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Specific antigen targeting to surface IgE and IgG on mouse bone marrow-derived mast cells enhances efficiency of antigen presentation

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SUMMARY

The discovery that bone marrow-derived mast cells can express major histocompatibility complex class II molecules and act as antigen-presenting cells prompted us to evaluate this function when antigen is internalized through fluid-phase endocytosis or via specific uptake by using IgG and IgE antibodies. This study was performed using a specific T-cell hybridoma developed against Lol p 1, the major allergen of grass pollen *Lolium perenne*. Expression of FcγR and FcεRI by mast cells led us to investigate the influence of IgG- and IgE-targeted antigen on the antigen-presenting function of mast cells. Internalization of Lol p 1 through different specific IgG monoclonal antibodies (mAb) resulted in the activation of Lol p 1-specific T-cell hybridoma at concentrations about 100-fold less than that required for T-cell stimulation by uncomplexed antigen. IgE-complexed Lol p 1, which facilitates trapping of antigen by mast cells, induced an accelerated and more efficient antigen-presenting capacity of mast cells than that obtained with uncomplexed antigen. However, aggregation of anti-dinitrophenyl (DNP) IgE mAb by the irrelevant antigen DNP–human serum albumin did not substantially increase the capacity of mast cells to present Lol p 1 to T cells. This suggests that the mere aggregation of FcεRI is not sufficient for enhanced antigen presentation mediated by IgE. Tissue distribution and strategic location of mast cells at the mucosal barriers and their capacity to process the antigen through efficient fluid-phase pinocytosis as well as IgG- and IgE-dependent targeting of antigens provide mast cells with a prominent role in immune surveillance.

INTRODUCTION

Specific T cells recognize immunogenic peptides bound to major histocompatibility complex (MHC) class II molecules expressed by antigen-presenting cells (APC). Internalization by APC of complex antigens occurs either through non-specific fluid-phase pinocytosis or via membrane receptors. Surface immunoglobulin-mediated efficiency of MHC class II-restricted antigen presentation has been extensively analysed by using B cells, B-cell lymphomas and Epstein–Barr virus (EBV)-transformed B-cell lines.[1,2] Normal B cells are capable of antigen presentation that is further enhanced by antigen binding to specific surface immunoglobulin.[3,4] B cells can also use IgM and IgD, as well as MHC class II and class I molecules to internalize antigen and enhance antigen presentation.[5,6] One major implication is that a many-fold less antigen concentration is required for a maximal stimulation of T cells which in turn provide help for B-cell activation and differentiation. Expression of CD23/FcεRII, the low-affinity receptor for IgE, by a number of cells and the high-affinity IgE receptor FcεRI mainly by basophils, mast cells and Langerhans' cells, offers an additional role for IgE which can possibly mediate allergen/antigen presentation. Enhancement of the specific antibody response can be achieved *in vivo*[7,8] and *in vitro*[9,10] via IgE–CD23 interaction. Whereas implication of IgE and the low-affinity IgE receptor CD23 in facilitating antigen presentation has been extensively investigated, little is known about the IgE-dependent antigen presentation mediated by the high-affinity IgE receptor FcεRI. Amplification by allergen-specific IgE antibodies of the allergen presentation by monocytes was demonstrated in human allergic patients.[11] The most representative FcεRI-positive cells are mast cells and basophils, which are also known to produce a variety of cytokines, in particular interleukin-4 (IL-4), upon cross-linking through IgE.[12,13]

We and others have recently found that mouse bone marrow-derived mast cells (BMMC), as well as rat peritoneal mast cells, can express MHC class II molecules and present processed peptides to specific T cells.[14–16] So far, mast cells have been reported to present antigens internalized through non-specific fluid-phase pinocytosis. To comply with physiological conditions where tissue mast cells naturally occur with

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IgE and IgG antibodies bound on their surface, we investigated the role of such antibodies in the antigen-presenting function of mast cells. In this report, we demonstrate that BMMC which express both FcεRI and FcγR can efficiently process and present antigens complexed to IgE or IgG antibodies. Such mechanisms may contribute to the maintenance or the regulation of the specific allergic immune response, in particular when allergens are present at low concentrations, which is the case for naturally occurring allergens, or when antigens display weak immunogenicity.

