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Mechanism of Follicular Antigen Trapping

MIGRATION OF ANTIGEN–ANTIBODY COMPLEXES FROM MARGINAL ZONE TOWARDS FOLLICLE CENTRES

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Summary. In spleens of normal mice transport of immune complexes takes place from the marginal zone towards the follicle centres. Some hours after an injection with paratyphoid vaccine the marginal zone in the spleen of mice shows a significant lack of lymphoid cells. In such mice trapping of immune complexes in lymphoid follicles is severely inhibited or delayed. These results strongly support the idea that lymphoid cells carry immune complexes from the marginal zone towards follicle centres.

A strong follicle centre reaction induced with paratyphoid vaccine did not remove previously trapped immune complexes, confirming that once trapped, immune complexes are well fixed in follicle centres.

INTRODUCTION

The lymphoid follicles of the spleen show no specificity in the process of antigen trapping (van Rooijen, 1972), and as far as cells are involved in the process of antigen trapping, these cells are not monospecific for one antigen (van Rooijen, 1973a). The process of antigen trapping in lymphoid follicles is dependent on the presence of specific antibody in the circulation (Humphrey and Frank, 1967; van Rooijen, 1972). It involves transport of clumps containing antigen–antibody complexes from the periphery of the follicles towards their germinal centres (Nossal, Austin, Pye and Mitchell, 1966; van Rooijen, 1972) and results in long term retention of complexes in these centres (Nossal and Ada, 1971). Strong support for a lymphocyte-mediated transport of the immune complexes has been given by Brown, de Jesus, Holborow and Harris (1970) and de Jesus, Holborow and Brown (1972).

White pulp capillaries in the rabbit spleen discharge their contents in the marginal zone between white pulp and red pulp (van Rooijen, 1973b). It was found by Pettersen, Borgen and Graupner (1967) that lymphoid cells migrate from the marginal zone into the follicles of the rat spleen shortly after antigenic stimulation. When typhoid-paratyphoid vaccine was used as antigen the marginal zone was almost obliterated.

It was the purpose of the present study to investigate further whether lymphoid cells carry immune complexes from the marginal zone towards the follicle centres. If this were the case the process of follicular antigen trapping should be severely inhibited or delayed when antigen–antibody complexes are injected some hours after a paratyphoid injection,

because the immune complexes would be arriving in a marginal zone without lymphoid cells

A second purpose was to study the effect of injecting paratyphoid vaccine on antigen–antibody complexes previously trapped in germinal centres. It is well known that injection of paratyphoid vaccine results in strong follicle centre reactions (Langevoort, 1963), but the effect of this reaction on the presence of antigen–antibody complexes in the germinal centres is unknown. Lymphoid follicles in the spleen of animals previously stimulated with antigens contain immune complexes, but it is possible that these complexes disappear again under the influence of a new strong follicle centre reaction. If they do not it must be concluded that lymphoid follicles in the spleen generally contain different immune complexes resulting from different immune reactions.

Finally the localization in the spleen of large doses of Indian ink was compared with that of immune complexes, since Cohen, Vassalli, Benacerraf and McCluskey (1966) have reported that non-antigenic compounds such as colloidal carbon, when injected into the footpads of guinea-pigs, localize in the same manner as antigens in lymphoid follicles of the draining lymph nodes. If carbon localizes in the follicles of the spleen in the same manner as immune complexes it is unlikely that trapping of immune complexes in follicles is an immunological event as previously supposed.

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); human serum albumin (HSA from Behringwerke AG Marburg-Lahn, West Germany); human gamma-globulin (HGG from the 'Central Laboratory of the Blood Transfusion Service of the Netherlands Red Cross', Amsterdam, The Netherlands); ^{125}I-HGG, bovine gamma-globulin (BGG from Pentex, Kankakee, Illinois, U.S.A.) and ^{125}I-BGG.

BGG and HGG were labelled with ^{125}I (IMS-30 from the Radiochemical Centre, Amersham, Great Britain) using the Chloramine-T method.

Anti-sera

Rabbit anti-HSA and rabbit anti-BGG sera were obtained from Nordic (Tilburg, The Netherlands).

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. Spleens of mice injected with Indian ink were fixed in Zenker-formol.

Experimental design

Three experiments were performed: experiment 1, three groups of mice were injected

with 0·015 mg ^{125}I-BGG (0·1 mCi) together with an excess of anti-BGG antibodies (0·08 ml of the rabbit anti-BGG serum). Mice were killed and their spleens fixed 2 hours after injection.

