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SPLIT TOLERANCE AFFECTING DELAYED HYPERSENSITIVITY AND INDUCED IN MICE BY PRE-IMMUNIZATION WITH PROTEIN ANTIGENS IN SOLUTION

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SUMMARY

Adult immunocompetent mice vaccinated with protein antigens in water-in-oil emulsion so as to develop immediate and delayed hypersensitivities resist developing the latter if they are treated with the immunizing antigen in aqueous solution before or during the sensitization period. If the treatments are given during or after vaccination this resistance is directly proportional to their intensity and inversely proportional to the degree of hypersensitivity which has developed when they are begun. But when the treatments are given before vaccination such split tolerance is more pronounced and seems to be directly proportional more to the degree of humoral antibody production existing at the time of vaccination than to the intensity of treatments. The characteristics of this antigenically specific selective unresponsiveness suggest that it may result from a competitive maturation or differentiation of primitive immunocytes: upon exposure to protein antigens in forms not readily able to induce delayed hypersensitivity, the potential functions of these immunocytes for making circulating antibodies may be pre-empted at the expense of such capacity to develop into cells making the antibody of delayed hypersensitivity.

If mice with established immediate and delayed hypersensitivities to protein antigen are desensitized with an appropriate series of seven daily injections of antigen, they will recover both types of hypersensitivity within a day or two. But 3 or 4 weeks later they will again lose delayed and sometimes also immediate hypersensitivity, thus acquiring immunologic unresponsiveness which is specific and long-lived (Crowle, 1963). An important facet of this and related forms of immunologic tolerance in mice is that delayed hypersensitivity is more affected than immediate hypersensitivity; these types of tolerance tend to be selective or split rather than comprehensive as are the more widely studied varieties (Crowle, 1962a, 1963; Crowle & Hu, 1965b; and unpublished work). Examples of split tolerance may be

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fairly common, but they have not yet attracted widespread interest. The following experiments were begun in order to obtain more information on tolerance induced in adult immunocompetent mice. During their course we noted that injecting such mice with small quantities of protein antigen in aqueous solution elicited pronounced split tolerance, rendering the animals unable to develop delayed hypersensitivity while enhancing their capacity to develop immediate hypersensitivity.

MATERIALS AND METHODS

Animals

Female CF_1 strain mice (Carworth Farms, Inc., Congers Road, New City (Rockland County), New York) of 2–3 months of age were used in groups of ten to twelve. They were maintained on Rockland food pellets (Teklad, Inc., Monmouth, Illinois) and water.

Sensitization and testing

Two different antigens were utilized—thrice-crystallized chicken ovalbumin (OVA; Nutritional Biochemicals Corporation, Cleveland, Ohio) and crystallized bovine serum albumin (BSA; Pentex, Incorporated, Kankakee, Illinois). We have described elsewhere our techniques for using these antigens to induce and detect immediate and delayed hypersensitivities in mice (Crowle, 1962b) and therefore review them here only briefly. Hypersensitization was achieved by giving mice two subcutaneous injections 1 week apart of 0·1 ml of water-in-oil (w/o) emulsion, containing 0·25 mg of antigen but no bacterial adjuvant. Boosting injections, when used, were the same but given within the axillary region rather than in the inguinal areas used for the two initial injections. Hypersensitivity was detected by intracutaneous testing of clipped flanks, alternating these from one test to the next, using 0·02–0·03 ml volumes of 0·1% antigen dissolved in physiologic phosphate buffer. Resulting skin reactions were read at 3 and 24 hr, representing immediate and delayed hypersensitivities, respectively (Crowle, 1962b), in terms of diameter, skin thickness and presence or absence of petechiae and central necrosis. Mice were considered to have a reaction when the diameter of palpable swelling exceeded 3 mm; our results are recorded as proportions of reactors to avoid presenting excessive data.

Pre-immunization treatments and tolerogenic treatments given during the sensitization period consisted of injections in physiologic phosphate buffer either of free antigen or alum-precipitated antigen prepared by Carpenter's method (Carpenter, 1956).

EXPERIMENTS AND RESULTS

Timing and tolerance induction in sensitized mice

We have employed a series of seven daily injections with antigen to induce tolerance in adult sensitized mice (Crowle, 1963). These begin with 0·1 mg given on the first day and progress daily through, 1, 10, 20, 20, 40 and 40 mg, injected each time intraperitoneally in buffered physiologic saline. The following experiment was performed to study the effect that such a series of treatments would have when given at different intervals of the hypersensitization period.

