

MURINE FOLLICULAR DENDRITIC CELLS AND LOW AFFINITY Fc RECEPTORS FOR IgE (FcεRII)¹

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The present study was undertaken to determine whether mouse follicular dendritic cells (FDC) bear FcεRII (CD23) and whether IgE-immune complexes are retained by FDC. Mouse FcεRII was localized by both L and electron microscopy using the mAb B3B4. In lymph nodes of normal mice, FcεRII was low but detectable on FDC. By 14 days after *Nippostrongylus brasiliensis* infection, the level of FcεRII increased on B lymphocytes located in the cortex of draining mesenteric lymph nodes. However, the FcεRII level on FDC remained low. Although numerous IgE-producing plasma cells were seen at day 14, very little IgE was associated with FDC. By 26 days after infection, FcεRII was observed on FDC in increased levels and IgE binding was clearly associated with FDC. Unexpectedly, FDC of control mice immunized with albumin in CFA to elicit an IgG response showed intense labeling for FcεRII. In contrast, the B cells exhibited very little FcεRII. IgE immune complexes were observed in association with FDC in the CFA-immunized mice. When mice were given a hapten-specific monoclonal of the IgE isotype, hapten carrier complexes were trapped and retained on FcεRII-bearing FDC. In conclusion, FDC were clearly one of the major murine cell types bearing FcεRII. IgE immune complexes were found in association with FDC and FcεRII appeared to play a major role in trapping and retaining IgE immune complexes. FDC FcεRII was subject to regulatory control, but the FcεRII level on FDC was regulated very differently from the FcεRII level on B cells.

The low affinity FcR for IgE (FcεRII or CD23) is distinctly different from the high affinity FcR for IgE (FcεRI) on mast cells (1-3). This molecule may play important roles not only in binding IgE but also as a factor involved in B cell (and perhaps T cell (4)) proliferation and differentiation (2). In humans, FcεRII may be found on a variety

of cell types including FDC³ (1, 3-15). Several recent reports indicate that human FDC, which are located in germinal centers of secondary lymphoid tissues like murine FDC, may bear high amounts of FcεRII (2, 13-15). In mice, only B cells are known to be clearly reactive with the FcεRII-specific mAb B3B4 (9, 11). However, there is no information on FcεRII on mouse FDC or on the ability of FDC to bind IgE-immune complexes in this well defined experimental model.

We reasoned that mouse FDC might express FcεRII when stimulated to produce high levels of IgE and that the receptor might be functional in binding and retaining IgE immune complexes. In our study we tested this hypothesis by infecting mice with Nb to elicit production of high levels of IgE. The results confirmed that mouse B cells bear FcεRII and that FcεRII is markedly up-regulated on B cells from Nb-infected mice shortly after the time when IL-4 is known to be produced (16). We also discovered that FDC in normal mice bore low levels of FcεRII. Mice infected with Nb did have increased levels of FcεRII on their FDC but not until late in the response and this increase did not correlate with the peak production of IL-4 or IgE that occurs much earlier (16). Quite unexpectedly, control mice immunized with albumins in CFA to induce an IgG-dominated response, had remarkably high levels of FcεRII on FDC. Furthermore, this FcεRII is capable of binding and retained IgE immune complexes. However, the B cells of these mice immunized using CFA bore very little FcεRII. Clearly, FDC in mice can bear FcεRII and it may be present in high levels; however, FcεRII appears to be regulated very differently on mouse FDC than on mouse B cells.

MATERIALS AND METHODS

Mice. C3H/He, BALB/c, and CAF-1 mice were purchased from Charles River Breeding Laboratories Inc. (Wilmington, MA) or The Jackson Laboratory (Bar Harbor, ME). The animals were kept in standard cages and given food and water ad libitum. Animals receiving injections with ¹²⁵I-HSA were given water containing KI (600 mg/liter) a few days before challenge and maintained on this water to minimize uptake of radioactivity in the thyroid. As a further safety, the animals were given 200 μl isotonic KI 16 to 18 h before challenge with radioactive Ag.

Ag. Crystallized HSA or egg albumin (OVA) was used in native form, heat aggregated (17) or for certain experiments HSA was derivitized with TNP (18). In our experience the heat aggregation induces a better antibody response against HSA. For some experiments the HSA or TNP-HSA was radiiodinated using the chloramine-T method to 10-20 μCi/μg (19).

Infection with Nb. Nb was maintained as previously described by Huff and Justice (20). BALB/c mice were infected with Nb by a single

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³ Abbreviations used in this paper: FDC, follicular dendritic cells; HSA, human serum albumin; Nb, *Nippostrongylus brasiliensis*.

s.c. injection of 1000 L3 infective larvae and were killed at various times after injection and their mesenteric lymph nodes excised.

Active immunization. Initially animals were injected s.c. behind the nape of the neck with 1 mg of an Ag (HSA or OVA) in CFA. Then 2 wk later, the mice were boosted in the footpads and behind the neck with 500 µg of Ag in CFA. Approximately 1 mo later, the mice were either used without further boosting (i.e., day 0 lymph nodes) or given a second booster injection (20 µg) without CFA in each footpad and behind the neck. The draining lymph nodes (popliteal, inguinal, axillary, brachial, and cervical) were obtained from groups of three mice at 1, 3, 5, and 9 days and 4 mo after antigenic challenge.

