CHARACTER OF STAPHYLOCOCCAL INFECTION IN GUINEA PIGS WITH DELAYED-TYPE HYPERSENSITIVITY TO STAPHYLOCOCCAL α-TOXIN

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Reactions of increased delayed-type hypersensitivity (IDTH) to the pathogenic agent frequently accompany staphylococcal infection [9], and aggravate the course of the underlying disease [5, 11] or, conversely, correlate with antibacterial immunity [4, 12]. There are many reasons for the different views on the role of IDTH in the pathogenesis of staphylococcal infection and, in particular, realtions between the level of activation of the immune system and the intensity of immunodepressive influences on it. During staphylococcal sensitization the immune response is aimed primarily against antigens of the cell wall [10], but ability to induce IDTH is a property not only of individual somatic staphylococcal antigens [8], but also of the toxins of this microorganism and, in particular, its α-hemolysin [9].

The aim of the present investigation was accordingly to study the effect of IDTH, induced by α-toxin (AT), on subsequent staphylococcal infection in guinea pigs.

EXPERIMENTAL METHOD

Native staphylococcal AT (initial concentration 7 L_h/ml) was used together with toxoids: native AT inactivated by heating (56°C for 30 min) and native commercial α-toxoid (initial concentration 10 fixation units/ml) produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology. A highly purified homogeneous preparation of AT was obtained by the method in [6], which included precipitation of the toxin from the cultural filtrate (*Staphylococcus aureus* strain L-15) with ammonium sulfate in the 60% saturation zone, dialysis (4°C for 24 h) against 5 mM Tris-HCl buffer, pH 8.0, followed by purification by ion-exchange chromatography on DEAE-cellulose 32 and gel-filtration on a column with Sephadex G-100. The purity of the resulting AT fraction was estimated by disk electrophoresis. The specific hemolytic activity of the preparation was 1000 hemolytic units (HU)/mg protein.

IDTH against the above preparations was induced in noninbred guinea pigs (350-370 g) by a single injection of a sensitizing dose, in a total volume of 100 μl of physiological saline, into the hind footpads. After 14 days the reacting dose of the preparation was injected in the same volume into the footpad of the right hind limb, physiological saline was injected into the left footpad, and the thickness of the footpads of both limbs was measured with a type MK-025 micrometer. A significant difference was observed 24 h after injection of the antigen (measurements were made before the reacting dose was given and 1 h after).

Animals of group 1 (n = 20) were sensitized with a subdermonecrotic dose of native AT (4 μl), and 2 μl of the preparation was used as the reacting dose. To compare the biological action of native AT with that of the highly purified preparation, the latter was used to induce IDTH in 5 guinea pigs. The subdermonecrotic sensitizing dose was 0.1 HU per animal, the reacting dose 0.05 HU.

In animals of groups 2 and 3 (n = 40) IDTH was induced against native inactivated toxin and toxoid respectively in doses equivalent to those of the animals of group 1. After the

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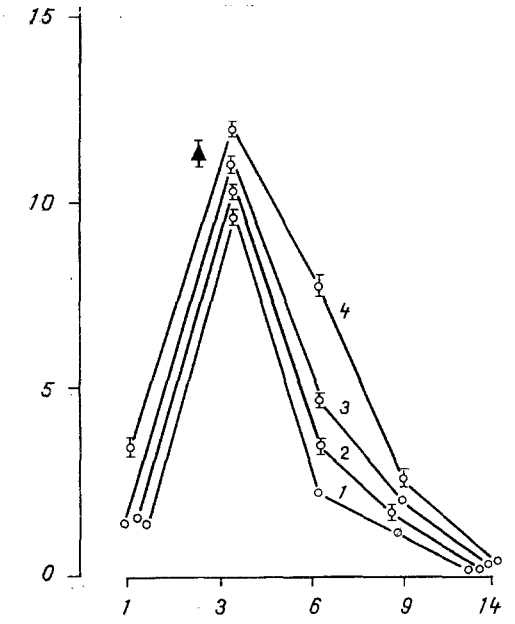

Fig. 1. Number of staphylococcal colonies per gram splenic tissue obtained by seeding from different groups of animals after infection with nonlethal dose of staphylococci (M ± m). Abscissa, time after infection (in days); ordinate, number of colonies × 10^3. 1) Animals of group 1 (IDTH to native toxin); 2) animals of group 2 (IDTH to inactivated toxin); 3) animals of group 3 (IDTH against toxoid); 4) animals of group 4 (control), infected only. Black triangle indicates group of animals (n = 5) with IDTH to purified AT.

