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# Suppression of Delayed Hypersensitivity by Antigen and Antibody

## IS A COMMON PRECURSOR CELL RESPONSIBLE FOR BOTH DELAYED HYPERSENSITIVITY AND ANTIBODY FORMATION?

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**Summary.** Delayed-type hypersensitivity and antibody formation to sheep erythrocytes (SRBC) was studied in rats. Two immunologically specific suppressive effects on the primary induction of delayed hypersensitivity were found: one was short-lasting and due to a direct action of intravenous antigen; the other was mediated by anti-SRBC antibody and demonstrable both as a later-occurring and indirect effect of active immunization and as occurring consequent to passive immunization. Either of these two forms of suppression only partially prevented primary induction of delayed hypersensitivity, but used together their effects were synergistic and completely suppressed development of delayed hypersensitivity. The secondary delayed hypersensitivity response was insusceptible to antibody inhibition.

The data concerning delayed hypersensitivity to SRBC in the rat were in some ways analogous to previous findings concerning induction of haemolytic plaque-forming cells to SRBC in the same animal. The interpretation that delayed hypersensitivity and humoral antibody formation could represent alternative responses of potential antibody-forming cells to immunological induction was considered.

## INTRODUCTION

Since the demonstration (Dienes and Schoenheit, 1929) that delayed hypersensitivity is not a reaction peculiarly directed against components of certain infectious agents, the possible relationship between it and humoral antibody formation has been a moot point. If such a relationship does not exist, the delayed hypersensitive state arises from the immunological induction of a cell population different from any population inducible to a humoral response. To examine this problem the concurrent and separate induction of humoral antibody formation and delayed hypersensitivity to sheep erythrocytes, and the effects of immunologically specific suppression on these two responses, were examined in the rat. In this system presence of circulating antibody did not appear to complicate assessment of delayed hypersensitivity.

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## MATERIALS AND METHODS

*Animals*

Adult female Sprague–Dawley rats of 175–275 g, matched for age and weight within each experiment, were used.

*Antigens and adjuvant*

Sheep erythrocytes were washed three times before use, and final concentrations checked by colorimetry. Human erythrocytes were drawn on the day of use. Freund's complete adjuvant (Difco) was used.

*Antisera*

Rats were bled by cardiac puncture. One-, 3- and 5-day serum pools were obtained after immunizing rats by vein with 1 ml of a 5 per cent SRBC suspension. Rats bled 8 and 4 days after primary sensitization with 5 per cent SRBC in Freund's complete adjuvant (FCA) provided 8- and 4-day pools used in attempted transfer of delayed hypersensitivity. 'Hyperimmune' rat anti-SRBC serum was obtained by first sensitizing with 20 per cent SRBC in FCA; 5 weeks later 1 ml of 10 per cent SRBC was given by vein, and serum pooled 8 days later.

The 1-, 3-, 4- and 8-day pools were used immediately; the rest were stored at $-20°$. The 5-day pool gave an agglutinin titre of 1:640 (1:20 after treatment with 2-mercaptoethanol). The hyperimmune pool titre was 1:5120 before and after treatment with 2-mercaptoethanol.

*Sensitization*

The term 'sensitization with erythrocytes' means intradermal injection of an emulsion of erythrocytes in saline and FCA. The erythrocyte concentration quoted is that in the saline suspension prior to emulsification.

Equal volumes of erythrocytes in saline and FCA were emulsified immediately before injection. The skin was shaved and a total of 0·5 ml of emulsion injected intradermally in three equal depots spaced at about 2·5-cm intervals along the nipple line.

*Challenge*

Via a 30-gauge needle, 0·1 ml of antigen was injected intradermally on the dorsal aspect of one hind paw.

*Assay of the delayed hypersensitivity response*

The method depended on the principle that an object immersed in a fluid displaces its own volume. If the object (of volume $V$ ml) has a lower specific gravity ($S_0$ g/ml) than that of the fluid ($S_f$ g/ml), the pressure ($P$ g) needed to achieve total immersion is given by the formula:

$$P = (S_f - S_0)\ V.$$

A Mettler K7 top-loading, single pan balance of 800-g capacity with an optical scale calibrated in 100-mg divisions was used. The reading for a small mercury-filled beaker was first obtained and then the additional deflection produced by immersing a rat's paw, to the level of a line drawn in the groove immediately distal to the lateral malleolus, was noted. Rats were anaesthetized and the hand immersing the paw rested on a firm bridge

just above the surface of the mercury. Occasionally the response was compared with a baseline reading obtained on the same paw just prior to challenge. However, as right and left hind paws of thirty untreated rats did not differ by more than 2 per cent in volume, the challenged paw was usually compared with the unchallenged contralateral hind paw.