Passive immunization. Ag trapping by FDC is dependent on immune complex formation and some mice were given passive antibody before immunization to study localization of immune complexes on FDC. Serum from animals immunized with HSA using CFA to give a dominant IgG response or anti-DNP mAb of the IgE isotype were used. Passive immunization with serum antibody was done by injecting 0.75 ml of HSA-specific rabbit antisera i.p. 1 day before the injection of Ag. Passive immunization with monoclonal anti-DNP was done by injecting 40 to 100 µg directly into the hind foot pad just before injecting Ag. The amount of IgE monoclonal in the total animal using this passive immunization procedure was clearly small (serum titers <75 µg/ml) and to generate high levels of DNP and TNP reactive anti-IgE, mice received injections with the heterocyclic ϵ -anti DNP-producing hybridoma H1 DNP- ϵ -26.82 obtained from F. T. Liu (21). The mice were prepared by an i.p. injection of 0.5 ml Pristane 13 and 3 days before the i.p. injection of 1.5×10^6 hybridoma cells. Within 1 wk IgE reactive with TNP could be detected in the serum and the mice developed ascites, but the degree of abdominal enlargement was variable from animal to animal. By 10 days the IgE serum titers varied widely but typically ranged from 0.1 to 1.0 mg/ml. The radiolabeled TNP derivatized immunogen used to study immune complex localization on FDC was injected 7 to 10 days after injecting the hybridoma cells. Mice received injections with 5 µg of HSA in the footpads and behind neck. The draining lymph nodes were excised between days 1 and 5 after Ag administration.

Antibodies. The mAb used in this study are listed in Table I. Each antibody was diluted with Dulbecco's modified PBS containing 1% BSA, Mac-2, anti-Thy-1.2, and normal rat IgG were used as controls for nonspecific binding to FDC.

Immunoperoxidase labeling for light microscopy. Cryostat sections were prepared from draining and nondraining lymph nodes. The sections were air-dried for 3 to 5 h, fixed using absolute acetone for 10 min at ambient temperature, and rinsed three times with PBS, pH 7.4. Endogenous peroxidase activity was blocked by incubating the sections in 5 mM periodate solution (no. p-7875, Sigma Chemical Co., St. Louis, MO) for 20 min at room temperature. The sections were rinsed with PBS three times and incubated in 5% BSA for at least 1 h to inhibit nonspecific binding of antibodies. Sections were incubated overnight at 4°C in optimally diluted primary antibody (Table I), rinsed three times (~3 min/rinse) using PBS, and incubated with peroxidase conjugated affinity purified F(ab')₂ fragments of mouse anti-rat IgG (H and L chain specific) at a 1/100 dilution (no. 212-036-082, Jackson ImmunoResearch Lab., West

Grove, PA or no. 756304-1, Pel-Freez Biologicals, Rogers, AK). The sections were washed twice in PBS for 3 min followed by a wash in 0.1 M cacodylate buffer, pH 7.4, and incubated in 0.03% 3,3'-diaminobenzidine (no. D-5637, Sigma) solution in 0.1 M cacodylate buffer with 0.01% H₂O₂ for development of peroxidase activity as described by Graham and Karnovsky (26). After an overnight incubation in 0.5% methyl green to counterstain, the sections were dehydrated in a graded series of ethanols, cleared in xylene, and mounted with Permount (Fisher Scientific Co., Fair Lawn, NJ) for light microscopy. Antibodies of the same isotype as B3B4 but of inappropriate specificity (see Table I) were used as controls. In addition, sections were examined for reactivity with secondary antibodies alone and endogenous peroxidase activity.

Immunoperoxidase staining for electron microscopy. To evaluate cellular labeling with B3B4 at greater resolution, tissues were prepared for electron microscopy. Actively immunized C3H mice (day 5 and 9 after challenge) received intracardiac perfusion with 50 ml of warmed heparinized Ringer's solution followed by 50 ml of fixative (periodate-lysine-4% paraformaldehyde solution) (27). The draining lymph nodes (popliteal, axillary, and brachial lymph nodes) were excised and further fixed by immersion in the same fixative for 6 to 10 h on ice. The lymph nodes were then washed overnight in PBS, embedded in 4% Noble agar, and sectioned at 50-µm thickness with an Oxford Vibratome Sectioning System. To block the endogenous peroxidase activity, the sections were incubated in 5 mM periodate solution for 20 min at ambient temperature. After three 2-min washes in PBS, the sections were incubated overnight at 4°C in 5% BSA-PBS solution to inhibit nonspecific binding of antibodies. Sections were then incubated for 24 to 48 h at 4°C in the mAb, antibodies of inappropriate specificity or PBS (controls) to check for nonspecific binding and endogenous peroxidase activity. After washing for 6 to 12 h in PBS, the sections were then incubated for 24 h at 4°C with the peroxidase conjugated secondary antibodies. After 12 to 24 h washing in PBS, the sections were further fixed using 1.0% glutaraldehyde solution for 20 min at 4°C. They were washed 12 to 24 h in PBS, incubated with 0.03% DAB in 0.1 M cacodylate buffer for 1 h at ambient temperature and then for an additional 0.5 h in the presence of 0.01% H₂O₂ to develop peroxidase activity. To select appropriate areas for electron microscopy, the sections were initially mounted in buffer on glass slides and examined by light microscopy. The slides were subsequently disassembled and the appropriate areas were cut out, postfixed in 2% OsO₄ in 0.1M cacodylate buffer for 4 h at 4°C, dehydrated in a graded series of ethanols and embedded in Polybed 812 (Polyscience, Carrington, PA) on vinyl slides (no. 144-06686, A. H. Thomas, Philadelphia, PA). After polymerizing, the specimens were cut out and mounted on blank epoxy stubs for ultramicrotomy on a LKB 2128. Unstained ultra thin sections were examined at 60KV on a Zeiss 10CA electron microscope.

