The Journal of Immunology

RESEARCH ARTICLE | MAY 15 2008

A Novel B Cell-Mediated Transport of IgE-Immune Complexes to the Follicle of the Spleen[1] FREE

Fredrik Hjelm; ... et. al

J Immunol (2008) 180 (10): 6604–6610.
https://doi.org/10.4049/jimmunol.180.10.6604

A Novel B Cell-Mediated Transport of IgE-Immune Complexes to the Follicle of the Spleen[1]

Fredrik Hjelm,* Mikael C. I. Karlsson,† and Birgitta Heyman[2]*

Ag administered i.v. to mice along with specific IgE or IgG2a induces higher Ab- and CD4$^+$ T cell responses than Ag administered alone. The IgE effect is completely dependent on the low-affinity receptor for IgE, CD23, whereas the IgG2a effect depends on activating FcγRs. In vitro studies suggest that IgE/Ag is presented more efficiently than Ag alone to CD4$^+$ T cells by CD23$^+$ B cells and that IgG2a/Ag is presented by FcγR$^+$ dendritic cells (DCs). In this study, we investigate in vivo the early events leading to IgE- and IgG2a-mediated enhancement of immune responses. OVA administered i.v. in PBS in combination with specific IgE binds circulating B cells after 5 min and is found in B cell follicles bound to follicular B cells (CD23^{high}) after 30 min. This novel B cell-dependent route of entry is specific for IgE because IgG2a-Ag complexes were trapped in the marginal zone. OVA-specific CD4$^+$ T cells were found at the T-B border in the T cell zones 12 h after immunization both with IgE/OVA or IgG2a/OVA and proliferated vigorously after 3 days. The findings suggest that IgE- and IgG2a-immune complexes are efficient stimulators of early CD4$^+$ T cell responses and that Ag bound to IgE has a specific route for transportation into follicles. ***The Journal of Immunology,*** **2008, 180: 6604–6610.**

Antibodies feedback regulate the Ab response to the Ag with which they form an immune complex (1). A well-known example of this is the ability of IgG to completely suppress the response to large particulate Ags like erythrocytes (2). The suppressive capacity of IgG is used successfully in the clinic to prevent rhesus D-negative women from becoming immunized against fetal rhesus D-positive erythrocytes transferred via transplacental hemorrhage (3, 4). IgG Abs have dual roles in feedback regulation and can also enhance Ab responses. An important factor in this decision appears to be whether the Ag is a large particle, which usually results in suppression, or whether it is a soluble protein, in which case IgG preferentially enhances the Ab responses (5). Enhancement by IgG3 is dependent on C (6) whereas IgG1-, IgG2a-, and IgG2b-mediated enhancement relies on activating FcγRs expressed on a bone marrow-derived cell (7, 8). The inhibitory receptor for IgG, FcγRIIB, exerts a negative influence on enhancement mediated by IgG1, IgG2a, or IgG2b as demonstrated by the "superenhancement" caused by these isotypes in mice lacking FcγRIIB (7, 9). IgG enhances not only primary Ab responses, but also secondary Ab responses (10, 11), the formation of germinal centers (GCs)[3] (9, 12), somatic hypermutation (13), and proliferation of specific CD4$^+$ T cells (9).

A more recently discovered feedback mechanism is the ability of specific IgE to cause a several 100-fold up-regulation of the response to soluble protein Ags administered in vivo to mice (14–16). The enhanced Ab response takes place in the absence of adjuvants and is constrained to antigenic determinants within the Ab-Ag complex (14, 15). IgE administered with Ag enhances the production of specific primary IgG1, IgG2a, IgM, as well as IgE (15, 17) and also induces a more efficient immunological memory than Ag alone (15). The IgG response peaks as early as 6 days after a primary immunization and remains high for several weeks (17). IgE-mediated enhancement is abrogated in mice lacking the low-affinity receptor for IgE (FcεRII, CD23) or when CD23 is blocked by CD23-specific mAbs (14–18), thus demonstrating the complete dependence on CD23. Splenic OVA-specific T cells proliferate vigorously in vivo after immunization with IgE anti-2,4,6-trinitrophenyl (TNP)/OVA-TNP complexes and the T cell proliferation is followed by increased production of OVA-specific IgG (19). In mice, CD23 expression has been demonstrated on mature B cells (20, 21), where it is lost after activation and switching (21), on follicular dendritic cells (FDCs) (22), and on enterocytes, where the recently described CD23b isoform mediates transepithelial transport of Ag (23). In addition to playing a role in IgE-mediated enhancement of T and B cell responses, CD23 mediates negative regulation of IgE and IgG1 responses against Ags administered in alum (24). Interestingly, the latter effect seems to operate independently of IgE and is clearly distinct from IgE-mediated enhancement of Ab responses (25).

Several observations suggest that CD23$^+$ B cells are the effector cells in IgE-mediated enhancement of Ab and T cell responses in vivo. Studies in chimeric mice showed that CD23 had to be expressed on bone marrow-derived cells (i.e., B cells), but not on FDCs, in order for IgE to enhance Ab responses (18) and isolated CD23$^+$ B cells, transferred to CD23$^{-/-}$ mice, rescued the ability of IgE to enhance Ab and T cell responses in these animals (19). It has been shown in vitro that CD23$^+$ human and murine B cells can capture IgE/Ag via CD23, endocytose and present Ag to specific T cells (25–29), and one hypothesis is that this mechanism explains IgE-mediated enhancement.

*Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden; and †Department of Medicine, Karolinska Institute, Clinical Allergy Research Unit, Stockholm, Sweden

Received for publication November 13, 2007. Accepted for publication March 3, 2008.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by Uppsala University, the Swedish Research Council, Ellen, Walter and Lennart Hesselman's Foundation, Hans von Kantzow's Foundation, King Gustaf V's 80 Years Foundation, and Ollie and Elof Ericsson's Foundation.

[2] Address correspondence and reprint requests to Dr. Birgitta Heyman, Department of Medical Biochemistry and Microbiology, Uppsala University, SE-75123, Uppsala, Sweden. E-mail address: birgitta.heyman@imbim.uu.se

[3] Abbreviations used in this paper: GC, germinal center; TNP, 2,4,6-trinitrophenyl; FDC, follicular dendritic cell; DC, dendritic cell; HEL, hen egg lysozyme; FO, follicular; MZ, marginal zone; PNA, peanut agglutinin.

An important question in the initiation of Ab responses is how Ag gains access to the B cell follicles of secondary lymphoid organs (reviewed in Ref. 30). Data suggest that neither soluble Ag nor Ag in the form of immune complexes have free access to the follicles (31, 32), and the exact mechanisms for transport of Ag to follicles are unknown. In the lymph nodes, follicular (FO) B cells may acquire Ag indirectly from DCs having bound Ag via FcγRIIB (33, 34) or via direct diffusion of soluble Ag from the subcapsular sinus (35). Specific B cells, located in the follicle-subcapsular sinus boundary of the lymph nodes, also seem to be able to transport Ag into the follicle (36). In addition, macrophages in the subcapsular sinus of lymph nodes can capture IgG-immune complexes and deliver these to the follicle where B cells in a complement-receptor 1/2 (CD35/CD21)-dependent manner transport them to FDCs (37).