The method clearly detected paw volume differences of 0·02 ml. The degree of paw swelling seen in delayed hypersensitivity reactions was probably limited by the degree of delayed hypersensitivity rather than by *pari-passu* increases of local tissue tension since potent non-specific inflammatory stimuli could, within 24 hours, provoke paw swellings of twice the maximum values obtained in the delayed hypersensitivity reactions. Results are given as the percentage increase of paw volume consequent to challenge; they are not essentially different if expressed as absolute volume increases, but recording percentages tended to diminish slight variations due to animals in different experiments not always being of similar sizes.

*Antibody assays*

Sera stored at $-20°$ were heated to 56° for 30 minutes prior to titration. Doubling dilutions in saline started at 1:10. To 0·5 ml of diluted serum 0·5 ml of 0·25 per cent SRBC was added, tubes shaken, left at room temperature for 2 hours, and placed in a cold-room at 4° overnight. For titres following 2-mercaptoethanol treatment, serum diluted 1:5 in saline was added to an equal volume of 0·1 M 2-mercaptoethanol, incubated at 37° for 30 minutes, and titrations then done as for total antibody. The titre was recorded as the number of the last tube showing a reaction of 1+ or more (Rowley and Fitch, 1964); if no tube showed such a reaction the titre was taken as 0.

## RESULTS

### ANTIGEN DOSES AND TIMES OF OBSERVATIONS

SRBC concentrations ranging from 0·4 to 40 per cent were tried for sensitization and challenge, and responses 5, 9, 10 and 15 days after sensitization were measured. In all cases the severest challenge response was between 8 and 11 days after sensitization. Delayed hypersensitivity responses for all doses of sensitizing antigen were similar but individual variability tended to increase when the sensitizing concentration was as low as 0·4 per cent. Serial measurements of individual paw responses showed that maximum paw swelling occurred between 20 and 24 hours after challenge. When the challenge concentration of SRBC was varied, responses to concentrations of 5 per cent or more were not detectably different though reducing the concentration to as little as 1 per cent gave diminished responses.

In consequence, the antigen concentrations selected were 7·5 per cent for challenge and, unless otherwise stated, 5 per cent for sensitization. Animals were challenged 9 days after sensitization and their responses measured 21 hours later. Injecting SRBC into paws of normal rats resulted in slight non-specific swelling 24 hours later. Each experiment, therefore, included non-sensitized animals which were challenged. The mean response of the appropriate challenge control group has been subtracted to arrive at the figures shown for all individual experiments, though not for the collated results given in Table 5.

### THE NATURE OF THE INFLAMMATORY RESPONSE PRODUCED BY CHALLENGE OF SENSITIZED RATS

*Timing*

When the paw response was followed by serial measurements during the first 42 hours after challenge no detectable difference between non-sensitized control and sensitized groups occurred for 10 hours; paws of the sensitized rats became maximally swollen 20–24 hours after challenge; by 42 hours the swelling was about 40 per cent of its peak value.

*Histology*

Reactions were characterized by intense mononuclear cell infiltrates and oedema; degranulation of mast cells seemed absent. Appearances were identical with those previously described for a delayed hypersensitivity response produced in the rat paw (Rowley, Chutkow and Attig, 1959).

*Suppression by total-body X-irradiation*

Eight hundred and fifty rad, 7 days after sensitization and 48 hours prior to challenge completely abolished the paw response.

*Transferability*

Serum from sensitized rats was obtained both early after sensitization and at the time of maximal delayed hypersensitivity. Twenty recipient rats each received 3 ml of serum by vein and 3 ml intraperitoneally. No recipient gave a positive paw response to challenge 48 hours later.

*Correlation between delayed and humoral responses*

No correlation between the intensity of the paw response and the agglutinin titre was found within any group of identically treated animals. Further, when very low doses of antigen were used for sensitization a maximal paw response was demonstrable prior to detectable circulating antibody.