Autoradiography. Three days after mice received injections with ¹²⁵I-HSA or ¹²⁵I-TNP-HSA, their draining popliteal and inguinal lymph nodes were removed and frozen in O.C.T. compound. The 6-µm thick cryostat sections were prepared and placed on gelatin-coated slides (prepared by dipping clean microscope slides in 0.5% gelatin solution and drying vertically in a 60°C oven). The sections were fixed by immersion in absolute acetone for 10 min followed by

TABLE I
Characteristics of mAb used

Antibodies (Clones)	Isotype	Reactivity	Working Dilution	Source	Reference
B3B4	Rat IgG2a	Mouse FcεRII	1/10-1/50	Dr. Conrad	9, 11
2.4G2	Rat IgG1	Mouse FcγRII	1/20-1/50	Dr. Unkeless	22
MK-1	Rat IgG2a	Mouse FDC, ATC HEV etc.	1/10-1/50		a
R35-92	Rat IgG1	Mouse IgE	1/50	PharMingen	
B1E3	Rat IgG1	Mouse IgE (Cε4)	1/50	Dr. Conrad	23
23G3	Rat IgG1	Mouse IgE (Cε3)	1/20	Dr. Conrad	23
R1E4	Rat IgG2a	Mouse IgE (Cε3)	1/20	Dr. Conrad	23
Mac-2	Rat IgG2a	Subsets of mouse macrophages	1/10-1/20		24
53-2.1	Rat IgG2a	Mouse Thy-1.2 Ag	1/50	PharMingen (Rm-Thy-1A)	25

^a Maeda, K., M. H. Kosco, G. F. Burton, A. K. Szakal, and J. G. Tew. A monoclonal antibody reactive with mouse follicular dendritic cells. Manuscript in preparation.

air drying. Localization of radiolabeled Ag in the section was detected by using Kodak's NTB3 emulsion. The emulsion was exposed for 1 wk at 4°C in boxes containing small packets of silica gel crystals to absorb moisture and then developed in D-19 developer at 19°C for 6 min, rinsed, and fixed in Kodak Fixer for 10 min at 19°C and allowed to dry at room temperature in a vertical position. Finally the sections were counterstained with methyl green and examined using light microscopy. The grains in the emulsion over the sections were most easily seen using a dark field condenser and the localization of the retained Ag was most apparent.

RESULTS

Distribution of Fc ϵ RII and IgE in mesenteric lymph nodes of Nb-infected mice. The major hypothesis to be tested was that infection with Nb would lead to high levels of Fc ϵ RII on mouse B cells and FDC. In normal mice some areas in the outer cortex of mesenteric lymph nodes could be found that were clearly Fc ϵ RII positive (Fig. 1a). However, most areas in the outer cortex were weak to negative and this was generally the case in other lymph nodes like the popliteal. The FDC in normal mice were not negative but Fc ϵ RII labeling was very weak compared with the most reactive B cells (Fig. 1a). Labeling of an adjacent section with 2.4G2 ((anti-Fc γ RII) that is present at very high levels on FDC) revealed the extent of the FDC-reticulum or network made up by the FDC and their processes. The next section in the series (Fig. 1c) was labeled with anti-IgE and it appeared that the immune complexes associated with the FDC-reticulum contained a detectable but low level of IgE.

By 2 wk after injecting Nb, a dramatic enlargement of the mesenteric lymph nodes was apparent and numerous well developed germinal centers were seen. Immunohistochemistry using B3B4 revealed that the Fc ϵ RII level had clearly increased. Numerous B cells in the outer cortex bore high levels of Fc ϵ RII but the light zone (FDC area) of the germinal center was still only weakly labeled (Fig. 2a). Despite the weak labeling of FDC with B3B4, 2.4G2 showed an intense reticular labeling in the light zone of these well developed germinal centers indicating FDC were present (Fig. 2b). The location of FDC was also confirmed using MK-1 that also labels FDC intensely (data not shown). Labeling of FDC in germinal centers with anti-IgE mAb was difficult suggesting that very little IgE immune complex was being retained (Fig. 2c). Nevertheless, IgE was present in the lymph node as indicated by the presence of numerous IgE producing plasma cells in the cortex and medulla (Fig. 2c). With time the intensity

of Fc ϵ RII labeling on FDC increased and by 26 days after infection the FDC were clearly labeled (locate FDC area in Fig. 3b and compare with Fig. 3a). This coincided with an increase in IgE localization on FDC (Fig. 3c). In popliteal, brachial, axillary, inguinal, and cervical lymph nodes of Nb-infected mice, only a few small germinal centers and primary follicles were observed. In these follicles FDC were only weakly labeled with B3B4 and very little if any IgE was localized to the FDC.

Distribution of Fc ϵ RII and IgE in draining lymph nodes of mice actively immunized using CFA. As a control to be contrasted with the IgE-dominated response induced by Nb, mice were immunized with OVA or HSA in CFA to elicit an IgG-dominated response. Indeed levels of albumin-specific IgG were in the range of 1 to 3 mg/ml serum. The serum IgE levels were also slightly elevated in CFA immune mice—490 ng IgE/ml average in normal mice vs 800 ng IgE/ml average in CFA-immunized mice. In contrast, Nb infection elicits 10 to 100 times these background levels of serum IgE. The draining popliteal lymph nodes from these animals immunized in the hind feet with CFA displayed several (3 to 7), well-developed germinal centers in their cortical areas. Surprisingly, histochemistry with B3B4 revealed an intense reticular labeling in the light zone of the germinal centers indicating that high levels of Fc ϵ RII were present on FDC (Fig. 4a). This interpretation was supported by the fact that the labeling pattern corresponded with the 2.4G2 pattern and an obvious localization of IgE (Fig. 4, b and c). In contrast, the small lymphocytes in the mantle zone and interfollicular areas were only weakly labeled and B lymphocytes in the dark zones of germinal centers appeared negative. This pattern was present from day 3 to 9 and even 4 mo after challenge the same pattern was still apparent although the intensity of FDC labeling was reduced.

