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The materno-foetal transfer of carrier protein sensitivity in the mouse

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Summary. The anti-dinitrophenol (DNP) antibody response was examined in the 18-week-old offspring of female mice primed to bovine serum albumin (BSA) and in control unprimed animals. Comparisons were made of the response to DNP–BSA, DNP–chicken gamma-globulin (CGG) and DNP–purified protein derivative (PPD) after all offspring had been primed with DNP–CGG at 12 weeks of age. An enhanced anti-DNP antibody response was found to DNP-BSA in mice whose mothers had received BSA. It is suggested that this phenomenon demonstrates the transfer of carrier protein sensitivity from mother to foetus.

INTRODUCTION

A critical examination of the evidence for the transfer of cells from mother to foetus during pregnancy suggests that the balance lies in favour of such an occurrence, at least in some species, such as man and the mouse (Stern, 1975). Attempts to assess whether allergic priming is transferred from mother to foetus (Gill, Kunz & Bernard, 1971; Field and Caspary, 1971) have given results which are open to varying interpretations, either because of technical difficulties in repeating experiments, or because of alternative explanations of the results.

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The hapten–carrier system provides a means of dissecting the immune response in terms of subpopulations of lymphocytes able to react either with carrier protein or with hapten; together able to mount an anti-hapten antibody response by means of cell co-operation (Mitchison, 1971).

This experiment was designed to explore the possibility that a transfer of T-cell help from mother to foetus might occur in the mouse.

MATERIALS AND METHODS

(1) *Experimental design*

The mice used in this study were from an inbred CBA strain. The structure of the study is illustrated diagrammatically in Fig. 1. Test dams were immunized intraperitoneally (i.p.) with 200 μg alum-precipitated BSA, given with 1×10^8 *Bordetella pertussis* organisms (Burroughs-Wellcome Ltd.) These animals were age-matched with unimmunized controls. Three months later, both groups of animals were mated with syngeneic CBA males, the offspring being segregated into test and control groups.

At 3 months of age, all the animals in both groups of offspring were injected with 200 μg alum-precipitated DNP–CGG with 1×10^8 *B. pertussis* organisms i.p. Some excess control animals were removed and given a second challenge with DNP–CGG to check its antigenicity and produced satisfactory anti-DNP antibody responses. Six weeks later, test and control

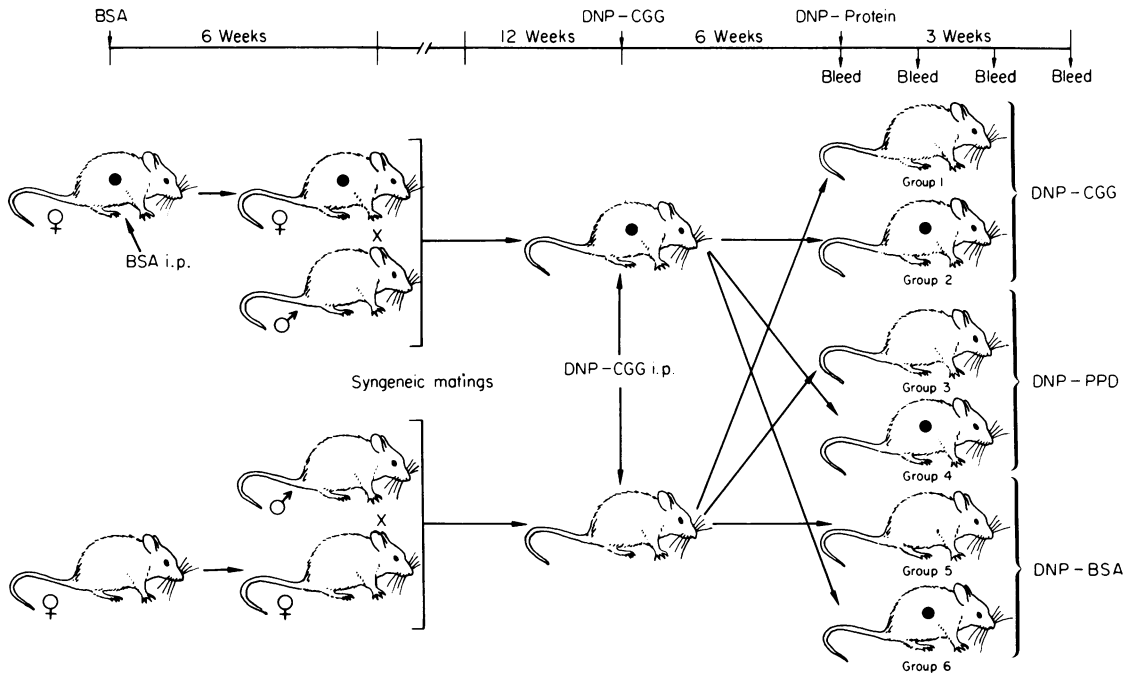

Figure 1. Experimental design. Mice marked with black spots are those from test groups.

offspring were divided into three pairs of groups and each pair of groups challenged with a different DNP–protein conjugate: 1 and 2 with DNP–CGG; 3 and 4 with DNP–PPD and 5 and 6 with DNP–BSA. All the animals were bled before the final immunization and on the 6th, 15th and 22nd days after immunization. All the sera were titrated together at the end of the experiment. Ten animals died during the course of the experiment as a result of anaesthesia.