Thus, although the paw responses elicited in the experiments which follow generally occurred in the presence of circulating antibody, the above findings are taken to indicate that such antibody does not mediate these responses, which are therefore regarded as delayed-type hypersensitivity reactions.

### PRIMARY HUMORAL AND DELAYED HYPERSENSITIVITY RESPONSES TO VARYING DOSES OF ANTIGEN IN ADJUVANT

Three groups of five rats were sensitized with 0·4 per cent, three groups with 4 per cent, and three groups with 40 per cent SRBC. For each sensitizing dose, one group was challenged 5, one 10 and one 15 days after sensitization. Blood for titres was taken immediately after reading challenge responses.

The results are shown in Fig. 1 in which the degree, development, and decline of hypersensitivity is closely similar for all doses of sensitizing antigen. The concurrently evoked humoral responses differ considerably from each other both in rate and extent. These findings illustrate dissociation of humoral and delayed hypersensitivity responses.

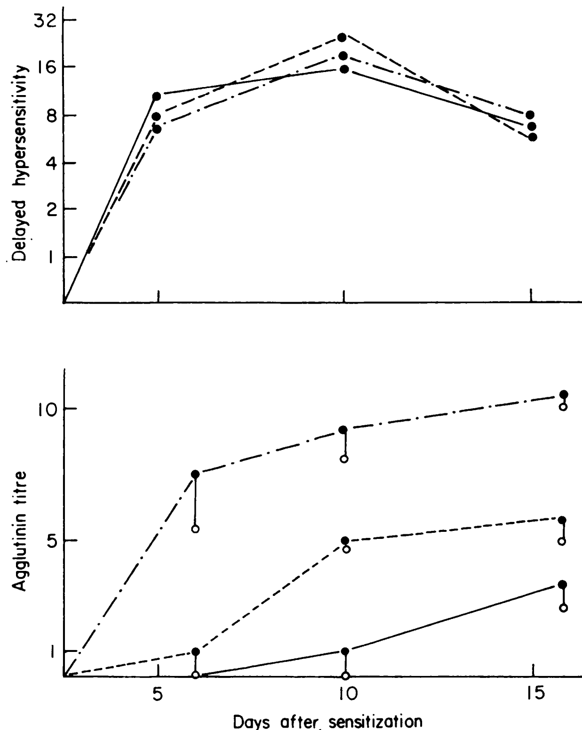


FIG. 1. Concurrent delayed hypersensitivity and humoral responses to three differing doses of SRBC (—, 0·4 per cent; – – –, 4 per cent; –·–·–, 40 per cent). Indicated doses refer to the sensitizing concentration of SRBC. All points indicate mean group responses, each group comprising five rats. Delayed hypersensitivity responses are shown as per cent paw volume increases 21 hours after challenge; these are scaled in doubling increments for comparison with the agglutinin scale which shows numbers of positive tubes in a double-dilution titration. On the agglutinin graph, solid circles (●) indicate titre before incubation of sera with 2-mercaptoethanol, and open circles (○) the titre after such treatment.

## EFFECT OF INTRAVENOUS ANTIGEN ON SENSITIZATION WITH ANTIGEN IN ADJUVANT

Suppression of adjuvant-dependent responses by antigen administered without adjuvant is well recognized (Asherson and Stone, 1965; Dvorak, Billote, McCarthy and Flax, 1965; Loewi, Holborow and Temple, 1966) and has been termed 'immune deviation' (Asherson and Stone, 1965). In the present system the effects of 1 ml of 5 per cent SRBC given intravenously prior to primary sensitization were tested. Table 1 shows the mean responses given by groups of five rats when such intravenous antigen was given 1, 4, 16 or 38 days prior to sensitization.

Intravenous antigen given 1, 16 or 38 days before sensitization partially inhibited development of delayed hypersensitivity; antigen given 4 days prior to sensitization was without effect. These findings were confirmed repeatedly, and suggested that the inhibition was due to two mechanisms; one ceasing to be operative a few days after intravenous antigen and the other only coming into play after the lapse of more than 4 days.

Suppression produced by antigen given 1 day prior to sensitization seemed specific. Intravenous injection of human erythrocytes inhibited the development of delayed hypersensitivity to human erythrocytes but did not affect the development of delayed hypersensitivity to SRBC. The details of this experiment are shown in Table 2.