Seven groups of ten mice each were used. All were sensitized with OVA at the same time. Six groups received in addition to their two sensitizing injections the series of seven daily injec-

tions. The treatments of each of these six groups started at a different time. Mice of group 1 were treated for the week preceding the first sensitizing injection, those of group 2 were treated for the week immediately following this injection, and so on with each group beginning treatments 1 week later than the preceding one with mice of group 6 not being treated until the fourth week following initial injection. The seventh group of vaccinated mice remained untreated as a positive control group. All of these mice received a boosting injection of OVA in w/o emulsion during the fifteenth week of the experiment to test the strength of any tolerance developed.

Skin tests performed on these various animals several times during the 20-week course of the experiment indicated that the antigen injections affected development of immediate

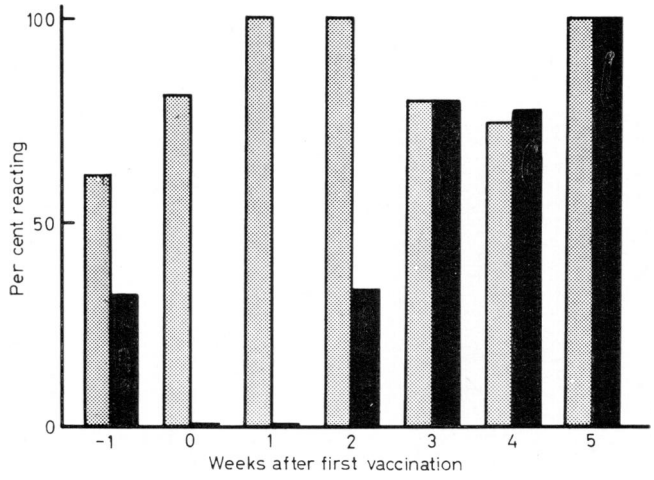

FIG. 1. Proportions of immediate (stippled bars) and delayed (solid bars) hypersensitivities in mice, 19 weeks after vaccination, which had received 1 week of tolerance-inducing treatments with antigen solution begun at different times. This distinction between groups is shown along the abscissa, indicating that treatments began 1 week before vaccination (−1), on the day of vaccination (0), 1 week after vaccination (1), etc. As shown, delayed hypersensitivity did not develop in the second and third groups of mice.

hypersensitivity only slightly, more delaying than preventing it, but that they strongly suppressed both development and maintenance of delayed hypersensitivity. This was especially evident in mice treated early in the sensitization period. These findings are seen best in the data obtained from the 19-week skin-testing, that is, the skin-testing given 4 weeks after boosting and therefore indicating the permanency as well as frequency of tolerance seen in the various groups.

These data, summarized in Fig. 1, show that a split tolerance affecting induction of delayed but not immediate hypersensitivity had been achieved in four of the groups, that it was a very stable tolerance capable of resisting the effects of boosting, and that it was induced best by treatments given during the first 2 weeks of sensitization. When this intensive course of treatments was given during the week before initial vaccination, no better tolerance

was induced, and it may not even have been as good. The effects of these earlier treatments with antigen differ from those applied to mice already hypersensitive (Crowle, 1963) in that these earlier treated mice never developed delayed hypersensitivity at all.

Effects of intensity of treatments during optimal time for tolerogenesis

Having found the time when injections with antigen solution could most readily induce this striking form of split tolerance, we set up an experiment to determine next what intensity of treatment is necessary, during this optimal time for its application, for this effect. A large

