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Part B: Monitoring and Treatment of Shock

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Contents

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Contents of Part A: Pathophysiological Role of Mediators and Mediator Inhibitors in Shock

1. THE PATHOPHYSIOLOGICAL ROLE OF MEDIATORS AND INHIBITORS THEREOF IN SHOCK

1.1. Complement—Granulocytes

1.2. Proteases

First Vienna Shock Forum, Part B: Monitoring and Treatment of Shock, pages 51-58

STUDIES OF GRANULOCYTE FUNCTION (CHEMILUMINESCENCE RESPONSE) IN POSTOPERATIVE INFECTION

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INTRODUCTION

Due to their ability to phagocytose soluble and solid agents polymorphnuclear granulocytes (PMN) are predominating in the nonspecific defense system. Activation of PMN phagocytosis, intracellular killing of microorganisms and digestion of foreign bodies proceed with increasing hexose monophosphate shunt activity and non-mitochondrial oxygen consumption (Becker et al. 1958, Sbarra and Karnovsky 1959). During this "respiratory burst" highly reactive oxygen derivates (O_2^-, H_2O_2, $\cdot OH$, $^1O_2$, OCl^-) are generated, which are responsible factors for intracellular microbicidal activity (Babior et al. 1973) and which can be assayed in diluted whole blood by luminol amplified chemiluminescence (CL) (Kato et al 1981). We studied the CL-response to in vitro-stimulation in whole blood samples of surgical patients in comparison to the disease state.

MATERIAL AND METHODS

Patients: 7o men, mean age 58,5 years, and 43 women, mean age 61.8 years, with manifest infections or at high risk for developing infectious complications were prospectively studied. They were adjoined daily to a severity group I - IV according to the clinically detectable degree of complications: Group I: 6o patients without postoperative complications. Group II: 16 patients with slight or moderate infections (wound infections, regionally limited pe-

ritonitis, basal pneumonia). Group III: 1o patients with severe postoperative infections (locally not limited soft tissue infection, 1 - 2 quadrant peritonitis, extensive broncho-pneumonia). Group IV: 27 patients with sepsis (positive blood culture, ensured infection focus and remote organ failure). Measurement of CL in Diluted Whole Blood: The reaction mixture contained 0.1 ml diluted blood (5o µl EDTA blood + 7oo µl phosphate buffered saline solution with 0.1 % glucose), 1.6 ml Veronal buffer (pH 7.2, containing Ca^{++}, Mg^{++} + 1 % glucose and human albumin each) and 0.2 ml luminol (7 x $1o^{-4}$ M in phosphate buffer, pH 7.4). Following 1o min. incubation at 37^{o}C the reaction was started by addition of o.1 ml opsonized (15 min. at 37^{o} C with pooled normal serum) zymosan solution (2o mg/-ml). The CL (counts per min.) was measured at 37^{o} C (Biolumat 95o5, Fa. Berthold, Wildbad, FRG, Apple II e computer) and calculated as the CL-integral over 3o min. for the whole sample (= total activity, counts per 3o min.). Specific activity was calculated from total activity as counts per 3o min. and $1o^{3}$ PMN. Data are indicated as mean ± SEM.

RESULTS

The specific CL activity of human granulocytes in response to the in vitro-stimulus zymosan was slightly diminished by the anesthesiologic-operative trauma (279 ± 43 counts) followed by an increase up to 844 ± 83 counts at the 3. postoperative day and a decrease to preoperative activity within the next 4 days. The primary reduction of specific CL activity however was more than compensated by the postoperative leukocytosis so that total CL activity increased already in the early postoperative phase reaching its maximum at the 1. day (Fig.1).

In patients with microbial-infectious complications the total CL activity increased according to the clinical severity of the inflammation. This was due to an enhanced specific CL response as well as to an increase of WBC. Interestingly, even slight to moderate bacterial infections (group II) induced a mean increase of specific CL comparable to that of severe infection (group III), whereas only during sepsis an additional enhancement of the CL response per granulocyte was found (Table 1). This behaviour is more clearly demonstrable in the follow up of severe infection or sepsis throughout the course of the disease (Fig. 2). Patients dying in the later phase showed a