In the spleen, blood-borne Ag arrives in the marginal zone (MZ) and can be transported into follicles by poorly defined myeloid Ag transport cells (38–40) or, if complexed to IgM and C, by MZ B cells (41–43). MZ B cells are only found in the spleen and in a semiactivated state remain stationary in the MZ (44). Because they do not recirculate, they will not encounter Ag until it is present in the MZ. In contrast, mature B cells continuously recirculate from the blood to secondary lymphoid organs and back again and have access to follicles. Since these cells express high levels of CD23, we hypothesized that they could bind IgE/Ag and, on their passage through the white pulp of the spleen, deliver Ag into splenic follicles. We followed localization of Ag as well as Ag-specific $CD4^+$ T cells in the spleen during the first hours after immunization with IgE/Ag and IgG2a/Ag as well as the ensuing proliferation of specific T cells, development of GCs, and production of specific Abs.

Materials and Methods

Mice

BALB/c mice were obtained from Bommice. $CD23^{-/-}$ mice (16) were backcrossed to BALB/c for 10 generations (19). DO11.10 mice, carrying rearranged transgenic TCRα and TCRβ genes encoding a TCR specific for OVA peptide 323–339 bound to $I\text{-}A^d$ class II molecules (45), backcrossed to BALB/c for >15 generations, were obtained from Dr. L. Westerberg (Karolinska Institute, Stockholm, Sweden). Animals were bred and maintained in the animal facilities at the National Veterinary Institute (Uppsala, Sweden) and were matched for age and sex within each experiment. All experiments were approved by the local ethical committee.

Antigens

OVA and TNP (picrylsulfonic acid/hydrate) were obtained from Sigma-Aldrich. TNP was conjugated to OVA and stored as previously described (19). The number of TNP residues/OVA was determined (19) and a TNP:OVA ratio of 1.1 and 2 was used. OVA-TNP was biotinylated using 1.6 mg of sulfosuccinimidyl-6-(biotinamido)hexanoate (Sulfo-NHS-LC-Biotin; Pierce) per 10 mg of OVA-TNP according to the manufacturer's recommendations. The reaction took place at room temperature for 30 min and free biotin was eliminated by passage over a PD-10 column (GE Healthcare). Fractions containing the highest biotin-OVA-TNP concentration (determined by absorbance at 280 nm) were sterile filtered and stored at 4°C until use.

Antibodies

Monoclonal IgE anti-TNP (IGELb4) (46) and IgG2a anti-TNP (C4007B4, 7B4) (11) were purified and stored as previously described (9, 19). For flow cytometry and immunostainings, the following mAbs from BD Pharmingen were used: PE-labeled anti-CD4 (GK1.5), PE- or FITC-labeled anti-CD23 (B3B4), FITC- or PE-labeled anti-CD45R/B220 (RA3-6B2), FITC-labeled anti-CD19 (1D3), allophycocyanin- or R-PE-labeled streptavidin, and PE-labeled anti-CD21 (7G6). The DO11.10-transgenic TCR was detected with a FITC- or biotin-labeled KJ1-26 mAb (Caltag Laboratories) specific for this particular TCR heterodimer.

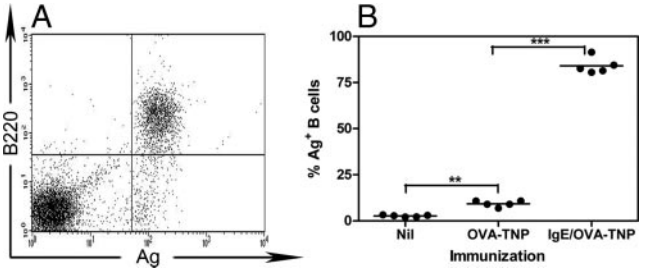

FIGURE 1. IgE/Ag is found on B cells in peripheral blood early after immunization. BALB/c mice were immunized with 50 μg of IgE anti-TNP and 150 μg of biotinylated OVA-TNP or with biotinylated OVA-TNP alone. The percentage of cells staining positive for Ag among $B220^+$ B cells in peripheral blood 5 min after immunization (*A* and *B*) was determined in flow cytometry. A representative of two experiments is shown.

Immunizations

Mice were immunized in the tail veins with 20 μg of OVA-TNP in 0.1 ml of PBS alone or 1 h after immunization with 50 μg of IgE anti-TNP or

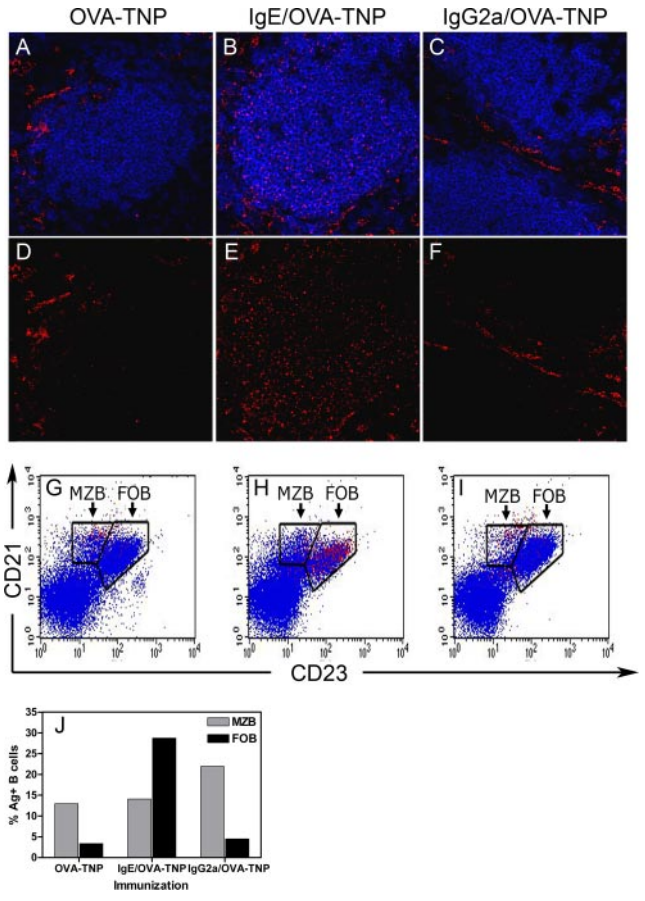

FIGURE 2. IgE/Ag is found primarily on FO B cells in the spleen. BALB/c mice were immunized with 50 μg of IgE anti-TNP or IgG2a anti-TNP and 150 μg of biotinylated OVA-TNP or with biotinylated OVA-TNP alone. Thirty minutes after immunization, half of each spleen was prepared for confocal microcopy (*A–F*; *D–F* shows the same images as *A–C*, but with the blue color subtracted; OVA-TNP is stained red and $B220^+$ B cells blue). The other half of the spleens was used to determine the percentage of Ag^+ cells within the MZ B and FO B cell populations, gated as indicated in *G–I* and as described in *Materials and Methods*. Ag^+ cells were defined as cells staining positive for streptavidin (red in *G–I*) which binds to the OVA-TNP-biotin used as Ag in the immunization. Data from *G* to *I* are summarized in *J*. Representative of four experiments (with a total of seven mice per immunization).

