The Defective Stimulus-Response Coupling for Oxidative Reactions in Neutrophils from Patients with Polycythemia vera

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We read with interest the report by Piva et al. [1] describing an impaired function of the NADPH oxidase, assessed as the reduction of nitroblue tetrazolium (NBT) after stimulation with phorbol myristate acetate (PMA), in patients with various stages of myelodysplastic syndromes and different chronic myeloproliferative disorders. In that paper, the authors concluded that PMA-induced NBT reduction was reduced in a few patients with polycythemia vera (PV) that were investigated. Since our group in a series of studies has come to different conclusions regarding the oxidative burst in polymorphonuclear neutrophil granulocytes (PMN) in PV, we would like to briefly comment on the part of the paper by Piva et al. [1] that concerned PV. A more detailed review of our findings has recently been published [2].

Prior to 1988, all studies of oxidative metabolism in PV PMN had been performed with latex beads as stimulus. Cooper et al. [3] reported an increased oxygen consumption and NBT reduction, whereas other studies showed normal [4] or reduced NBT reduction [5, 6]. In the article by Piva et al. [1], our first study [7] was quoted as showing that 'in other myeloproliferative disorders, most authors have described normal or even activated neutrophils in PV. Though it is true that spontaneous as well as N-formyl-methionyl-leucyl-phenylalanine (fMLP)-induced adherence and chemotaxis was normal, our study demonstrated for the first time a stimulus-specific defect in the oxidative metabolism of PV PMN, assessed as chemiluminescence (CL), after stimulation with surface-receptor-dependent stimuli, i.e. fMLP and leukotriene B₄ (LTB₄). However, in contrast to the findings by Piva et al. [1], we found that CL induced by PMA was normal in PV PMN. Furthermore, in a later study [8], microscopic NBT reduction was similar in both unstimulated and PMA-stimulated PMN, but significantly reduced after fMLP stimulation. We subsequently showed that the generation of superoxide anions was significantly impaired in PV PMN after stimulation with fMLP, but once again, the response to PMA was normal [9]. Finally, we could show that CL induced by fMLP and PMA was normal in PMN from patients with chronic myelogenous leukemia (CML) and essential thrombocythemia (ET) [10]. Moreover, studies of hydrogen peroxide generation ($^1_8\text{C}^1_8$) in single PMN and monocytes by flow cytometry revealed that resting and PMA stimulated $^5_8\text{C}^1_8$ generation was normal in PV, CML and ET, as was fMLP induced $^7_8\text{C}^1_8$ generation in ET. In contrast, fMLP induced $^7_8 < ^3_4$

production was significantly impaired in PV PMN and monocytes. A less pronounced reduction of fMLP stimulated H_2O_2 production was also noted in CML [10].

In an effort to elucidate the biochemical basis of the impaired oxidative response to fMLP, detailed studies of the different steps in the stimulus-response coupling were performed. They showed that the amount and affinity of fMLP receptors, as well as fMLP- and PMA-induced activation of phospholipase C, measured as the generation of 1,4,5-inositoltrisphosphate and the subsequent rise in intracellular calcium, were normal in PV PMN [9]. On the other hand, the activation of phospholipase D (PLD) was impaired, since the generation of phosphatidylethanol (PET) was significantly reduced after fMLP stimulation, whereas the response to PMA, once again, was normal. The mechanism behind impaired PLD activation is currently under investigation by our group.

Thus, a series of studies from our laboratory [7-10] have shown that PMN from PV patients exhibit an impaired oxidative response measured as CL, NBT reduction, superoxide anion and hydrogen peroxide generation after stimulation with fMLP, whereas PMA stimulation has consistently elicited a normal response. Our results therefore seem to be in conflict with the results published by Piva et al. [1]. However, their microscopic NBT reduction test was performed on whole blood, whereas we have used pure PMN preparations, and as Piva et al. pointed out, interactions between PMN and other blood cells can have influenced their results. Furthermore, only 2 PV patients were studied by the microscopic method. Pure PMN from 4 PV patients were investigated photometrically by Piva et al. and the difference in NADPH oxidase activity in this test was less pronounced, and could conceivably be compatible with our findings of normal PMA-enhanced NADPH oxidase activity, if more patients had been studied. Finally, as stated above, we have also found that the pattern of oxidative response varies greatly within the group of myeloproliferative disorders, which leads us to conclude that statistical calculations regarding oxidative metabolism in PMN should be performed on results obtained in a specific disease entity, and not on a group of patients with various myeloproliferative disorders.

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Reply

The letter of Samuelsson and Palmblad has been helpful in prompting further discussion of our findings, which nonetheless do not appear to contradict their own. The respiratory burst oxidase (NADPH oxidase complex) catalyzes the production of O_2^- by activated phagocytes. This activation depends on multiple signal transduction pathways. Failure to produce O_2^- is characteristic of chronic granulomatous disease, an inherited phagocyte function disorder and of other diseases, some hematologic malignancies in particular. When we assessed NADPH oxidase activity by the photometric method, no significant differences were found between findings in polycythemia vera (PV) patients and those in control subjects, whether the polymorphonuclear leukocytes (PMN) were stimulated or not (resting PMN).

The only stimulus employed in our published study was phorbol myristate acetate (PMA). The activation of the respiratory burst oxidase by PMA is thought to be mediated through the protein-kinase-C-catalyzed phosphorylation of p41phox and possibly other oxidase subunits [1], whereas N-formyl-methionyl-leucyl-phenylalanine (fMLP) showed protein-kinase-C-independent pathways and receptor-mediated activation. In our paper, the first study by Samuelsson et al. [2] was quoted as showing normal burst oxidase activity in PV PMN, assessed by chemiluminescence and in response to PMA stimulation: this finding was in agreement with ours obtained with the photometric method. Moreover, in the same study, those authors found that the oxidative metabolism in PV patients was subnormal only after fMLP stimulation, suggesting the presence of a stimulus-response coupling defect and in a more recent detailed studies on the different biochemical steps performed they demonstrated phospholipase-D-mediated activation impairment [3]. In our paper, we found that patients with myelodysplastic syndromes (MDS) and those with chronic myeloproliferative disorders (cMPD) had similarly reduced scores when evaluated by microscopic NBT reduction, whereas the photometric method showed a cMPD reduction only in patients susceptible to infection. The photometric method was therefore more specific than the microscopic one, this explaining some apparently contradictory findings. Furthermore, since microscopic NBT reduction was performed in whole blood, the interaction

between PMN, platelets, monocytes, lymphocytes and their environment should be further investigated.

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Letter to the Editor

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