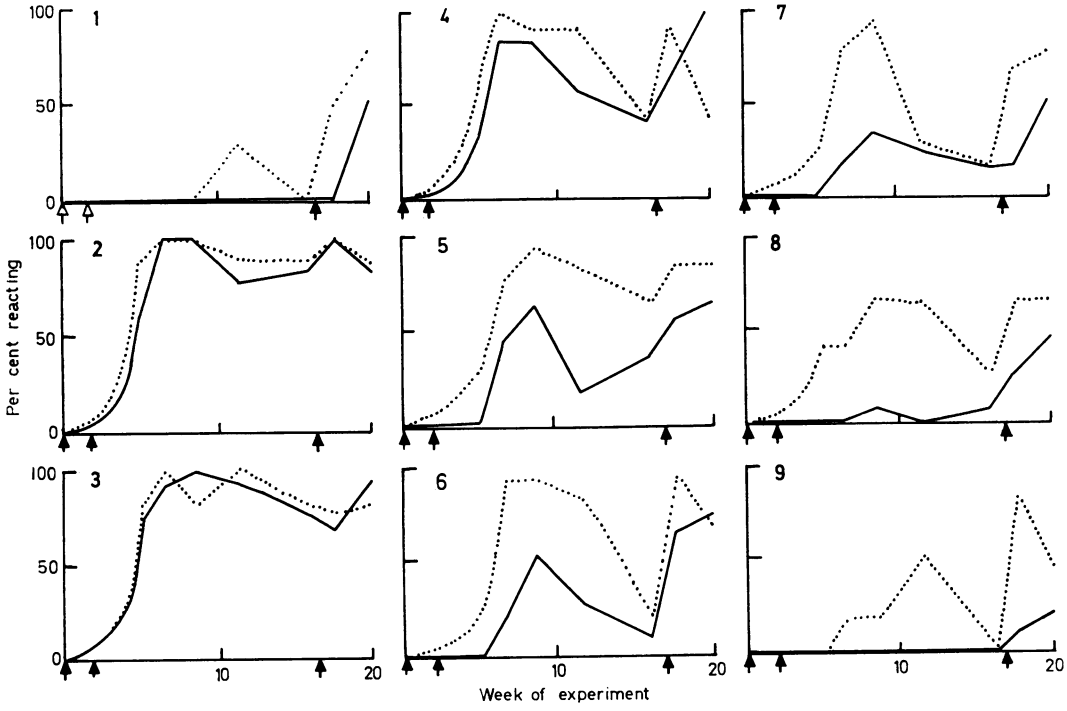

FIG. 2. Development with time of immediate (broken line) and delayed (solid line) hypersensitivities in nine groups of mice in an experiment showing the successively greater effectiveness of more intensive early treatments with antigen in solution in suppressing development of hypersensitivity. Groups 1 and 2 did not receive these early treatments, these being unsensitized and sensitized control groups, respectively. Group 3 was given one early treatment, group 4 was given two treatments, and so forth through to group 9 which received the full course of seven treatments (see text). Solid arrows along the abscissa indicate injection of antigen in w/o emulsion (i.e. sensitizing vaccination); hollow arrows designate similar injection, but with w/o emulsion containing no antigen.

number of mice was vaccinated with OVA in w/o emulsion. These mice then were divided into groups of ten, one serving as an untreated positive control group and the others as test groups, according to treatments to be given them in the period between first and second vaccinations. The first test group (number 3 of Fig. 2) received only a single intraperitoneal injection of 0·1 ml of OVA in buffer on the day of the first vaccination; the second test group

received this and a second treatment of 1 mg on the second day; the third received three succeeding daily treatments of 0·1, 1·0 and 10 mg, and so on through the last group (number 9 in Fig. 2), which received the full series of seven daily injections described above. A separate group of ten unsensitized control mice (group 1 in Fig. 2) were neither vaccinated nor treated; but they received a 'boosting' later in the experiment along with the other animals. Mice in all groups were skin-tested regularly and at the same intervals.

A chart summarizing results from a series of skin tests done with these various groups of animals is given in Fig. 2. It shows that the number and intensity of treatments proportionally increased inhibition of sensitization of both immediate and delayed types, especially of the latter. Such suppression is evident as a lowering of the proportion of reactors, a shortening

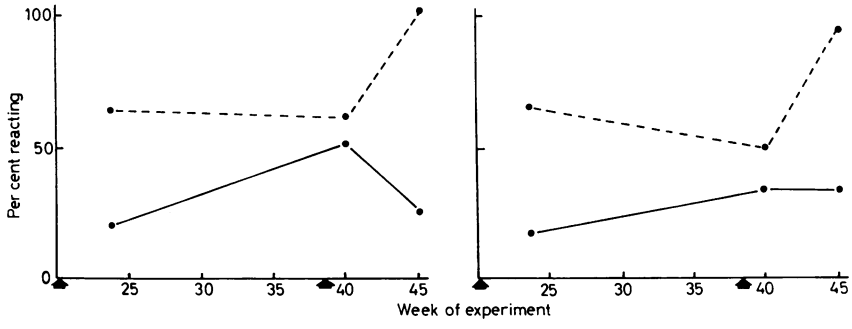

FIG. 3. Effectiveness of tolerance in two groups of tolerant mice from experiment shown in Fig. 2. Mice whose reactivities are summarized in the left graph were pooled from animals in group 1, Fig. 2, which had not developed delayed hypersensitivity by the twentieth week of the experiment; results for the second graph were obtained from mice similarly unresponsive but pooled from groups 3–8 of Fig. 2. The basic difference between the two groups was that in the left group tolerance was induced unintentionally only by previous skin testings, whereas in the right group it had been induced purposely by early treatments with antigen solution, but both groups nevertheless resist similarly the development of delayed hypersensitivity following vaccination with antigen in w/o emulsion (arrows along abscissa).