MATERIALS AND METHODS

Mice

DBA/2, BALB/c, C57BL/6 and C3H/HeOU mice (8–12 weeks old) were purchased from Janvier (Laval, France).

Recombinant Lol p 1 and Lol p 1 fragments

The gene encoding Lol p 1 was cloned from genomic cDNA of ryegrass pollen following a procedure described earlier.[17] Briefly, genomic DNA was extracted from perennial ryegrass pollen and the coding sequence for Lol p 1 protein was amplified using a polymerase chain reaction (PCR) with the following primers: 5′ GTAAGATATCTTCCT-GGGCAGCGCGCATGGC 3′ and 5′ GATTGGATC-CTCACTTGGCCGAGTAGGAAG 3′. Truncated Lol p 1 polypeptides were engineered by excising C-terminal fragments using either PCR amplification or restriction enzymes. Overlapping fragments N100 (26 amino acids), N200 (63 amino acids), N300 (1–103), N400 (1–132), N500 (1–170) and Lol p 1 (1–240), were produced by PCR amplification using the 5′ oligoprimer JB11 and the 3′ oligoprimers JB13, JB14 and JB15, respectively. The sequence of each construct was verified using a T7 sequencing kit (Pharmacia, Uppsala, Sweden). Subsequently, these fragments were blunt-ended and digested by the restriction enzyme *Eco*RV and ligated to the pMAL-c plasmid in the dephosphorylated *Stu*I restriction site. After transformation of *Escherichia coli* strain TB1 with the recombinant plasmid, the fusion protein was expressed and purified by affinity chromatography using amylose. The recombinant fusion protein was cleaved using factor Xa, and the resulting fragments were separated from maltose-binding protein fragments using an amylose column.

Reagents, antibodies and cell lines

Recombinant mouse IL-3, IL-4 and granulocyte–macrophage colony-stimulating factor (GM-CSF) were purchased from Immugenex (Los Angeles, CA). Ovalbumin (OVA) grade VII was purchased from Sigma (St Louis, MO) and OVA peptide 323–339 from Neosystem (Strasbourg, France). Anti-Thy-1 monoclonal antibody (mAb) from clone 9.37 was given by Dr U. Hammerling and S. Kimura (Sloan-Kettering Institute, NY). Anti-CD4 mAb was prepared from clone GK1.5 [American Type Culture Collection (ATCC), Rockville, MD]. Culture media of the hybridoma Hi-DNP-ε-26.82[18] producing α-dinitrophenyl (DNP)-specific IgE were provided by Dr J. Rivera (NIAMS, National Institutes of Health, Bethesda, MD). Anti-Lol p 1 mAb 1H5, 4F7 and 8H5, all of them of the IgG1 subclass, were obtained from the fusion of Sp2/O-Ag14 cells and spleen cells of mice immunized with Lol p 1.[17] All these mAb were purified from ascitic fluids or cell culture supernatants by affinity chromatography on protein G (Gibco-BRL, Gaithersburg, MD). Biotin-labelled anti-mouse I-Ab/d mAb was prepared from clone 25-9-17S (ATCC). The rat mAb 2.4G2 (ATCC), kindly provided by Dr M. Daëron (Institut Curie, Paris, France), is specific for the mouse FcγRII/III. lipopolysaccharide (LPS) from *Neisseria meningitidis* was a gift from Dr M. Caroff (Institut de Biochimie, Orsay, France). The 2,4-dinitrobenzenesulphonic (DNBS) acid was purchased from Aldrich, Milwaukee, WI. A20 (ATCC) is a B-lymphoma cell line originated from the BALB/c mouse strain. These cells of H-2d haplotype are used as conventional APC.