One group consisted of five normal mice. Another group consisted of five mice which had received paratyphoid vaccine (0·2 ml, 10^9 formol-killed organisms in saline) with the object of depleting lymphoid cells from the marginal zones (Pettersen, Borgen and Graupner, 1967). The vaccine was given 22 hours and again 6 hours before injection of the labelled antigen. A third group consisted of three mice injected with 2·3 mg HSA together with 0·33 ml rabbit anti-HSA serum, 1·5 hour before injection of the labelled antigen.

Experiment 2, nine mice received 0·022 mg ^{125}I-HGG (0·2 mCi). They had been preimmunized with 1 mg HGG 4 and 2 months before. Three mice were killed 2 weeks after injection of the labelled antigen, and six were injected with paratyphoid vaccine at this time. Three mice of the latter group were killed 4 days after the paratyphoid injection; the three others were killed 8 days after. Experiment 3, nine mice were injected with 0·2 ml undiluted Indian ink (Pelikan Specialtusche C11/1431a, Günther Wagner). Three of these mice were killed after 2 hours, three after 1 day and three after 3 days.

RESULTS

EXPERIMENT 1

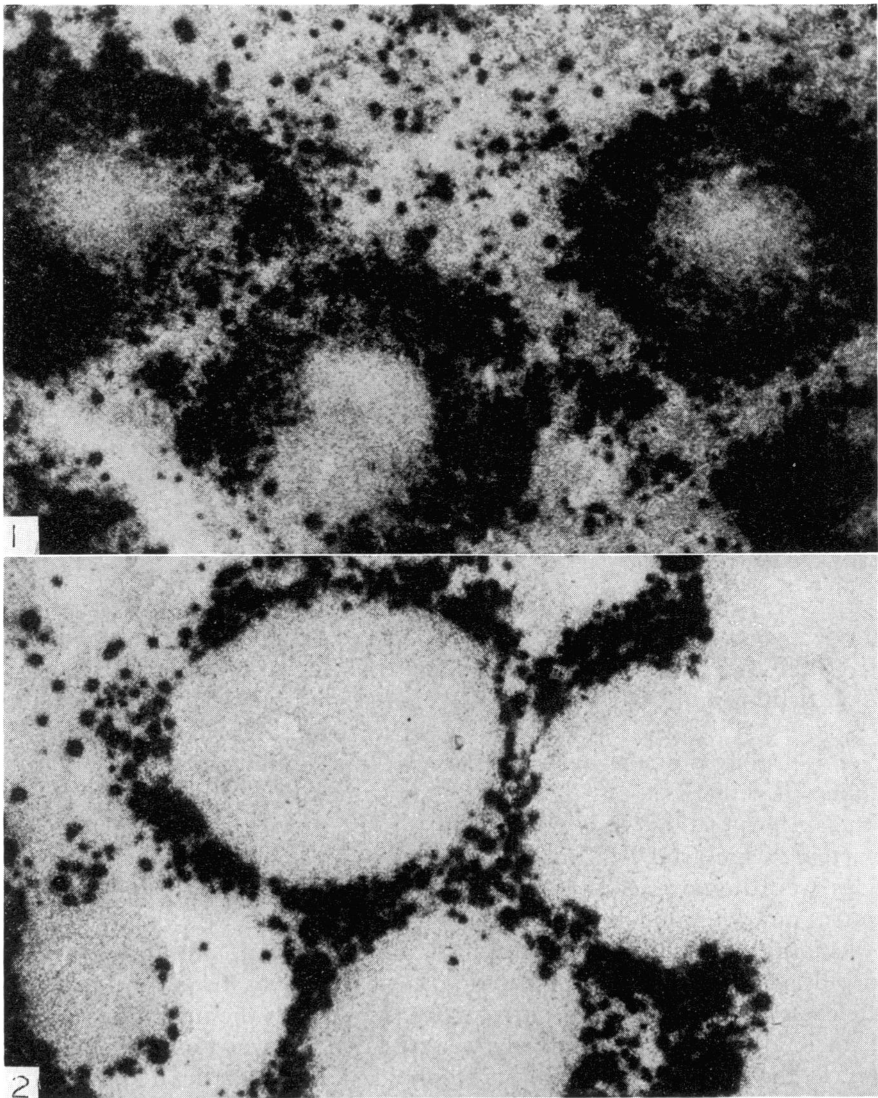
The aim of this experiment was to see whether the injection of paratyphoid vaccine or HSA–anti-HSA antibody complexes inhibits the migration of subsequently injected ^{125}I-BGG–anti-BGG antibody complexes from the marginal zone towards the follicle centre.