of the longevity of hypersensitivity, and an increase in the proportion of mice which were unable to respond to a boosting injection of OVA in w/o emulsion given 16·5 weeks after the experiment began. No tolerance was induced by the single 0·1 mg injection,* but the two-injection treatment did induce some, showing that in this critical period of the experiment only very mild treatments with antigen solution are needed to produce split tolerance in some adult mice. Striking inhibition was obtained by progressively more intensive treatments, the 6- and 7-day treatments completely inhibiting development of delayed hypersensitivity and strongly suppressing that of immediate hypersensitivity.

* Such lack of inhibition by early treatment with antigen does not conflict with our previous observations of inhibition caused by intradermal injection of antigen a few days after vaccination (Crowle, 1962a), because in the present experiment the first skin tests were done 5 weeks after vaccination when the general retardation, rather than prevention, of delayed-type hypersensitization effected by these early treatments no longer would be evident.

Effectiveness of tolerance induced

We noticed that unsensitized skin-test control mice in this experiment responded poorly to boosting with OVA in w/o emulsion: less than half responded to develop delayed hypersensitivity within 4 weeks; had they been previously untreated, all should have become hypersensitive. Their previous skin tests may have induced a split tolerance. To examine this possibility as well as to determine the longevity of tolerance induced in test animals, this experiment was continued for another 25 weeks totalling forty-five in the following manner.

All test group mice which had been unresponsive to boosting, as verified by a skin-testing on the twenty-fourth week, were pooled and responsive ones were discarded. The collected unresponsive mice along with separately caged unresponsive mice of the original unsensitized control group were injected once again with OVA in w/o emulsion during the thirty-eighth week of the experiment. Then 2 and 6 weeks later they were skin-tested.

The results of these tests, shown in Fig. 3, indicate that while both sets of mice responded readily to boosting to develop immediate hypersensitivity the majority of animals in each remained unable to develop delayed hypersensitivity. Thus, split tolerance apparently can be induced by much less intensive treatments than originally expected, providing sufficient time is allowed for it to develop.

Inducing split tolerance by pre-immunization with small amounts of antigen

We have observed this apparent tolerogenicity of pre-treatments using small amounts of antigen in aqueous solution in various other unpublished experiments, and similar phenomena have been reported by others (see 'Discussion'). To verify these findings more formally we performed an experiment with six groups of mice treated according to the following outline:

Group 1: Mice injected with w/o emulsion only (unsensitized controls).

Group 2: Mice pre-immunized with alum-precipitated BSA.

Group 3: Mice pre-immunized with BSA solution.

Group 4: Mice pre-immunized with alum-precipitated BSA and later immunized with BSA in w/o emulsion.

Group 5: Mice pre-immunized with BSA solution and later immunized with BSA in w/o emulsion.

Group 6: Mice immunized with BSA in w/o emulsion (positive controls).

Pre-immunizations were given on days -28, -27, -26, -21, -20 and -19. The first three BSA injections were intravenous; the second three and all of those with alum-precipitated BSA were intraperitoneal. For each such injection 1 mg of BSA was injected in 0·1 ml of buffer. Immunizations were given on days 0 and 7 and were repeated twice again later on weeks 11 and 23. Skin tests were performed at various intervals during the experiment. Their results are plotted in Fig. 4. According to these results, pre-immunization of either type alone induces immediate but not delayed hypersensitivity; on the contrary, but as might be expected from the data discussed above, such pre-immunization prevents development of delayed hypersensitivity in most of the animals treated and shortens its span in those mice which do develop this type of hypersensitivity. For example, pre-immunization with alum-precipitated BSA prevented 70% of the animals from developing

delayed hypersensitivity, and the remaining 30% which did acquire such hypersensitivity lost it within 4 weeks. The unresponsiveness elicited by pre-immunization with alum-precipitated BSA not only was potent but also was long-lasting: 60% of mice so treated failed to develop delayed hypersensitivity even after the two later boostings. Unresponsiveness in mice pre-immunized with BSA in solution was somewhat less potent.

