EFFECT OF *Staphylococcus aureus* ANTIGENS ON SPLENIC LYMPHOCYTES OF IMMUNIZED GUINEA PIGS

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The effect of staphylococcal antigens on immunocompetent cells has not been adequately studied [1, 7]. All that is known is that these antigens stimulate the lymphocytes of man and some animals [6, 8, 9]. However, there are no data on the mitogenic effect of *Staphylococcus aureus* antigens on the lymphocytes of guinea pigs and mice, which are the principal experimental models.

It was accordingly decided to study the action of protein A and of *Staph. aureus* strains Cowan-1 and Wood-46 on the proliferative activity of splenic lymphocytes after immunization of guinea pigs with protein A and with whole cells of *Staph. aureus* Cowan-1. The characteristics of DNA synthesis in the splenic lymphocytes of these animals in response to T and B cell mitogens also were investigated.

EXPERIMENTAL METHOD

Guinea pigs weighing 350-450 g were immunized with protein A from *Staph. aureus* Cowan-1 (2352, with a high protein A content) or with killed cells of this strain. Protein A, obtained and purified as in [3], in a dose of 100 μg, and *Staph. aureus* Cowan-1 freed from formalin [3], in a dose of 2×10^8 cells, were injected into the footpads of the guinea pigs' hind limbs together with Freund's incomplete adjuvant (from Difco). The analogs were revaccinated 7-9 days later, using antigen without adjuvant and in a dose an order of magnitude lower. The guinea pigs were killed 1 week later and their spleens removed; cells were isolated from the spleens for study in the lymphocyte blast transformation test (LBTT) against protein A, *Staph. aureus* strains Cowan-1 and Wood-46 (2351; protein-free), *Salmonella typhimurium* lipopolysaccharide (LPS, from Sigma), phytohemagglutinin P (PHA, from Difco), and concanavalin A (con A, from Sigma). Cells were isolated from the spleen for the LBTT, media were prepared, the concentration of the ingredients in the media and conditions for culture of the suspensions were established, and incorporation of ^{3}H-thymidine (HT) into lymphocytes when added 16 h before the end of a 48-h period of culture in a dose of 0.5 μCi per microdisk well (Falcon Plastics) was estimated as described previously [4].

EXPERIMENTAL RESULTS

To rule out any nonspecific binding of protein A with serum class G immunoglobulin [2] (since the antigen–antibody complex formed has a nonspecific action on DNA synthesis) 5% embryonic calf serum was added to the culture medium for the LBTT. It was virtually free from IgG, as shown by the absence of precipitation of protein A with the serum in the double immunodiffusion in agar test. DNA synthesis in the spleen cells of the immune animals was significantly higher ($P < 0.05$) than in intact guinea pigs (Fig. 1). Incorporation of ^{3}H-thymidine into DNA in the lymphocytes of guinea pigs immunized with protein A in medium containing protein A (12.5-50 μg/ml), strain Cowan-1 and Wood-46 (2×10^8 cells/ml), LPS (50 and 100 μg/ml), PHA (0.025-0.1 μg/ml), and con A (2.5 and 25 μg/ml), was statistically significantly higher ($P < 0.05$) than in cultures from intact animals (Fig. 1, shaded and unshaded columns). It must be pointed out that both in intact guinea pigs and in animals immunized with protein A, addition of further protein A to the culture medium led to activation of DNA synthesis in lymphocytes not responding to LPS and PHA, but responding to con A.

A different effect was observed in animals immunized with whole Cowan-1 cells. For instance, DNA synthesis in splenic lymphocytes of these guinea pigs was higher in response to protein A, it was unchanged in response to strain Cowan-1 and Wood-46, and lower to PHA (0.05-0.1 μg/ml), con A, and LPS than in intact animals. Sharp differences in proliferation of the spleen cells of guinea pigs immunized with protein A and Cowan-1 were observed in response to the staphylococcal antigens, LPS, and PHA, but not to con A (Fig. 1, unshaded and shaded columns). Cells of animals immunized with Cowan-1 reacted weakly in response to protein A, LPS, and PHA unlike the suspension of spleen cells from guinea pigs immunized with protein A. These differences may be due to the greater immunogenicity of the corpuscular *Staph. aureus* cell than protein A, evidently resulting in more substantial changes in the cell subpopulations (a decrease in the