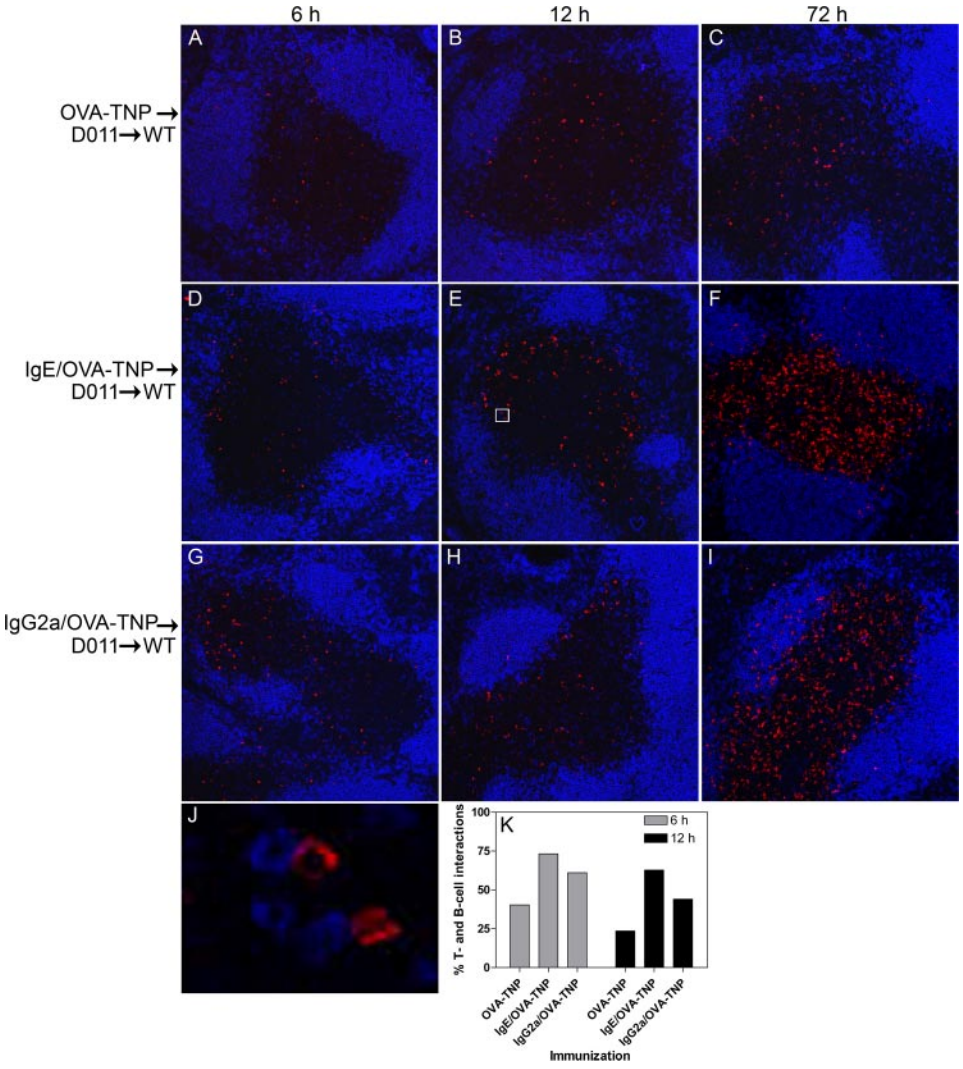

FIGURE 3. Ag-specific $CD4^+$ T cells localize to the edge of the T cell zone 12 h after immunization with IgE/Ag and IgG2a/Ag. BALB/c mice were adoptively transferred with 8×10^6 $CD4^+KJ1\text{-}26^+$ cells from DO11.10 mice. Twenty-four hours later, two mice per group were immunized with 50 μg of IgE anti-TNP and 20 μg of OVA-TNP, 50 μg of IgG2a anti-TNP and 20 μg of OVA-TNP, or OVA-TNP alone. The spleens were frozen, sectioned, and stained for $B220^+$ cells (blue) and $KJ1\text{-}26^+$ T cells (red) (*A–I*). A representative of nine (IgE) or two (IgG2a) experiments are shown. The number of $KJ1\text{-}26^+$ T cells in close contact with B cells (*J*) were counted and divided with the total number of $KJ1\text{-}26^+$ T cells (*K*). WT, Wild type.

IgG2a anti-TNP i.v. in 0.1 ml of PBS. For localization of Ag and Ag^+ cells, 150 μg of biotinylated OVA-TNP in 0.1 ml of PBS was used.

Flow cytometry

Spleen cell suspensions were prepared and RBC were depleted by hypotonic lysis in 5 ml of ACK lysing buffer (0.15 M NH_4Cl (Merck), 1.0 mM $KHCO_3$ (Sigma-Aldrich), and 0.1 mM Na_2EDTA (Sigma); pH 7.3) for 2 min at room temperature. Cells were washed once in PBS, resuspended in 5 ml of PBS, and counted in a Bürker chamber (Marienfeld). Fluorescence staining was performed at 4°C in 100 μl of PBS containing $2–4 \times 10^6$ cells incubated with predetermined optimal amounts of labeled Ab. After 30 min, the cells were washed twice in PBS containing 1% BSA (Sigma-Aldrich) and 0.1% NaN_3 (Sigma-Aldrich). When biotinylated Ag or Abs were used, the procedure was repeated with fluorescently labeled streptavidin. One hundred fifty thousand events, acquired by gating on all cells with the forward and side scatter properties of lymphocytes, were collected on a FACSort or FACSCalibur flow cytometer from BD Biosciences. Data were analyzed using CellQuest version 3.3 software (BD Biosciences). FO B cells were defined as $CD23^{high}CD21^{low}$ and MZ B cells as $CD23^{low}CD21^{high}$ (47, 48).

Adoptive transfers

DO11.10 spleen cell suspensions were depleted of RBC by hypotonic lysis (see above) and suspensions containing $3–8 \times 10^6$ OVA-specific $CD4^+KJ1\text{-}26^+$ cells, determined by flow cytometry, were transferred i.v. to unirradiated recipients in 0.1 ml of PBS. In some experiments specified in the figures, $CD4^+$ cells from DO11.10 spleens were enriched by labeling with anti-CD4-conjugated magnetic beads (MACS anti-mouse CD4; Miltenyi Biotec). Labeled cells were collected using LS columns (Miltenyi Biotec) according to the manufacturer's instructions. Isolated cells were >97% $CD4^+$ and were transferred to recipients in 0.1 ml of PBS i.v. All mice were immunized the day after cell transfers.