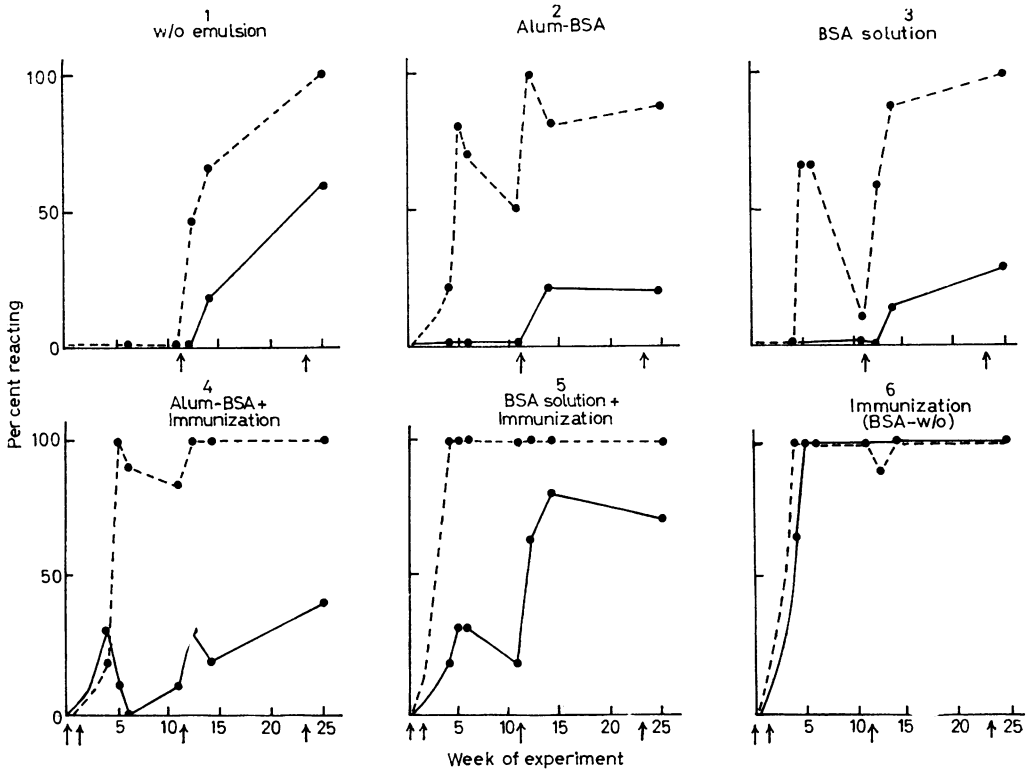

FIG. 4. Inhibitory effects of pre-immunization with alum-precipitated BSA (group 4) or with BSA solution (group 5) on development of delayed hypersensitivity (solid line) as induced by vaccinations with BSA in w/o emulsion (solid arrows along abscissa). Pre-immunization alone (groups 2 and 3) induced some immediate hypersensitivity (broken line) but no delayed hypersensitivity, and its inhibitory effect is evident even when vaccinations are given 11 and 23 weeks afterward (groups 2 and 3). Both types of hypersensitivity developed normally in the positive control group 6 and did not develop in the initial part of the experiment in the negative control group 1.

A striking inhibition is evident in the results of skin tests presented for responses of those mice in groups 2 and 3 which were at first only pre-immunized but got the later boostings with BSA in w/o emulsion. Nearly all of these animals developed good immediate hypersensitivity, but in 70–80% delayed hypersensitivity failed to appear. This split tolerance, though of lesser proportion, also is evident in group 1, confirming that skin tests themselves are tolerogenic relative to delayed hypersensitivity.

Specificity of split tolerance

If the tolerance induced by pre-immunization were an immunologic phenomenon, then it should be antigenically specific. This was demonstrated by an experiment testing OVA and BSA for potential cross-tolerogenicity.

In this experiment there were nine groups of mice including unsensitized and sensitized controls, mice only pre-immunized with alum-precipitated antigen, and mice pre-immunized with OVA or BSA and later vaccinated once with one or the other antigen in w/o emulsion (see Table 1). These mice were skin-tested at 3, 4 and 5 weeks after vaccination with appropriate antigen. Table 1 summarizes results from the last of these testings only, since they are qualitatively similar to those from the earlier tests but more complete. They confirm that this pre-immunization-induced split tolerance selectively suppresses the development of delayed hypersensitivity and also demonstrate that the effect is antigenically specific.