(2) *Antigens*

(i) *BSA* (Armour Pharmaceutical Company Limited), twice recrystallized, was used for the initial maternal priming and for conjugation in the preparation of DNP–BSA described below.

(ii) *DNP conjugates* were made to three protein carriers (Cross, personal communication), namely BSA, PPD and CGG. PPD was obtained from the Ministry of Agriculture, Fish and Food Central Veterinary Laboratory, Weybridge, Surrey. CGG was prepared by four successive precipitations of normal chicken serum with half-saturated ammonium sulphate solution and repeated dialysis against water. PPD was dialysed against three changes of water to remove low molecular weight fragments; and BSA was dissolved in water. DNP conjugation was carried out using a standard fluorodinitrobenzene method: the DNP:protein ratios aimed at being 10:1 for CGG and BSA and 1:1 for PPD. The resultant DNP: protein ratios were calculated spectrophotometrically and shown to be about equal to the values above. Each conjugate was assayed for its ability to generate an anti-DNP antibody response in control mice and proved satisfactory in this respect, although a larger dose of DNP–BSA was needed compared with the other conjugates.

(3) *Anti-DNP assay system*

(i) Preparation of DNP–$F(ab')_2$ anti-ox erythrocyte antibody.

Sheep, immunized with 10 per cent ox red blood cells in Freund's complete adjuvant and boosted 5 weeks later by a further injection of the same cells in Freund's incomplete adjuvant (Difco), were bled out and the serum collected. This antiserum gave a titre of 1:2048 against 0·5 per cent ox erythrocytes. Peptic digestion to yield a $F(ab')_2$ antiserum (Nisonoff, Wissler, Lipman and Woernley, 1960) was carried out as follows. A 'slow' IgG fraction was obtained by DEAE fractionation and this was

digested with pepsin in 0·2 M glycine–HCl, pH 3·2. After neutralization, a protein was precipitated by half-saturated ammonium sulphate solution which upon immunoelectrophoresis (IEP) and SDS gel analysis proved to be a homogeneous $F(ab')_2$ compound. DNP conjugation of this material was carried out as in 2, ii above, which produced DNP_{13}–$F(ab')_2$ sheep IgG. At 2 mg/ml, this preparation agglutinated 0·5 per cent ox erythrocytes to a dilution of 1 : 32, but the addition of rabbit anti-sheep IgG increased the titre to 1 : 512. Treatment of the cells from this last titration with 1 : 20 guinea-pig complement further raised the titre to 1 : 2048 of the DNP–$F(ab')_2$ sheep IgG.

(ii) *Assay of anti-DNP antibody.* Two per cent ox erythrocytes in phosphate-buffered saline, pH 7·2 (PBS) were sensitized by the addition of a 1 : 81 dilution of DNP_{13}-$F(ab')_2$ anti-ox erythrocyte sheep IgG for 30 min at 37°C. Fifty microlitres of 0·5% sensitized ox erythrocytes were added to 50 μl of the serum under test and allowed to sediment at room temperature for 2 h before reading the agglutination titre. The cell pellet was then resuspended and one drop of a 1 : 20 dilution of guinea-pig complement was added. The mixture was then incubated at 37°C for 2 h before reading the lytic titre (complement-mediated). A standard CBA mouse anti-DNP antiserum gave consistent agglutination titres of 1 : 729 and lytic titres of 1 : 2178 by this method. No agglutination or lysis was seen with normal mouse serum.

(4) *Analysis of variance*

In order to test the significance of differences between groups of animals it was necessary to assess the data by analysis of variance. The Biomedical Data program, BMD10V, was chosen, written by P. Sampson, of the Health Sciences Computing Facility, University College of Los Angeles (Kempthorne, 1961). The data were analysed as a two-way analysis of variance with unequal replication. The analysis of variance table generated was used to obtain a probability (P) value by reference to standard tables (Pearson and Hartley, 1972). The data were submitted for computation essentially in the form laid out in Table 1, but substituting the actual replicated values of anti-DNP antibody titre for each animal in place of the cell means. The data were $\log_3$ transgenerated before computation. The program was run on the IBM 7600 computer as part of the University of London Computing Centre Library. Further statistical details are available from the author.

