDS-PAGE. Protein expression was quantified by densitometry.

Results: We previously found that recombinant PBEF primes PMN for increased oxidative burst in response to fMLP; pre-treatment with insulin or an inhibitor of IR attenuates the priming effect. Both PBEF and insulin induced phosphorylation of IR in PMN (Figure). Both Erk and p38 MAPK's were phosphorylated following rPBEF stimulation, but not following insulin stimulation. To confirm the role of these MAPK's in rPBEF priming of oxidative burst, we used specific inhibitors to these MAPKs and found that rPBEF priming was blocked (see chart, values are mean · standard error of mean channel fluorescence). Finally, to determine whether the effects of rPBEF were actually



occurring via its ability to recycle NAD, we tested whether NAD or nicotinamide, the substrate for PBEF, could induce priming and found no priming occurred (fMLP · 2193.8 · 75.3; rPBEF · fMLP · 5804.0 · 74.7^a; nicotinamide · fMLP · 2185.8 · 169.5^{b,c}; NAD · fMLP · 1418.8 · 76.2^b). Additionally, an inhibitor of PBEF enzymatic activity yielded no change in its priming ability (rPBEF · fMLP · 2906.8 · 81.6; FK · rPBEF · fMLP · 2803.3 · 53.6^d). Finally, we found activation of IR was not observed upon stimulation with nicotinamide or NAD alone.

a. p· 0.05 vs. fMLP; b. p· 00.5 vs. rPBEF · fMLP; c. N.S. vs. fMLP; d. N.S. vs. rPBEF · fMLP

Conclusion: rPBEF primes for PMN oxidative burst through the IR and downstream MAPKs in PMN; its activity is competitively blocked by insulin. Priming does not require NAD-generating enzymatic activity of PBEF. Since PBEF expression is increased in sepsis and inflammatory conditions, competitive interactions between PBEF and insulin may play an important role in regulating the inflammatory function of PMN.