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Restoration of the Antibody Response to IgE/Antigen Complexes in CD23-Deficient Mice by CD23\(^{+}\) Spleen or Bone Marrow Cells\(^1\)

Susanne Gustavsson,\(^2\) Sara Wernersson, and Birgitta Heyman

Mice immunized with IgE/Ag complexes produce significantly more Ag-specific Abs than mice immunized with Ag alone. The enhancement is mediated via the low-affinity receptor for IgE (Fc\(\epsilon\)RII or CD23), as shown by its complete absence in mice pretreated with mAbs specific for CD23 and in CD23-deficient mice. Because the constitutive expression of murine CD23 is limited to B cells and follicular dendritic cells (FDCs), one of these cell types is likely to be involved. One of the suggested modes of action of IgE/CD23 is to increase the ability of B cells to present Ag to T cells, as demonstrated to take place in vitro. Another possibility is that FDCs capture the IgE/Ag complexes and present these directly to B cells. The purpose of the present study was to determine whether CD23\(^{+}\) B cells or FDCs are responsible for the IgE/CD23-mediated enhancement of specific Ab responses in vivo. We show that the enhancement is completely restored in irradiated CD23-deficient mice reconstituted with CD23\(^{+}\) spleen or bone marrow cells. In these mice, the B cells are CD23\(^{+}\) and the FDCs are presumably CD23\(^{−}\) because the FDCs are radiation resistant and are reported not to be replaced by donor cells after this type of cell transfer. In contrast, enhancement was not restored in irradiated wild-type mice reconstituted with CD23\(^{−}\) cells. These results indicate that CD23\(^{+}\) B cells, and not FDCs, are the cells that capture IgE/Ag complexes and induce enhancement of Ab responses in vivo. (The Journal of Immunology, 2000, 164: 3990–3995.)

In murine in vivo systems, IgE Abs have the capacity to enhance Ab responses against soluble protein Ags that they recognize (1, 2). The enhancing effect is strictly Ag specific and affects the primary IgM, IgG, and IgE response as well as the memory response (2). The capacity of IgE to enhance the Ab response is abolished when mice are pretreated with mAbs specific for the low-affinity receptor for IgE (Fc\(\epsilon\)RII or CD23) (1, 2). Targeting of Ag to CD23 by covalent binding of Ag to CD23-specific mAbs also induces a strong response, both in vivo and in vitro (3), further emphasizing the role of CD23 in Ab responses. In addition, it has been shown in vitro that IgE/Ag complexes are more efficiently presented to T cells by murine splenic B cells (4, 5) and EBV-transformed human B cells (6–8) than Ag alone. Also in these studies, the enhancing effect of IgE is inhibited with CD23-specific mAbs (4–7). Three groups have produced CD23-deficient mice (9–11). These mice displayed normal lymphocyte differentiation and could mount normal Ab responses to thymus-independent Ags and after infection with *Nippostrongylus brasiliensis*. The effect on the Ab response to thymus-dependent Ags was investigated in two of the studies. One group found no difference between the response of wild-type and CD23-deficient mice (9), whereas another group found increased IgE levels in CD23-deficient mice (11). Moreover, the ability of DNP-specific IgE to enhance the Ab response to OVA-DNP was severely impaired in CD23-deficient mice (9).

In naive mice, the constitutive expression of CD23 is limited to B cells (12) and follicular dendritic cells (FDCs)\(^3\) (13). This murine CD23 is probably a human CD23a counterpart, because human CD23a is expressed constitutively on B cells (14). Another human isoform, CD23b, is induced on a wide variety of hematopoietic cell types after IL-4 stimulation (14, 15). Low levels of murine CD23b mRNA are found in B cells and non-B cells after activation with LPS and IL-4 (16). However, only one form of murine CD23, the one analogous to human CD23a, is expressed at detectable protein levels (17). Involvement of the murine CD23b isoform in IgE-mediated enhancement of in vivo Ab responses is unlikely because IgE/Ag complexes induce unperturbed enhancement in mice deficient in IL-4, thereby demonstrating that the constitutively expressed CD23 is sufficient (18). This finding suggests that either CD23\(^{+}\) B cells or FDCs (or both) are the cells that capture the IgE/Ag complexes and initiate enhancement of Ab responses.

During the primary response to a T cell-dependent Ag, activated B cells produce factors required for maturation of the FDC network. The FDCs capture and retain native Ag in complex with Ab and complement at their surface for long periods of time. It is believed that the trapped Ag can be endocytosed by B cells. These B cells become highly efficient APCs that present processed Ag to T cells. The T cells, together with FDCs, then support further proliferation and differentiation of the B cells. During the secondary response to an Ag, the kinetic of the germinal center reaction is much quicker. Abs from the primary response are present and form complexes with Ag. These Ab/Ag complexes are more efficiently trapped by FDCs than free Ag (reviewed in Refs. 19 and 20).

