Enhancement by Normal Cells of an Immunological Response.* (27683)

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Sensitization procedures which employ a diversity of materials to enhance immunologic responses have had extensive application in immunology. The mechanisms of adjuvant effects have not yet been entirely elucidated. Certain factors such as increased antigen retention, increased activity of the reticuloendothelial system, lymphoid proliferation, and the creation of inflammatory foci are thought to be involved in adjuvant activity(1,2,3). Effects similar to those induced by known adjuvants may occur, *pari pasu,* in experiments which have as their primary purpose the study of the function of cellular materials as a source of antigen or antibody(2,4,5). The nature of cellular material itself introduces into such studies a complexity of biochemical substances which may have an effect on the sensitization processes. The present study indicates that some normal cells can exert an adjuvant effect in animals immunized with a known antigenic stimulus.

Procedure. Normal white male guinea pigs weighing 400-800 g were randomly divided into 2 groups. The animals of Group I all received 0.4 μg AgN bovine plasma albumin (BPA) (Armour & Co.) diluted in either Hanks' solution or in a suspension of 0.4 ml packed normal guinea pig cells prepared as previously described(6). All antigens and mixtures were made up to a volume of 1.0 ml and administered by intramuscular injection. The animals of Group II all received 1 μg of egg albumin (EA) (Pentex) diluted in either Hanks' solution, Freund's adjuvant, or in a suspension of normal guinea pig cells or an aliquot of cells disrupted by 3 cycles of freezing and thawing. Five days after the sensitizing injection all of the animals were challenged with an intracutaneous injection of either 0.4 mg BPA or 0.4 mg EA in 0.1 ml saline. The skin tests were observed at ½, 1, 2, 6, 8, 24, and 48 hours, and degree of reaction was recorded. The observed reactions were characterized by faint to marked areas of erythema ranging from 0.5 cm to several cm in diameter, slight edema and, with few exceptions, minimal induration or necrosis. Less than 5 mm of reaction was considered negative. Positive skin tests of 5 to 14 mm diameter were considered one-plus reactions, and those greater than 14 mm diameter were considered 2-plus reactions. The skin test when administered to unsensitized control guinea pigs was uniformly negative.

Results. The results at the time of the skin test 5 days after the initial injection of either BPA or EA in various combinations are given in Fig. 1. Injection of either 0.4 mg BPA or 0.4 mg EA intracutaneously in the appropriate animal did not elicit an observable reaction in the animals during the first 8 hours. Frequently, positive reactions were noted at 24 hours. All 9 animals receiving complete adjuvants developed positive reactions. Twenty-five of 39 animals receiving spleen cell suspension with BPA and 31 of 34 animals receiving spleen cell suspension with EA also showed positive reactions. Twelve of 29 animals receiving the BPA alone and 25 of 53 receiving EA alone developed a positive reaction. The reactions in the animals sensitized with antigen alone were thus less frequently elicited. They were also, on the average, less intense by any of the criteria used than were the reactions in the groups receiving antigen and spleen. It appears that normal homologous spleen cells, particularly when intact, enhanced the sensitivity of animals immunized to an antigen when compared to the sensitivity resulting from immunization with antigen alone. Known adjuvants, as expected, also enhanced sensitivity.

In certain groups where immunizing and skin test injections were repeated 2 additional times at weekly intervals, subsequent skin tests were positive more frequently. At the second and third skin tests positive reactions were manifest sooner after testing. By the

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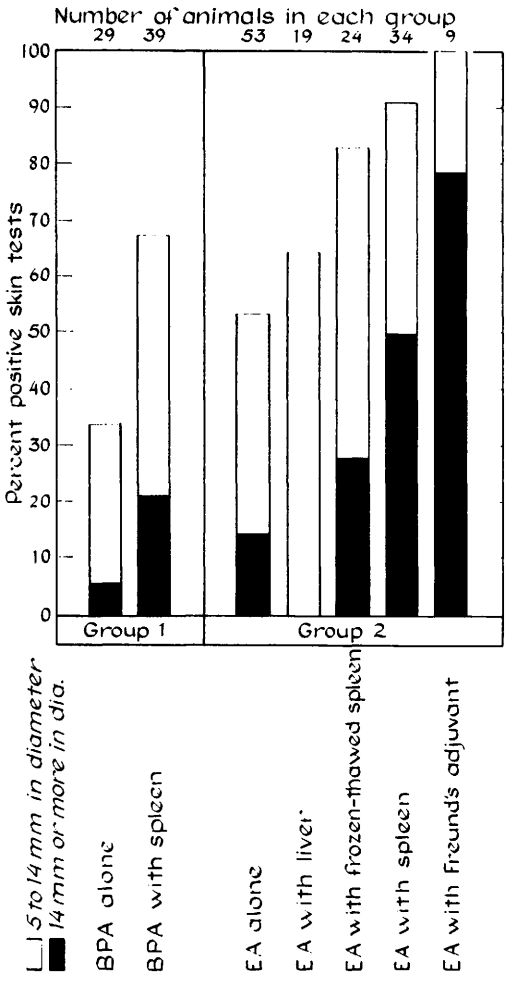

FIG. 1. Skin test readings at 24 hr in experimental groups and controls. Length of the bar indicates per cent of positive reactions in each group.

third test most of the animals, including those controls whose only exposure to antigen occurred during repeated skin testing, showed such pronounced dermal reactivity that differences in degree between groups were difficult to assess.