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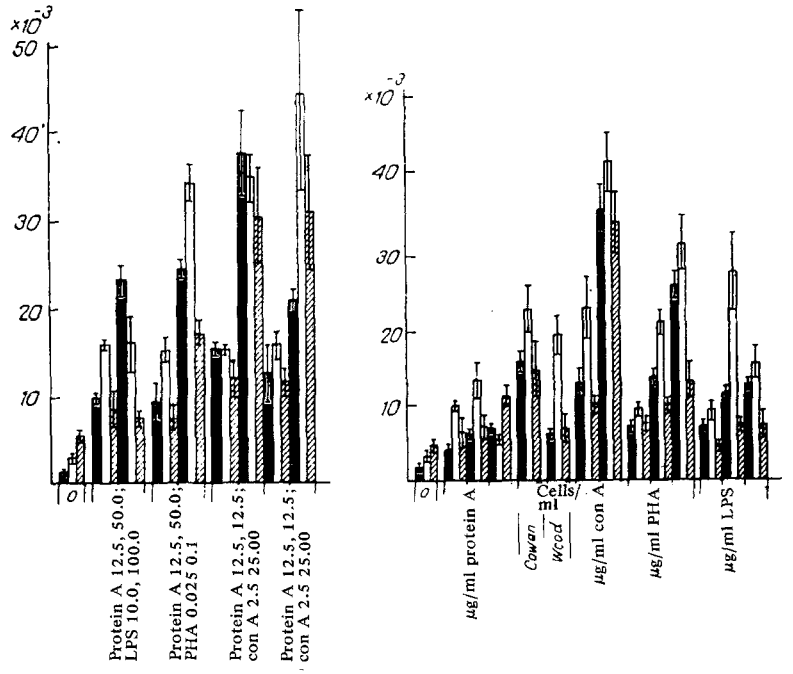

Fig. 1. DNA synthesis in splenic lymphocytes of guinea pigs. A) Protein (in μg/ml), B) *Staph. aureus* (A – Cowan-1, b – Wood-46; cells/ml), C) con A (in μg/ml), D) PHA (in μg/ml). Abscissa, reagent used to activate DNA synthesis in guinea pig spleen cells: intact (black columns), immunized with protein A (unshaded columns), or immunized with *Staph. aureus* Cowan-1 (obliquely shaded columns); ordinate, radioactivity of spleen cell culture, cpm.

Fig. 2. Incorporation of ^{3}H-thymidine into DNA of splenic lymphocytes of guinea pigs in response to combined effect of protein A and B and T mitogens. Abscissa, reagents added together with guinea pig spleen cells; ordinate, radioactivity of spleen cell cultures, cpm. Legend as to Fig. 1.

number of target cells) in the spleen and/or in their sensitivity to LPS and PHA. Protein A, which is nonspecifically bound *in vivo* with serum immunoglobin [2, 5], may perhaps trigger proliferation of B cells to a lesser degree than the corpuscular antigen, which contains other substances also that have a mitogenic effect on B lymphocytes [8]. Evidence in support of this possibility is given, first, by differences in spontaneous ^{3}H-thymidine incorporation into the cells after injection of protein A and *Staph. aureus* Cowan-1 and, second, its mitogenic action on cell proliferation in animals immunized with Cowan-1 in response to Wood-46.

With the above facts in mind it can reasonably be assumed that after immunization of guinea pigs with protein A, more target cells remain in the spleen to react with LPS and PHA and to react again with protein A *in vitro* than after antigenic stimulation with whole Cowan-1 cells. That this hypothesis is correct is confirmed by experiments to study the combined action of suboptimal doses of protein A and LPS and of protein A and PHA on proliferation of spleen cells of immune animals (Fig. 2). Analysis of these results showed that after combined treatment with protein A and B or T mitogen (PHA) the mitogenic effect on DNA synthesis in the spleen cells underwent summation in animals immunized with protein A (Fig. 2, unshaded columns), but not with whole Cowan-1 cells (Fig. 2, obliquely shaded columns). The summation effect was absent in cell suspensions to which doses of protein A and LPS (50 and 100 μg/ml, respectively) or protein A and PHA (50 and 0.1 μg/ml, respectively) were added *in vitro*, obtained from animals immunized with staphylococcal antigens. This result is evidence that the doses of reagents used were in excess of optimal, so that an effect of inhibition of DNA synthesis was observed more frequently in animals immunized with corpuscular antigen (Fig. 2, shaded columns) than with protein A (Fig. 2, unshaded columns).

Antigens of *Staph. aureus* thus differ in their action on DNA synthesis in the cells of immune and intact animals. The proliferative response of spleen cells to the reagents studied depended on the submolecular organization of the *Staph. aureus* antigens used for immunization, as a result of which immunization of animals with protein A isolated from *Staph.*

aureus and with whole *Staph. aureus* Cowan-1 cells caused a different redistribution of immunocompetent cells and target cells and a different change in their sensitivity to protein A, LPS, and PHA. Constant sensitization of lymphoid and hematopoietic cells by staphylococcal antigens, causing changes in the functional activity of immunocompetent and A cells may lead to the formation of immunologic tolerance, which sharply inhibits antistaphylococcal immunity *in vivo*. This must be taken into consideration in clinical practice when the mechanism of action of staphylococcal antigens on lymphoid and hematopoietic tissue is analyzed.

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