Immunology 1984 **53** 713

Suppression of follicular trapping of antigen-antibody complexes in mice treated with anti-IgM or anti-IgD antibodies from birth

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Accepted for publication 20 July 1984

Summary. Mice were treated from birth with either goat anti-mouse IgM or with a monoclonal anti-IgD antibody. When they were 8 weeks old, cohorts of these mice were given ^{125}I-labelled antigen, either by itself, or in an antigen-antibody complex. Anti-IgM-treated mice, which did not develop follicular structures in their spleens, failed to retain immune complexes on follicular dendritic cells in the characteristic pattern. Anti-IgD-treated mice, which had small follicles consisting of IgM^+ IgD^- B cells in their spleens, retained substantially smaller amounts of immune complexes than normal.

These results support the concept that B lymphocytes transport antigen-antibody complexes to follicular dendritic cells. Furthermore, in the mouse it seems likely that this is mediated by both IgM^+ IgD^+ and IgM^+ IgD^- B cells.

INTRODUCTION

The existence of a unique accessory cell–the follicular dendritic cell (FDC)–in follicles of both spleen and lymph nodes has been recognized for many years (reviewed by Nossal & Ada, 1971). These cells retain antigens in the form of antigen-antibody (Ag-Ab) complexes on their voluminous, ramifying processes for extremely long periods. This retained antigen has been shown to play a central role, both in the generation of B memory cells, and in feedback control of humoral immunity (reviewed by Klaus *et al.*, 1980; Tew, Phipps & Mandel, 1980).

Follicular dendritic cells are sessile cells which are not bone marrow-derived, and which turn over very slowly (Humphrey, Grennan & Sundaram, 1984). The mechanisms by which Ag-Ab complexes reach these cells are therefore of considerable interest. Follicular trapping is abolished in decomplemented animals (Papamichail *et al.*, 1975; Klaus & Humphrey, 1977), and also by procedures which deplete lymphocytes, such as X-irradiation or cyclophosphamide treatment (Brown *et al.*, 1973; Gray *et al.*, 1984). It is therefore likely that lymphocytes, and presumably B cells, actually transport Ag-Ab complexes to FDC (Brown *et al.*, 1973; van Rooijen, 1973). The present study addresses this question directly by examining the effects of procedures known to selectively deplete B cells on follicular trapping of immune complexes in the spleen of the mouse.

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MATERIALS AND METHODS

Experimental animals

C3H/He mice derived from specific-pathogen-free mothers and bred at NIMR were used for this study.

Reagents

Anti-IgM antibodies were from a goat immunized alternately with T183 (IgM, κ) and M104E (IgM, λ) myeloma proteins. A large pool of serum was rendered μ chain specific by repeated absorption on columns of MPC21 (IgG1, κ) and APC5 (IgG2a, κ) linked to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden). A 50% ammonium sulphate precipitate was then prepared, and reconstituted to the original volume. Anti-IgD was the monoclonal antibody 10.4.22 (Oi *et al.*, 1978): this is an IgG2a antibody directed against the Ig-5a allele. The antibody was purified by affinity chromatography on protein A-coupled Sepharose 4B (Pharmacia). $F(ab')_2$ fragments of rabbit antibodies specific for either μ or δ chains used for immunoperoxidase staining were purified as described by Chayen & Parkhouse (1982).

The preparation of radiolabelled (^{125}I) dinitrophenylated keyhole limpet haemocyanin (DNP-KLH), and of the IgG2a anti-DNP monoclonal antibody Hy1.2 have been described previously (Enriquez-Rincon & Klaus, 1984).

B-cell suppression

Anti-IgM: mice received their first injection (50 μl) of anti-IgM (i.p.) within 16–20 hr after birth, and this was repeated daily during the first week. During the second week, they received 100 μl anti-μ on alternate days, and thereafter 3 weekly doses until the time of the experiment, when the animals were some 8 weeks old.

Anti-IgD: Comparable litters received 20 μg of 10.4.22 antibody on alternate days during the first 10 days of life, 40 μg on alternate days from day 10 until day 20, and finally 50 μg three times weekly until the time of the experiment.

Experimental design

Groups ($n = 4$–8) or 8-week-old mice received 10 μg ^{125}I-DNP-KLH either alone (Ag), or as an immune complex made *in vitro* at a 1:5 (w/w) ratio with antibody Hy1.2 (Ag-Ab) (both given i.v.). After 24 hr, the mice were killed and their spleens were counted for ^{125}I. One-half of each spleen was then fixed in Carnoy's solution and processed for autoradiographical histology, using previously published methods (Klaus & Humphrey, 1977).