Four different mAb against mouse IgE (R35-92, B1E3, 23G3, and R1E4) were used and all antibodies revealed the same FDC localization pattern that corresponded to labeling with B3B4. In addition to FDC, many scattered plasma cells in the medulla of draining lymph nodes were labeled indicating production of IgE (Fig. 3c). The reactivity with R1E4 was especially interesting because it binds an epitope on IgE that would not be available if it were bound to Fc ϵ RII. Apparently, the IgE is part of the immune complex mass retained by the FDC but not all IgE is

Figure 1. Fc ϵ RII, Fc γ RII, and IgE on cells in the follicles of a normal mesenteric lymph node. These light micrographs show the immunoreactivity of B3B4 (a), 2.4G2 (b), and anti-mouse IgE (c) in serial sections. Note the B3B4 labeling for Fc ϵ RII on the small lymphocytes located in the mantle zone and interfollicular areas and the very faint reticular reactivity in the FDC area (light zone of germinal center (GC) indicated by arrows). In contrast, 2.4G2 labels Fc γ RII on FDC intensely (arrows) in b. IgE in c was very faint on cells in the FDC area of the germinal center (arrows). However, IgE was localized to some plasma cells, generally outside the follicles (arrowheads), indicating that the IgE-specific label was working. This example is typical of follicles in three animals. (Indirect immunoperoxidase staining, counterstained by methyl-green, a, b, and c, X130.)

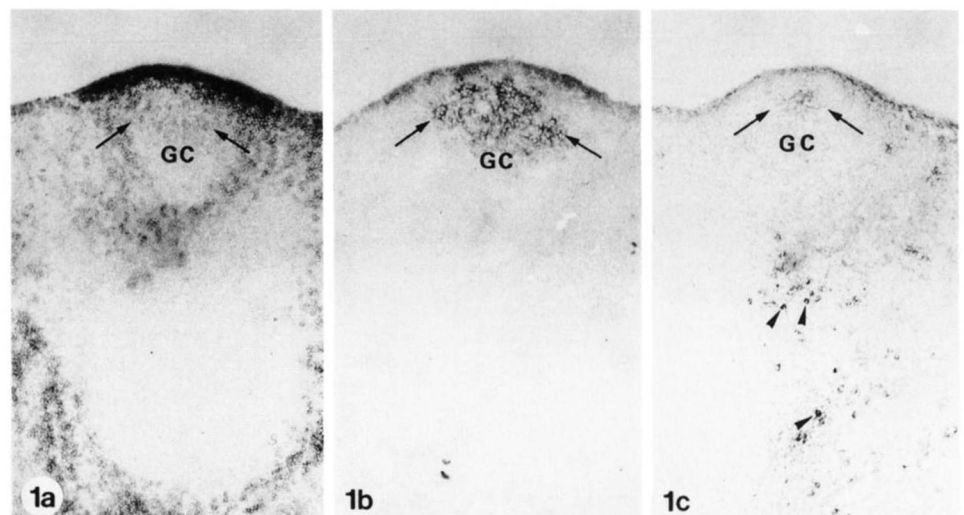


Figure 2. Fc ϵ RII, Fc γ RII, and IgE on cells in the follicles of the mesenteric lymph node of a mouse 14 days after infection with Nb. The light micrographs show the typical immunoreactivity of B3B4 with Fc ϵ RII (a), 2.4G2 reactive with Fc γ RII (b), and anti-mouse IgE (c) in serial sections. Note the intense B3B4 labeling of the small lymphocytes located in the mantle zone and inter-follicular areas but not in the "FDC" area in the light zone of the germinal center (indicated by arrows). In contrast, 2.4G2 intensely labels FDC (arrows) in b. IgE in c was very faint on cells in the FDC area of the germinal center (arrows). However, IgE was localized to numerous plasma cells, generally outside the follicle (arrowheads), indicating that the IgE specific label was working. This example is typical of follicles from four animals. (a, b, and c, X120.)

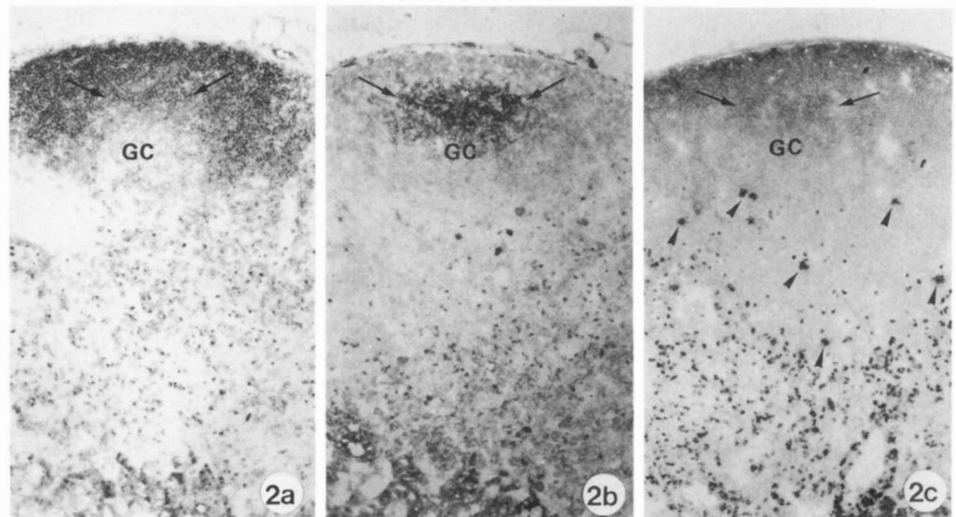


Figure 3. Fc ϵ RII, Fc γ RII, and IgE on cells in the follicles of the mesenteric lymph node of a mouse 26 days after infection with Nb. The light micrographs show the typical immunoreactivity of B3B4 for Fc ϵ RII (a), 2.4G2 reactive with Fc γ RII (b), and anti-mouse IgE (c) in serial sections. Note the intense labeling of the small lymphocytes located in the mantle zone in a. However, as indicated by the localization of FDC in b by the Fc γ RII labeling, the FDC in a bear Fc ϵ RII. IgE is now clearly localized to the FDC area (arrows) as well as to a number of scattered plasma cells that are generally outside the follicle (arrowheads). This example is typical of three animals. (a, b, and c, X100.)

