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Immune Complexes in the Spleen

THE DIFFERENCE BETWEEN COMPETITIVE INHIBITION OF IMMUNE COMPLEX TRAPPING IN SPLEEN FOLLICLES AND INHIBITION BY PARATYPHOID VACCINE

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Summary. Paratyphoid vaccine injected between 4 days and 3 hours before injection of labelled immune complexes (^{125}I-labelled BGG–anti-BGG), inhibits follicular trapping of these complexes in the mouse spleen. Inhibition is maximal when paratyphoid vaccine is given 1 day before, almost no label being found in the spleen follicles.

No inhibition of follicular trapping of the complexes occurred when paratyphoid vaccine was injected simultaneously with the labelled immune complexes. Competitive inhibition was found when unlabelled immune complexes were given together with labelled immune complexes. Simultaneous injection of mice with paratyphoid vaccine and labelled immune complexes resulted in an additional form of localization of the labelled immune complexes in the white pulp, heavily labelled clumps also appearing in the periarteriolar lymphocyte sheaths and follicles. The results are discussed in relation to the mechanism of immune complex trapping in spleen follicles.

INTRODUCTION

Earlier studies have established that as soon as both antigen and specific antibody are present in the circulation, antigen–antibody complexes begin to concentrate in the periphery of the follicles in the spleen (van Rooijen, 1972a). Subsequently, complexes move from here towards the follicle centres.

Evidence has been presented that this movement is mediated by lymphoid cells (Brown, Harris, Papamichael, Sljivic and Holborow, 1973; van Rooijen, 1973a), and that these cells transfer the complexes (van Rooijen, 1974) to dendritic reticulum cells in the follicle centres (Hanna and Szakal, 1968; Nossal, Abbot, Mitchell and Lummus, 1968).

Neither lymphoid cells nor dendritic reticulum cells are monospecific for immune complexes formed by one particular antigen (van Rooijen, 1973b), since Fc portions of the immune complexes seem to be responsible for their binding to the cells (Herd and Ada, 1969). Complement may play a role in the follicular trapping of immune complexes (Dukor, Bianco and Nussenzweig, 1970; Gajl-Peczalska, Fish, Meuwissen, Frommel and Good, 1969).

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Recently we found that immune complex trapping in spleen follicles was severely inhibited or delayed when injection of labelled immune complexes was preceded by injection of paratyphoid vaccine given 22 and 6 hours before injection with the complexes and the spleen was taken 2 hours afterwards (van Rooijen, 1973a).

In such spleens the marginal zone which surrounds the spleen follicles is depleted of lymphoid cells (Pettersen, Borgen and Graupner, 1967; Abe and Ito, 1972). These lymphoid cells have entered the follicles (Pettersen *et al.*, 1967; Veerman, 1974), so that when immune complexes arrive (van Rooijen, 1972b) in the marginal zone no lymphoid cells are present to mediate transport towards the follicle centres.

It was the purpose of the present investigation to study whether paratyphoid vaccine really suppresses follicular trapping of injected immune complexes or only delays the trapping process. Suppression would be expected if complexes have been removed from the serum by other mechanisms (e.g. phagocytosis) by the time the follicular trapping mechanism has recovered.

The influence of a single injection of paratyphoid vaccine on the trapping of immune complexes in mouse spleen was studied when it was given before, simultaneously with or after the administration of the immune complexes. This was done in order to find out what interval between injections gives the most complete inhibition of follicular trapping.

Finally, competitive inhibition of immune complex trapping in spleen follicles was studied and compared with inhibition by paratyphoid vaccine.

MATERIALS AND METHODS

Animals

Young male Swiss mice were used for all experiments. They were fed a pellet diet and had water *ad libitum.*

Antigens

The following antigens were used: paratyphoid vaccine (from the 'Rijksinstituut voor de Volksgezondheid', Utrecht, The Netherlands); bovine gammaglobulin (BGG from Pentex, Kankakee, Illinois); ^{125}I-labelled BGG. BGG was labelled with ^{125}I (IMS-30 from the Radiochemical Centre, Amersham, Bucks.) using the chloramine-T method.