The other half of each spleen was frozen in a bath of dry ice in ethanol: 6–8 μm cryostat sections were fixed in $CHCl_3$:CH_3OH (1:1), and were then incubated for 3 hr with 10 μg of $F(ab')_2$ fragments of either rabbit anti-δ or anti-μ antibodies. After washing, the sections were similarly incubated with 20 μg horseradish peroxidase-coupled $F(ab')_2$ fragments of goat anti-rabbit Ig antibodies. Finally, the peroxidase reaction was revealed by 2,4 diamino-benzidine (1 mg/ml), containing 0·1% hydrogen peroxide and 0·03% cobalt chloride.

RESULTS

Localization of antigen-antibody complexes in spleens of B-cell-suppressed mice

Figure 1 shows the percentages of injected radiolabel retained in the spleens, and Fig. 2 shows the representative autoradiographs of splenic sections from control and experimental groups given either Ag or Ag-Ab complexes. Both anti-IgD-treated and control mice given Ag alone had about 0·5% of the injected label remaining in their spleens 24 h after injection, and none of this was in follicles (not shown). In marked contrast, control mice given Ag-Ab retained about 10-fold more label, and this was localized in areas of FDC within follicles, typically in a crescentic distribution around their periphery (Fig. 2a).

Anti-IgM treated mice given Ag-Ab complexes did not retain more label than controls receiving Ag alone. Histologically, although periarteriolar lymphatic sheaths (PALS) were discernible, follicular structures were not (Fig. 2c). Anti-IgD-treated mice which had received Ag-Ab complexes showed quite a different picture. These retained significantly more ^{125}I than mice given Ag, but only 30% of the amount in controls given Ag-Ab. Histologically, labelling of autoradiographs was appreciably lighter, although small follicles were recognizable by virtue of their accumulated label, which was considerably more circumscribed than in the controls (Fig. 2b).

Immunohistological features of B-cell-depleted mice

Figures 3, 4 and 5 illustrate representative immunoperoxidase-stained sections of spleens from the mice used in the experiment summarized above, in each case stained with either anti-μ or anti-δ antibodies.

The spleens of control mice showed the characteristic staining of follicular areas in the white pulp with both antibodies (Fig. 3). IgM^+ B cells are abundant throughout the follicles, whereas the heaviest staining with anti-IgD was in the depths of the follicles

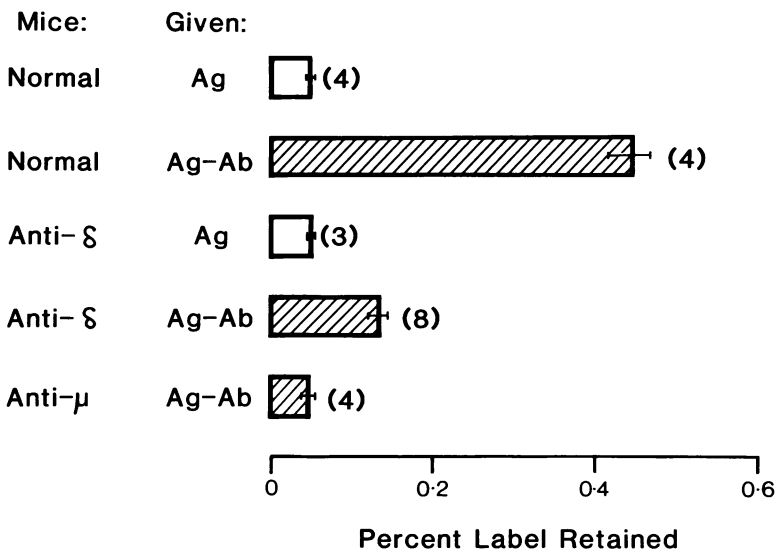

Figure 1. Retention of ^{125}I-labelled DNP-KLH (Ag), or DNP-KLH-anti-DNP antibody complexes (Ag-Ab) in the spleens of normal or B-cell-depleted mice. Groups (*n* in brackets) of mice received 10 μg of free or complexed Ag. Spleens were removed after 24 hr and counted for residual ^{125}I. Bars represent the arithmetic means ± SEM of the percentages of injected radiolabel retained in the spleens.

adjoining the PALS. No well-defined areas of IgM$^+$ IgD$^-$ B cells were discernible in the marginal zones, as occur in the rat (Gray *et al.*, 1982).