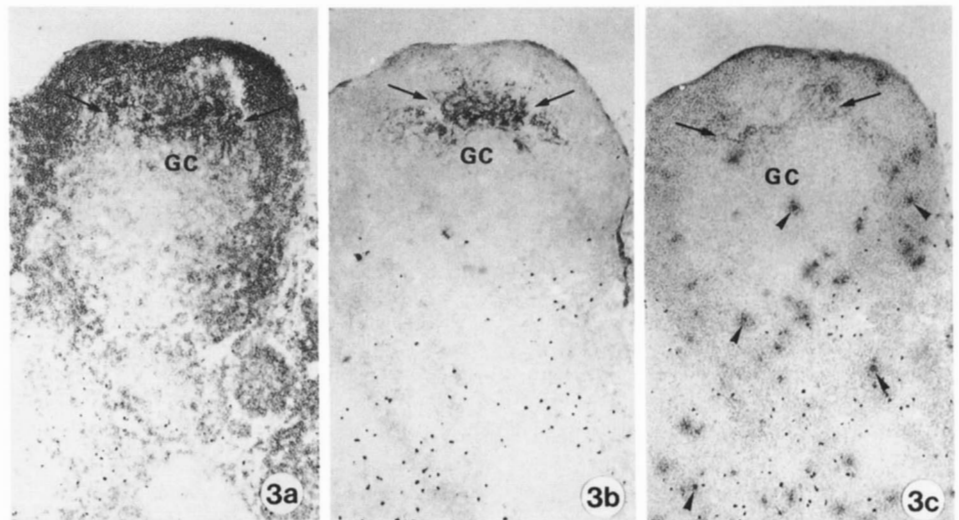
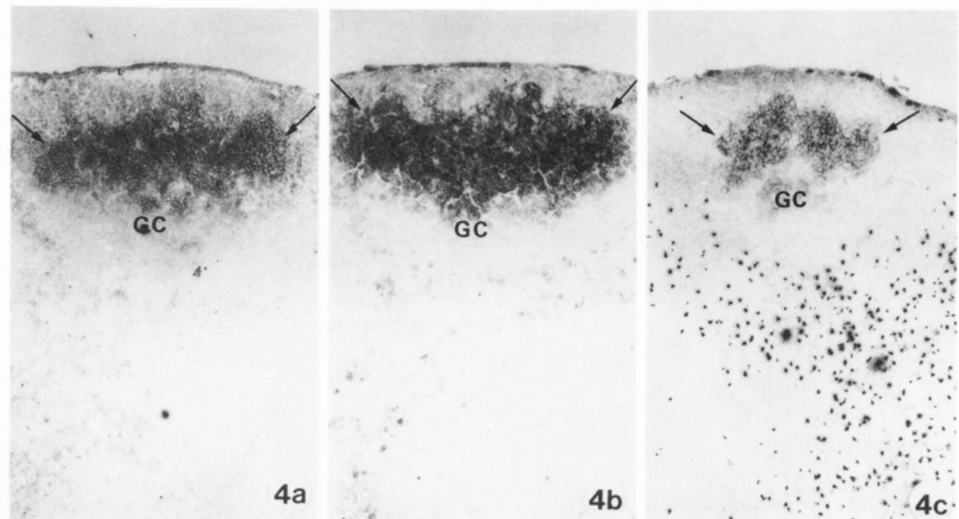


Figure 4. Fc ϵ RII, Fc γ RII, and IgE on cells in the follicles of the draining popliteal lymph node of an actively immunized mouse 9 days after secondary challenge with HSA. The light micrographs show the typical immunoreactivity of B3B4 for Fc ϵ RII (a), 2.4G2 for Fc γ RII (b) and anti-mouse IgE (c) in serial sections of these animals immunized using CFA. Note the intense B3B4 labelling in the FDC area of a (arrows) and the relatively lighter labeling on the small lymphocytes surrounding the FDC area. Distinct IgE accumulations are apparent in the FDC area (arrows) and several scattered plasma cells were also seen outside the follicle (arrowheads) (c). This example is typical of follicles from 12 animals. (a, b, and c, X80.)

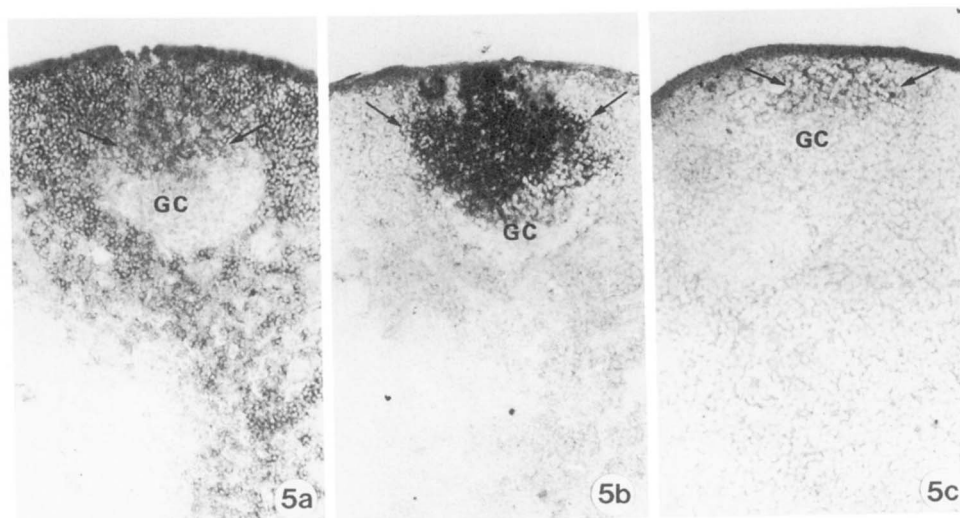


associated with FcR.