Production of T-cell hybridoma

BALB/c mice were immunized subcutaneously (s.c.) at the tail base and in the hind footpad with 100 μg of recombinant Lol p 1 emulsified in complete Freund's adjuvant (CFA; Sigma). Ten days later, cells from popliteal, periaortic and inguinal lymph nodes were removed and stimulated *in vitro* at 4×10^6 cells/ml with 20 μg/ml of Lol p 1. After 3 days of activation, cells were mixed one-to-one for fusion with the thymoma cell line BW5147. The cells were pelleted and gently resuspended in 0·5 ml of polyethylene glycol 1500 (Boehringer Mannheim, Germany). After a centrifugation at 120 ***g*** for 4 min, the pellet was resuspended in 8 ml of serum-free RPMI-1640 and the cell suspension was brought to a final volume of 40 ml of enriched medium containing 50% of conditioned medium made from M12 cell incubated for 48 hr in RPMI containing 20% fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, and 4×10^{-5} M 2-mercaptoethanol (2-ME). The cells were then plated in flat bottom 96-well plates at 100 ml/well. The day after, 20 ml of sixfold concentrated selective medium, containing a mixture of hypoxanthine–azaserine, was added to each well. Hybridoma which started to appear 6–10 days later were expanded in 24-well plates and then 100 μl of confluent cells were cocultured with 5×10^4 appropriate APC in the presence of Lol p 1. Positive hybridomas were subcloned by the limiting dilution method and tested for their capacity to produce IL-2 in response to serial dilutions of antigen in the presence of different APC. In a final volume of 200 μl, T-cell hybrids (5×10^4) were cocultured with 10^5 mast cells or 3×10^5 B cells or 5×10^4 A20 cells with or without varying doses of specific antigens. After 24 hr incubation at 37°, 50-μl aliquots of supernatants were removed, frozen and later assayed for IL-2. The IL-2 in 24-hr culture supernatant was titrated using the IL-2-dependent cell line, CTL-L2. Supernatants were incubated for 24 hr with 10^4 CTL-L2 cells and 0·25 μCi [^{3}H]TdR/well was added 8 hr before cell harvesting.

Preparation of B cells

B cells were prepared by incubating spleen cells at 5×10^7/ml in the presence of anti-Thy-1 and anti-CD4 antibodies for 30 min at 4°, the cells were pelleted and resuspended in 1/10 dilution of fresh low-tox rabbit serum complement (Cedarlane, Hornby, Ont., Canada), and incubated for 45 min at 37°. The complement lysis of T cells was repeated twice. The purity of B cells was consistently more than 95–98%. Resting B cells are poor APC because they lack costimulatory activity.[19] In order to activate B cells for efficient antigen presentation,

10^6 B cells/ml were incubated for 24 hr at 37° in the presence of 5 μg/ml of LPS.

Generation of BMMC

BMMC were prepared as described by Razin *et al.*[20] and modified by us.[15] After 3 weeks of culture in the presence of 3 U/ml of recombinant IL-3 (rIL-3), the cells were harvested for experiments and consisted of over 98% mast cells as assessed by toluidine blue staining on day 21. Consistent with our previous reports, non-specific esterase staining, immunofluorescence staining for Mac-1, NLDC-145,[21] and B220 cell surface antigens indicated that the mast cell preparations were not contaminated with macrophages, dendritic cells, or B cells, respectively. For effective antigen-presenting capacity, BMMC were cultured for the last 48 hr before harvest in the presence of 3 U/ml rIL-3, 100 U/ml IL-4, and 3 U/ml GM-CSF.[15]

Antigen presentation and IL-2 assay.