ELISA

Mice were bled from the tail vein and sera were tested in ELISA for OVA-specific IgG as described previously (19).

Immunohistochemical staining for GCs

Spleens were flash frozen in OCT embedding medium (Miles) and 7-μm sections were cut using a cryostat, air dried, and fixed onto Super Frost plus glass slides (Mentzel-Gläser) in ice-cold 50% acetone for 30 s and then 100% acetone for 5 min. Sections were rehydrated and washed in an excess of PBS for 15 min, blocked with 0.1% BSA in PBS for 20 min, incubated at room temperature for 1 h with 1 μg/ml biotinylated peanut agglutinin (PNA; Sigma-Aldrich) and then washed in PBS for 10 min. PNA was detected with preformed avidin-biotin-coupled HRP (Vector Elite ABC; Vector Laboratories) and a diaminobenzidine substrate kit (Vector Laboratories). The reaction was followed in a microscope until desired staining was obtained. Slides were washed and counterstained with methyl green (Vector Laboratories) for 2 min at 65°C in ethanol (Kemetyl) followed by xylene (Pierce and Nordic Biolabs) and mounted in Pertex mounting medium (Cell HistoLab Products).

Immunofluorescence staining for confocal microscopy

Spleens were frozen as described above. Eight-micrometer-thick sections were air dried onto Super Frost plus glass slides for 1 h and then frozen at −70°C. The slides were fixed with 50% acetone for 30 s and then 100% acetone for 5 min. Sections were rehydrated for 15 min in PBS and blocked with 5% horse sera (SVA) in PBS for 15 min at room temperature. Excessive blocking solution was poured off. B cells were detected using 100

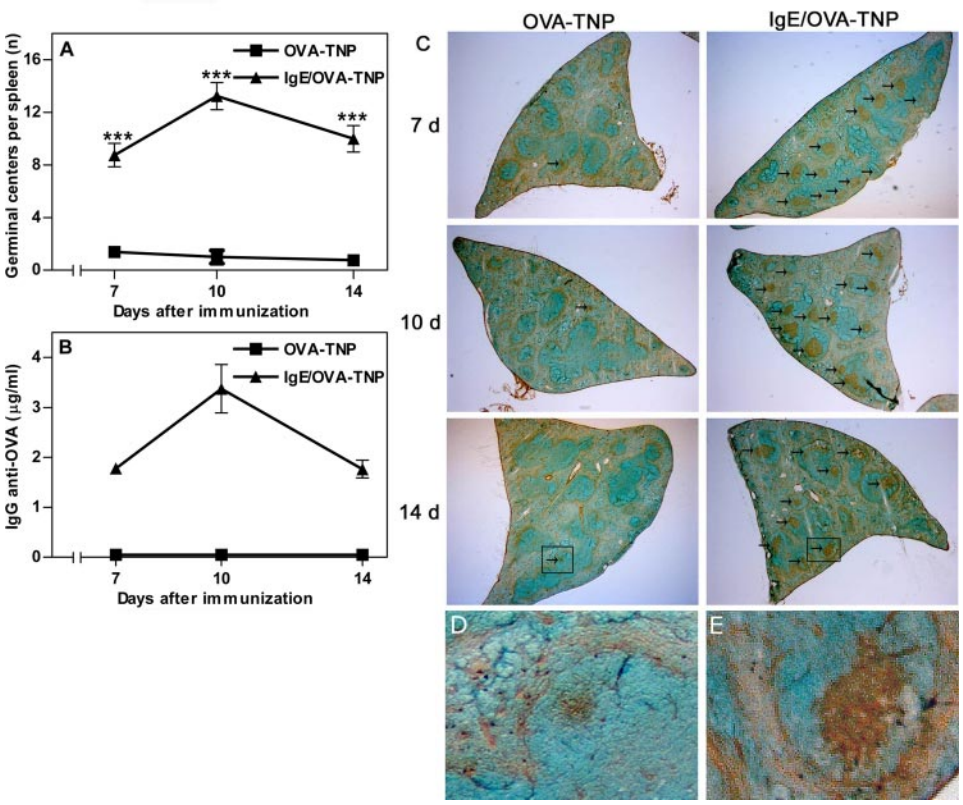

FIGURE 4. IgE enhances GC formation and Ab production. BALB/c mice were immunized with 50 μg of IgE anti-TNP and 20 μg of OVA-TNP or with 20 μg of OVA-TNP alone ($n = 2$). The mice were bled and sacrificed 7, 10, and 14 days later. Four nonconsecutive sections of each spleen were stained for GCs with PNA (brown). The average number of GCs per spleen (*A*) and spleen sections containing a typical number of GCs (*C–E*) are shown with arrows indicating GCs. Mice immunized with IgE anti-TNP alone or left unimmunized had similar numbers of GCs as mice immunized with OVA-TNP alone (data not shown), indicating that one to 2 GCs per spleen section represents background levels. IgG anti-OVA levels in serum were assayed in ELISA (*B*). A representative of two experiments is shown.

μl of FITC-labeled B220 (2 μg/ml) and DO11.10 CD4$^+$ cells with 100 μl biotinylated KJ1-26 mAb (0.4 μg/ml). Slides were washed twice in PBS for 5 min. One hundred microliters of allophycocyanin-labeled streptavidin (1.7 μg/ml) was added and slides were washed twice and mounted in Fluoromount G (Southern Biotechnology Associates).

Statistical analysis

Statistical differences between the control and the experimental groups were determined by Student's *t* test. The results were: NS, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; or ***, $p < 0.001$.

Results

IgE-Ag complexes are bound to circulating B cells after 5 min and are found on FO B cells in the splenic B cell follicles after 30 min

To determine whether IgE promotes binding of Ag to B cells in vivo, mice were immunized with biotinylated OVA-TNP alone or along with IgE anti-TNP. In the latter mice, >80% of the B220$^+$ cells in peripheral blood stained positive for Ag after 5 min (Fig. 1, *A* and *B*). To visualize the splenic localization of Ag, mice were immunized with biotinylated Ag alone, IgE/Ag, or IgG2a/Ag. Immune complexes containing IgG2a were included for comparison because IgG2a enhances T cell proliferation and Ab production in a similar manner as does IgE, but uses activating FcγRs instead of CD23 (9, 11). In IgE/Ag-immunized mice, essentially all Ag localized to the primary follicles within 30 min (Fig. 2, *B* and *E*). After 60 min, the amount of Ag in the follicles was maximal and after 4 h most of it had disappeared (data not shown). In mice given Ag alone or IgG2a-Ag complexes, the Ag remained outside the primary follicles at all times tested (30 min to 4 h; Fig. 2, *A*, *C*, *D*, and *F*, and data not shown). Analysis of single-cell suspensions, obtained from the other halves of these spleens 30 min after immunization, showed that IgE drastically increased the amount of Ag bound to FO B cells, whereas Ag administered alone or with IgG2a primarily bound to MZ B cells (Fig. 2, *G–J*). In summary, IgE increased binding of Ag to circulating B cells in peripheral blood within minutes. IgE also caused massive entry of Ag into the splenic B cell follicles, clearly detectable after 30 min. This way of entry to the follicle was specific for IgE, since IgG2a immune complexes instead got trapped in the MZ of the spleen.