The spleens of anti-IgD-treated animals contained no IgD-bearing B cells. Substantial numbers of IgM$^+$ cells were apparent, although the follicles were significantly smaller than in the controls (Fig. 4). In sharp contrast, spleens from anti-IgM-treated mice contained essentially no recognizable follicles. The one structure which resembled a tiny follicle is illustrated in Fig. 5, and this consisted of a small number of weakly staining IgM$^+$ cells in the centre, with a few IgD$^+$ B cells around the periphery. Otherwise, only occasional stained cells were visible in the spleens from this group, mostly in a ring around the PALS, in both the anti-μ and the anti-δ stained sections.

DISCUSSION

These experiments show that treatment of mice from birth with either anti-IgM or anti-IgD antibodies abolishes, or markedly reduces, the localization of Ag-Ab complexes on splenic FDC (Fig. 2). These results therefore provide further compelling evidence for the concept that immune complexes are transported to FDC by lymphocytes and, indeed, that these are B cells (Brown *et al.*, 1973; van Rooijen, 1973). Several investigators have shown that procedures which deplete lymphocytes, but which do not affect FDC, such as X-irradiation (Brown *et al.*, 1973), administration of endotoxin (van Rooijen, 1975), or of cyclophosphamide (Gray *et al.*, 1984) abolish the trapping of Ag-Ab complexes in follicles. The recent study by Gray *et al.* (1984) showed that a dose of cyclophosphamide, which selectively kills marginal zone B cells in the rat spleen, abolished the trapping phenomenon. They therefore suggest that the non-recirculating IgM$^+$ IgD$^-$ B cells in the marginal zone are responsible for immune complex transport, a conclusion in line with earlier work in the mouse (Brown *et al.*, 1973; van Rooijen, 1973, 1975). Our results show that the small follicles composed of IgM$^+$ IgD$^-$ B cells (Fig. 4) present in the spleens of anti-IgD-treated mice, only trapped relatively small amounts of immune complexes (Figs. 1, 2). It thus appears that in the mouse IgM$^+$ IgD$^+$ B cells are also involved in the transport of Ag-Ab complexes. This probably reflects a species difference between the mouse and rat, since in the former the compartmentalization of IgM$^+$IgD$^-$ B cells into broad marginal zones does not occur. Gray *et al.* (1984) also showed