The T-cell stimulation assay was carried out in flat-bottom 96-well plates (GIBCO) using RPMI-1640 supplemented with 5% (v/v) FCS (Boehringer), 5×10^{-5} M 2-ME, 2 mM L-glutamine, 0·1 mM non-essential amino acids (Flow Laboratories, Rickmansworth, UK), penicillin 100 U/ml and streptomycin 0·1 mg/ml (Eurobio, Les Ulis, France). In a final volume of 200 μl/well, T-cell hybridomas (5×10^4) were cocultured with 10^5 mast cells or 3×10^5 B cells with or without varying doses of Lol p 1. In some experiments where internalization of antigen through FcγR was performed, 3 μg/ml of anti-Lol p 1 mAb were allowed to react with antigen for 1 hr at 37° prior to addition to the cultures. After 24 hr incubation at 37°, 50-μl aliquots of supernatants were removed, frozen and later assayed for IL-2. The IL-2 in 24-hr culture supernatant was titrated using the IL-2-dependent cell line, CTLL-2. Supernatants were incubated for 24 hr with 10^4 CTLL-2 cells and 0·25 μCi [^{3}H]TdR/well was added 8 hr before cell harvest. The results are given as the mean counts per minute (c.p.m.) of duplicate cultures from two to four experiments.

Kinetics of antigen presentation

Mast cells (2×10^6 in 0·5 ml of RPMI-1640) were incubated at 37° for various times with 10 μg of Lol p 1. When indicated for IgE-mediated antigen internalization, mast cells incubated for 3 days with anti-DNP IgE mAb (5 μg/ml) were pulsed with DNP–Lol p 1 conjugate. In some experiments, Lol p 1-pulsed mast cells and B cells were fixed with 0·15% paraformaldehyde for 0·5 min at room temperature. After washing in RPMI-1640, aldehyde-free groups were saturated by incubating cells in RPMI-1640 containing 0·5% glycine for 30 min at 37°. After the indicated times of incubation, mast cells (10^5/well) were then cocultured with T-cell hybridoma (5×10^4/well) in a final volume of 200 μl. After 18 hr of culture, supernatants were tested for the presence of IL-2 as previously described.

Fluorescence-activated cell sorter (FACS) analysis

Mast cells (10^6/ml) which express low-affinity receptors FcγRIIa and FcγRIIb1 were labelled for 30 min on ice with rat antimouse FcγR 2.4G2 mAb or with anti-DNP IgE mAb in phosphate-buffered saline (PBS) containing 1% FCS. Subsequently, cells were washed three times in PBS/1% FCS and incubated with either fluorescein isothiocyanate (FITC)-labelled goat anti-rat IgG or with biotin-labelled rat anti-mouse IgE mAb (Biosys, Compiègne, France). After washing, cells were reincubated for 30 min on ice with streptavidin–phycoerythrin (SA–PE) (Dako, Glostrup, Denmark) for detection of bound IgE. After washing, cells were resuspended in 0·5 ml of PBS, kept on ice and analysed in a FACScan cytofluorograph (Beckton-Dickinson, San Jose, CA). Dead cells were gated out by mean of forward scatter and sideward scatter. Fluorescence of 10^4 cells was recorded on a logarithmic scale. Data were normalized with respect to percentage of cells labelled with SA–PE or FITC-goat anti-rat IgG alone as controls.

Lol p 1-DNP conjugation

Coupling of DNP to Lol p 1 was performed to allow binding of Lol p 1–DNP to DNP-specific mAb. Briefly, 2 mg of Lol p 1 were allowed to react with 2 mg of DNBS in a total volume of 2 ml of 0·2 M Na_2CO_3 at room temperature for 5 hr. The uncoupled hapten was removed by dialysis against PBS. Human serum albumin (HSA) (Sigma) was coupled to DNP as for Lol p 1.

RESULTS

Characterization of T-cell hybridoma specific for Lol p 1

BALB/c mice were immunized with Lol p 1, the major allergen of *Lolium perenne*, a structurally well-known molecule against which mAb directed to distinct epitopes are available.[17] We selected a T-cell hybridoma G8A5.2, used throughout this work, which was obtained from hybridization of anti-Lol p 1 T-cell blasts from BALB/c mice with BW5147 thymoma cells. This hybridoma was selected from numerous other T-cell hybrids obtained from this fusion because of its strong responsiveness to low antigen concentrations. To map the Lol p 1 sequence recognized by G8A5.2, this T-cell hybridoma was assayed for its ability to be stimulated to produce IL-2 in response to Lol p 1 as well as five other Lol p 1-derived recombinant overlapping polypeptides spanning the entire Lol p 1 molecule. The length and location of the different fragments are the following: N100 (1–26), N200 (1–63), N300 (1–103), N400 (1–132), N500 (1–170) and Lol p 1 (1–240). As shown in Fig. 1, G8A5.2 responded uniformly to all antigens when the A20 B-cell line as well as BMMC were used as APC. No antigen by itself at any concentration could activate T cells in the absence of APC. The minimal structure recognized by G8A5.2 is the fragment N100, indicating that the specific peptide is comprised within the first 26 amino acids. It is worth noting that G8A5.2 consistently reacted much better when antigens were presented by mast cells.