Specific T cells localize to the periphery of the T cell zone 12 h after immunization with IgE/Ag or IgG2a/Ag and proliferate vigorously after 72 h

To study the subsequent effects of immune complexes on immune responses, BALB/c mice were adoptively transferred with CD4$^+$ T cells from DO11.10 mice and immunized with IgE/Ag, IgG2a/Ag, or Ag alone. Twelve hours after immunization with IgE/Ag or IgG2a/Ag, the OVA-specific T cells were found around the border to the primary follicle and few T cells remained in the center of the T cell zone (Fig. 3, *E* and *H*). This pattern was highly reproducible, observed in nine of nine experiments with IgE and in two of two with IgG2a. The number of OVA-specific T cells in close contact with B cells, analyzed from Fig. 3, *A*, *B*, *D*, *E*, *G*, and *H*, was increased in spleens where IgE or IgG2a was present (Fig. 3*K*). The *inset* (Fig. 3*J*) shows a typical example of T-B cell interactions. In agreement with previous data (9, 19), OVA-specific T cells proliferated vigorously 72 h after immunization with IgE/Ag (Fig. 3*F*) or IgG2a/Ag (Fig. 3*I*). No T cell proliferation was seen in CD23$^{-/-}$ recipients immunized with IgE/Ag (data not shown). These observations suggest that both IgE-Ag and IgG2a-Ag complexes have similar effects on the early stages of specific T cell activation, although achieving this via different mechanisms.

IgE/Ag induces a significant increase in GC formation and Ab production

Immunization with thymus-dependent Ags in adjuvant results in formation of GCs, known to be involved in affinity maturation and generation of memory B cells (49). Whether administration of

Table I. *Consecutive events during IgE-mediated enhancement of immune responses*

Time after First Immunization with IgE/Ag	Effect on the Immune Response	Fig./Ref.
5 min	Ag found on peripheral B cells	Fig. 1*B*
30 min	Ag in splenic B cell follicles on FO B cells	Fig. 2
6–12 h	Ag-specific $CD4^+$ T cells localize close to the T-B border in spleen	Fig. 3, *D* and *E*
3 days	Massive proliferation of Ag-specific $CD4^+$ T cells in the T cell zone	Fig. 3*F*/Ref. 19
7–14 days	Increase in size and number of GCs	Fig. 4, *A* and *C*
7–123 days	Enhanced first Ab response	Fig. 4*B*/Refs. 14–19 and 54)
138 days	Enhanced recall response 14 days after boost with Ag alone	Ref. 15

IgE-Ag complexes in physiological solutions without adjuvant also enhances the formation of GCs has not been studied previously. In spleens from the immunized mice, we found that IgE/Ag induced a 6-, 13-, and 13-fold increase in the numbers of GCs as compared with Ag alone 7, 10, and 14 days after immunization (Fig. 4, *A* and *C–E*). As expected, there was a concomitant increase in the OVA-specific IgG response (Fig. 4*B*). Thus, IgE enhanced the GC formation and promoted Ab production.

Discussion

Specific IgE, administered in small amounts along with soluble protein Ags, have dramatic consequences for the B and T cell responses to this Ag. We have here studied the early stages in the chain of events previously shown to result in a peak in the T cell proliferation on day 3 and enhanced primary and secondary Ab responses during many weeks following immunization (see Table I for summary). The most striking finding is the rapid appearance of Ag on FO B cells in splenic follicles where IgE/Ag was found 30 min after immunization while Ag administered alone or with IgG2a remained in the MZ (Fig. 2). The majority of peripheral B cells carried IgE/Ag on their surface 5 min after immunization (Fig. 1), and the most likely route of entry of IgE/Ag into follicles is as passengers on recirculating $CD23^+$ B cells which enter the white pulp via the MZ and then migrate to the B cell follicles. To our knowledge, there are no other reports of such a rapid transport of Ag into the B cell follicle as demonstrated here with IgE/Ag. When localization of IgG-Ag complexes in lymph nodes was studied, most of the Ag remained in the subcapsular sinus 2 h after immunization (37), and hen egg lysozyme (HEL) administered i. v. remained in the MZ of the spleen after 30 min (50). Ag complexed with IgM and C given i. v. was initially found on the surface of MZ B cells, bound to the C receptor CD21. After 1 h, still only small amounts of Ag were found in the B cell follicle and not until after 4 h had the MZ B cells deposited a significant amount of their Ag load onto FDCs (43). Because MZ B cells express high levels of CD21 and low levels of CD23, whereas the reverse is true for FO B cells (44), it seems logical that IgE-Ag and IgM-C-Ag complexes bind to different B cells. An intact C system is vital for a normal Ab response (51) and one of the explanations is increased deposition of Ag on FDCs in B cell follicles, both in spleen (43, 52, 53) and lymph nodes (37). Our observations indicate that there exists an additional route for transport of Ag into splenic B cell follicles using IgE and $CD23^+$ B cells. This may explain why mice lacking CD21, generally exhibiting poor Ab responses, respond perfectly well to IgE-Ag complexes (54). In this situation, C-dependent transport of Ag to follicles would not be a limiting factor because this role is taken over by IgE/CD23.

The next step in the chain of events leading to an immune response to IgE/Ag and IgG2a/Ag studied here is the early migration of specific $CD4^+$ T cells in the spleen. Others have shown that transgenic HEL-specific B cells adoptively transferred to wild-type mice moved to the splenic T-B boundary to interact with cognate T cells 6–8 h after immunization with HEL (50, 55). We found that the OVA-specific T cells in mice immunized with IgE/Ag or IgG2a/Ag, but not with Ag alone, had moved to the periphery of the T cell zone, close to the T-B boundary, 12 h after immunization (Fig. 3, *E* and *H*). This agrees well with the idea that these T cells have interacted with APCs in the T cell zone and received activation signals causing them to move toward the B cell zone to help Ag-specific B cells. After 72 h, the T cell zones in IgE/Ag- and IgG2a/Ag-immunized mice were filled with OVA-specific T cells (Fig. 3, *F* and *I*), previously shown to express activation markers and to have divided several times (19).

These findings show that both IgE-Ag and IgG2a-Ag complexes induce much more efficient proliferation of specific T cells than Ag alone. Although the T cell migration patterns (Fig. 3, *E* and *H*), the magnitude and kinetics of T cell proliferation (Fig. 3, *F* and *I*, and Refs. 9 and 19) as well as the ability to enhance Ab responses (9, 19) are similar after immunization with IgE- and IgG2a-complexed Ag; the two Ab classes clearly use different mechanisms to achieve these effects. As described previously, IgE is dependent on CD23 expressed on B cells (18, 19) and IgG2a requires activating FcγRs expressed on a bone marrow-derived cell other than the B cell (which lacks activating FcγRs) (7–9). Another difference, first described herein, is that IgG2a-Ag complexes are not transported as rapidly into the follicles as IgE-Ag complexes (Fig. 2, *B*, *C*, *E*, and *F*). A possible explanation for how they, despite this, can "catch up" with the IgE-Ag complexes in stimulating T cells is that DCs, which are the most likely effector cells involved in presentation of IgG2a/Ag, handle Ag more efficiently than B cells, which are the likely APCs in IgE-mediated enhancement.