IDTH had been estimated, all animals were infected by intramuscular injection of a nonlethal dose (10^{10} bacterial cells/ml) of a 24 h culture of *Staph. aureus* (strain 1B). All animals thereupon developed a primary suppurative focus followed by generalization (positive culture of the spleen). The severity of infection was estimated by the number of staphylococcal colonies per gram of splenic tissue, obtained by seeding it on protein-salt agar, from three animals of each group, 1, 3, 6, 9, and 14 days after infection. The percentage of active T lymphocytes (T_{act}) in the peripheral blood was determined at intervals by the rosette-formation test with rabbit's erythrocytes, the percentage of B lymphocytes by the EAC-rosette formation test, and the percentage of lymphocytes with receptors for nonpathogenic staphylococci (RFC_{staph}) by methods described previously [2]. Stimulation of neutrophils was estimated by means of a histochemical test of bactericidal power based on reduction on nitro-BT by the method in [7]. The intensity of the reaction was expressed as the percentage of neutrophils containing deposits of diformazan (the reduced form of nitro-BT). All the results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

In animals effected in a state of IDTH (groups 1-3) the course of the infection was milder than in control guinea pigs which were only infected (group 4; n = 20): The number of positive splenic cultures was less at all times of the investigation than in the control. Differences also were found between the groups of experimental animals (Fig. 1). In the guinea pigs of group 1 (IDTH to native toxin), for instance, the number of positive cultures obtained was significantly less than in the animals of group 2 (IDTH to inactivated toxin) and, in particular, compared with those of group 3 (IDTH to native toxoid), especially at the climax of the infectious process (3rd-6th days after infection). During sensitization and investigation before infection the relative percentages of the various subpopulations of lymphocytes and active neutrophils in the peripheral blood was unchanged in both experimental and control groups of animals, whereas after infection and, in particular, at the peak of generalization of the supprative process (3rd-6th days), activation of lymphocytes and neutrophils was higher in the experiment than in the control. This was characteristic of the animals of