TABLE 1. Effect of homologous and heterologous antigen pre-immunization on the development of delayed hypersensitivity

Group	Pre-immunization	Immunization	5-week test Homologous antigen	
			I*	D
1	—	w/o	0	0
2	—	BSA–w/o	90	60
3	—	OVA–w/o	89	100
4	Alum–BSA	—	0	0
5	Alum–OVA	—	14	0
6	Alum–BSA	BSA–w/o	100	0
7	Alum–BSA	OVA–w/o	90	70
8	Alum–OVA	BSA–w/o	100	89
9	Alum–OVA	OVA–w/o	87	13

* I, Immediate; D, delayed; results are given as per cent reacting to antigen homologous with the immunizing antigen 5 weeks after vaccination.

Pre-immunization with OVA inhibited delayed hypersensitization to OVA but not BSA (13% *vs.* 89% delayed hypersensitivity, respectively); pre-immunization with BSA inhibited delayed hypersensitization to BSA but not to OVA (0 *vs.* 70% delayed hypersensitization, respectively). In none of these groups was development of immediate hypersensitivity suppressed by pre-immunization.

DISCUSSION

In the course of amplifying previous findings (Crowle, 1963) that existing immediate (Arthus) and delayed (cellular-antibody) hypersensitivities to protein antigens in adult mice can be replaced with specific tolerance, we have learned that the effectiveness of tolerance-inducing treatments is inversely proportional to the degree of hypersensitivity already existing in the treated mice. But, as we have found before (Crowle, 1962a, 1963; Crowle & Hu, 1965a), tolerance specifically affecting delayed hypersensitivity is disproportionately

easier to induce than that affecting immediate hypersensitivity, sufficiently so as to suggest some inverse connection between the inductions of immediate and delayed hypersensitivities. This suggestion is supported by the present observations that pre-immunizing mice with a small amount of a protein antigen in aqueous solution specifically primes them to develop Arthus hypersensitivity but at the same time nullifies their erstwhile normal capacity to develop delayed hypersensitivity to that antigen, that is, pre-immunizing induces selectively a split tolerance.

Such selective split tolerance probably is not uncommon; it seems evident in the results reported by several investigators. Chase (1959a) noted that antigenic stimulation which induces development of humoral antibodies made difficult later attempts to induce delayed hypersensitivity. He, and other workers following his leads, have shown repeatedly that guinea-pigs fed or injected with contactants can develop anaphylactic or Arthus hypersensitivity to the contactant while becoming tolerant for contact (i.e. delayed) hypersensitivity to it (Chase, 1959b; Battisto & Miller, 1962; Bowser & Baer, 1963; Chase, Battisto & Ritts, 1963; Frey, de Weck & Geleick, 1964a, b). Boyden (1957) found some time ago that guinea-pigs pre-treated with unheated tuberculoprotein develop Arthus hypersensitivity to it but become uncommonly resistant to later development of delayed hypersensitivity to this protein following injection of normally allergenic entire tubercle bacilli, and Arima, Yamamoto & Takahashi (1959) have made similar observations. Selective tolerances have been induced with the aid of drugs (Schwartz, 1965), for prolongation of the life of homografts apparently not involving the separate unresponsiveness phenomenon of enhancement (Hardin & Werder, 1955; Katsh, Talmage & Katsh, 1964), and by pre-immunization to protect against experimental autoimmune disease (Good, 1959; Shaw *et al.*, 1962; Alvord *et al.*, 1965). For example, pre-immunizing guinea-pigs with appropriate quantities of the purified antigen which is used to induce experimental allergic encephalomyelitis makes them strongly resistant both to development of delayed hypersensitivity to that antigen and of encephalomyelitis while appreciably enhancing their capacity to produce humoral antibody to that antigen (Shaw *et al.*, 1965). Very recently Dvorak *et al.* (1965) and Asherson & Stone (1965) have reported results from guinea-pig experiments which are remarkably similar to those which we describe here for mice, and which substantiate directly the existence of selective split tolerance especially affecting delayed hypersensitivity.