Suppression produced by antigen 1 day before sensitization did not appear dependent on a humoral fàctor. Sera obtained 1, 3 and 5 days after intravenous SRBC were given by vein to seperate groups of rats; each animal received four 1-ml injections over a 3-day period commencing 12 hours prior to sensitization with SRBC in adjuvant. None of these sera affected the development of delayed hypersensitivity.

Table 1

Effect of intravenous antigen on the primary induction of delayed hypersensitivity

| Sensitization* | Intravenous immunization† | Interval (days) between i.v. immunization and subsequent sensitization | Individual delayed hypersensitivity responses (per cent)‡ | | | | | Group mean response (per cent) |
|---|---|---|---|---|---|---|---|---|
| + | 0 | — | 25 | 24 | 20 | 19 | 18 | 21 |
| + | + | 38 | 12 | 12 | 9 | 6 | 2 | 8 |
| + | + | 16 | 15 | 13 | 11 | 11 | 4 | 11 |
| + | + | 4 | 21 | 20 | 19 | 18 | 10 | 18 |
| + | + | 1 | 16 | 13 | 9 | 6 | 5 | 10 |

* 0·5 ml intradermally of 1:1 emulsion of 5 per cent SRBC in saline and Freund's complete adjuvant.
† 1 ml of 5 per cent SRBC in saline by vein.
‡ Per cent paw volume increase 21 hours after a challenge on the 9th post-sensitization day.

Thus the inhibitory effect of antigen given 1 day prior to sensitization appears due to a directly mediated and immunologically specific action of the injected antigen.

Table 2

Comparison of delayed hypersensitivity to human (HRBC) and sheep erythrocytes (SRBC): specificity of suppression with intravenous antigen

| Sensitizing antigen* | Intravenous antigen† | Individual delayed hypersensitivity responses‡ (per cent) | | | | | Group mean response (per cent) |
|---|---|---|---|---|---|---|---|
| SRBC | Nil | 24 | 22 | 20 | 19 | 19 | 21 |
| SRBC | HRBC | 28 | 25 | 24 | 22 | 9 | 22 |
| SRBC | SRBC | 13 | 10 | 9 | 8 | 5 | 9 |
| HRBC | Nil | 42 | 32 | 25 | 25 | 23 | 29 |
| HRBC | HRBC | 22 | 13 | 10 | 5 | 4 | 11 |

* Sensitizing antigen used at 5 per cent concentration, human erythrocytes were Group O Rh positive.
† Given intravenously as 1 ml of a 5 per cent suspension in saline, 24 hours prior to sensitization.
‡ Per cent increase in paw volume 21 hours after challenge with 0·1 ml of a 7·5 per cent erythrocyte suspension in saline. Challenges were given 9 days after sensitization. Individuals were challenged with the same species of erythrocytes as that which had been used for their sensitization.

### SUPPRESSION OF DELAYED HYPERSENSITIVITY BY ANTIBODY

Passive immunization with serum obtained early in the primary response to SRBC had not influenced the development of delayed hypersensitivity but the effect of serum obtained later after immunization remained to be tested. Five rats each received four 1-ml

injections of hyperimmune anti-SRBC serum, spaced over a period of 3 days, commencing 12 hours prior to sensitization with SRBC. Their delayed hypersensitivity responses were compared with those of a concurrently sensitized group which received no serum. The results, given in Table 3, show development of delayed hypersensitivity in the passively immunized group was partially inhibited. This was confirmed in other experiments of

TABLE 3

EFFECT OF PASSIVE IMMUNIZATION ON THE PRIMARY INDUCTION OF DELAYED HYPERSENSITIVITY TO SHEEP ERYTHROCYTES

| Sensitization | Passive immunization* | Individual delayed hypersensitivity responses (per cent)† | | | | | Group mean response (per cent) |
|---|---|---|---|---|---|---|---|
| + | 0 | 25 | 25 | 17 | 16 | 11 | 19 |
| + | + | 9 | 8 | 7 | 5 | 0 | 6 |

* Hyperimmune anti-SRBC serum given intravenously in 1-ml quantities at the following times: 12 hours prior to sensitization, 12, 36 and 60 hours after sensitization.

