CIRCULATING IMMUNE COMPLEXES AND HETEROPHILIC HEMOLYSINS IN BURNS

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An important factor in the mechanism of development of burns is the relationship between toxic and immunodepressive influences on the immune system and the level of its stimulation by microbial antigens.

Depression of the immune response in burns has been measured mainly by studying the effector function of phagocytes and also the general level of proliferative and, to some degree also, the regulatory function of lymphocytes relative to specific microbial antigens [2, 3, 13].

However, in the pathogenesis of burns an important role may be played not only by the agent as such, but also by its biologically active degradation products, burn toxin, and other products of tissue destruction and alteration. All these factors are capable, on the one hand, of forming specific and nonspecific complexes with immunoglobulins and complement (C), causing various severe immunopathologic changes [1, 14], and on the other hand, of affecting stimulation of lymphocytes by microbial antigens, more especially the production of polyclonal immunoglobulins, the second line of defense against infection after phagocytosis.

The aim of the present investigation was to study the relation between the level of circulating immune complexes (CIC) and polyclonal activation of lymphocytes as one parameter of the developing immune response [5, 11], and their connection with the stage of the course of burns.

EXPERIMENTAL METHOD

Blood serum from 30 healthy donors, five patients with mild burns (I-IIIA degree, 10-20% of the body surface), and with local infection, 10 patients with moderately severe burns (II-IIIAB degree, 20-40%) and with local infection, and 15 patients with severe burns (IIIAB-IV degree, 40-65%) and with burn sepsis, of whom eight recovered and seven died, was used in the investigation. In 86% of the burned patients, staphylococci were seeded from the wound and (or) blood in the form of a monoculture or as a component of microbial associations.

The CIC level was determined by precipitation with polyethylene-glycol (PEG, mol. wt. 6000) by the method described in [6] in the writers' modification: 4 ml of patient's serum diluted 1:25 with borate buffer (pH 8.4, 0.1M) was mixed with 4 ml of 7% PEG in the same buffer. The mixture was incubated for 18 h at 4°C, then centrifuged for 20 min at 20,000g and the residue dissolved in 5 ml of 0.1N NaOH. The results were read on an FEK-56M photoelectric colorimeter, with No. 1 filter, wavelength 315 ± 5 nm.

Depending on the degree of hemolytic activity of the serum, detected in hemolysis tests with sheep's red blood cells (SRBC), one of the manifestations of polyclonal activation of B lymphocytes in the patients as a result of burn trauma and exposure to microbial antigens could be estimated.

Since in response to specific stimulation of immunocompetent cells by bacterial antigens, possessing common (cross-reacting) determinants with antigens of mammalian erythrocytes, B lymphocytes produce broad-spectrum antibodies, including some directed toward SRBC, it can be tentatively suggested that the production of human hemolysins against SRBC, revealed by

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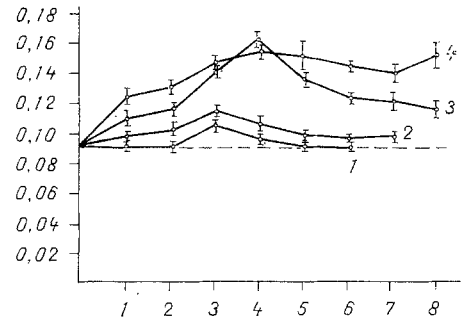

Fig. 1

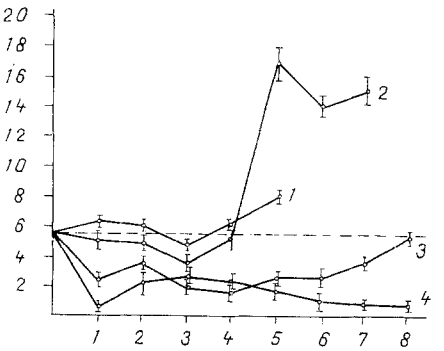

Fig. 2

Fig. 1. Dynamics of changes in CIC level in burned subjects. 1) Patients with mild burns and local infection; 2) patients with moderately severe burns and local infection; 3) patients with severe burns and burn sepsis with favorable outcome; 4) patients with severe burns and burn sepsis, with unfavorable outcome; broken line — healthy blood donors. Abscissa, time of investigations (in weeks); ordinate, CIC level.

