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Specific Immunologic Unresponsiveness to Delayed Hypersensitivity Elicited in Adult Mice.* (27545)

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During experimentation on factors affecting induction of delayed hypersensitivity to protein antigens in mice, we have encountered a form of specific immunologic unresponsiveness affecting this delayed hypersensitivity, of the tuberculin type, but not immediate hypersensitivity, and which is induced in immunologically mature animals. The way in which it is elicited is unique and apparently has not hitherto been described. It is to treat animals which have received a hypersensitizing injection with a solution of antigen during a critical short period after that injection. Preliminary description of this finding is presented here because it aids in distinguishing mechanistically between immediate and delayed hypersensitivities, and because its experimental application to various immunological problems may help to explain the events which occur after vaccination and lead to development of humoral and cellular antibody allergies.

Materials and methods. CF_1 white female mice 6 to 8 weeks old maintained on water and Rockland mouse pellets were employed. The experimental antigen was twice-crystallized chicken ovalbumin (OVA) purchased from Nutritional Biochemicals Corp. Living, avirulent H37Ra strain tubercle bacilli cultured on the surface of Kirchner synthetic medium were utilized as mycobacterial adjuvant. For the hypersensitizing injection these two materials were incorporated in a water-in-oil (w/o) emulsion of a composition and by technics described elsewhere(1). Skin tests were performed by intracutaneous injection of 0.02 ml volumes of 1% OVA freshly prepared in physiologic phosphate buffer of pH 7.4(2). Skin reaction diameters were read with calipers 3 hours and 24 hours after tests were performed representing, respectively, immediate and delayed hypersensitivities(1, 3), and results are expressed in mean millimeters of reaction diameter for each group of animals. These reactions are evident as skin thickenings, edema for immediate hypersensitivity and induration for delayed hypersensitivity. In mouse skin erythema commonly is absent from such reactions(2).

Experiments and results. The mice were divided into 2 large groups. One group was given no sensitizing injection but was subdivided into several smaller groups of 10 to 12 mice each to provide nonallergic skin-test control animals. Each mouse in the second group received a single subcutaneous injection into the groin of 0.1 ml of w/o emulsion containing 0.25 mg of OVA (dry weight) together with 0.25 mg of bacilli (moist weight). Then this group was divided into 5 subgroups, also of 10 to 12 mice each, so that reactions to primary skin tests could be measured at 4, 8, 12, 18, and 22 days after vaccination. Each group after it had received its first test was skin-tested again at every succeeding test period. For example, mice in the group tested 4 days after vaccination also were tested at 8, 12, 18, and 22 days. Succeeding skin tests

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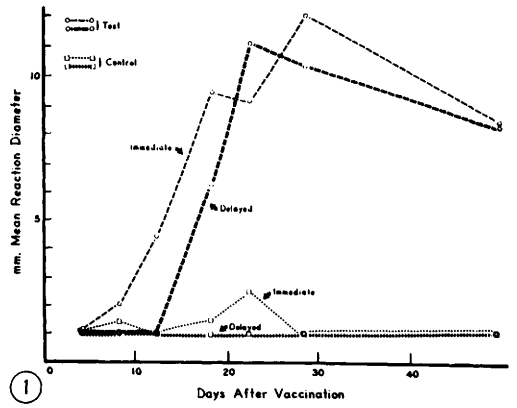

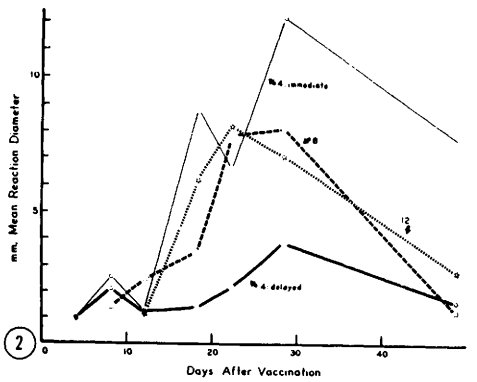

FIG. 1. Normal tempo of development of delayed and immediate hypersensitivities in mice vaccinated once subcutaneously with w/o emulsion of ovalbumin ("test"). Unvaccinated mice ("control") remain unreactive throughout experiment.

FIG. 2. Inhibition of development of delayed hypersensitivity in mice first skin-tested 4 days ("4") after subcut. vaccination with ovalbumin in w/o emulsion. Immediate hypersensitivity developed normally in these mice. Inhibition of development of delayed hypersensitivity becomes successively less marked in mice first tested 8 and 12 days ("8" and "12," respectively) after vaccination.

were performed on alternating sides of the animals. Finally, all groups, both experimental and control, were skin-tested 28 and 49 days after vaccination.