† Per cent increase in paw volume 21 hours after a challenge on the 9th post-sensitization day.

similar design. Adjuvant had been employed in raising the hyperimmune serum used, but passive immunization with serum obtained from rats which had received both primary and secondary immunizations of SRBC without adjuvant was also effective. Serum from rats immunized with an adjuvant–saline emulsion alone showed no inhibitory action. Thus suppression by passive immunization would appear specifically due to anti-SRBC antibody.

The antibody found effective in partially inhibiting the primary induction of delayed hypersensitivity occurred late in the response to SRBC immunization and was, by the criterion of 2-mercaptoethanol sensitivity, predominantly '7S'. Rats receiving intravenous antigen 16 or more days prior to primary sensitization all had produced, by the time of sensitization, SRBC agglutinins in a titre of 1:80 or more—agglutinins which by the above criterion were also predominantly '7S' (Table 1). Thus inhibition of delayed hypersensitivity in these animals was presumably mediated by antibody, in contrast to the group receiving SRBC by vein 1 day prior to sensitization which were suppressed by an immediate action of antigen. Neither this antigen action nor the antibody-mediated form of inhibition was demonstrable in the accompanying group of rats which received intravenous SRBC 4 days prior to sensitization.

### COMPLETE SUPPRESSION OF PRIMARY SENSITIZATION

Administration of antibody or of intravenous antigen only partially inhibited the primary induction of delayed hypersensitivity, but when both suppressive measures were used inhibition of delayed hypersensitivity proved complete, as shown in the following experiment.

Four groups of five rats were sensitized with SRBC. One group received intravenous SRBC 1 day prior to sensitization, and a total of four 1-ml intravenous injections of hyperimmune rat anti-SRBC serum over a period of 3 days, commencing 12 hours prior

TABLE 4

THE COMBINED SUPPRESSIVE EFFECT OF INTRAVENOUS ANTIGEN AND PASSIVE IMMUNIZATION ON THE PRIMARY INDUCTION OF DELAYED HYPERSENSITIVITY TO SHEEP ERYTHROCYTES

| Sensitization | Intravenous antigen* | Passive immunization† | Individual delayed hypersensitivity responses (per cent)‡ | | | | | Group mean response (per cent) |
|---|---|---|---|---|---|---|---|---|
| + | 0 | 0 | 31 | 27 | 21 | 19 | 15 | 23 |
| + | + | 0 | 12 | 8 | 7 | 5 | 3 | 7 |
| + | 0 | + | 17 | 14 | 12 | 5 | 5 | 11 |
| + | + | + | 1 | 1 | 0 | 0 | 0 | 0 |

* Given by vein as 1 ml of a 5 per cent suspension in saline 24 hours prior to sensitization.
† Hyperimmune anti-SRBC serum given by vein in 1-ml quantities at the following times: 12 hours prior to sensitization, 12, 36 and 60 hours after sensitization.
‡ Per cent increase in paw volume 21 hours after a challenge on the 9th post-sensitization day.

to sensitization. A second group received hyperimmune serum as above but no intravenous antigen, and a third group intravenous antigen but no serum. These three groups were compared with a fourth, which received neither intravenous antigen nor anti-SRBC serum.

The results, listed in Table 4, show that the *partial* inhibition obtainable by the use of either intravenous antigen or passive immunization becomes *complete* when intravenous antigen is followed by passive immunization.

TABLE 5

PRIMARY INDUCTION OF DELAYED HYPERSENSITIVITY TO SHEEP ERYTHROCYTES: COLLATED RESULTS

| Group | No. of animals | Mean response (per cent) | Standard deviation | Standard error of mean | Minimum individual response | Maximum individual response | Significant group difference from groups:* | No significant difference from groups:* |
|---|---|---|---|---|---|---|---|---|
| A: sensitized controls | 142 | 20·5 | 7·0 | 0·6 | 5 | 46 | B, D, E, G, H | C, F |
| B: i.v. antigen 1 day before sensitization | 51 | 8·3 | 4·4 | 0·6 | 0 | 18 | A, C, G, H | |
| C: i.v. antigen 4 days before sensitization | 20 | 16·2 | 7·0 | 1·5 | 2 | 26 | B | A |
| D: passive immunization with hyperimmune serum | 15 | 9·4 | 5·0 | 1·3 | 0 | 17 | A, G, H | |
| E: i.v. antigen 16 or more days before sensitization | 21 | 9·4 | 4·4 | 1·0 | 2 | 19 | A, G, H | |
| F: passive immunization with 3- or 5-day primary immunization serum | 10 | 18·2 | 6·1 | 1·9 | 8 | 27 | | A |
| G: 'combined suppression' | 27 | 2·0 | 2·2 | 0·4 | −1 | 7 | A, B, D, E | H |
| H: challenge controls | 92 | 2·0 | 2·3 | 0·2 | −2 | 9 | A, B, D, E | G |