Antiserum

Rabbit anti-BGG serum was prepared in our laboratory. Its anti-BGG titre was 2^{15} according to the haemagglutination technique (Stavitsky, 1954).

Histological procedures and autoradiography

For autoradiography spleens were fixed in a mixture of alcohol and acetic acid (3:1) and embedded in paraffin. Sections of 5 μm thickness were made. Sections were prepared for autoradiography using Kodak AR 10 stripping film. After an exposure time of 6 weeks the autoradiographs were developed, fixed and stained with methyl green and pyronin.

Experimental design

Four experiments were performed in which all injections were given intravenously.

Experiment 1. Eighteen mice were injected with paratyphoid vaccine (0·2 ml, 10^9 formol-killed organisms in saline). Sixteen hours later they received a similar injection. Again

6 hours later they received an injection with 0·01 mg of ^{125}I-labelled BGG (0·15 mCi) together with 0·015 ml of the rabbit anti-BGG serum. At each of the following intervals after the last injection (with labelled immune complexes) two mice were killed and their spleens were excised at 2 hours, 4 hours, 8 hours, 16 hours, 1 day, 1·5 days, 2 days, 3 days and 5 days.

Experiment 2*a*. Fourteen mice were injected with 0·01 mg of ^{125}I-labelled BGG (0·15 mCi) together with 0·015 ml of the rabbit anti-BGG serum. Eight days, 4 days, 2 days, 1 day, 12 hours, 6 hours and 3 hours respectively before this injection pairs of these mice had been injected with paratyphoid vaccine (0·2 ml, 10^9 formol-killed organisms in saline). All mice were killed and their spleens were excised 2 hours after injection of the labelled immune complexes.

Experiment 2*b*. Ten mice were injected with 0·01 mg of ^{125}I-labelled BGG (0·185 mCi) together with 0·05 ml of the rabbit anti-BGG serum. Pairs of these mice received paratyphoid vaccine (0·15 ml, $7{\cdot}5 \times 10^8$ formol-killed organisms in saline), 1 hour before, 0·5 hours before, simultaneously with, 0·5 hours after or 1 hour after injection of the labelled immune complexes. All mice were killed and their spleens were excised 2 hours after injection of the complexes.

Experiment 3. Ten mice were injected with 0·01 mg of ^{125}I-labelled BGG (0·185 mCi) together with 0·0025 ml of the rabbit anti-BGG serum. Two of the mice received at the same time 0·02 mg of unlabelled BGG and 0·005 ml serum, two received 0·09 mg of unlabelled BGG and 0·0225 ml of serum, two received 0·29 mg of unlabelled BGG and 0·0725 ml of serum and two mice received 0·99 mg of unlabelled BGG and 0·2475 ml of serum. Both the total volume (0·3 ml) and the total radioactivity (0·185 mCi) administered were the same for all mice. Two days after injection all mice were killed and their spleens were excised.

RESULTS

DURATION OF THE EFFECT OF PARATYPHOID VACCINE ON IMMUNE COMPLEX TRAPPING IN SPLEEN FOLLICLES

When the ^{125}I-labelled BGG–anti-BGG injection was preceded by injections of paratyphoid vaccine 22 and 6 hours before, and spleens were excised at different intervals after giving the labelled immune complexes (experiment 1), the following results were obtained.

In seven mice no trapping of the label in the follicles was seen. In seven other mice some of the follicles were very weakly labelled. In three mice weakly labelled follicles were found and in only one mouse a moderate trapping of label in the follicles had occurred (Table 1). There was no relation between the degree of labelling of the follicles and the time interval after the ^{125}I-labelled BGG–anti-BGG injection. The sites where label was found were initially in more peripheral and later in more central parts of the follicles. This is in agreement with earlier work (van Rooijen, 1972a).