Fig. 2. Dynamics of changes in HL titers in burned subjects. Ordinate, HL titer. Remainder of legend as to Fig. 1.

a micromethod in hemolysis tests, is one of the consequences of stimulation of B lymphocytes by microbial antigens. Its level is determined by titers of hemolysins to SRBC. Since this lysis is complement-dependent, the level of complement and of anticomplementary factors in the patient's blood serum can be estimated to a certain degree in such a system (see below).

Double dilutions of fresh patient's serum were prepared in Ringer's physiological saline from 1 to 1/512, after which the diluted serum was transferred in a volume of 2 μl by means of a microsyringe into the corresponding wells of microchambers for immunologic tests under mineral oil, and the same volume of thrice washed 1% SRBC was added. The microchambers were then incubated for 1 h at 37°C and centrifuged for 2 min at 20g. Only 100% hemolysis was counted. Three types of tests were set up with the patients' sera:

1. SRBC + double dilutions of serum — to estimate the degree of activity of heterophilic hemolysins (HL), in titers, in a test system. To each well was added 2 μl of physiological saline for correct comparison of the results with the second type of test.

2. SRBC (2 μl) + double dilutions of serum (2 μl) + exogenous guinea pig C (2 μl) — to estimate the presence of C in the patient's serum, for if after this procedure activity of the hemolysins increased (in the absence of anticomplementary activity of the serum), this indicated a low serum C level.

3. After incubation of serum with SRBC for 1 h, it was removed by shaking from the wells of the microchambers, after which exogenous C was added to the system, incubation for 30 min followed at 37°C and, after centrifugation of the microchambers at 20g for 2 min the results of hemolysis were read (an increase in HL activity after removal of the serum is evidence that it contained anticomplementary factors, interfering with estimation of the height of the HL titers).

All the results were subjected to statistical analysis by Student's test. Only data differing significantly with a probability of possible error $P < 0.05$ are described in this paper.

EXPERIMENTAL RESULTS

The CIC level in healthy subjects was 0.09 ± 0.004 (extinction reading on the photoelectric colorimeter) and the HL titers were 5.53 ± 0.42.

The results were compared at intervals during 8 weeks after infection of burns (Fig. 1). In mild burns accompanied by local infection the CIC level was indistinguishable from normal. In moderately severe burns with local infection the CIC level rose during treatment and fell toward the time of recovery. In severe burns and burn sepsis CIC remained at a high level throughout the period of investiation and fell toward its end in patients whose outcome was

favorable, by contrast with patients who died, but it remained higher than in patients with mild and moderately severe burns. The CIC level in patients who died was higher at all times of the investigation than in patients with mild and moderately severe burns at the same times.

The opposite trend was observed when polyclonal activation of B lymphocytes by microbial antigens in the burned patients was studied (Fig. 2). For instance, HL titers in mild and moderately severe burns were indistinguishable from normal (in healthy subjects) and rose toward the time of recovery. This was particularly noticeable in moderately severe burns. In patients with severe burns HL activity was depressed throughout the period of investigation but rose to normal toward its end in patients whose outcome was favorable, whereas in patients who died the low level of HL activity fell even more.

The experiments showed that depression of HL activity in burns takes place mainly not because of true depression of lymphocyte stimulation. The evident fall in HL titer in sera of patients in a serious condition was found to depend on the anticomplementary activity of the serum and (or) on the low C level since HL activity increased after removal of the serum from the wells or addition of exogenous C to the test system. The low C level in burns may be due either to a decrease in synthesis and an increase in catabolism of the components of C or to a rise in the level of their inhibitors. In some patients a true fall of HL titers was observed, i.e., true depression of polyclonal activation of B lymphocytes. This depression may be caused either by inadequate antigenic stimulation or by disturbance of regulation of polyclonal activation: T-B helpers and suppressors participate in this process [4, 8, 10] together with macrophage suppressors, acting on T helpers [9] or B lymphocytes [12]. Consequently, four probable reactions can be observed in burned patients.