* One-way analysis of variance with differences between groups investigated by 99 per cent confidence bounds computed according to the Scheffe method of multiple comparisons. Analysis was performed on a square-root transformation of data, which served to stabilize variances sufficiently for valid use of the Scheffe method.

These results were substantiated by further experiments, the collated results of which are included in Table 5, where the data obtained in relation to the primary induction of delayed hypersensitivity to SRBC is summarized.

### COMPARISON OF THE PRIMARY AND SECONDARY INDUCTION OF DELAYED HYPERSENSITIVITY

Preceding experiments have dealt with primary induction of delayed hypersensitivity. In the following experiment the primary response is compared with the secondary.

Four groups of five rats were used. The first group was challenged 5, the second 9, the third 15 and the fourth 35 days after primary sensitization. At this latter time the first three groups received a secondary sensitization; the first group was re-challenged 5, the second 9 and the third 15 days later. Administration of multiple challenges to a single group was avoided, as evocation of a delayed hypersensitivity response temporarily diminishes the response obtainable to a subsequent challenge. Blood for agglutinin titres was taken from each group after reading the paw responses of that group, and from all groups on the 35th day following primary sensitization.

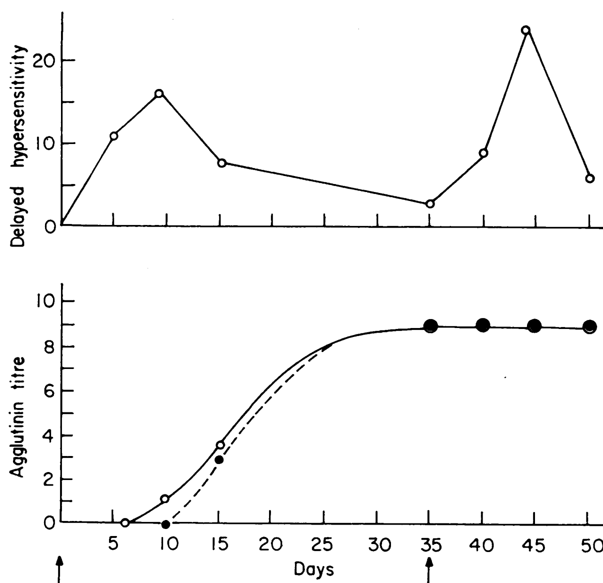


FIG. 2. Concurrent delayed hypersensitivity and humoral responses following primary and secondary sensitization with SRBC. Times of sensitization are indicated by the vertical arrows ( ↑ ). All points represent mean group responses, each group comprising five rats. The concentration of antigen used for sensitization was 0·4 per cent. Delayed hypersensitivity response is plotted as the per cent paw volume increase 21 hours after challenge. The agglutinin titre obtained by double-dilution titrations is shown both before (○) and after incubation of the sera with 2-mercaptoethanol (●).

The results are plotted as group mean values in Fig. 2. In experiments which employed higher concentrations of SRBC for sensitization but were otherwise of identical design, the initial humoral response was more vigorous but the pattern of the delayed hypersensitivity responses essentially the same.

These results show that: (1) following primary sensitization the level of delayed hypersensitivity reaches a peak in about 9 days and then rapidly declines; and (2) secondary

sensitization results in a full and rapid reappearance of the delayed hypersensitive state despite the presence of high titres of circulating antibody, whereas such antibody has previously been shown to suppress the *primary* induction of delayed hypersensitivity.

## 'SUSTAINED' SUPPRESSION OF DELAYED HYPERSENSITIVITY

Rats whose response to primary sensitization had been completely suppressed, by the combined action of intravenous antigen and anti-SRBC antibody, gave a partial response to a second sensitization 4 weeks later. These partial responders had circulating, at the time of their second sensitization, moderate titres of 2-mercaptoethanol-resistant anti-SRBC agglutinins. This suggested that initially completely suppressed rats eventually become capable of a response which is in fact a primary response and as such partially inhibitable by antibody. To ascertain the period for which sensitization in 'combined suppressed' animals is unable to evoke any delayed hypersensitive response, and the feasibility of extending this period, the following experiment was performed.