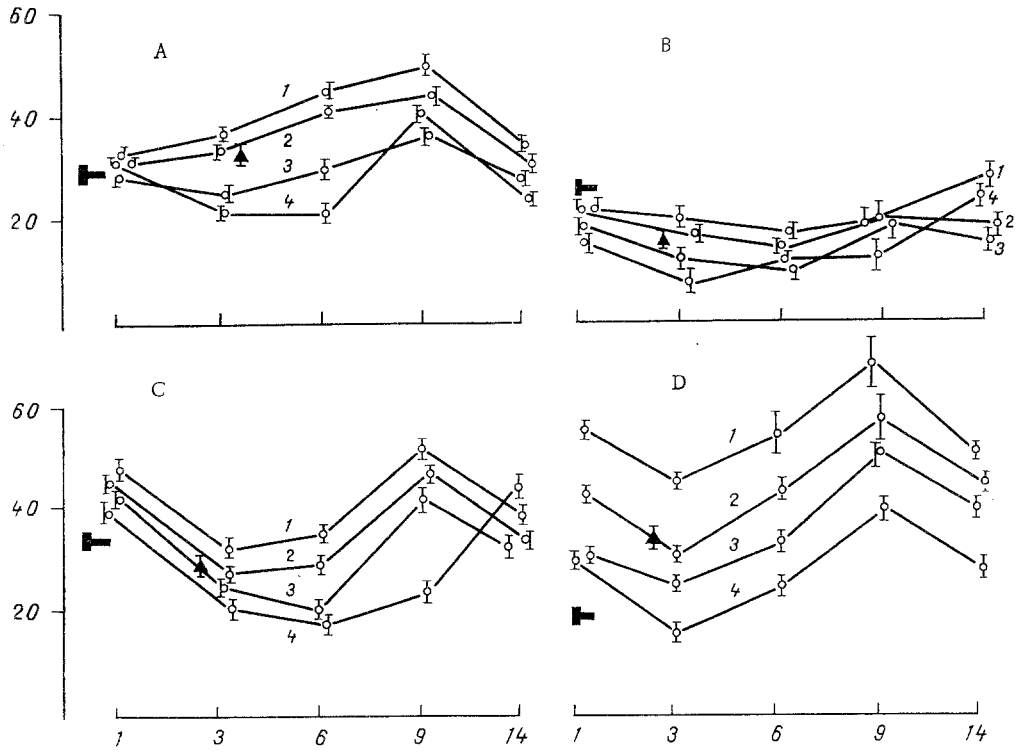

Fig. 2. Trend of immunologic parameters in animals of different groups after infection with a nonlethal dose of staphylococci (M ± m). Ordinate, relative percentage of T_{act} (A), B lymphocytes (B), RFC_{staph} (C), and activated neutrophils (nitro-BT; D). Vertical line T-shaped symbol indicates immunologic parameters (in percent) of animals before infection. Remainder of legend as to Fig. 1.

group 1 but less marked in those of group 2 and, in particular, of group 3, in which the trend of the immunologic parameters in some cases did not differ significantly from the control, even though the course of the infection in the control group was more severe (Fig. 2).

Injection of the reacting doses of the various preparations into intact guinea pigs and also into the control animals on the 14th day after infection did not reveal IDTH, whereas IDTH to all preparations was still preserved in the groups of experimental animals at the same time after infection.

Comparative analysis of the biological action of native and purified toxins, undertaken at the height of generalization (3rd day after infection), showed that the number of positive splenic cultures obtained from guinea pigs with IDTH to purified toxin was less than in the control, but greater than in animals with IDTH to the native preparation. The level of activation of lymphocytes and neutrophils in guinea pigs with IDTH to purified toxin was correspondingly lower than in animals of group 1 (IDTH to native toxin), but higher than in the control (infection only). This may be due to the presence of traces of leukocidin in native AT, obtained from production strain 0-15. Preparation of toxoid contaminated with leukocidin are known to be more effective for immunization for both prophylactic and therapeutic purposes than highly purified toxoids [3].

It follows from the results that IDTH to the preparations used does not aggravate the course of the underlying disease but, on the contrary, it has a protective action against subsequent infection, which may be connected with activation of the immune system on account of the antigenic properties of the toxin. In this case the immune response directed toward these antigens, by inducing IDTH, in fact increases resistance to the infection. The possibility cannot be ruled out that antigens of the toxins simultaneously induce IDTH and, independently of it, mobilization of the protective forces of the organism, manifested as activation of the system of phagocytes and (or) lymphocytes. Treatment of neutrophils with AT in doses not damaging the cell (under 10 HU/ml), or addition of AT to a test system (neutrophils + suspension of *Staph. aureus*) has been shown to enhance phagocytic activity and to po-

tentiate the bactericidal power of the neutrophils [13]. The system of lymphocytes, predominantly the T-dependent population [1], also is stimulated by small doses of AT; this stimulation, moreover, is not connected with its hemolytic properties [14]. Consequently, antigenic determinants of AT are not connected with the hemolytic center of the molecule, in agreement with out data showing a protective effect of IDTH not only against the toxin, but also against its toxoids. However, this effect was less marked in the latter, evidence rather in support of definite changes in the antigenic structure of the polypeptide chain of the toxin molecule during treatment by heat and (or) formalin.

In nonlethal staphylococcal infection, IDTH to AT thus correlates with the state of resistance, and this is reflected in the lower percentage of positive cultures obtained from the spleen and the higher level of stimulation of lymphocytes and neutrophils.

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