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\(^{2}\) Abbreviations used in this paper: FDC, follicular dendritic cells; BM, bone marrow; TNP, 2,4,6-trinitrophenyl.
An early and enhanced germinal center formation has been demonstrated in mouse regional lymph nodes or spleens following primary injection of Ab/Ag complexes as compared with Ag alone (21, 22). In addition, the immune complex-induced appearance and growth of germinal centers preceded, or paralleled, an observed rapid and enhanced Ab response (21, 22). Most likely the germinal center reaction is involved in IgE/CD23-mediated enhancement of Ab responses. One possibility is that IgE/CD23 increase the ability of B cells to take up and present Ag to T cells. A mutually not exclusive explanation is that FDCs capture the IgE/Ag complexes via CD23 and present them directly to B cells.

The origin of FDCs has not been unequivocally established. A number of studies suggest that FDCs differentiate from local stromal cells with a fibroblastic or mesenchymal morphology, whereas others suggest that they are derived from hematopoietic cells. There is also speculation that germinal centers may display two types of FDCs of different origin (reviewed in Refs. 23–25). Early studies with mouse chimeras demonstrated that host FDCs are not replaced by donor cells after transfer of BM cells (26, 27). This is in agreement with a more recent study demonstrating that organized clusters of FDCs are absent from the spleen in mice deficient in lymphotoxin α. When these mice are reconstituted with CR1/2-deficient bone marrow (BM) cells, organized CR1+ FDCs are identified (28), suggesting that FDCs are not transferred by BM cells, but rather mature from non-BM precursor cells. Further, transfer of mouse allogeneic spleen cells into SCID mice (also lacking organized clusters of FDCs) resulted in the generation of FDCs of host origin (29). However, Kapasi et al. (30) observed development of both mouse and rat FDCs in SCID mice reconstituted with BM or fetal liver cells from rat. In the present study we use transfer experiments to evaluate the contribution of CD23+ cells and FDCs in IgE/CD23-mediated enhancement of in vivo Ab responses.

Materials and Methods

Mice

Because all H-2b mice have an I-Aβ-linked low responsiveness to IgE/BSA-TNP and IgG/BSA-TNP complexes (31, 32), male CD23-deficient founder mice on a H-2b background (9) were mated with female responder CBA/J (H-2b) mice and the offspring were repeatedly backcrossed to the CBA/J strain. The CD23 genotype was analyzed in a PCR using one sense primer and either of two antisense primers: P1 (5′-AAC GCA CGG GTG TTT GCT CGT TTG-3′), P2 (5′-GCT TTC ACA CTT GCA GTC CTC-3′), and P3 (5′-TGA GAC ATT CTG CTC CCA TCC-3′). Gene amplification was done in 50 μl 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM (P1/P2) or 1.5 mM (P2/P3) MgCl2, 0.2 mM dNTPs, 0.4 μM of primer, and 2.5 U AmpliTag DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT) for 40 cycles (1 min at 94°C, 1 min at 58°C, 1 min at 74°C). P1/P2 PCR gave 2.5 U AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT) for 40 cycles (1 min at 94°C, 1 min at 58°C, 1 min at 74°C). P1/P2 PCR gave a 862-bp fragment, whereas P2/P3 PCR gave a fragment of 1007 bp. By intercross of the fifth generation, mice with homozygous CD23 gene defects and wild-type littermates were obtained. Groups of four to eight male or female mice, 2–5 mo of age, were used and matched for age and sex only within each experiment. The mice were bred in the animal facilities at the Department of Genetics and Pathology, Uppsala University.

Abs and Ags

The origin and purification of mAbs from the hybridoma cell lines IGELb4 (mouse IgE anti-TNP) (33) and C4007B4 (7B4, mouse IgG2a anti-TNP) (34) has been described before (1, 35). BSA, OVA, and TNP were obtained from Sigma (St. Louis, MO), and TNP was conjugated to BSA in 0.2 M cacodylate buffer, pH 6.9 (cacodylic acid, sodium salt; Sigma). After 70 min at room temperature the reaction was stopped by an excess of glycylglycin, 1 mg/ml (Merck, Darmstadt, Germany). The number of incorporated TNP residues per BSA molecule was 23 as determined according to Ref. 36. The proteins were dialyzed against PBS and stored at 4°C as sterile solutions.