Many clumps of heavy label were found in the red pulp. At 2 and 4 hours after the ^{125}I-labelled BGG–anti-BGG injection these were mainly arranged in a narrow rim around the white pulp, especially around the follicles and to a lesser extent in the rest of the red pulp. The rim of clumps was less sharply defined at later intervals, leaving clumps distributed randomly in the red pulp. Apart from changes in their arrangement the clumps in the red pulp gradually decreased in number and in intensity of labelling with time after

TABLE 1

DEGREE OF LABELLING OF SPLENIC FOLLICLES IN PAIRS OF MICE IN THE DIFFERENT GROUPS OF EXPERIMENTS 1, 2a AND 3

Experiment 1. Paratyphoid vaccine given 22 and 6 hours before ^{125}I-labelled BGG–anti-BGG

Time after ^{125}I-labelled BGG–anti-BGG injection:	2 hours	4 hours	8 hours	16 hours	1 day	1·5 day	2 days	3 days	5 days
Mouse 1	±	+	±	±	+	++	±	±	+
Mouse 2	−	±	−	−	±	−	−	−	−

Experiment 2a. Paratyphoid vaccine given at different intervals before ^{125}I-labelled BGG–anti-BGG. Spleens excised 2 hours after injection of ^{125}I-labelled BGG–anti-BGG

Interval between paratyphoid vaccine and ^{125}I-labelled BGG–anti-BGG:	3 hours	6 hours	12 hours	1 day	2 days	4 days	8 days
Mouse 1	+++	++	+	±	+	+++	++++
Mouse 2	++	±	±	−	+	++	+++

Experiment 3. Competition between immune complexes for follicular localization. Different doses of BGG–anti-BGG complexes injected together with a constant amount of ^{125}I-labelled BGG–anti-BGG complexes. Spleens taken 2 days after injection

Total quantity of injected BGG:	10 μg	30 μg	100 μg	300 μg	1000 μg
Mouse 1	++++	+++	++	+	±
Mouse 2	+++	++	+	±	±

− = No trapping of labelled complexes; ± = very weak trapping; + = weak trapping; ++ = moderate trapping; +++ = heavy trapping; ++++ = very heavy trapping.

^{125}I-labelled BGG–anti-BGG injection. At 5 days only occasional slightly labelled clumps were found in the red pulp.

Part of the label was distributed homogeneously in the red pulp, especially in the sinuses and to a lesser extent in the white pulp. Label in this form also gradually diminished and hardly any remained at 5 days.

INHIBITION OF IMMUNE COMPLEX TRAPPING BY PARATYPHOID VACCINE GIVEN BEFORE INJECTION OF IMMUNE COMPLEXES

When a single paratyphoid vaccine injection was given at different intervals before ^{125}I-labelled BGG–anti-BGG injection and spleens were excised 2 hours after injection of the latter (experiment 2a), the following results were obtained.

As far as label was trapped in the follicles, it was found in the peripheral parts of the follicles in all mice. When paratyphoid vaccine was given 8 days before the labelled immune complexes, very heavily labelled follicles were found in the spleens; label was present in a ring around the germinal centres of the follicles stimulated by paratyphoid vaccine (Fig. 1). The intensity of the labelling in the follicles gradually decreased when paratyphoid vaccine was given respectively 4, 2 or 1 days before the labelled immune complexes, but progressively increased again when the vaccine was given 12, 6 or 3 hours before the complexes (Table 1). Trapping of the immune complexes was maximally inhibited when paratyphoid vaccine was given 1 day before the labelled immune complexes (Fig. 2).

Clumps of heavy label were found in the red pulp. These clumps were randomly distributed in the red pulp and formed a ring around the white pulp. The majority of the clumps were in a narrow rim around the white pulp, especially around the follicles, when paratyphoid vaccine was given 1 day or 12 hours before the immune complexes (Fig. 2). Usually the rim was narrowest in mice in which follicular trapping of the labelled immune complexes was maximally inhibited.