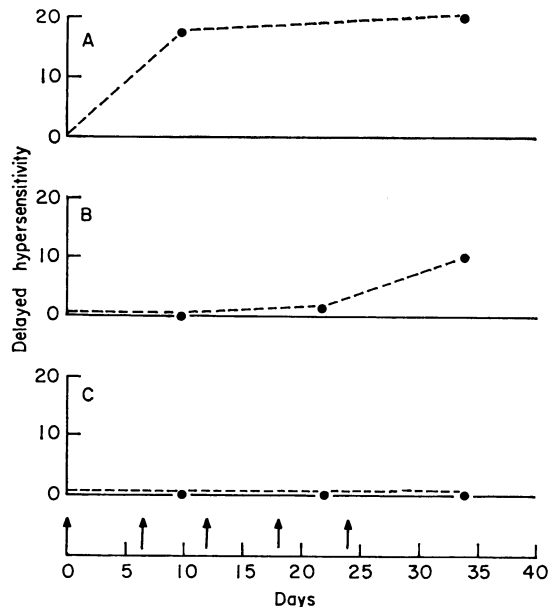


FIG. 3. Complete suppression of delayed hypersensitivity: duration of unresponsiveness. Points show group mean responses as per cent paw volume increase 21 hours after challenge, each group comprising six rats. Sensitization (↑) with 5 per cent SRBC in FCA to all groups (A, B and C) at 6-day intervals. 'A' are sensitized controls; 'B' received 'combined suppression' (as detailed in Table 4) with the initial sensitization only; 'C' in addition received 1 ml 5 per cent i.v. SRBC 24 hours before each subsequent sensitization.

Four groups of six rats were used. All groups were sensitized repeatedly, at 6-day intervals. It had previously been established that rats receiving repeated sensitizations at 6-day intervals maintained a maximum level of delayed hypersensitivity rather than evidencing the decline seen after a single sensitization. One group ('B' in Fig. 3) received 'combined suppression' in association with the initial sensitization only; another ('C') in

addition to such 'combined suppression' received a further 1 ml of 5 per cent SRBC by vein 24 hours prior to each subsequent sensitization; the remaining two groups served as sensitized controls—the one being challenged 9 days after the first sensitization and the other at the end of the experiment—these groups are collectively shown as group 'A' in Fig. 3.

Fig. 3 shows paw responses during the experiment. The 'combined suppression' initially given to group B rendered these rats totally unresponsive to attempted induction of delayed hypersensitivity for only about 3 weeks. Continued complete suppression is achieved in group C where, in addition to the presence of circulating antibody at the time of each sensitization, there has been exposure to intravenous antigen prior to *each* sensitization.

## DISCUSSION

The relationship of delayed hypersensitivity to humoral antibody formation remains speculative. Differing doses of SRBC in adjuvant evoked similar degrees and similar rates of development and decline of the delayed hypersensitive state, with concurrent humoral responses that were considerably different for each dose of antigen employed. In fact whenever agglutinin and paw responses to sensitization were considered in a group of identically treated animals, individual variation of these two responses showed no correlation. Such a lack of correlation was also found in a series of undescribed experiments in which a wide range of SRBC doses without adjuvant resulted in a variety of humoral responses but a uniform failure to produce detectable delayed hypersensitivity—regardless of the immunizing route employed. Such observations suggest humoral and delayed hypersensitivity responses are independently induced. They do not, however, necessitate that delayed hypersensitivity results from stimulation of an immunocompetent cell which initiates such reactions and was originally possessed of no other immunological potential. The delayed hypersensitive state could be determined by a special form of induction resulting in a cell, otherwise capable of giving rise to a humoral antibody-forming cell, undergoing an alternative pathway of differentiation. Such alternative pathways, entailing differences in the requirements both for their realization and the eventual expression of the form of immunity they generate, might be expected to result in delayed hypersensitivity and humoral antibody formation appearing as largely independent responses. If, moreover, both responses depended on a common precursor cell, similarities of behaviour should also be evident.