Adaptive transfer and immunization

Single cell suspensions of donor spleens were prepared in PBS by pressing cells through a mesh, whereas BM cells were harvested by flushing the femurs of donor mice with PBS. Recipient mice were irradiated with 600 rad, and 5 × 107 spleen or 1 × 107 BM cells in 0.2 ml of PBS were injected i.v. 24 h after the irradiation. After 1 day (spleen cells) or 42 days (BM cells) the recipient mice received 50 μg TNP-specific IgE or IgG (IGELb4 or 7B4) together with 20 μg BSA-TNP and 20 μg OVA (as a specificity control) in 0.1 ml of PBS. Ab/Ag complexes were formed by incubating Ag and Ab (or Ag and PBS for controls) for 1 h at 37°C immediately before injection.

ELISA

Mice were bled from the tail veins, and isolated sera were analyzed by ELISA as described (2). Hyperimmune antisera against BSA or OVA, affinity purified on BSA- or OVA-Sepharose, were used as standards. Standard curves and calculations were done by the use of a Softmax program (Molecular Devices, Menlo Park, CA). Statistical differences were determined by Student’s t test.

Flow cytometry

Spleen cells were suspended in PBS containing 0.5% FCS, PE-labeled B3B4 (rat anti-mouse CD23) and FITC-labeled 1D3 (rat anti-mouse CD19) from PharMingen (San Diego, CA) were incubated with 107 cells for 30 min at 4°C. After incubation, cells were washed and data of 10,000 cells were collected and analyzed using a FACSort flow cytometer (Becton Dickinson, Mountain View, CA).

Results

Impaired IgE-mediated enhancement of Ab responses in CD23-deficient mice

It has been reported that CD23-deficient mice display impaired IgE-mediated enhancement of Ab responses (9). To confirm this finding in our system, IgE/BSA-TNP complexes were injected into CD23-deficient mice and wild-type controls. Since it has been shown previously that anti-hapten Abs modulate the Ab response to all determinants on an Ag (1, 2, 9, 34, 37), the BSA-specific response was determined. No enhancement was seen in CD23-deficient mice, whereas wild-type controls had a 50- to 400-fold enhancement of the BSA-specific Ab response (Fig. 1, a and b). We conclude that the IgE-mediated enhancement is mediated exclusively via CD23 and not via any other IgE receptor. To investigate whether our CD23-deficient mice were able to produce Abs in other situations, they were immunized with IgG/BSA-TNP complexes also known to enhance Ab responses in vivo (34, 38). CD23-deficient mice were indeed capable of mounting an Ab response to IgG/BSA-TNP and even showed a higher response than wild-type controls (Fig. 1, c and d). Both IgE- and IgG-mediated enhancement of Ab responses is strictly Ag specific (1, 2, 38). To confirm the specificity in the present study, mice were always immunized with unspecific Ag (OVA) in addition to the specific Ag (BSA-TNP). Although the specific response was significantly enhanced, the response to unspecific Ag was never enhanced (data not shown).

Transfer of CD23+ BM cells to CD23-deficient mice, but not transfer of CD23− BM cells to wild-type mice, restores IgE-mediated enhancement of Ab responses

To investigate whether the impaired response to IgE/BSA-TNP complexes in CD23-deficient mice was due to absence of the receptor on B cells or FDCs, irradiated CD23-deficient mice were reconstituted with CD23+ BM cells from wild-type controls. In BM chimeras, the B cells are of donor origin and the FDCs of host origin because FDCs are radiation resistant (26, 39) and are not replaced by donor cells after transfer of BM cells to irradiated hosts (26–28). The cell surface expression of CD19 and CD23 on BM cells from donors and on spleen cells from reconstituted
recipients was analyzed by flow cytometry. In a representative experiment, wild-type donor BM cells consisted of 7.2% CD19^+CD23^+ cells and 29% CD19^-CD23^- cells, whereas only 0.7% CD19^-CD23^- cells were detected. CD23-deficient donor BM cells consisted of 56% CD19^-CD23^- cells and 0.4% CD23^+ cells (data not shown). Thus the CD23-deficient donor mice were indeed CD23 negative. Representative histograms from recipients 42 days after adoptive transfer are shown in Fig. 2. CD19^-CD23^- cells constituted 44% of the cells in wild-type mice and 45% of the cells in CD23-deficient mice reconstituted with CD23^- cells (Fig. 2, a and c). Spleen cells from CD23-deficient and wild-type mice reconstituted with CD23^- cells had $\approx 2.3\%$ CD19^-CD23^- cells (Fig. 2, b and d). There were $\approx 1.1\%$ CD19^-CD23^- cells in all the cell suspensions tested by flow cytometry. These findings indicate that 1) in spleen cell suspensions from naive mice, only CD19^- cells (i.e., B cells) express CD23, and 2) CD23-deficient mice are efficiently reconstituted with CD23^- B cells.