What is then the explanation for the ability of IgE to, via $CD23^+$ B cells, induce potent Ab and T cell responses? The only murine hematopoietic cells expressing CD23 are B cells and FDCs (20–22). Since CD23 expression on B cells, but not on FDCs, is required for IgE-mediated enhancement (18, 19), a mechanism involving transfer of IgE/Ag from FO B cells to FDCs, as shown to take place with IgM- and IgG-immune complexes (37, 43), seems unlikely. The increased concentration of Ag in the follicles may in itself suffice to induce the enhanced immune responses observed. Close collaboration between B cells and DCs during the early phases of immune responses has been demonstrated in other systems (56) and DCs perhaps pick up Ag from FO B cells for subsequent presentation to T cells. The high concentration of Ag in the B cell follicles will also lead to increased chances for cognate B cells to encounter Ag and present it to T cells. In line with in vitro findings, it can also be hypothesized that the $CD23^+$ FO B cells, in addition to capturing and concentrating IgE/Ag in the follicles, internalize and present Ag to $CD4^+$ T cells. Human EBV-transformed B cells and murine splenic B cells, cultured with specific IgE, Ag, and specific T cells, induce enhanced T cell proliferation as compared with cells cultured with Ag alone (25–29). This

mechanism is CD23 dependent. Especially when using EBV-transformed B cell lines, which should be free of contaminating cell types, contribution of DCs to the T cell stimulation/Ag presentation is excluded (26–28). This, taken along with the fact that human B cells have been shown to internalize IgE/Ag via CD23 (57, 58), strongly suggest that $CD23^+$ B cells can indeed present IgE-Ag complexes to specific T cells in vitro. Whether this also takes place in vivo is more difficult to ascertain. The findings herein and in a previous report (19) are compatible with, but do not prove, this hypothesis. Apart from the in vitro parallel, an indication that Ag presentation explains IgE-mediated enhancement is that we detect an increased number of T-B cell interactions in the T cell zones of mice given IgE/Ag (Fig. 3, *G* and *H*). There is accumulating evidence from many laboratories that specific B cells play an important role in presenting Ag and activating $CD4^+$ T cells in vivo (50, 59–63). Notably, also unspecific B cells present Ag in vivo when the Ag, for example, is coupled to CpG (64) or targeted to CD19 on the B cell (65). Should the mechanism behind IgE-mediated enhancement of immune responses be enhanced Ag presentation to specific T cells by B cells, two interesting conclusions can be drawn. First, not only MZ B cells (50), but also FO B cells, are able to present Ag. Second, although the majority of the Ag-presenting B cells would be unspecific (because they capture Ag via CD23 and not via the B cell receptor), the ensuing enhanced Ab response is strictly Ag specific (14, 15), demonstrating that cognate interactions between $CD4^+$ T cells and B cells are required also in this situation.

The biological role of IgE-mediated enhancement of immune responses is enigmatic. Only extremely low concentrations of IgE are found in the serum of healthy individuals and these levels may not suffice to form the critical amount of immune complexes. It has however been hypothesized that this pathway plays a role in perpetuating allergies, when serum concentrations of allergen-specific IgE are high (66), and there is accumulating experimental evidence that the IgE/CD23-mediated allergen presentation indeed plays a role in atopic disease. Sera from allergic patients were shown to contain sufficient amounts of allergen-specific IgE to induce presentation of the allergen by $CD23^+$ EBV-transformed B cells in vitro (27, 67) and IgE/CD23-mediated allergen presentation is decreased in patients undergoing successful specific immunotherapy or anti-IgE treatment (67). Perhaps not only allergens, but also certain pathogens, induce high levels of specific IgE, which may then aid in the induction of an efficient protective immune response.

Disclosures

The authors have no financial conflict of interest.