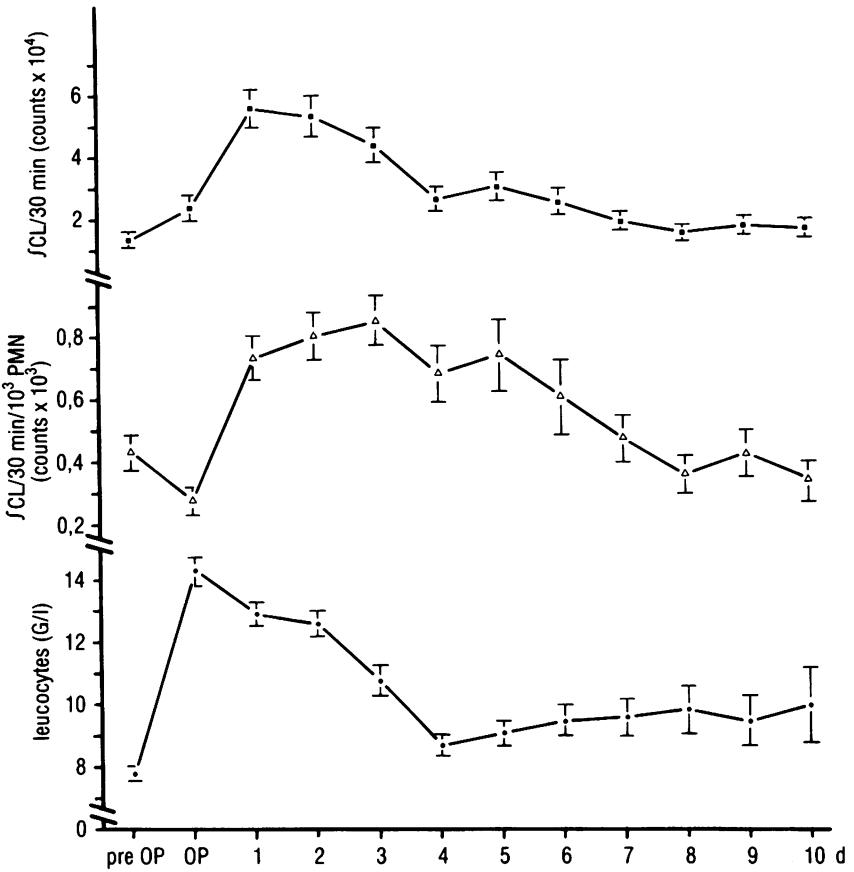

Fig.1: Total CL-activity (•——•), specific CL-activity (△——△) and number of leucocytes (•———•) in the control group.

distinctly higher total and an intensified specific in vitro-excitability to CL already one day before the clinical manifestation of the fatal complication. In contrast, in surviving patients diagnosis of severe infection coincided with the maximum of the CL response. During the first days of the follwing inflammatory course the slightly decreasing CL values in both groups did not show any significant difference. In the later phase, however, a clear dis-

Table 1: Total and specific CL-activity in group I - IV patients ($\bar{X} \pm$ SEM)

CL	preop.	I	II	III	IV
Total activity	13575 ±1778	34298 ±3o45	51782 ±9162	62633 ±13135	118564 ±2o762
Specific activity	433 ±57,3	573 ±4o,3	1o71 ±258	1o91 ±181	15o3 ±21o

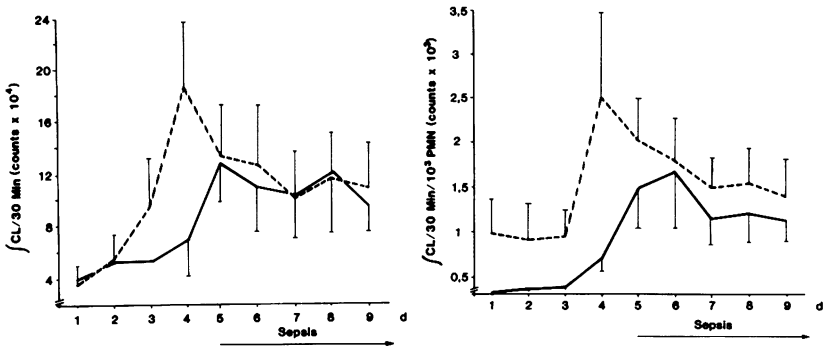

Fig. 2. Total CL-activity (left) and specific CL-activity (right) in patients developing sepsis.----- patients dying in the later course, ——— survivers.