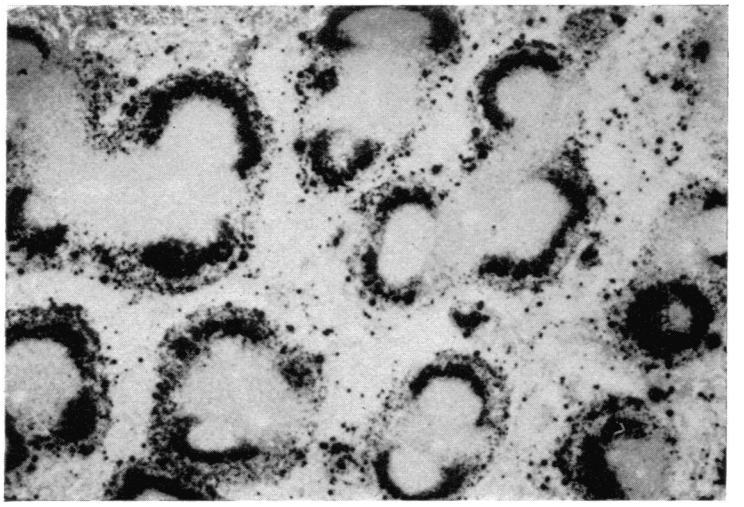

FIG. 1. Autoradiograph of the spleen of a mouse 2 hours after an intravenous injection of ^{125}I-labelled BGG–anti-BGG complexes. The mouse had been injected with paratyphoid vaccine 8 days before the labelled complexes (experiment 2a). A ring of heavy label is shown in the follicles and clumps of label are present in the marginal zone and in the red pulp.

Part of the label in the spleens of all mice was distributed homogeneously in the red pulp and to a lesser extent also in the white pulp.

THE INFLUENCE OF PARATYPHOID VACCINE GIVEN SHORTLY BEFORE, SIMULTANEOUSLY WITH OR SHORTLY AFTER IMMUNE COMPLEXES ON FOLLICULAR TRAPPING

When a single paratyphoid vaccine injection was given 1–½ hour before, simultaneously with or ½–1 hour after the labelled immune complexes and the spleen was excised after 2 hours (experiment 2b), follicles in the spleens of all the mice were very heavily labelled; the label was present in the outer zone of the follicles.

In the red pulp clumps of heavy label were present, part of it forming a ring around the white pulp. This was especially conspicuous in mice receiving paratyphoid vaccine 1 or ½ hour before, or simultaneously with, the labelled immune complexes.

In the white pulp clumps of heavy label were also randomly distributed, in periarteriolar lymphocyte sheaths as well as in follicles. They were especially numerous in mice receiving paratyphoid vaccine simultaneously with the labelled immune complexes (Fig. 3), but were sparser in mice receiving vaccine before or after.

Homogeneously distributed label in red and white pulp was found in all mice.

COMPETITION BETWEEN IMMUNE COMPLEXES FOR FOLLICULAR LOCALIZATION

When ^{125}I-labelled BGG–anti-BGG complexes were given to mice together with different doses of unlabelled BGG–anti-BGG complexes and the spleens were taken 2 days later, a small amount of label was distributed homogeneously in the spleen or present in the form of clumps in the red pulp, but most of it was in the follicles, as was to be expected 2 days after injection.