RESULTS

Table 1 and Fig. 2 show the mean $\log_3$ reciprocal anti-DNP agglutination titres for the six groups of mice at the four times of assay, numerically and dynamically respectively. Fig. 3 shows the equivalent data for the complement mediated lytic anti-DNP titres. $\log_3$ Transformation of reciprocal titres was employed because the dilutions of antiserum employed in successive tubes were three-fold.

A comparison between paired test and control groups was made using an unpaired 'Student's' t-test (Gosset, 1908). This showed that only groups 5 and 6 differed significantly from each other, on days 6, 15 and 21 of the experiment, at $P < 0{\cdot}02$.

Comparisons of the anti-DNP antibody production between all groups of mice were made by analysis of variance (see Materials and Methods section 4). Table 3 displays the variance data for agglutination and lysis and the derived probability values.

Table 1. Mean $\log_3$ reciprocal anti-DNP agglutination titres of mice

Day	Group 1* (DNP–CGG)†	Group 2 (DNP–CGG)†	Group 3* (DNP–PPD)†	Group 4 (DNP–PPD)†	Group 5* (DNP–BSA)†	Group 6 (DNP–BSA)†
0	0·286(7)‡	0·375(8)	0·143(7)	0·250(8)	0·286(7)	0·125(8)
6	0·857(7)	1·000(8)	0·429(7)	0·250(8)	0·286(7)	1·000(8)
15	3·833(6)	4·125(8)	1·667(6)	1·125(8)	1·286(7)	3·500(8)
22	2·750(4)	3·000(6)	2·600(5)	2·167(6)	1·833(6)	3·250(8)

* The offspring of dams not immunized against BSA.
† DNP–protein challenge given to each group finally.
‡ The numbers of animals tested at each stage.

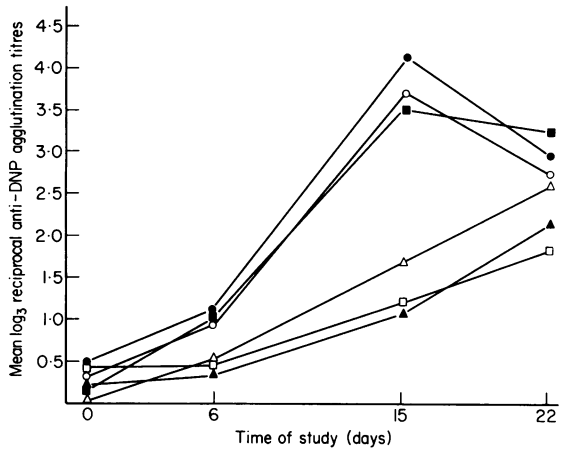

Figure 2. Dynamic anti-DNP antibody response of different mouse groups measured by agglutination of sensitized ox erythrocytes. Solid symbols show groups from BSA-primed dams. Open symbols show groups from control dams. (○) DNP–CGG challenge; (△) DNP–PPD challenge; (□) DNP–BSA challenge.

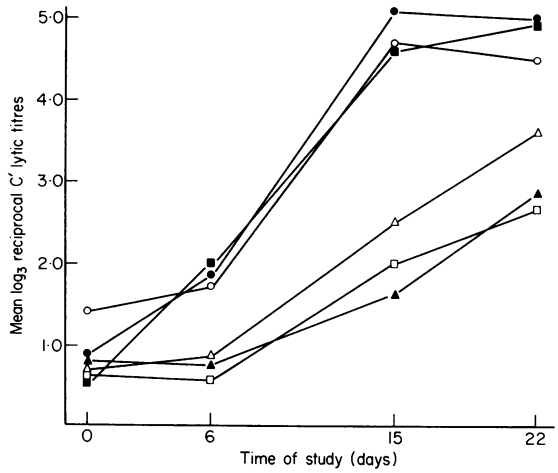

Figure 3. Dynamic anti-DNP antibody response of different mouse groups measured by complement-mediated lysis of sensitized ox erythrocytes. Solid symbols show groups from BSA-primed dams. Open symbols show groups from control dams. (○) DNP–CGG challenge. (△) DNP–PPD challenge. (□) DNP–BSA challenge.

Table 2. Variance analysis of the agglutination data shown in Table 1 and the complement-mediated lysis displayed in Fig. 3

Hypothesis tested*	Degrees of freedom	Frequency value		Probability (P)	
		Agglutination	Lysis	Agglutination	Lysis
1	1	820·038	977·645	<0·001	<0·001
2	5	18·667	20·317	<0·001	<0·001
3	3	141·155	102·948	<0·001	<0·001
4	15	5·711	3·372	<0·001	<0·001
5	1	85·799	93·739	<0·001	<0·001
6	1	0·153	0·053	n.s.	n.s.
7	1	34·670	39·707	<0·001	<0·001
8	1	66·483	25·254	<0·001	<0·001
9	1	2·703	0·111	<0·100 n.s.	n.s.
Error	144	—	—	—	—

n.s. = Not significant.