Irradiated wild-type mice reconstituted with CD23^+ BM cells (Fig. 3a) as well as irradiated CD23-deficient mice reconstituted with CD23^- BM cells (Fig. 3c) responded to IgE/Ag complexes with a 100- to 2000-fold enhancement. Irradiated CD23-deficient mice reconstituted with CD23^- BM cells (Fig. 3b) showed only marginal enhancement of the Ab response. To further analyze the involvement of FDCs, the converse experiment was performed. Irradiated wild-type mice were reconstituted with CD23^- BM cells (Fig. 3d), resulting in an animal where the B cells were CD23^- and the FDCs were CD23^- . These mice had very low Ab responses indistinguishable from those in irradiated CD23-deficient mice reconstituted with CD23^- BM cells (Fig. 3b).

Despite the abundance of data indicating that FDCs are not derived from BM, this is still a controversial area (reviewed in Refs. 23–25). To avoid any possible differentiation of transferred BM cells into FDCs, spleen cells instead of BM cells were used in the following experiments. Single cell suspensions of donor spleens were prepared by pressing cells through a mesh. This rough treatment destroys the fragile FDCs (22, 40) but leaves the B cells unharmed. In addition, it has been reported that host FDCs are not replaced by donor cells after transfer of spleen cells (29). Therefore, we assume that the B cells are of donor origin and the radiation-resistant FDCs of host origin in spleen cell chimeras. The expression of CD19 and CD23 on spleen cells from donors and reconstituted recipients was measured by flow cytometry. In a representative experiment, wild-type donor spleen cells consisted of 34% CD19^-CD23^- cells, 6.1% CD19^-CD23^- cells, and 1.1% CD19^-CD23^- cells. CD23-deficient donor spleen cells consisted of 34% CD19^-CD23^- cells and 0.9% CD23^- cells (data not shown). Representative histograms from recipients 42 days after adoptive transfer are shown in Fig. 4. The number of CD19^-CD23^- cells observed in wild-type and CD23-deficient mice reconstituted with CD23^- cells ranged from 35 to 41% (Fig. 4, a and c). CD23-deficient and wild-type mice reconstituted with CD23^- cells were consistently CD23 negative, i.e., $\approx 1.9\%$ of the cells were CD23^- (Fig. 4, b and d).

Irradiated wild-type mice reconstituted with CD23^- spleen cells (Fig. 5a) as well as irradiated CD23-deficient mice reconstituted with CD23^- spleen cells (Fig. 5c) responded to IgE/Ag complexes with a 20- to 500-fold enhancement. As expected, irradiated CD23-deficient mice reconstituted with CD23^- spleen cells (Fig. 5b) showed no enhancement of the Ab response. In the converse experiment, irradiated wild-type mice reconstituted with
CD23− spleen cells (Fig. 5d) had a similar lack of Ab responses as irradiated CD23-deficient mice reconstituted with CD23− spleen cells (Fig. 5b).

Discussion

We have presented data confirming and extending previous reports that CD23 is required for IgE-mediated enhancement of in vivo Ab responses. Immunization with IgE/BSA-TNP induced up to a 400-fold enhancement of the BSA-specific response in wild-type mice, whereas the response in CD23-deficient mice was indistinguishable from that in nonimmunized mice or in mice receiving BSA-TNP alone. In contrast, immunization with IgG/BSA-TNP immune complexes that do not bind to CD23 induced a BSA-specific response that was in the same order of magnitude in CD23-deficient and wild-type mice. It is known from previous studies that the IgG response in CD23-deficient mice is normal (9) or even higher (11) than in wild-type mice. It is also known that surface Ig (sIg), specific for the Ag component, is not a prerequisite for mere binding to CD23. Moreover, CD23-dependent signals without involving the Ag presentation pathway. Immunization of nude mice with IgE/Ag do not induce Ab responses (2), and therefore direct signaling to the B cell without involving the Ag presentation pathway. Immunization of nude mice with IgE/Ag do not induce Ab responses (2), and therefore direct signaling to the B cell without involving the Ag presentation pathway. Immunization of nude mice with IgE/Ag do not induce Ab responses (2), and therefore direct signaling to the B cell without involving the Ag presentation pathway. Immunization of nude mice with IgE/Ag do not induce Ab responses (2), and therefore direct signaling to the B cell without involving the Ag presentation pathway.