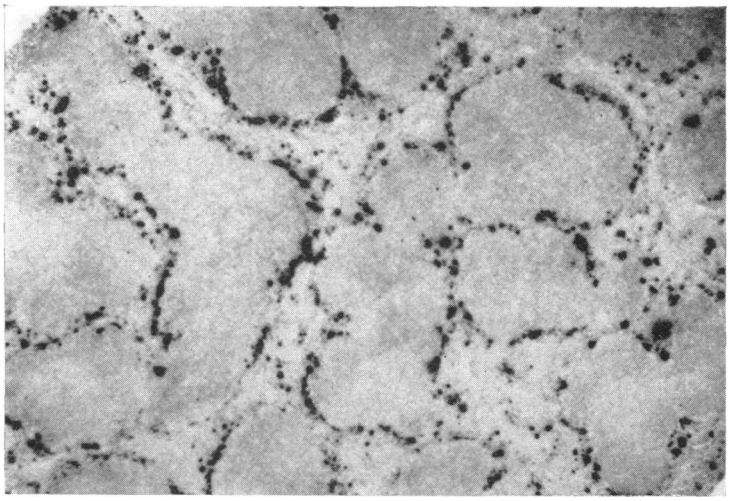

FIG. 2. Autoradiograph of the spleen of a mouse 2 hours after a similar intravenous injection of ^{125}I-labelled BGG–anti-BGG complexes as given to the mouse in Fig. 1. Paratyphoid vaccine was given to this mouse 1 day before the labelled immune complexes (experiment 2a). Clumps of label are present in a narrow rim around the white pulp. Almost no label is seen in the follicles.

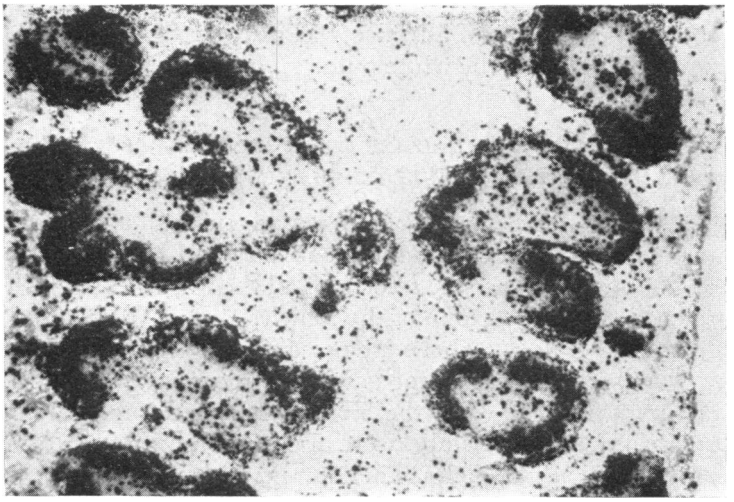

FIG. 3. Autoradiograph of the spleen of a mouse 2 hours after a simultaneous injection of ^{125}I-labelled BGG–anti-BGG complexes and paratyphoid vaccine (experiment 2b). A ring of heavy label is shown in the follicles and clumps of label are present in the marginal zone and in the red pulp. Clumps of heavy label are also present in the white pulp.

The intensity of labelling in the follicles, however, steadily decreased as more unlabelled immune complex was mixed with the labelled complex (Table 1).

DISCUSSION

The results confirm those of earlier studies which showed that injections of para-

typhoid vaccine given 22 and 6 hours before injection of labelled immune complexes inhibited or delayed follicular trapping of these complexes for at least 2 hours (van Rooijen, 1973a).

In the present study the effect of paratyphoid vaccine on follicular trapping of immune complexes was investigated during the period of 5 days after injection of the labelled immune complexes. It appeared that trapping did not improve during these 5 days. Clearly trapping of immune complexes in the follicles was prevented long enough to allow nearly all the immune complexes to be removed from the circulation by other mechanisms (e.g. phagocytosis). It may be concluded that inhibition by paratyphoid vaccine does not merely delay the trapping process.

Follicular trapping of antigen–antibody complexes is the only mechanism known by which small amounts of antigen are preserved in the body for a long time (Nossal and Ada, 1971). We intend to investigate whether prevention of such antigen conservation will have effects on the late immune response.

It appears that strong inhibition of immune complex trapping is obtained when paratyphoid vaccine is given 6 hours to 2 days before the labelled immune complexes (Table 1). Veerman (1974) has shown that at this time after paratyphoid vaccine the lymphoid cells have disappeared from the marginal zone. Thus a correlation seems to exist between the disappearance of lymphoid cells from the marginal zone and the inhibition of immune complex trapping in spleen follicles. Veerman (1974) found that the minimum concentration of lymphoid cells in the marginal zone was reached somewhat earlier than maximal inhibition of immune complex trapping occurred in the present study. The correlation between the two supports our previous conclusion that paratyphoid vaccine inhibits immune complex trapping by removing lymphoid cells from the site where they take up immune complexes, i.e. the marginal zone. Evidence has been adduced that these cells transfer the immune complexes to dendritic cells after migration from the marginal zone towards the follicle centres (van Rooijen, 1974).

