© 1990 Elsevier Science Publishers B.V. (Biomedical Division)
Advances in analytical cellular pathology. G. Burger et al, eds.

CELL BIOCHEMICAL PARAMETERS OF NEUTROPHIL ACTIVATION IN INTENSIVE CARE PATIENTS MEASURED BY FLOW CYTOMETRY

G. ROTHE[1], W. KELLERMANN[2], G. VALET[1]
[1]Arbeitsgruppe Zellbiochemie, Max-Planck-Institut für Biochemie, D-8033 Martinsried, FRG
[2]Institut für Anästhesiologie, Klinikum Großhadern, D-8000 München 70, FRG

INTRODUCTION

Neutrophils are essential for the antibacterial host defence, but are also mediators of trauma- and sepsis-related respiratory and cardiovascular organ failure due to the release of reactive oxygen metabolites and proteolytic enzymes. A flow cytometric study in 47 patients of an intensive care unit (ICU) has shown that functional parameters of neutrophils phagocytosing *E. coli* can be used for the recognition and prediction of the four diseases states: septic organ failure, posttraumatic organ failure, intermediate state and stable organ function after recovery.[1] Now the analysis of the neutrophil intracellular pH, cytosolic free calcium and spontaneous and stimulated oxidative product formation was used for the further cell biochemical characterization of the functional alterations of neutrophils during the septic and posttraumatic state of ICU patients.

METHODS AND RESULTS

Intracellular pH (1,4-diacetoxy-2,3-dicyano-benzene),[2,3] cytosolic free calcium (indo-1/AM),[4] the intracellular oxidation of the peroxidase-sensitive dihydrorhodamine 123 (DHR)[5] and 2',7'-dichlorofluorescin diacetate (DCFH-DA)[6] and the superoxide anion-sensitive hydroethidine (HE)[6] in neutrophils in resting state or stimulated by phagocytosis of *E. coli* bacteria, by phorbol 12-myristate 13-acetate (PMA) or *N*-formyl-Met-Leu-Phe (FMLP) were each measured together with electrical cell volume in a FLUVO-II flow cytometer in 76 serial blood samples from 17 patients of an operative ICU.

Increases of cell volume, intracellular pH, cytosolic free calcium, and spontaneous oxidative activity of neutrophils sensitively discriminated blood samples from patients with posttraumatic and septic organ failure from samples obtained during stable organ function following recovery (Table I).

TABLE I. SPONTANEOUS ACTIVITY OF NEUTROPHILS FROM SEPTIC AND POSTTRAUMATIC ICU PATIENTS

Clinical classification	Cell volume (μm^3)	Intracellular pH	Cytosolic free calcium (F_1/F_2 ratio)	DHR oxidation (arbitrary green	DCFH oxidation or red fluorescence units)	HE oxidation
Posttraumatic (n = 8-22)	374 ± 24*	7.46 ± .06	14.5 ± .7	.024 ± .003	.068 ± .010	.076 ± .008
Septic (n = 5-28)	381 ± 14	7.64 ± .05	14.3 ± .9	.022 ± .002	.065 ± .007	.081 ± .007
Intermediate (n = 47-119)	350 ± 5	7.56 ± .02	14.2 ± .2	.021 ± .001	.054 ± .003	.078 ± .003
Normal (n = 16-27)	306 ± 6 (p < .001)**	7.37 ± .06 (p = .001)**	13.8 ± .5 (p = .507)	.021 ± .001 (p = .529)	.046 ± .004 (p = .064)	.074 ± .019 (p = .721)

* Mean ± SEM. ** p < .05; Kruskal-Wallis one-way analysis of variance.

Stimulation of neutrophils obtained posttraumatically either through incubation with viable *E. coli* or direct stimulation of protein kinase C with PMA showed decreased oxidation of the peroxidase-sensitive substrates DHR and DCFH but unchanged oxidation of HE (Table II).

Neutrophils from septic patients showed decreased activation by bacteria but unchanged respiratory burst activity following stimulation by PMA suggesting alterations of surface receptor expression or intracellular signal transduction.

TABLE II. RESPIRATORY BURST OF NEUTROPHILS FROM SEPTIC AND POSTTRAUMATIC PATIENTS

Clinical classification	DHR + *E. coli*	DHR + PMA	DCFH + *E. coli*	DCFH + PMA	HE + *E. coli*	HE + PMA
			(% of control)			
Posttraumatic (n = 8)	934 ± 159*	1525 ± 240	394 ± 70	481 ± 71	198 ± 9	350 ± 19
Septic (n = 5)	712 ± 161	1892 ± 376	280 ± 51	723 ± 112	150 ± 10	361 ± 16
Intermediate (n = 47)	996 ± 71	2085 ± 93	451 ± 28	650 ± 33	196 ± 7	351 ± 8
Normal (n = 16)	1140 ± 107 (p = .234)	2076 ± 174 (p = .179)	484 ± 50 (p = .141)	722 ± 61 (p = .058)	207 ± 9 (p = .055)	328 ± 10 (p = .384)

* Mean ± SEM. ** $p < .05$; Kruskal-Wallis one-way analysis of variance.