An interesting possibility is that uptake of IgE/Ag via CD23 on B cells followed by processing and presentation of antigenic peptides to T cells explains IgE-mediated enhancement of Ab responses in vivo. In vitro, mouse or human B cells were shown to present Ag in complex with IgE to T cells much more efficiently than Ag alone (4–8). This effect was dependent on CD23. An alternative explanation for the in vivo effect of IgE/Ag is that CD23, acting as a coreceptor, initiates positive signaling to the B cell without involving the Ag presentation pathway. Immunization of nude mice with IgE/Ag do not induce Ab responses (2), and therefore direct signaling to the B cell through CD23 would not abrogate the need for T cell help. IgE/BSA-TNP complexes bind to the majority of naive splenic B cells ex vivo (32), suggesting that surface Ig (sIg), specific for the Ag component, is not a prerequisite for mere binding to CD23. Moreover, CD23-dependent endocytosis and presentation of IgE/Ag complexes to T cells is conducted by non-Ag-specific B cells in vitro (6–8). It has been proposed that activation of these "bystander" B cells by allergen-specific type 2 T-helper cells would cause the deterioration of single allergies into multiple allergies (41).

FIGURE 3. IgE-mediated enhancement of Ab responses is restored by transfer of CD23+/− BM cells to CD23-deficient mice, but not by transfer of CD23 BM cells to wild-type mice. Groups of four male irradiated wild-type mice reconstituted with CD23+/− BM cells (a), irradiated CD23-deficient mice reconstituted with CD23+/− BM cells (b), irradiated CD23-deficient mice reconstituted with CD23+/− BM cells (c), or irradiated wild-type mice reconstituted with CD23+/− BM cells (d) were immunized i.v. with 20 μg BSA-TNP and 20 μg OVA (□) or 50 μg TNP-specific monoclonal IgE together with 20 μg BSA-TNP and 20 μg OVA (■). Serum levels of OVA- and BSA-specific IgG were determined by ELISA 11, 14, and 21 days after immunization. The level of BSA-specific IgG in normal mouse serum was 0.28 μg/ml. Data are representative of two independent experiments. Statistical differences: ***p ≤ 0.001; **p ≤ 0.01; *p ≤ 0.05; NS, p > 0.05.

FIGURE 4. Cell surface expression of CD19 and CD23 in spleen cell recipients. Spleen cell suspensions were prepared from recipients 42 days after adoptive transfer and analyzed by flow cytometry. Staining performed with anti-CD23-PE in combination with anti-CD19-FITC is expressed as log10 fluorescence intensity.

As mentioned, it is unlikely that other cell types in naive mice than B cells and FDCs express CD23. The purpose of the present study was to determine whether CD23 expressed on B cells or FDCs are responsible for the IgE-mediated enhancement of specific Ab responses in vivo. Although some FDCs may be derived from BM (30), donor FDCs are not developed in normal irradiated mice after BM reconstitution using our protocol (26–28), nor do they survive conventional preparations of spleen cell suspensions (22, 40). This information was used in the construction of chimeric mice expressing CD23 on spleen cells or BM-derived cells (B cells) but not on radiation resistant cells (FDCs), or, inversely, mice expressing CD23 on FDCs but not on B cells. Chimeric mice expressing CD23 on B cells, but not on FDCs, produced high Ab titers after immunization with IgE/Ag complexes, whereas mice expressing CD23 on FDCs, but not on B cells, did not. Our interpretation of these observations is that CD23+/− B cells are required and sufficient for IgE-mediated enhancement of Ab responses in vivo, and that CD23+/− FDCs alone are not sufficient for induction of enhancement.
Ags within the complex is enhanced (present study and Refs. 1 and 2). Therefore, the mechanism behind IgE-enhanced Ab production must encompass a step that prevents unspecific B cells from being activated, even though they may take up IgE/Ag complexes via CD23. Independent ligation of CD23 and sIg cannot sufficiently explain why mice immunized with IgE-anti-TNP/BSA-TNP and an unspecific Ag (hen egg lysozyme (HEL), keyhole limpet hemocyanin (KLH), or OVA) do not produce Abs recognizing HEL, KLH, or OVA (present study and Ref. 2). A possible explanation for our observations is that CD23 and sIg must be co-cross-linked to trigger the stimulatory events leading to increased Ab production in vivo. These events may be connected with Ag presentation and/or with signaling through the B cell receptor complex. Our studies do not allow the distinction between the two, but given the abundance of in vitro studies showing increased presentation of IgE/Ag complexes by B cells, we find it most likely that this is also an in vivo mechanism. Clearly, however, a safety catch preventing all CD23+ B cells from activating T cells must exist and the definition of the molecular mechanism behind this remains an important task.

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