1. High polyclonal activation without participation of its inhibitors — this is a favorable sign, not requiring treatment.

2. Weak polyclonal activation, i.e., a true fall of HL titers (removal of anticomplementary activity or addition of C to the system does not lead to an increase in HL activity — this is an unfavorable sign, requiring measures to correct function of the immune system.

3. High polyclonal activation after removal of anticomplementary activity — this is an indication that detoxicating treatment is necessary and, in particular, hemoperfusion.

4. Low initial activity independent of anticomplementary activity and stimulated by addition of C to the system — this is an indication that the patient's C level is too low and requires appropriate correction.

Whatever the case, the fall in HL activity in autologous serum may reflect the actual situation *in vivo*: The opsonizing properties of these immunoglobulins, which are mainly responsible for their antimicrobial action, cannot be exhibited without C. The presence of immunoglobulins with such low activity also explains the existence of a high CIC level.

The conditions of CIC formation probably differ depending on the degree of the burn. For instance, in severe burns this parameter rose in the early stages after trauma, before infection developed, and this can be attributed to burn toxemia. In moderately severe burns the rise in CIC took place later, after addition of infection, and was thus due to a combination of the action of burn and microbial toxemia.

On recovery the CIC level fell to normal and HL activity rose, evidently in connection with correction of function of the immune system during treatment. This was more characteristic of moderately severe burns, for in mild burns the immune system is virtually unaffected, whereas in patients with severe burns it is burdened by the consequences of burn sepsis, and this naturally interferes with such correction. In patients who died, HL activity was very low (0.83 ± 0.40) a few days before death, mainly on account of the anticomplementary activity of serum, whereas the CIC level was high (0.136 ± 0.007) compared with HL activity (4.5 ± 0.48) and the CIC level (0.105 ± 0.006) in patients who survived, on discharge from hospital ($P < 0.05$). This is an unfavorable prognostic sign, calling for detoxicating therapy to lower the high CIC level and to create conditions for enhancement of phagocytic activity of macrophages and neutrophils. HL activity in patients who survived, while it did not differ from HL titers of healthy blood donors before their discharge from hospital, was nevertheless depressed on account of anticomplementary activity, for after removal of the latter, HL activity in these patients rose even more and true HL titers within the range from 1/8 to 1/16 were observed.

To remove CIC two factors must probably be present: either components of C, which dissolve CIC, or active phagocytes, but since burns are characterized by dysfunction of phagocytosis, and solubilizing and opsonizing components of C bind with necrotic tissues [7], these circumstances prevent degradation of CIC and lead to their accumulation in the circulating blood.

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IMMUNOMODULATING ACTIVITY OF p-HYDROXYPHENYL-LACTIC AND ASCORBIC ACIDS

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p-Hydroxyphenyl-lactic acid (PHPLA) is formed from p-hydroxyphenylpyruvic acid which, in turn, is a transamination reaction product of tyrosine. PHPLA is converted into homogentesic acid in the presence of ascorbic acid (AA). In vitamin C deficiency and also in patients with hemoblastoses the urinary PHPLA level rises sharply [3]. PHPLA has been shown to exhibit the properties of an endogenous carcinogen and, on systemic administration to animals, it induces increased formation of leukemias and hepatomas [1, 2].

In the investigation described below the effect of PHPLA and AA on cell proliferation was studied in a one-way mixed lymphocyte culture (MLC) from healthy blood donors and patients with carcinoma of the large intestine, who as a rule have AA deficiency [5].

EXPERIMENTAL METHOD

PHPLA in the culture medium and lymphocytes was determined on an MAT-311A chromatomass-spectrometer (Varian, West Germany), connected through an interface with a 3700 chromatograph (West Germany) [3]. AA was measured by a spectrophotometric method with phenylhydrazine. The cell proliferation index of MLC and in the blast transformation reaction to polyclonal mitogens was determined by the method described previously [3]. Splenocytes were obtained from mice with avitaminoses B_6 and A [6, 8].

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