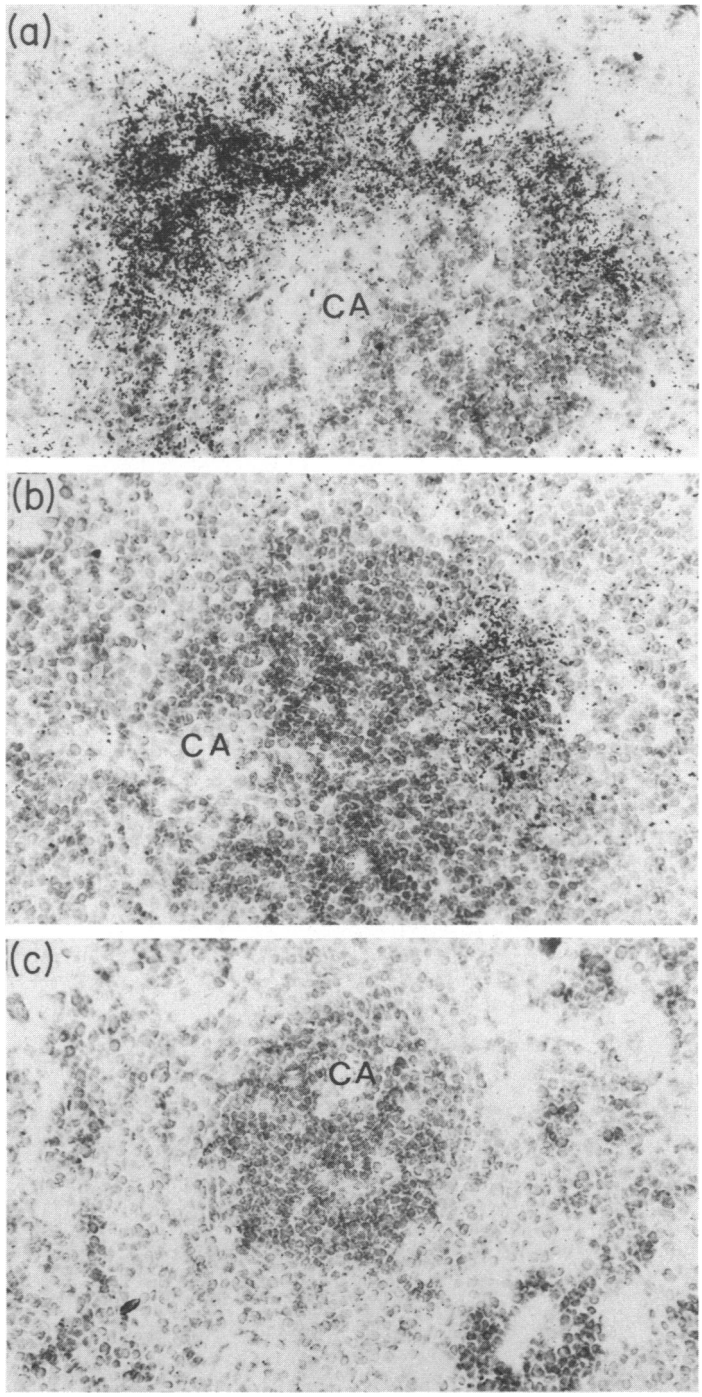

Figure 2. Follicular localization of ^{125}I-labelled Ag-Ab complexes in the spleens of (a) normal, (b) anti-IgD-treated, and (c) anti-IgM-treated mice. Methyl green-pyronin stained autoradiographs of representative spleen sections from mice used in the experiment summarized in Fig. 1. Each panel shows a white pulp island with the central arteriole (CA) in the periarteriolar lymphoid sheath labelled for orientation.

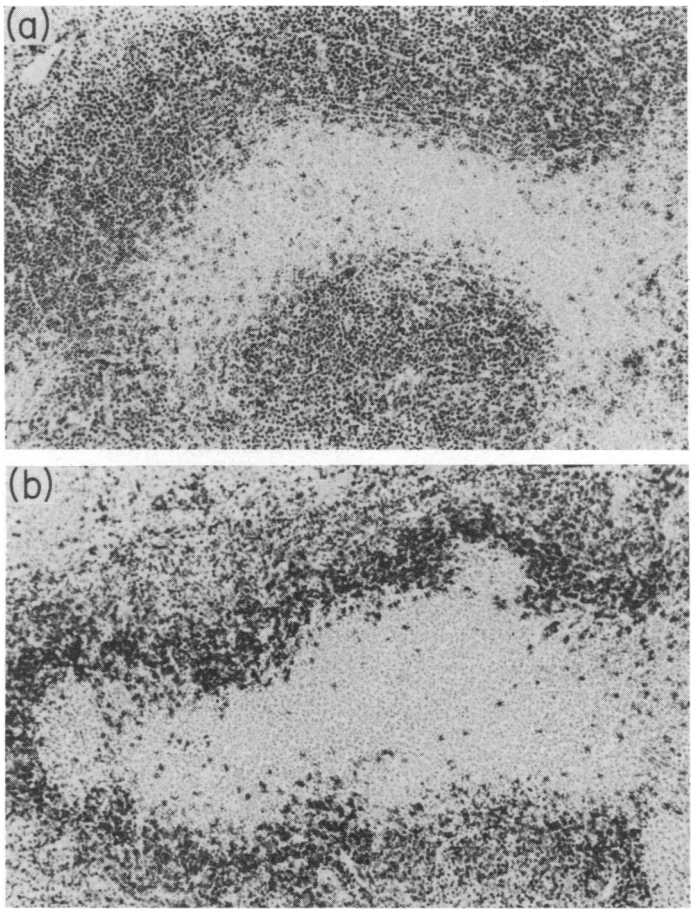

Figure 3. Immunoperoxidase-stained sections of spleens from normal mice used in the experiment shown in Fig. 1 and stained with (a) anti-IgM and (b) anti-IgD.

that marginal zone B cells in the rat carry receptors for C3b, C3bi and C3d, which may well explain the well-established complement-dependence of trapping (Papamichail *et al.*, 1975; Klaus & Humphrey, 1977).

The absence of follicular localization in anti-IgM-treated mice is hardly surprising, since the spleens of these mice were essentially devoid of lymphoid follicles (Figs. 2b, 5). It is therefore possible that they may also lack FDC, although more detailed histological studies would be required to investigate this point. Unfortunately, little is known about the origins of FDC, or about the factors that govern their development. Earlier experiments by Williams & Nossal (1966) suggested that, in the rat, the follicular trapping mechanism appears earlier in ontogeny than the colonization of follicular anlagen by lymphocytes. More recently, however, Dijkstra, van Tilburg & Dopp (1982) have found that the characteristic trapping pattern does not appear before there are recognizable follicles. This suggests that microenvironmental factors in the developing follicle stimulate the differentiation of FDC, perhaps from reticulum cells in the stroma (Dijkstra, Kamperdijk & Dopp, 1984).