If a common precursor cell determined both delayed hypersensitive and humoral responses, its immediate alternative to the delayed hypersensitivity pathway might be IgM synthesis. There are at least four reasons for suggesting this.

(1) In the phylogenetic development of immunity, acquisition of humoral and cellular forms appear to coincide; however, many lower vertebrates, which can be shown to develop cellular immunity (Papermaster, Condie, Finstad and Good, 1964), produce only, or predominantly, macroglobulin antibody (Pollara, Finstad and Good, 1966).

(2) Ontogenetically, there is evidence that the ability to develop cellular and IgM responses antedates that for other immunoglobulin responses to immunization (Miller, 1966).

(3) Chronologically, following immunization both delayed hypersensitivity and IgM responses tend to appear before evocation of other specific immunoglobulins is evident

(Pappenheimer, Scharff and Uhr, 1959; Uhr and Finkelstein, 1963; Nossal, Szenberg, Ada and Austin, 1964; Wortis, Taylor and Dresser, 1966).

(4) In bursectomized fowl showing severely impaired immunoglobulin synthesis, there is not a proportionally severe diminution of either the ability for delayed hypersensitivity responses or of IgM levels (Cooper, Peterson, South and Good, 1966; Cooper, Peterson and Good, 1965).

Thus, in seeking similarities between the induction of delayed hypersensitive and humoral responses, it might be most pertinent to consider on the humoral side the induction of IgM synthesis. The direct Jerne plaque technique (Jerne and Nordin, 1963) provides an extremely sensitive method of assessing at a cellular level what is predominantly an IgM response to immunization with SRBC. The immunocompetent precursor cells responsible for the primary humoral response to SRBC in the rat have been termed 'potential antibody-forming cells' (Rowley and Fitch, 1964), and the primary and secondary humoral responses to SRBC (Rowley and Fitch, 1964, 1968)—as assessed by the direct Jerne plaque technique—provide some interesting similarities to the presently reported findings regarding the primary and secondary delayed hypersensitivity response to SRBC in the rat. Thus: (1) Both delayed hypersensitive and humoral (plaque-forming cell) primary responses reach a peak in a few days and then rapidly decline. (2) Both types of primary responses can be inhibited by passive immunization; complete suppression of the primary plaque-forming cell response can be obtained by this measure (Rowley, unpublished data). However, induction of delayed hypersensitivity entailed presentation of antigen with adjuvant and when the primary plaque-forming cell response to antigen plus adjuvant was studied the inhibition obtainable with passive immunization was also only partial (Rowley, Fitch and Solliday, 1967). (3) For both delayed hypersensitivity and humoral antibody formation a secondary response is demonstrable. In both cases this reaches a peak in a few days and then rapidly declines. (4) Induction of neither the secondary delayed hypersensitivity nor the secondary plaque-forming cell response is readily inhibitable by circulating antibody.

Another aspect of delayed hypersensitivity to SRBC might also be interpreted as consistent with a potential antibody-forming cell giving rise to the 'delayed hypersensitivity cell', namely the suppressive effect of 'free antigen' (i.e. antigen without adjuvant) on induction of delayed hypersensitivity with antigen in adjuvant. This has been termed 'immune deviation' (Asherson and Stone, 1965). In the present study on sheep erythrocytes the 'deviating' action of free antigen given 1 day prior to sensitization would appear direct and immunologically specific. Although alternative mechanisms might account for such 'deviation', the phenomenon presently seems compatible with the hypothesis that humoral and cellular immunity to SRBC are dependent on the same precursor cell, and that immunization with SRBC in the absence of adjuvant results in commitment of such a precursor cell to a pathway leading to humoral antibody formation. If following such commitment, restoration of the small initially uncommitted population was consequent to the cessation of the direct plaque-forming cell proliferative response, it may be significant that SRBC in the dose here used for 'deviation' evoke a primary plaque-forming cell response which begins to decline following the fourth post-immunization day—the time when the suppressive effect of such intravenous immunization on the primary induction of delayed hypersensitivity to SRBC disappears.

In view of the foregoing it is suggested that the rat's immune response to sheep erythrocytes, as evidenced by the development of delayed hypersensitivity and direct haemolytic

plaque-forming cells, might be dependent on a common immunocompetent precursor cell—a potential antibody-forming cell with the capacity for differentiating along alternative pathways: the one resulting in delayed hypersensitivity and the other in humoral antibody formation.

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