The functional heterogeneity of neutrophils became apparent upon stimulation with the chemotactic bacterial peptide FMLP (Fig. 1).

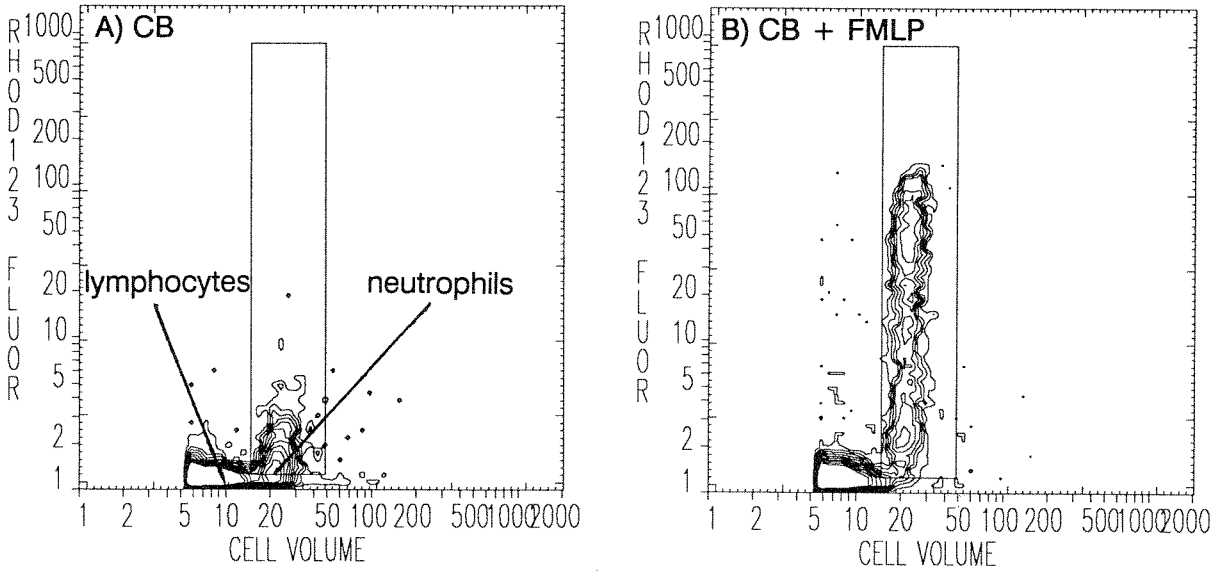

Fig. 1. Intracellular oxidation of DHR by neutrophils from a septic patient in the presence of cytochalasin B without (A) or with (B) further stimulation by FMLP.

CONCLUSIONS

Cell biochemical parameters of neutrophil activation can be analyzed in resting cells as well as in stimulated cells. They are of interest for the recognition[1] and pathophysiological analysis of septic or posttraumatic organ failure and acute or chronic infection, rheumatoid arthritis, or HIV-related immune defects.

REFERENCES

1. Rothe G, Kellermann G, Valet G (1990) J Lab Clin Med *in press*
2. Valet G, Raffael A, Moroder L, Wünsch E, Ruhenstroth-Bauer G (1981) Naturwissenschaften 68:265-6
3. Rothe G, Valet G (1988) Cytometry 9:316-24
4. Valet G, Raffael A, Rüssmann L (1985) Naturwissenschaften 72:600-2
5. Rothe G, Oser A, Valet G (1988) Naturwissenschaften 75:354-5
6. Rothe G, Valet G (1990) J Leukocyte Biol *in press*

Supported in part by SFB 207, Universität München, project G6.

ADVANCES IN ANALYTICAL CELLULAR PATHOLOGY

Proceedings of the First Conference of the European Society for Analytical Cellular Pathology, Schloss Elmau, F.R.G., 12–17 November 1989

Editors

Georg Burger
Gesellschaft für Strahlen- und Umweltforschung mbH
Institut für Strahlenschutz
Neuherberg, F.R.G.

Martin Oberholzer
Institut für Pathologie der Universität Basel
Basel, Switzerland

G. Peter Vooijs
University Hospital Sint Radboud Hospital
Nijmegen, The Netherlands

1990

EXCERPTA MEDICA, Amsterdam – New York – Oxford