When paratyphoid vaccine was given simultaneously with the labelled complexes, no inhibition of immune complex trapping was observed. Thus the effect of paratyphoid vaccine cannot be competitive, as is the case when labelled immune complexes are given simultaneously with unlabelled immune complexes.

Cell movements in the marginal zone after paratyphoid vaccine administration are attributable to the endotoxin component (Kool, Veerman, Flørenes, Nitter and Langevoort, 1967). Similar changes were obtained when endotoxin was administered together with bovine gamma-globulin (BGG) but not with BGG alone (Pierce, 1966; Kool *et al.*, 1967). It is very probable that the effect of paratyphoid vaccine on immune complex trapping is also due to endotoxin.

Since in the present work labelled antigen was injected simultaneously with specific antibody, which is necessary for trapping of the antigen (Humphrey and Frank, 1967; van Rooijen, 1972), the possibility is excluded that the effect of paratyphoid vaccine on the follicular trapping mechanism was due to a decreased capacity of certain cells to produce opsonins or specific antibodies. The effects of whole body X-irradiation and chronic thoracic duct drainage on the follicular trapping mechanism were explained in this way by Williams (1966a, b).

It is unlikely that paratyphoid vaccine has a direct effect on the dendritic reticulum cells, because it produces no change when immune complexes are already trapped in the follicle centres (van Rooijen, 1973a). Nettesheim and Hammons (1970) have shown that

immunosuppressive drugs also inhibited follicular trapping effectively only when given before the substances to be trapped.

Our studies and those of Brown *et al.* (1973) point to a role for lymphocytes in carrying immune complexes or aggregated gamma-globulin from the periphery of the follicles towards the follicle centres. In the studies of Brown *et al.* (1973), X-irradiation or anti-lymphocyte serum appeared to destroy this capacity of lymphocytes, and the effects of these treatments were clear-cut, whether given before, simultaneously with or some hours after aggregated gamma-globulin. In our studies inhibition by paratyphoid vaccine occurred when it was given 2 days to 6 hours before the labelled immune complexes. However, in contrast with inhibition by anti-lymphocyte serum or X-irradiation, paratyphoid vaccine given shortly before, simultaneously with or after the immune complexes did not inhibit the follicular trapping mechanism. This difference is explained by the fact that X-irradiation and anti-lymphocyte serum have a destructive effect on the lymphoid cells involved in the transport of immune complexes towards follicle centres, but when immune complexes have once been transferred from lymphoid cells to dendritic cells, X-irradiation and anti-lymphocyte serum are unable to reverse the trapping of immune complexes in lymphoid follicles. The endotoxin effect of paratyphoid vaccine, on the other hand, causes a temporary disappearance of lymphoid cells from the site where they pick up immune complexes, i.e. the marginal zone.

When paratyphoid vaccine was given together with labelled immune complexes to mice, many clumps of heavy label were randomly distributed in the white pulp, and in the periarteriolar lymphocyte sheaths as well as in the follicles. When it was given shortly before or shortly after the labelled immune complexes, a lesser number of clumps was found. Without paratyphoid vaccine only occasional clumps of heavy label were seen in the white pulp, apart from those contributing to the follicular trapping of immune complexes. Thus the simultaneous injection of paratyphoid vaccine causes cells to enter the white pulp, either before or after taking up complexes. It has been shown by Abe and Ito (1972) that polymorphonuclear leucocytes enter the white pulp after a typhoid–paratyphoid vaccine injection, and it is probable that it is these cells that account for the clumps of heavy label found in the white pulp when paratyphoid vaccine and labelled immune complexes are given simultaneously.

Clumps of heavy label in the red pulp, seen 12–24 hours after injection of the paratyphoid vaccine were mainly arranged in a narrow rim around the white pulp and may represent uptake by polymorphonuclear leucocytes as well as by macrophages.

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