References

1. Heyman, B. 2000. Regulation of antibody responses via antibodies, complement, and Fc receptors. *Annu. Rev. Immunol.* 18: 709–737.
2. Karlsson, M. C. I., S. Wernersson, T. Diaz de Ståhl, S. Gustavsson, and B. Heyman. 1999. Efficient IgG-mediated suppression of primary antibody responses in Fcγ receptor-deficient mice. *Proc. Natl. Acad. Sci. USA* 96: 2244–2249.
3. Clarke, C. A., W. T. A. Donohoe, J. C. Woodrow, R. Finn, J. R. Krevans, W. Kulke, D. Lehane, and P. M. Sheppard. 1963. Further experimental studies on the prevention of Rh haemolytic disease. *Br. Med. J.* 1: 979–984.
4. Bowman, J. M. 1988. The prevention of Rh immunization. *Transfus. Med. Rev.* 2: 129–150.
5. Enriques-Rincon, F., and G. G. B. Klaus. 1984. Differing effects of monoclonal anti-hapten antibodies on humoral responses to soluble or particulate antigens. *Immunology* 52: 129–136.
6. Diaz de Ståhl, T., J. Dahlström, M. C. Carroll, and B. Heyman. 2003. A role for complement in feedback-enhancement of antibody responses by IgG3. *J. Exp. Med.* 197: 1183–1190.
7. Wernersson, S., M. Karlsson, J. Dahlström, R. Mattsson, J. S. Verbeek, and B. Heyman. 1999. IgG-mediated enhancement of Ab responses is low in FcRγ chain deficient mice and increased in FcγRII deficient mice. *J. Immunol.* 163: 618–622.
8. Diaz de Ståhl, T., and B. Heyman. 2001. IgG2a-mediated enhancement of antibody responses is dependent of $FcR\gamma^+$ bone marrow-derived cells. *Scand. J. Immunol.* 54: 1–7.
9. Getahun, A., J. Dahlström, S. Wernersson, and B. Heyman. 2004. IgG2a-mediated enhancement of Ab- and T-cell responses and its relation to inhibitory and activating FcγRs. *J. Immunol.* 172: 5269–5276.
10. Klaus, G. G. B. 1979. Generation of memory cells: III. Antibody class requirements for the generation of B-memory cells by antigen-antibody complexes. *Immunology* 37: 345–351.
11. Coulie, P., and J. Van Snick. 1985. Enhancement of IgG anti-carrier responses by IgG2-anti-hapten antibodies in mice. *Eur. J. Immunol.* 15: 793–798.
12. Song, H., X. Nie, S. Basu, and J. Cerny. 1998. Antibody feedback and somatic mutation in B cells: regulation of mutation by immune complexes with IgG antibody. *Immunol. Rev.* 162: 211–218.
13. Nie, X., S. Basu, and J. Cerny. 1997. Immunization with immune complex alters the repertoire of antigen-reactive B cells in the germinal centers. *Eur. J. Immunol.* 27: 3517–3525.
14. Heyman, B., T. Liu, and S. Gustavsson. 1993. In vivo enhancement of the specific antibody response via the low affinity receptor for IgE. *Eur. J. Immunol.* 23: 1739–1742.
15. Gustavsson, S., S. Hjulström, T. Liu, and B. Heyman. 1994. CD23/IgE-mediated regulation of the specific antibody response in vivo. *J. Immunol.* 152: 4793–4800.
16. Fujiwara, H., H. Kikutani, S. Suematsu, T. Naka, K. Yoshida, K. Yoshida, T. Tanaka, M. Suemura, N. Matsumoto, S. Kojima, et al. 1994. The absence of IgE antibody-mediated augmentation of immune responses in CD23-deficient mice. *Proc. Natl. Acad. Sci. USA* 91: 6835–6839.
17. Westman, S., S. Gustavsson, and B. Heyman. 1997. Early expansion of secondary B cells after primary immunization with antigen complexed with IgE. *Scand. J. Immunol.* 46: 10–15.
18. Gustavsson, S., S. Wernersson, and B. Heyman. 2000. Restoration of the antibody response to IgE/antigen complexes in CD23-deficient mice by $CD23^+$ spleen or bone marrow cells. *J. Immunol.* 164: 3990–3995.
19. Getahun, A., F. Hjelm, and B. Heyman. 2005. IgE enhances antibody and T cell responses in vivo via $CD23^+$ B Cells. *J. Immunol.* 175: 1473–1482.
20. Rao, M., W. T. Lee, and D. H. Conrad. 1987. Characterization of a monoclonal antibody directed against the murine B lymphocyte receptor for IgE. *J. Immunol.* 138: 1845–1851.
21. Waldschmidt, T. J., D. H. Conrad, and R. G. Lynch. 1988. The expression of B cell surface receptors: I. The ontogeny and distribution of the murine B cell IgE Fc receptor. *J. Immunol.* 140: 2148–2154.
22. Maeda, K., G. F. Burton, D. A. Padgett, D. H. Conrad, T. F. Huff, A. Masuda, A. K. Szakal, and J. G. Tew. 1992. Murine follicular dendritic cells and low affinity Fc receptors for IgE (FcεRII). *J. Immunol.* 148: 2340–2347.
23. Yu, L. C. H., G. Montagnac, P.-C. Yang, D. H. Conrad, A. Benmerah, and M. H. Perdue. 2003. Intestinal epithelial CD23 mediates enhanced antigen transport in allergy: evidence for a novel splice form. *Am. J. Physiol.* 285: G223–G234.
24. Gould, H. J., B. J. Sutton, A. J. Beavil, R. L. Beavil, N. McCloskey, H. A. Coker, D. Fear, and L. Smurthwaite. 2003. The biology of IgE and the basis of allergic disease. *Annu. Rev. Immunol.* 21: 579–628.
25. Carlsson, F., F. Hjelm, D. H. Conrad, and B. Heyman. 2007. IgE enhances specific antibody and T cell responses in mice overexpressing CD23. *Scand. J. Immunol.* 66: 261–270.
26. Pirron, U., T. Schlunck, J. C. Prinz, and E. P. Rieber. 1990. IgE-dependent antigen focusing by human B lymphocytes is mediated by low-affinity receptor for IgE. *Eur. J. Immunol.* 20: 1547–1551.
27. van der Heijden, F. L., R. J. J. van Neerven, M. van Katwijk, J. D. Bos, and M. L. Kapsenberg. 1993. Serum-IgE-facilitated allergen presentation in atopic disease. *J. Immunol.* 150: 1–8.
28. Bheekha Escura, R., E. Wasserbauer, F. Hammerschmid, A. Pearce, P. Kidd, and G. C. Mudde. 1995. Regulation and targeting of T-cell immune responses by IgE and IgG antibodies. *Immunology* 86: 343–350.
29. Kehry, M. R., and L. C. Yamashita. 1989. Low-affinity IgE receptor (CD23) function on mouse B cells: role in IgE-dependent antigen focusing. *Proc. Natl. Acad. Sci. USA* 86: 7556–7560.
30. Okada, T., and J. G. Cyster. 2006. B cell migration and interactions in the early phase of antibody responses. *Curr. Opin. Immunol.* 18: 278–285.
31. Gretz, J. E., C. C. Norbury, A. O. Anderson, A. E. Proudfoot, and S. Shaw. 2000. Lymph-borne chemokines and other low molecular weight molecules reach high endothelial venules via specialized conduits while a functional barrier limits access to the lymphocyte microenvironments in lymph node cortex. *J. Exp. Med.* 192: 1425–1440.
32. Szakal, A. K., K. L. Holmes, and J. G. Tew. 1983. Transport of immune complexes from the subcapsular sinus to lymph node follicles on the surface of nonphagocytic cells, including cells with dendritic morphology. *J. Immunol.* 131: 1714–1727.
33. Bergtold, A., D. D. Desai, A. Gavhane, and R. Clynes. 2005. Cell surface recycling of internalized antigen permits dendritic cell priming of B cells. *Immunity* 23: 503–514.
34. Delamarre, L., M. Pack, H. Chang, I. Mellman, and E. S. Trombetta. 2005. Differential lysosomal proteolysis in antigen-presenting cells determines antigen fate. *Science* 307: 1630–1634.
35. Pape, K. A., D. M. Catron, A. A. Itano, and M. K. Jenkins. 2007. The humoral immune response is initiated in lymph nodes by B cells that acquire soluble antigen directly in the follicles. *Immunity* 26: 491–502.