In Fig. 1 are plotted mean skin reaction readings for succeeding previously untested groups of mice during the first 22 days of the experiment as well as averages of the means of all test and control groups for 28- and 49-day tests. This graph depicts the normal development in mice of immediate and delayed hypersensitivities to protein antigens; it agrees closely with graphs which we have obtained from similar experiments in mice with bovine serum albumin and tuberculoprotein (1). Thus, both types of allergy appear within 2 to 3 weeks after a single subcutaneous vaccination, the immediate type arising somewhat more rapidly, and both peak about 4 weeks after vaccination. Also evident from this graph and supported by several other experiments is the fact that skin tests themselves given in any of the various combinations provoke neither immediate nor delayed hypersensitivity in mice measurable by our technic.

For contrast Fig. 2 shows means for 3 individual groups of vaccinated mice, including the means for both immediate and delayed hypersensitivity of the group first skin-tested 4 days after vaccination and the means of delayed skin reactions for mice given their primary tests at 8 and 12 days. It is obvious that the 4-day skin test prevented delayed hypersensitization but did not affect development of immediate hypersensitivity. This effect is much less evident in mice not given a test until the 8th day after vaccination, and nearly absent from those first tested 12 days after vaccination. In both of the latter 2 groups the effect is most obvious in the rapid drop of that delayed allergy which did develop to an insignificant level by the 49th day. Not shown is the fact that mice tested 18 or 22 days after vaccination developed greater allergy and had not lost any by the 49-day test. Immediate hypersensitivity developed nearly equally in all groups and also remained relatively unchanged in all from the third week through the end of the experiment.

Discussion. Immunologic unresponsiveness affecting development of circulating antibodies has received considerable attention (4). It is induced, usually in animals with undeveloped or injured immunologic apparatus, by injecting replicating antigens in moderate or nonreplicating antigens in very large quantities. Conditions required for such unresponsiveness to immediate hypersensitivity were not met in the present experiment, for treated mice developed this kind of allergy normally. Consequently the immunologic apparatus whose sequence of activities was disturbed must in one or more basic respects have been different from that which goes into

action after antigenic stimulation to produce circulating antibodies.

Immunologic unresponsiveness to delayed hypersensitivities has received scant notice, unless one includes in this area homograft sensitivity. Although such inclusion may be justified, understanding of the basic mechanisms of this sensitivity remains clouded by the complexity of homograft reactions by which such sensitivity is studied. An interesting kind of immunologic unresponsiveness inducible in adult guinea pigs to contact sensitivity first was demonstrated by Chase(5). Animals fed allergen when later given a hypersensitizing stimulation with it developed delayed hypersensitivity poorly or not at all. This finding has been extended recently to show that small quantities of allergen injected into the mesenteric veins of guinea pigs elicit the same effect, but the unresponsiveness evoked also extends to anaphylactic sensitivity(6). Adult mice can be prevented from developing either immediate or delayed hypersensitivity to ovalbumin by treating them frequently with large quantities of this antigen is aqueous solution beginning at the time of vaccination(7). Later, when these repeated injections are stopped, anaphylactic hypersensitivity develops slowly but completely and in all mice, but delayed hypersensitivity appears much more slowly and, in many animals, not at all.

We have noticed the type of unresponsiveness described here also to occur against contact sensitivity when the allergen chlorodinitrobenzene is applied during the first few days after a sensitizing exposure (unpublished). Whether and how this phenomenon can be related to that observed by Chase or to the unresponsiveness induced by repeated injections of much antigen is not yet clear. Characteristics common to all 3 are that they are effective against delayed hypersensitivity and that they can be provoked in adult animals by treatments with allergen alone. As to how the unresponsiveness described here may arise, the necessity for giving the interfering dose of antigen shortly after vaccination during the induction period suggests that at this time the allergy induction apparatus has been stimulated and is passing through a stage of development at which it has acquired immunologic specificity but is vulnerable in some way to destruction by fresh antigen injected into the animal. Further work will be necessary to examine this idea and to relate this unresponsiveness phenomenon to others. If the proposed explanation is true it could help to explain hitherto puzzling events in the induction of allergic and immune phenomena, and it might lead to practical ways of controlling development of delayed hypersensitivity reactions, such as those to homografts.

Summary. Immunologic unresponsiveness directed against delayed but not immediate hypersensitivity to protein antigen can be elicited in adult mice by exposing them to a buffer solution of antigen within a certain short, critical time after they have been vaccinated with the same antigen in water-in-oil emulsion. Further study of this phenomenon may improve understanding of and control over the mechanisms of induction of delayed and immediate hypersensitivities.

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