Some of the animals, negative at the first skin test, were first noted to be positive when read 24 hours after a second skin test. For a particular animal the initial dermal sensitivity noted was characteristically delayed in onset. There was individual consistency of response in the 3 skin tests. Strong reactors to the initial test remained strong reactors on subsequent tests, though the nature of the reaction changed from one of delayed kind to one more akin to the Arthus type. The degree of dermal sensitivity manifested by an animal seemed to parallel the ultimate titers of serum antibody in groups where such determinations were carried out. Most of the animals showed serum antibody when tested 28 days after the initial injection of antigen. The serum antibody response was variable and low in the present study, and quantitative procedures did not offer a sufficiently sensitive tool, with the limited amounts of serum available, to measure any enhancement of serum antibody response by the normal cells tested.

Discussion. The addition of either intact or disrupted spleen cells to extraneous antigens had the apparent effect of enhancing the development of the early-appearing delayed hypersensitivity sometimes referred to by the eponym "Jones-Mote" hypersensitivity(7). Liver cells were much less, if at all, effective. Bloch has recently reported that tubercle bacillus PPD injected with normal peritoneal exudate cells is more likely to cause delayed sensitivity in guinea pigs than is antigen alone (8).

Such results may be of significance in interpretation of experiments concerned with the passive transfer by cells of "delayed hypersensitivity"(4). Further, the diverse capacities of tissues to induce homograft rejection may in part be caused by differences in adjuvant capacity rather than wholly by qualitative or quantitative differences in antigenic constitution(4,9). Small amounts of antigen, too small to elicit sensitivity, may be transferred with cells. It is conceivable that in such circumstances certain cells or cell fractions may cause recognizable sensitivity to the antigen because of the adjuvant properties of the cells. The enhancement may come about in a variety of ways. Since a host of extraneous substances is filtered through the spleen, small amounts of materials derived from the degradation of the few bacteria fortuitously present may contribute to the observed adjuvant effect. Since the liver did not cause the same degree of enhancement, and preliminary experiments with brain tissue do show the enhancement, we do

not think such an explanation is completely satisfactory. An alternative explanation is offered by Berglund and Fagraeus to explain the restoration by intact spleen cells of antibody producing capacity in rats previously treated with cortisone(10). These authors suggest that added cells or cell fractions may contribute to a preliminary processing of antigen to enhance its employment by immunologically competent cells.

Summary. Normal guinea pig spleen cells, when injected with bovine plasma albumin or egg albumin, appear to enhance the delayed sensitivity to the antigen which appeared early in the course of the immunologic response in guinea pigs.

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Ascites Tumor Fluid as a Source of Antibodies in Mice. (27684)

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Recent studies by Munoz(1) have shown that antigens mixed with Freund's adjuvant and injected intraperitoneally into mice caused the development of large amounts of peritoneal fluid containing specific antibody in high concentrations. Difficulty is observed in that ascites formation does not always occur particularly with mice of certain strains (2) and time of production of ascites and amount formed is variable(1).

Herrmann and Engel(3) showed that ascites fluid induced by Sarcoma 180 cells in mice immunized with a viral preparation contained antiviral antibodies. Lieberman *et al.* (4) has employed *Staphylococcus aureus* strain 18-adjuvant mixtures in mice for ascites formation.

Since many transplantable ascites tumors are available in mice and since mice implanted with ascites tumors invariably develop ascites with large amounts of fluid, mice should prove very useful in producing antisera to small quantities of antigen. Use of ascites tumor has the advantage over the use of adjuvant alone in that ascites can be produced with greater certainty, and by regulation of cell dosage the time of ascites formation can be regulated. The results of a study of the production of precipitating antibodies by this procedure are reported here.

Methods. Mice of the Swiss/Webster and the C_3H/Heston strain and of both sexes were injected intraperitoneally with 0.25 ml of an adjuvant antigen mixture, reinjected with a second 0.25-ml dose 2 weeks later, then at the appropriate time injected with approximately 35 million Ehrlich ascites cells or Gardner lymphosarcoma ascites cells. Animals were tapped starting one week after ascites cells were introduced.

The adjuvant contained 50 ml Bayol F, 100 mg mycobacterium butyricum and 25 ml Arlacel 80 and was sterilized in an autoclave before use. The antigen preparation contained 2 volumes of antigen solution mixed with 3 volumes of adjuvant.* The antigens

* The procedure is similar to that described by Munoz(1) with slight changes in materials.