crimination in the CL activity was observed, although both groups showed clinically an equally severe degree of inflammation at this observation period. The specific CL response of PMN of surviving patients stayed in the range of 11oo whilst the excitability of the granulocytes in patients dying later on increased up to two-fold. Depending on the significantly higher decline of circulating leukocytes in the latter patients, the total CL activity in their blood samples was similar to those shown by surviving subjects at the last day of severe infection or sepsis (Fig. 3). During the following recovery period the CL response to zymosan further decreased gradually to normal values due to decline of specific CL activity and leuko-

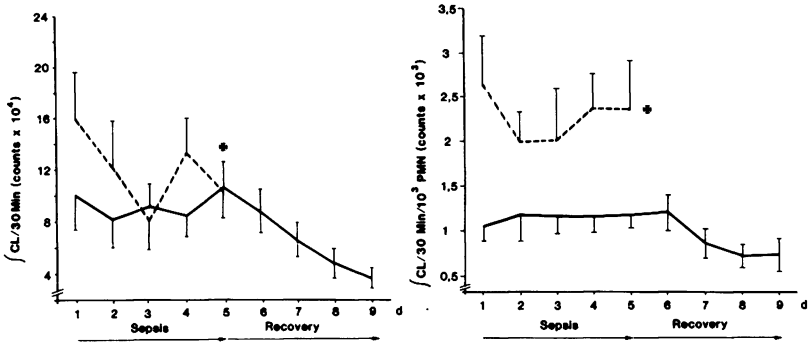

Fig. 3. Total CL-activity (left) and specific CL-activity (right) from dying patients (- - - - - - -) and survivers in the recovery phase (———).

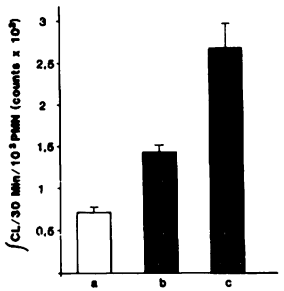

Fig. 4: Specific CL-activity in group IV patients: Survivers (a), patients dying in the later course (b) and those dying within 1o days (c).

cyte numbers as well.

DISCUSSION

Measurement of the CL response of whole blood samples to in vitro-stimulation seems to be a reliable assay for estimation of phagocytotic capacity (Ewetz et al. 1981, Tono-Oka et al. 1983). Use of highly diluted blood and op-

sonized particles provides information exclusively about the phacocytotic capacity of the PMN cells. Facing clinical employment of the assay, time consuming cell separation methods are no longer necessary, which may also impair granulocytic function (Ogle et al. 1985). Moreover, falsification of CL values by high erythrocyte numbers (quench effect) may be limited, if highly diluted blood samples are used (Allen et al. 1982, Redl et al. 1983, Szczeponik 1986).

With the assay procedure applied in this study a depressing influcence of anesthesiologic-operative trauma to the excitability of PMN granulocytes could be clearly demonstrated. In this respect, longer operation times induced also a slower increase of specific CL capacity following the primary decrease (data not shown). Nonbacterial inflammation as part of each wound healing elicited excitability of the PMN cells corresponding to the healing course.

An increased CL response of the granulocytes to microbial inflammation has been described recently (Barbour et al. 198o, Allen et al. 1982, Tono-Oka et al. 1983). Interestingly, in our study blood samples either from moderate or severe inflamed patients showed an equally elevated specific CL response. This is in contrast to results shown by Allen et al. 1982 and Tono-Oka et al. 1983. Latter authors only found an increased total excitability during infection answer. Enhancement of total CL activity elicited by an exogenous stimulus according to the degree of inflammation primarily reflected a rise of granulocyte numbers in peripheral blood and only in fatal sepsis a further increase of specific CL activity. In the later course of clinical similarly severe sepsis granulocytes of non-survivers have been considerably more excitable than those of survivors. Highest CL values were observed in patients, who died very early throughout the septic course (Fig. 4). This observation might suggest an overshooting in defense power in the latter patients. By that means, hyperreactivity of phagocytes to stimuli like microbes, cell debris or other foreign substances in the organism may lead locally to a tremendous release of toxic oxygen species as well as lysosomal enzymes greatly overstressing the regulatory inhibitor potential with fatal consequences (Jochum et al. 1986).

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Index