Finally, the relevance of the present findings to the known immunological effects of anti-IgD treatment bear comment. Mice treated from birth with anti-IgD can mount surprisingly normal antibody responses to both T-dependent and T-independent antigens, despite a virtual absence of IgD-bearing B cells (Metcalfe *et al.*, 1981; Layton *et al.*, 1978; Jacobson *et al.*, 1981).

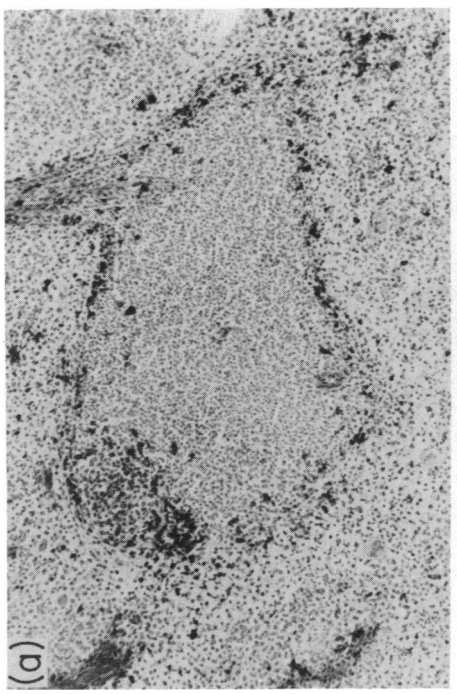

Figure 5. Immunoperoxidase-stained serial sections of the spleen of a mouse treated with anti-IgM from birth, and stained with either (a) anti-IgM or (b) anti-IgD.

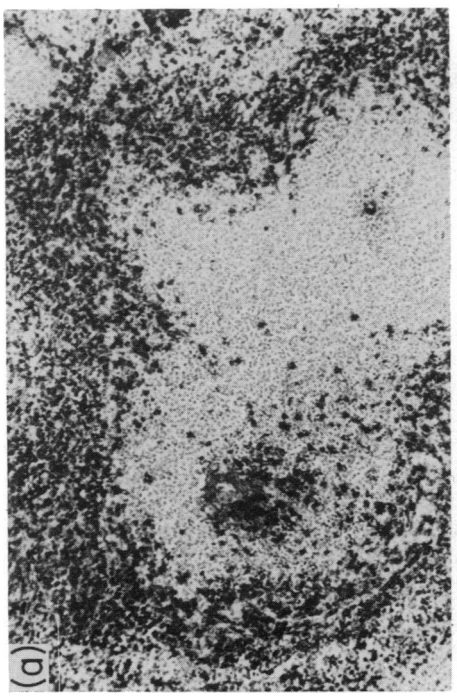

Figure 4. Immunoperoxidase-stained serial sections of the spleen from a mouse treated from birth with anti-IgD, and stained with (a) anti-IgM and (b) anti-IgD. CA: central arteriole.

This appears to reflect an essentially normal antibody-forming capacity in the spleen, while responses of lymph nodes to local immunization are depressed (Baine *et al.*, 1982). We have previously shown that follicular localization of Ag-Ab complexes is intimately related to the generation of B memory cells (Klaus *et al.*, 1980). With one exception (Layton *et al.*, 1978) the studies of IgD-suppressed mice have indicated that these animals produce normal secondary antibody responses (Metcalfe *et al.*, 1981; Jacobson *et al.*, 1981). It therefore seems likely that the residual IgM^+ IgD^- B cells present in such mice can transport sufficient AG-Ab complexes into follicles to induce germinal centre formation and memory cell development. This is supported by the presence of active germinal centres in IgD-suppressed mice (Jacobson *et al.*, 1981). It remains possible, however, that careful antigen dose-response studies might reveal a more profound defect in memory generation in anti-IgD treated mice than has hitherto been found.

ACKNOWLEDGMENTS

F. Enriquez-Rincon was supported by a fellowship from Consejo Nacional de Ciencia y Technologia, Mexico. We thank Niel Almond for supplying the absorbed anti-IgM antiserum.

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