Active immunization with CFA appeared to increase the typical background level of Fc ϵ RII on FDC in the nondraining mesenteric lymph nodes (Fig. 5). The B cells in most follicles contained comparatively low levels of Fc ϵ RII. However, the B cells a few follicles of the mesenteric lymph nodes contained relatively high levels of Fc ϵ RII and the follicle shown in Figure 5 was selected to

illustrate that relatively high levels of Fc ϵ RII can be on FDC and B cells in the same follicle at the same time. Nevertheless, the general lack of correlation between the Fc ϵ RII on B cells and FDC suggests that the mechanism for inducing this receptor is probably different in the two cell types. IgE was also localized on the FDC in these mesenteric lymph nodes although relatively few IgE producing plasma cells were present.

Figure 5. FcεRII, FcγRII, and IgE on cells in the follicles of the nondraining mesenteric lymph node of an actively immunized mouse 9 days after secondary challenge with HSA. The light micrographs show the typical immunoreactivity of B3B4 (a), 2.4G2 (b) and anti-mouse IgE (c) in serial sections. Note the B3B4 labeling of the small lymphocytes located in the mantle zone. The FDC also appeared to bear FcεRII but bear much less than the B cells (a, b, and c, X120.)



Distribution of FcεRII and IgE in lymph nodes of passively immunized mice. To determine if the presence of immune complexes alone would up-regulate FcεRII, mice were passively immunized and challenged with HSA. Histochemistry revealed that the FcεRII level on FDC in the draining lymph nodes of these mice was indistinguishable from the normal controls. It would appear that memory cells with their lymphokine producing potential are involved in the up-regulation of FcεRII on FDC.

Follicular localization of Ag in mice passively immunized with IgE mAb. IgE localization always correlated with FDC-bearing FcεRII and this result is consistent with the concept that FcεRII may play a major role in binding and retaining IgE immune complexes. However, specific IgG was present in the systems studied and we reasoned that the actual Ag retention by the FDC might be mediated by IgG. The IgE was clearly a part of the immune complex but the FDC may not bind IgE Fc or such binding could simply be of no significance in the retention of the complex. To check this more rigorously, we passively immunized mice with varying amounts of the IgE mAb reactive with the hapten TNP. TNP-derivitized protein was injected but little if any Ag retention was apparent on FDC. Our major concern was whether we had enough IgE in the draining lymph node to maintain Ag on FDC over the 3-day period we allowed to get clearance of Ag from the macrophages. To circumvent this problem we injected the hybridoma cells to get a high level of IgE circulating throughout the animals. Then 7 to 10 days later, ^{125}I -labeled TNP HSA was injected into the hind foot pads and 3 days later the draining popliteal lymph nodes were collected, sectioned, and set up for autoradiography. Four of six animals primed by injecting CFA to get expression of FcεRII on FDC had clear and convincing localization of TNP-HSA when given the hybridoma cells (Fig. 6a). In contrast, none of the 14 controls run in this experiment, or a large number of animals used in previous experiments when IgE was simply passively transferred, showed localization (Fig. 6b). The controls for this study included: six animals injected with ^{125}I -HSA not conjugated to hapten, two animals given the hybridoma but not given CFA to induce FcεRII on FDC, two CFA-treated animals not given the hybridoma cells, and four animals receiving injections with B3B4 before Ag to block binding of the immune complex.

Electron microscopy. To confirm that it was indeed the FDC that bore FcεRII, the reactivity of B3B4 was examined at the ultrastructural level. Draining popliteal lymph nodes were taken 7 and 9 days after antigenic challenge of HSA immune mice. Morphologically a typical FDC can be recognized by its indented or lobated euchromatic nucleus, prominent nucleolus, scanty cytoplasm with scattered mitochondria and highly convoluted dendritic processes (Fig. 7). In the central portion of the light zone of the germinal center, B3B4 labeled the cell membranes of FDC intensely and the well developed convoluted processes were most intensely labeled (Fig. 7). The surrounding germinal center lymphocytes appeared to be negative. In contrast, at the peripheral portion of the light zone, FDC were not labeled around the cell body but the cytoplasmic processes that retain immune complexes were still labeled (Fig. 8a). A lymphocyte with the typical B3B4 labeling around the cell body is included for comparison (Fig. 8b).

DISCUSSION

The hypothesis that mouse FDC bear FcεRII and that Nb infection leads to up-regulation of mouse FcεRII was supported by the results reported here. However, the increased level of FcεRII on FDC occurred late in the response elicited by Nb and did not correlate with FcεRII levels on B cells. The most remarkable finding in the present study was the unanticipated high level of FcεRII on FDC in draining lymph nodes of mice immunized with CFA for an IgG response and the contrast with B cells which displayed very little FcεRII. Clearly both mouse B cells and FDC can display different levels of FcεRII and the regulation of FcεRII levels was very different in the two cells. Presumably, IL-4 was produced in the draining mesenteric lymph nodes of Nb-infected mice and helped induced expression of the FcεRII by the B cells. Work using this Nb model has shown that IL-4 production by cells in the mesenteric lymph node peaks at 8 days after infection and IL-4 production continues at day 11 but by day 14 and beyond, IL-4 production is at background levels (16). The increased level of FcεRII on B cells by day 14 is consistent with the production of IL-4 a few days earlier. In contrast, the late increased level of FcεRII on FDC is not easy to explain if IL-4 alone were the inducer. Clearly, the regulatory events that led to the high levels of FcεRII on mouse B cells by day 14 did not result in high

Figure 6. Follicular localization of IgE immune complexes in lymph nodes of mice with high levels of Fc ϵ RII on FDC. These autoradiographs were obtained using sections of popliteal lymph nodes from CFA immune mice injected with ^{125} I-TNP HSA after passive immunization with a TNP-reactive IgE mAb. The grains in the emulsion and the follicular localization were more easily seen using dark field than bright field microscopy. Note the localization of the radiolabeled Ag within follicles in *a* and the lack of follicular localization in *b*. Both animals were CFA immunized and injected with ^{125} I-TNP-HSA but the animal used in *b* was injected with B3B4 to block the Fc ϵ RII receptor 2 h before challenge with the radioactive TNP-HSA. These results are typical of animals exhibiting follicular localization of Ag and controls lacking IgE mediated Ag localization. (*a* and *b*, X150.)