These and other examples of split tolerance possibly appertaining only to varieties of humoral antibody formation (Dresser, 1962) appear to be legitimate unresponsiveness phenomena induced quite differently from the better-known more comprehensive forms of immunologic tolerance. They are characterized by appearing in adult immunocompetent animals usually several days after an inducing treatment, which consists of applying an immunizing dose of antigen in a form normally unable to induce the type of hypersensitivity affected by the resulting tolerance but able to induce a possibly competing variety of hypersensitivity. Therefore, comprehensive definitions of immunologic unresponsiveness stressing as cardinal features that it is induced and maintained by excesses of antigen in relation to the number of immunocompetent cells potentially responsive to the antigen (Medawar, 1959; Brent & Gowland, 1963; Dorner & Uhr, 1964; Eisen & Karush, 1964) need to be qualified to recognize not only antigen-induced split tolerance but also antiserum-induced forms of unreactivity (i.e. enhancement; Brent, 1958; Hăsek, Lengerová & Hraba, 1961; Arnason & Waksman, 1964; Crowle & Hu, 1965b). If these various phenomena are

recognized as varieties of immunologic unresponsiveness, then only the nonreactivity itself remains a basic feature of immunologic unresponsiveness. This state can be achieved in at least three different ways: (1) eradicating all immunocompetent cells potentially responsive to a given antigen; (2) eliminating, possibly competitively, the type of immunocyte clone responsible for one kind of antibody formation to an antigen or preventing its development; and (3) blocking formation of antibody or differentiation of potentially antibody-making cells. Consequently, the various unresponsiveness phenomena that are now recognized should probably not be considered simply as varieties of a mechanistically uniform occurrence.

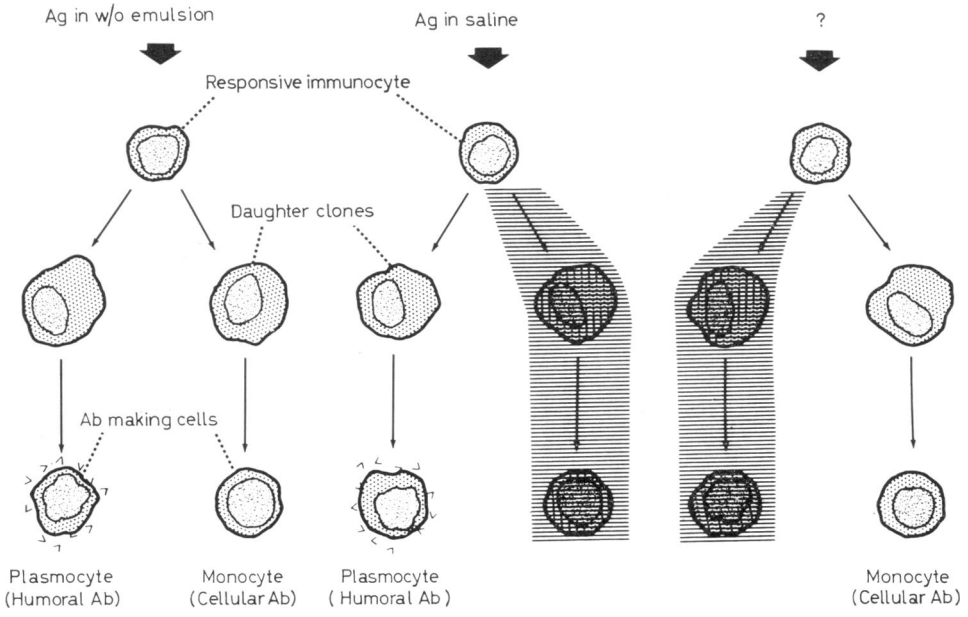

FIG. 5. Speculative explanation for split tolerance in mice affecting one kind of hypersensitivity more than another. Shaded areas are events which appear to have been suppressed. The question mark indicates we have not yet observed split tolerance affecting immediate but not delayed hypersensitivity, although it may exist.

The far-sighted question asked by Waksman (1959): 'Is it not possible that treatment which leads the immunologically competent cell to make humoral antibody makes this cell unable to develop delayed hypersensitivity?' leads us to speculate that the split tolerance in mice which we describe here may be due to selective or competitive differentiation of immunocytes. By framing this speculation with some other popular ideas on the nature of antibody formation (Hăsek *et al.*, 1961; Smith, 1961; Talmage & Pearlman, 1963; Asherson & Stone, 1965; Schwartz, 1965) we envision the events depicted diagrammatically in Fig. 5. A clone of primitive immunocytes appropriately stimulated, with protein antigen in w/o emulsion, can develop irreversibly into offspring daughter clones of secondary immunocytes. These clones have two basic functions, to reproduce themselves and to produce tertiary terminal and further differentiated antibody-producing cells. The kind of antibody manufactured by

the terminal cells will depend upon the kind of secondary cell begetting it, these secondary cells being committed to produce either humoral or cellular antibody-making terminal cells.