36. Carrasco, Y. R., and F. D. Batista. 2007. B cells acquire particulate antigen in a macrophage-rich area at the boundary between the follicle and the subcapsular sinus of the lymph node. *Immunity* 27: 160–171.
37. Phan, T. G., I. Grigorova, T. Okada, and J. G. Cyster. 2007. Subcapsular encounter and complement-dependent transport of immune complexes by lymph node B cells. *Nat. Immunol.* 8: 992–1000.
38. Martinez-Pomares, L., M. Kosco-Vilbois, E. Darley, P. Tree, S. Herren, J. Y. Bonnefoy, and S. Gordon. 1996. Fc chimeric protein containing the cysteine-rich domain of the murine mannose receptor binds to macrophages from splenic marginal zone and lymph node subcapsular sinus and to germinal centers. *J. Exp. Med.* 184: 1927–1937.
39. Berney, C., S. Herren, C. A. Power, S. Gordon, L. Martinez-Pomares, and M. H. Kosco-Vilbois. 1999. A member of the dendritic cell family that enters B cell follicles and stimulates primary antibody responses identified by a mannose receptor fusion protein. *J. Exp. Med.* 190: 851–860.
40. Yu, P., Y. Wang, R. K. Chin, L. Martinez-Pomares, S. Gordon, M. H. Kosco-Vibois, J. Cyster, and Y. X. Fu. 2002. B cells control the migration of a subset of dendritic cells into B cell follicles via CXC chemokine ligand 13 in a lymphotoxin-dependent fashion. *J. Immunol.* 168: 5117–5123.
41. Pozdnyakova, O., H. K. Guttormsen, F. N. Lalani, M. C. Carroll, and D. L. Kasper. 2003. Impaired antibody response to group B streptococcal type III capsular polysaccharide in C3- and complement receptor 2-deficient mice. *J. Immunol.* 170: 84–90.
42. Whipple, E. C., R. S. Shanahan, A. H. Ditto, R. P. Taylor, and M. A. Lindorfer. 2004. Analyses of the in vivo trafficking of stoichiometric doses of an anti-complement receptor 1/2 monoclonal antibody infused intravenously in mice. *J. Immunol.* 173: 2297–2306.
43. Ferguson, A. R., M. E. Youd, and R. B. Corley. 2004. Marginal zone B cells transport and deposit IgM-containing immune complexes onto follicular dendritic cells. *Int. Immunol.* 16: 1411–1422.
44. Martin, F., and J. F. Kearney. 2002. Marginal-zone B cells. *Nat. Rev. Immunol.* 2: 323–335.
45. Murphy, K. M., A. B. Heimberger, and D. Y. Loh. 1990. Induction by antigen of intrathymic apoptosis of $CD4^+CD8^+TCR^{low}$ thymocytes in vivo. *Science* 250: 1720–1723.
46. Rudolph, A. K., P. D. Burrows, and M. R. Wabl. 1981. Thirteen hybridomas secreting hapten-specific immunoglobulin E from mice with Ig^a or Ig^b heavy chain haplotype. *Eur. J. Immunol.* 11: 527–529.
47. Amano, H., E. Amano, T. Moll, D. Marinkovic, N. Ibnou-Zekri, E. Martinez-Soria, I. Semac, T. Wirth, L. Nitschke, and S. Izui. 2003. The Yaa mutation promoting murine lupus causes defective development of marginal zone B cells. *J. Immunol.* 170: 2293–2301.
48. Oliver, A. M., F. Martin, G. L. Gartland, R. H. Carter, and J. F. Kearney. 1997. Marginal zone B cells exhibit unique activation, proliferative, and immunoglobulin secretory responses. *Eur. J. Immunol.* 27: 2366–2374.
49. Wolniak, K. L., S. M. Shinall, and T. J. Waldschmidt. 2004. The germinal center response. *Crit. Rev. Immunol.* 24: 39–65.
50. Attanavanich, K., and J. F. Kearney. 2004. Marginal zone, but not follicular B cells, are potent activators of naive CD4 T cells. *J. Immunol.* 172: 803–811.
51. Carroll, M. C. 2004. The complement system in regulation of adaptive immunity. *Nat. Immunol.* 5: 981–986.
52. Papamichail, M., C. Gutierrez, P. Embling, P. Johnson, E. J. Holborow, and M. B. Pepys. 1975. Complement dependence of localisation of aggregated IgG in germinal centers. *Scand. J. Immunol.* 4: 343–347.
53. Youd, M. E., A. R. Ferguson, and R. B. Corley. 2002. Synergistic roles of IgM and complement in antigen trapping and follicular localization. *Eur. J. Immunol.* 32: 2328–2337.
54. Applequist, S. E., J. Dahlström, N. Jiang, H. Molina, and B. Heyman. 2000. Antibody production in mice deficient for complement receptors 1 and 2 can be induced by IgG/Ag and IgE/Ag, but not IgM/Ag complexes. *J. Immunol.* 165: 2398–2403.
55. Reif, K., E. H. Ekland, L. Ohl, H. Nakano, M. Lipp, R. Forster, and J. G. Cyster. 2002. Balanced responsiveness to chemoattractants from adjacent zones determines B-cell position. *Nature* 416: 94–99.
56. Huang, N. N., S. B. Han, I. Y. Hwang, and J. H. Kehrl. 2005. B cells productively engage soluble antigen-pulsed dendritic cells: visualization of live-cell dynamics of B cell-dendritic cell interactions. *J. Immunol.* 175: 7125–7134.
57. Yokota, A., K. Yukawa, A. Yamamoto, K. Sugiyama, M. Suemura, Y. Tashiro, T. Kishimoto, and H. Kikutani. 1992. Two forms of the low-affinity Fc receptor for IgE differentially mediate endocytosis and phagocytosis: identification of the critical cytoplasmic domains. *Proc. Natl. Acad. Sci. USA* 89: 5030–5034.
58. Karagiannis, S. N., J. K. Warrack, K. H. Jennings, P. R. Murdock, G. Christie, K. Moulder, B. J. Sutton, and H. J. Gould. 2001. Endocytosis and recycling of the complex between CD23 and HLA-DR in human B cells. *Immunology* 103: 319–331.
59. Constant, S., N. Schweitzer, J. West, P. Ranney, and K. Bottomly. 1995. B lymphocytes can be competent antigen-presenting cells for priming $CD4^+$ T cells to protein antigens in vivo. *J. Immunol.* 155: 3734–3741.
60. Milich, D. R., M. Chen, F. Schodel, D. L. Peterson, J. E. Jones, and J. L. Hughes. 1997. Role of B cells in antigen presentation of the hepatitis B core. *Proc. Natl. Acad. Sci. USA* 94: 14648–14653.
61. Constant, S. L. 1999. B lymphocytes as antigen-presenting cells for $CD4^+$ T cell priming in vivo. *J. Immunol.* 162: 5695–5703.
62. Rivera, A., C. C. Chen, N. Ron, J. P. Dougherty, and Y. Ron. 2001. Role of B cells as antigen-presenting cells in vivo revisited: antigen-specific B cells are essential for T cell expansion in lymph nodes and for systemic T cell responses to low antigen concentrations. *Int. Immunol.* 13: 1583–1593.
63. Crawford, A., M. Macleod, T. Schumacher, L. Corlett, and D. Gray. 2006. Primary T cell expansion and differentiation in vivo requires antigen presentation by B cells. *J. Immunol.* 176: 3498–3506.
64. Shirota, H., K. Sano, N. Hirasawa, T. Terui, K. Ohuchi, T. Hattori, and G. Tamura. 2002. B cells capturing antigen conjugated with CpG oligodeoxynucleotides induce Th1 cells by elaborating IL-12. *J. Immunol.* 169: 787–794.
65. Yan, J., M. J. Wolff, J. Unternaehrer, I. Mellman, and M. J. Mamula. 2005. Targeting antigen to CD19 on B cells efficiently activates T cells. *Int. Immunol.* 17: 869–877.
66. Mudde, G. C., T. T. Hansel, F. C. v. Reijsen, B. F. Osterhoff, and C. A. F. M. Bruijnzeel-Koomen. 1990. IgE: an immunoglobulin specialized in antigen capture? *Immunol. Today* 11: 440–443.
67. van Neerven, R. J., E. F. Knol, A. Ejrnaes, and P. A. Wurtzen. 2006. IgE-mediated allergen presentation and blocking antibodies: regulation of T-cell activation in allergy. *Int. Arch. Allergy Immunol.* 141: 119–129.