* Hypotheses tested: (1) tests the overall mean; (2) tests the comparison between all mouse groups; (3) compares differences at varying assay times; (4) tests interactions between (2) and (3); (5) compares mouse groups 1, 2 and 6 with groups 3, 4 and 5; (6) compares mouse group 6 with groups 1 and 2; (7) compares mouse group 6 with groups 3 and 4; (8) compares mouse groups 1, 2 and 6 with groups 3, 4 and 5 on day 15 of the response; and (9) makes the same comparison on the 22nd day of the response.

DISCUSSION

Table 1 shows that animals immunized with different DNP–protein conjugates (as shown in Fig. 1) all showed some rise in anti-DNP antibody production. However, mouse groups 1, 2 and 6 show a different dynamic change in their antibody response from groups 3, 4 and 5 by both assay systems, and this is illustrated by Figs 2 and 3. Groups 1, 2 and 6 show a secondary type of antibody response, whereas groups 3, 4 and 5 show a primary response. The comparison between groups by unpaired *t*-test shows that only one pair of groups, 5 and 6, differed significantly from each other over the course of the experiment.

Analysis of variance was used to test hypothetical differences between groups, and the results of this analysis are summarized in Table 3. This shows that group 6 behaved like groups 1 and 2 and not like groups 3, 4 and 5 and that this highly significant difference was most marked on day 15 of the response.

The conclusion which one is persuaded to draw from these experiments is that priming mothers with BSA leads to an enhanced ability on the part of their offspring to recognize BSA and to express this recognition as a secondary response to the hapten, DNP, when it is presented on BSA as a carrier protein.

There is little experimental data on the transfer of antigen sensitization from mother to foetus. Work by Gill and his colleagues (1971) suggested that such a phenomenon might occur, but ascribed the effect to the passage of complexed antigen to the foetus on the basis of the transfer of ^{125}I label which had been linked to the antigen. Unfortunately, they do not comment on the possibility that radiolabel released on the breakdown of antigen is likely to concentrate in the conceptus by virtue of the known high foetal avidity for iodine (Pickering and Kontaxis, 1961). Studies on human tuberculin sensitivity (Field and Caspary, 1971) show the materno-foetal transfer of PPD sensitivity, but merit confirmation.

Recently, Kindred and Roelants (1974) showed a restricted clonality of the response to DNP of the offspring of TNP- and DNP-primed dams, using bovine gamma-globulin as a carrier, compared to the responses of offspring born before maternal immunization. They felt that the responses might reflect the result of the induction of tolerance *in utero* to DNP, followed by a later response to antigen mediated by a few clones of DNP-responsive lymphocytes which had broken the tolerant state. They did not comment on the role of carrier protein sensitivity in their model.

There are several mechanisms which could explain these apparently conflicting data. It is possible that some BSA crossed into the foetus during pregnancy and was able to alter the foetal response in some way. However, to fit all the experimental results, BSA would need to have induced T-cell priming as well as B-cell tolerance, which, in the Kindred and Roelants experiments, would have been induced both to DNP and bovine gamma-globulin. Such a situation could lead to the observed results, but there is not yet sufficient data on this point. In view of the relatively long period between maternal priming and mating, antigen passage does not seem very likely.

Some maternal anti-BSA antibody probably crossed to the foetus in this experiment. It may be that such small amounts of antibody could enhance the DNP response by localizing on macrophages and dendritic cells and encouraging the early concentration of DNP–BSA. This mechanism is speculative and unlikely to operate here, since the amounts of antibody will be minute, albeit of relatively high avidity.

Finally, the foetus may have received immunocompetent carrier-primed cells or a transfer factor-like substance from the mother. There is disagreement among workers as to whether cells cross to the foetus during pregnancy. Work in mice (Tuffrey, Bishun and Barnes, 1969), using the T6 chromosome marker, has shown that materno-foetal lymphocyte traffic can occur, but it has proved difficult to repeat this work. The experimental system described here is syngeneic and so such cell co-operation could function adequately. Although Kindred and Roelants (1974) were unable to trace identical clones of anti-DNP antibody in their dams and post-immunization offspring, they remark that the identification of such clones was difficult. The possibility that their results could be explained by the transfer of a response involving clones of anti-DNP-responsive cells or their molecular equivalents cannot be ruled out. If such cells or molecules had crossed to the foetus in *this* experiment, one would expect to find an enhanced anti-DNP response to DNP–BSA, as was the case.

While it is not possible to differentiate between the role of antigen transfer or the passage of primed cells as the likeliest agent of these phenomena, further experiments are under way to attempt to unravel the immunological mechanisms involved.

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