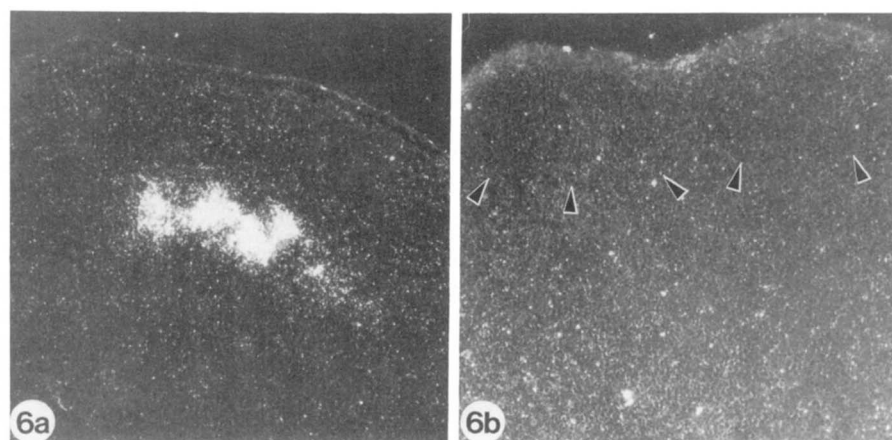
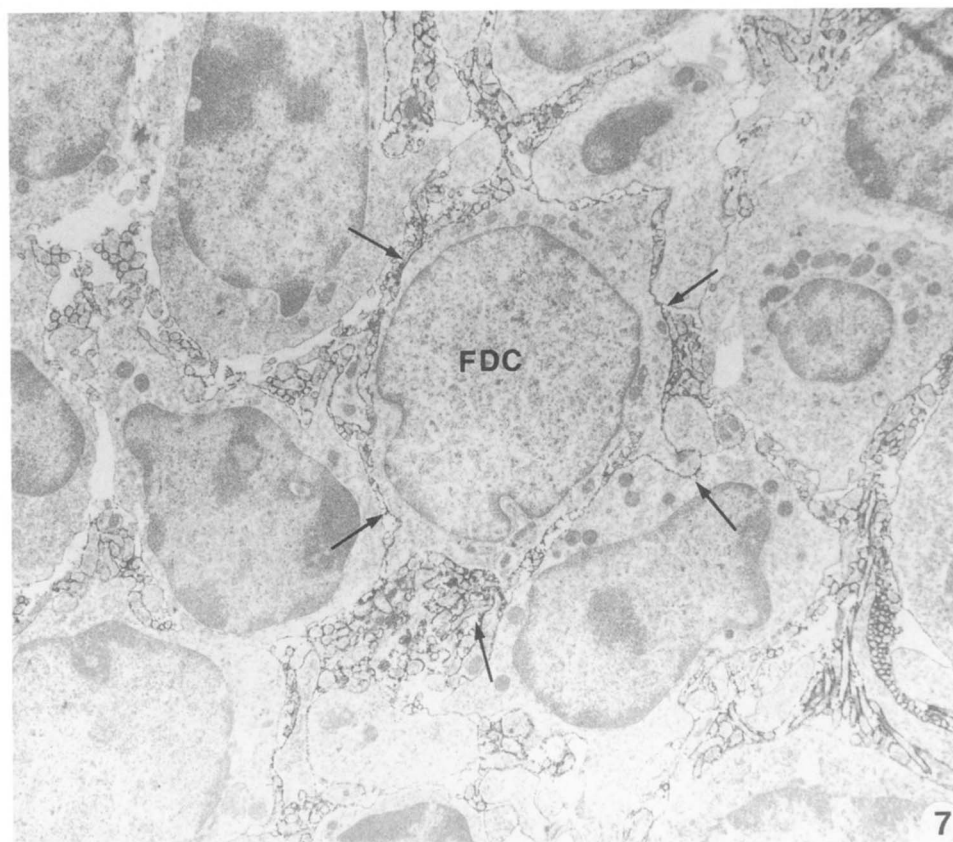


Figure 7. An electron micrograph demonstrating B3B4 labeling in the germinal center of a draining lymph node from an HSA actively immune mouse 5 days after booster immunization. An FDC cell body is apparent with the typical euchromatic nucleus and dendritic process extending among surrounding lymphocytes. The electron-dense (black) labeling on the FDC plasma membrane indicates the binding of B3B4 (arrows). The free surfaces of the lymphocytes in the area appear negative. (Non counterstained, X8300.)