If the primary immunocyte is stimulated with antigen in saline rather than in w/o emulsion, then it is pre-empted to produce irreversibly only the kind of secondary daughter clone which eventually gives issue to humoral antibody-making cells. Thus, the animals' original potentiality to develop delayed hypersensitivity to the antigen is destroyed not by eradication of a clone of cells but rather by selective differentiation of all the originally multipotent primary immunocytes responsive to that given antigen to secondary immunocytes able to respond to this antigen only by producing humoral antibody-making terminal cells; permanent split tolerance results. Conditions necessary to excite differentiation to exclusively cellular antibody-making cells producing a selective unresponsiveness in the opposite direction, if they exist, are unknown.

The absence of immunocytes capable of a given type of antibody response to an antigen, rather than their blockade by a competing immunologic response, is postulated in this diagram to explain the kind of split tolerance described in this paper, because in experiments still progressing we are finding that mice with this type of tolerance can be reconstituted by adoptive transfer of immunocompetent normal or sensitized isologous cells, even though the recipients already have apparently competitive Arthus hypersensitivity. Thus, this split tolerance differs mechanistically from enhancement-type forms of unresponsiveness due to effects of antiserum, although the two are similar in suppressive effects (Crowle & Hu, 1965b).

Unresponsiveness phenomena could act at three levels in this scheme. Comprehensive unresponsiveness would be effected by eradicating the primary clone of immunocytes; selective split unresponsiveness would result from causing one kind of secondary immunocyte clone to develop to the competitive exclusion of another; enhancement-like (antiserum-mediated) unresponsiveness occurs when differentiation of the secondary immunocyte to the tertiary one is blocked or antibody production by the terminal immunocyte is prevented by antiserum.

A few words on the practical meaning of our findings are called for. Once an animal has been exposed to antigen, even in small sensitizing quantities, it differs from the pristine animal not only, as commonly known, in being capable of subsequent magnified response to the antigen (anamnesis) but also, as not so widely recognized, in the opposite fashion of being less than normally able to develop some kinds of antibodies. Hence, the ultimate immunologic response of any immunized animal should be viewed not only as induction of one or another kind of antibody production but also as the outcome of a balance struck between possibly competing types of immunologic response and a given stage in immunocyte differentiation. Natural immunologic responses and their consequences should be viewed as determined to a significant degree by possible competitive interplay of stimuli by key antigens in different ways. For instance, this interplay could determine the outcome of infection with tubercle bacilli in the following manner.

Such infection can elicit one or more of four different significant immunologic responses: humoral antibodies to bacillary proteins, cellular antibodies to these antigens, humoral antibodies to an immunizing polysaccharide antigen (cf. Crowle & Hu, 1965c), and cellular antibodies to this immunogen. Humoral antibodies to the proteins would not directly be either beneficial or harmful, but cellular antibodies to the proteins would be very harmful in their capacity to produce cavities in the lungs (Crowle, 1962c). Since the results which

we have presented here suggest that the induction of humoral antibody formation may impair induction of cellular antibody formation, one may surmise that although humoral antibodies to tuberculoprotein are not themselves significant in the pathogenesis of tuberculosis, initiation of their manufacture and the resulting direct disablement of the induction of analogous cellular antibody formation should favour recovery from tubercular infection. On the other hand, since acquired immunity to tuberculosis seems to be a cellular antibody immunologic response (Lurie, 1964) probably directed against a polysaccharide antigen (Crowle & Hu, 1965c), one would hope to achieve maximum protection against the tubercle bacillus by developing cellular antibodies against this polysaccharide without interference by induction of humoral antibody formation against this antigen. Fortunately, this substance appears to have a very low propensity for inducing humoral antibody formation (Crowle & Hu, 1965c).

Similar balances between immunologic responses conceivably could determine whether or not autoimmunity would develop after exposure to a given antigen, except that the situation might be simpler because the competitiveness of responses might involve only one effect of antibodies—their destructiveness. For instance, autoimmunity caused by cellular antibodies would not develop if humoral antibody formation could be provoked earlier than cellular antibody formation, and conceivably such autoimmunity could be treated by temporarily abolishing all antibody formation (e.g. by X-irradiation) and then selectively re-inducing the relatively harmless but competitively protective humoral antibody response.

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