STAPHYLOCOCCAL INFECTION IN GUINEA PIGS WITH DELAYED-TYPE HYPERSENSITIVITY INDUCED BY SURFACE ANTIGENS OF *Staphylococcus aureus*

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KEY WORDS: delayed-type hypersensitivity; staphylococcal surface antigens; sublethal staphylococcal infection.

The connection between reactions of delayed-type hypersensitivity (DTH) to individual staphylococcal antigens and the presence of immunity to this microorganism remains virtually unstudied. There are only solitary references in the literature to negative or positive correlation between antistaphylococcal immunity and DTH induced by whole cells of this pathogenic agent [5-7].

The authors have studied correlation between the phenomenon of DTH to individual staphylococcal antigens with the intensity of the infectious process in guinea pigs infected with Staphylococcus aureus.

EXPERIMENTAL METHOD

Corpuscular antigens were used, namely inactivated whole cells of strains of Staph. aureus containing (Cowan 1) and not containing (2287) protein A, and individual surface antigens of Staph. aureus: cell wall (CW) and peptidoglycan (PG), obtained by the method [3] from strain 2287, and also protein A, isolated from the culture filtrate of stain A-676 and purified [4].

Noninbred guinea pigs were infected by intramuscular injection of a sublethal dose of Staph. aureus strain 1B (10^{10} microbial cells in 1 ml) after induction of DTH to the above-mentioned antigens. Assuming that each antigen separately cannot possess protective activity, complexes of surface antigens in different combinations with one another also were used to induce DTH.

DTH was induced by a single injection of a sensitizing dose of the preparation in 100 μl of physiological saline into the hind footpads: the dose injected was $2 \cdot 10^8$ microbial cells for corpuscular antigens and 100 μg per animal for CW and its components. On the 14th day of sensitization DTH was determined by the paw edema test in response to injection of a reacting dose of the preparation ($2 \cdot 10^7$ microbial cells or 50 μg respectively). A marked positive reaction was observed in all the animals after 24 h. The intensity of staphylococcal infection was judged from the results of seeding splenic homogenates on elective protein-salt agar, carried out at the peak of the infectious process (greatest number of positive cultures obtained from the test tissue), which corresponded to the 3rd day after infection. The number of active T lymphocytes, B lymphocytes, and lymphocyte receptors for staphylococci was determined, in percent, in the animals' peripheral blood [1]. Activity of neutrophils was determined by the nitro-BT reduction reaction [2].

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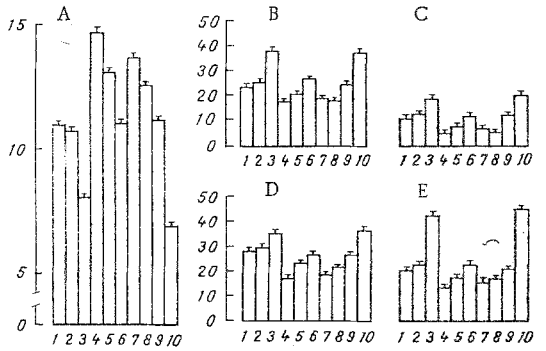

Fig. 1. Bacteriologic and immunologic parameters in various groups of guinea pigs at peak of infectious process (M ± m). Abscissa, groups of animals (eight animals in each group): 1) animals infected only (control); in remaining groups animals were infected after induction of DTH to corpuscular antigen containing (group 2) and not containing (group 3) protein A, to CW (group 4), PG (group 5), protein A (group 6), to CW + PG (group 7), to CW + protein A (group 8), to PG + protein A (group 9), and to CW + PG + protein A (group 10); ordinate, number of staphylococcal colonies (in thousands) per gram of splenic tissue (A), and relative percentages of active T lymphocytes (B), B lymphocytes (C), lymphocytes with receptors for staphylococci (D), and activated neutrophils (E).