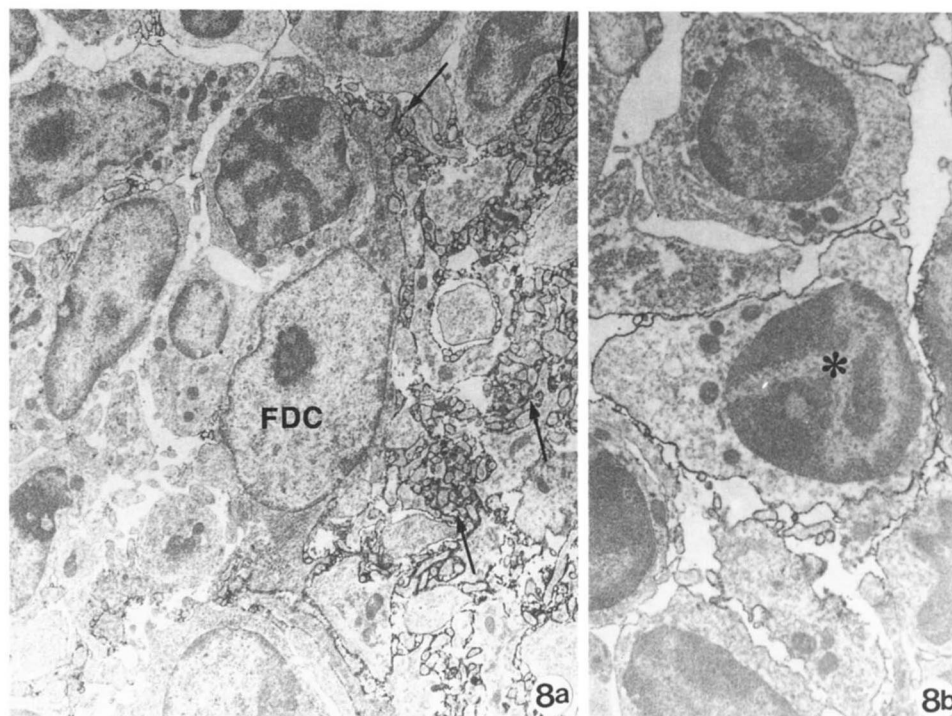


levels of Fc ϵ RII on FDC in the same area. Conversely, in mice immunized with CFA the FDC had remarkable levels of Fc ϵ RII although the B cells had low to undetectable levels of Fc ϵ RII.

The correlation between FDC-associated IgE and the presence of Fc ϵ RII was striking and consistent with the concept that Fc ϵ RII may be important in retaining immune complexes in IgE responses. Furthermore, the experiments using the IgE mAb reactive with TNP indicate that IgE immune complexes can be both trapped and retained by Fc ϵ RII-bearing FDC (Fig. 6). The reason why we were unable to find IgE immune complexes localized on two of the six animals in the experimental group is unknown but may relate to the Ag-antibody ratio of the immune complexes formed *in vivo*. This ratio would depend on the growth and secretion of IgE by the hybridoma and would probably have been different in each of the six animals. Retention of IgG immune complexes is facilitated by complement activation and the deposition of

C3b in the immune complexes (28). We would not expect IgE immune complexes to fix complement and to generate C3b to assist in FDC-mediated immune complex trapping and retention. Therefore, trapping and retention of IgE immune complexes by FDC may be more difficult and less consistent than the trapping and retention of IgG immune complexes. IgE immune complex retention appears to involve other features of the FDC beyond the simple presence of Fc ϵ RII. This comment is prompted by the observation that IgE immune complexes were clearly retained on FDC but were not apparent on B cells with similar or even greater levels of Fc ϵ RII. The results with RIE4 indicate that not all of the IgE molecules in the FDC-associated immune complexes were associated with Fc ϵ RII receptors. This conclusion is based on the specificity of RIE4 for an epitope of IgE that would not be available if all IgE were bound to Fc ϵ RII. It is possible that the IgE in the retained immune complex could provide a signal for the FDC to express more Fc ϵ RII because IgE

Figure 8. An electron micrograph demonstrating B3B4 labeling of an FDC located in the peripheral portion of the germinal center (a) and a lymphocyte located in the mantle zone (b). The FDC has the typical morphology but the reactivity with B3B4 is only apparent on the dendritic processes emanating toward the center of the germinal center (arrows). No distinct labeling is seen on the peripheral side of the FDC cell body. Compare the FDC labeling with labeling on the entire surface of the lymphoid cell (*, b). (Noncounterstained. a, X6,000; b, X11,000.)



immune complexes are known to upregulate FcεRII on other cells (29). It may also be possible that FcεRII on FDC may help stimulate B cells (2) and this may be more important than its role in the retention of IgE immune complexes.

FDC and B cells were the only cell types obviously recognized by the FcεRII-specific B3B4 mAb in murine lymphoid tissues. However, the existence of a small number of T cells, macrophages, or other cells bearing FcεRII has not been ruled out. Clearly a variety of human cell types bear FcεRII and there are reports of FcεRII on other cells in the mouse (1, 3, 7). These differences may relate to species differences and/or a possible heterogeneity among FcεRII molecules.

Ag retained on FDC is associated with the long term maintenance of B memory (30) and serum IgG antibody levels (31–33). The importance of IgE present in FDC-associated immune complexes has not been established. However, the presence of IgE in the immune complexes does raise the possibility that IgE on FDC may be related to the maintenance of the IgE response. Clearly IgE is hypersecreted in the mesenteric lymph nodes of Nb-infected mice about 2 wk after infection (16) and this occurred in the present study in the absence of increased levels of FcεRII and large amounts of IgE containing immune complexes on the FDC. Later in the response when FDC acquired more FcεRII and IgE, local IgE production was still taking place but at a much lower level. Thus the FcεRII and Ag in the IgE immune complex on FDC probably had little to do with the initial induction of the IgE response but could have been very important in the induction of B memory cells and the long term maintenance of the IgE response. This concept is supported by the known association between the long term maintenance of specific IgG in serum and the long term retention of specific Ag in the immune complexes on FDC (31–33). Most clinical problems associated with IgE hypersensitivity are encountered long after the initial sensitization. Therefore, if FDC are important in the main-

tenance of the specific IgE response, then FDC and the associated Ag are clearly clinically relevant. Interestingly, certain patients maintaining high levels of serum IgE are known to have high levels of IgE on FDC (14). A better understanding of the role of FDC in the maintenance and regulation of IgE responses in vivo may offer insight leading to new approaches to the management of IgE-mediated hypersensitivity.

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