The splenic histology of the normal mice was not significantly different from that of the mice injected with HSA–anti-HSA antibody complexes. Clear differences were found between the spleens of these two groups and the spleens of the mice injected with paratyphoid vaccine, which showed a marked lack of lymphoid cells in their marginal zones. The follicles were homogeneous in composition, not showing the normal division into follicle centres and lymphocytic coronae.

Label was found as clumps or patches in the marginal zone and to a lesser extent also in the red pulp of all mice. In the normal animals label was also found in the periphery of the follicles in the form of clumps or patches (Fig. 1). In the mice injected with HSA–anti-HSA antibody complexes relatively weaker labelling was found in the periphery of the follicles. In the mice injected with paratyphoid vaccine practically no label was found in the follicles (Fig. 2).

EXPERIMENT 2

This experiment was done to study the influence of a paratyphoid injection with the subsequently occurring follicle centre reaction upon the presence of ^{125}I-HGG–anti-HGG antibody complexes in these follicle centres. The spleens of mice 4 days and particularly 8 days after paratyphoid injection showed follicles with large active germinal centres. In the control mice not injected with paratyphoid vaccine label was present in many of the mostly small and inactive follicles. While some follicles were not labelled at all, the heaviest labelled follicles were nearly completely black with grains. Label was also



FIGS 1 and 2. Autoradiographs of the spleens of mice 2 hours after intravenous injection of ^{125}I-BGG (0·1 mCi/0·015 mg) together with an excess of anti-BGG antibodies.

Fig. 1. A normal mouse.

Fig. 2. A mouse preimmunized with paratyphoid vaccine 22 hours and 6 hours before the injection with labelled antigen. In both mice label is present in the marginal zone surrounding the white pulp. Only in the normal mouse part of the label has penetrated in the follicles.

found consistently in the active follicle centres of the mice 4 and 8 days after paratyphoid vaccine, although these were larger areas where the label was distributed in a less compact way than in the mice not injected with vaccine.

EXPERIMENT 3

The purpose of this experiment was to compare the localization of Indian ink in the spleen of normal mice with that of antigen–antibody complexes. The localization of the ink in the marginal zone and in the red pulp was not significantly different 2 hours, 1 day or 3 days after injection. At 2 hours the localization of the ink and of the ^{125}I-BGG–anti-BGG antibody complexes was similar, i.e. clumps in the marginal zone and to a lesser extent also in the red pulp. After 1 day only a very small amount of ink was found in the white pulp. This was present as small clumps in macrophages in the periarteriolar lymphocyte sheath and to a lesser extent in the follicles. Three days after injection of the ink the amount in the white pulp had somewhat increased, but in the follicles the amount of ink remained extremely small.

DISCUSSION

It was found that in the spleens of mice injected 22 and 6 hours before with paratyphoid vaccine the marginal zone showed a significant lack of lymphoid cells. This is in agreement with the findings of Petterson, Borgen and Graupner (1967). When the localization of ^{125}I-BGG–anti-BGG antibody complexes in normal mice was compared with that in mice injected with paratyphoid vaccine, no marked differences were observed in the marginal zone and in the red pulp. In the normal mice however some of the immune complexes had penetrated into the periphery of the follicles after 2 hours while no such distribution was observed in the periphery of the follicles of the paratyphoid injected mice. This is a strong indication that immune complexes are carried from the marginal zone towards the germinal centres by lymphoid cells. Presumably lymphoid cells had already left the marginal zone and entered the follicles by the time the immune complexes arrived in the marginal zone. According to Nieuwenhuis (1971) lymphoid cells of the marginal zone are B lymphocytes, which is in agreement with the conclusion of de Jesus, Holborow & Brown (1972) that it is B lymphocytes that carry immune complexes towards follicle centres.

The initial localization of injected Indian ink in the marginal zone and in the red pulp was the same as that of immune complexes. In the white pulp, however, the localization of the ink was completely different from that of immune complexes. While 3 days after injection some of the ink was found in macrophages in the periarteriolar lymphocyte sheaths, only a very small amount was seen in the follicles. These results are in agreement with those of Nossal, Austin, Pye and Mitchell (1966). It appears that a small amount of ink is transported by macrophages from the marginal zone into the periarteriolar lymphocyte sheath and occasionally into the follicles. Immune complexes generally leave the red pulp and the marginal zone within 1 day; the amount of ink in these areas is not markedly diminished after 3 days.

A very strong follicle centre reaction produced by paratyphoid injection did not remove previously trapped ^{125}I-HGG–anti-HGG antibody complexes to any extent. It is therefore probable that follicle centres generally contain immune complexes from different immune reactions. The non-specificity of the follicles in the process of antigen trapping has been previously demonstrated (van Rooijen, 1972).

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