EXPERIMENTAL RESULTS

A decrease in the intensity of the infectious process, i.e., a reduction in the number of positive seedings of staphylococci from splenic tissue and elevation of the level of activation of lymphocytes and neutrophils compared with their values in animals of the control group, subjected to infection only, was observed in guinea pigs infected after induction of DTH to corpuscular antigen not containing protein A, but especially to a combination of all the surface antigens used. In guinea pigs with DTH to protein A-containing corpuscular antigen, none of the immunologic parameters differed from the control (Fig. 1).

After induction of DTH to single surface antigens or to an incomplete combination of them, either the immunologic parameters were indistinguishable from the control, for example, in groups with DTH to protein A and to a combination of it with PG, or the number of splenic colonies was greater and the level of activation of lymphocytes and neutrophils lower than in the control, especially in animals after induction of DTH to CW or to a combination of CW + PG.

The maximal protective effect in these experiments was thus obtained against the background of DTH induced by a combination of all the surface antigens used. This evidently endows the antigenic complex with adjuvant properties, or a combination of these antigens performs the role of hapten–carrier conjugate, in which the carrier, by modifying the character of interaction of the immunocompetent cells with one of the surface antigens, enhances its immunogenicity. The possibility of a synergic effect of the preparations likewise cannot be ruled out. An incomplete combination of the antigens or each of the surface antigens separately had no protective activity, but induced cutaneous reactions of DTH.

Consequently, the fact that DTH develops in the present experiments is not itself a criterion of protection, for stimulation of lymphocytes and neutrophils actually correlated with increased resistance of the guinea pigs to nonlethal staphylococcal infection.

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EXPERIMENTAL STUDY OF EVANS' BLUE AS ADJUVANT FOR INDUCING DELAYED HYPERSENSITIVITY

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To induce delayed-type hypersensitivity (DTH) to soluble protein antigens experimentally, an oily adjuvant containing various microorganisms (BCG, Mycobacterium tuberculosis, M. butyricum, etc.) is usually used. Some adjuvants can only intensify developing DTH [3]. Recently much attention has been paid to water-soluble natural and artificial polyelectrolytes, which considerably stimulate the immune response, including DTH [4]. Crowle et al. [5, 6] suggested using the dye Evans' blue (EB) to induce DTH, because it is free from toxicity, can bind with blood proteins, and is used clinically to determine the circulating blood volume [1, 8]. Crowle and co-workers showed that 2-7 weeks after separate injections of EB and antigen into mice the animals developed hypersensitivity which, in its histologic picture and in the character of the skin tests, corresponded to DTH.

The aim of this investigation was to study the principles governing development of this DTH in the early stages of sensitization (in the first 2 weeks), to investigate dependence of DTH on the dose of EB and the dose and type of protein antigens, and to study its nature in experiments with passive transfer of sensitivity.

EXPERIMENTAL METHOD

CBA (male or female) mice weighing 14-18 g were used in the experiments, with five to ten animals in each group. The animals were sensitized subcutaneously in the interscapular region with antigens: bovine serum albumin (BSA) from Serva (West Germany) or of USSR origin, methylated BSA (MBSA) or ovalbumin (OA), from Serva. A solution of EB (from Serva, or from Reanal, Hungary) was injected simultaneously into the same region. In the positive control group the animals were sensitized with antigen mixed with Freund's complete adjuvant, in a ratio of 1:1 with antigen solution, and with a total volume of mixture of 0.2 ml.

To assess the intensity of the developing BTH the widely tested method of injecting antigen into the animals' paws [2, 7] was used. A solution of antigen was injected into the right hind footpad in a volume of not more than 0.04 ml. The same volume of distilled water or physiological saline, in which the antigens were made up, was injected into the contralateral (control) footpad. Different sensitizing doses of antigens were tested (from 50 to 2000 μg per mouse) with different doses of EB as adjuvant (from 50 to 2000 μg per mouse) and with different times of injection of the reacting dose (from the 2nd to the 14th day after sensitization). The local inflammatory reaction was assessed 24 h later, by measuring the difference in weight of the experimental (Pe) and control (Pc) paws of each mouse, and calculating the arithmetic mean and its error (M ± m). The reaction index (RI) was determined by the formula

$$\frac{P_e - P_c}{P_c} \times 100\%.$$

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