Kinetics of presentation of T-cell epitope of Lol p 1 by mast cells

To determine the kinetics of processing and presentation of antigen, Lol p 1 was incubated with mast cells for increasing periods of time. After the elapsed time, the cells were washed and either paraformaldehyde-fixed to prevent further processing or left unfixed to allow processing to proceed. The presence on the cell surface of the processed peptide was determined by incubation with the Lol p 1-specific T-cell hybridoma G8A5.2. Figure 2 shows that mast cells took only 30 min to generate a sufficient amount of peptide necessary

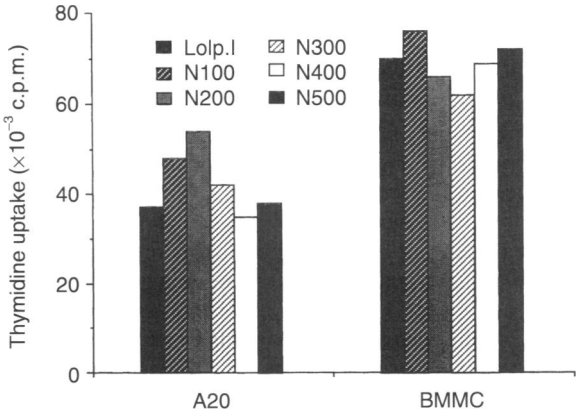

Figure 1. Characterization of Lol p 1-specific T-cell hybridoma. Antigen presentation was carried out by coculturing mast cells (10^5/well) or A20 cells (5×10^4/well) with the T-cell hybridoma G8A5.2 (5×10^4/well), in the presence of Lol p 1 or Lol p 1-derived fragments (8×10^{-5} M). All samples were performed in triplicate and differences between replicate wells were less than 10%. OVA peptide 323–339, used as control, could not be recognized by G8A5.2 hybridoma (less than 2000 c.p.m. for both BMMC and A20 cells). Results are representative of four independent experiments.

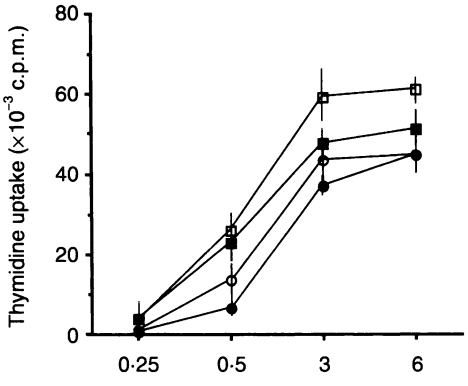

Figure 2. APC unfixed or fixed at different times after antigen pulse exhibit different stimulatory potencies for T-cell hybridoma. GM-CSF-treated mast cells (□, ■) and LPS-stimulated B cells (○, ●) were pulsed with 50 μg/ml of Lol p 1 and after different times of incubation at 37° were left untreated (□, ○) or fixed with paraformaldehyde (■, ●). The cells were washed and incubated at 10^5/well for mast cells and 3×10^5/well for B cells in the presence of 5×10^4 T-cell hybridoma G8A5.2. After 48 hr of culture, IL-2 production was assessed by the CTL-L2 proliferation assay for an additional 8 hr. All samples were performed in triplicate and similar results were obtained in four separate experiments. Each value represents the mean ± SD of triplicates.

for T-cell activation, and the maximal response was achieved after 3 hr of contact with antigen. There is only a minor difference in antigen-presenting capacity between paraformaldehyde-treated and untreated mast cells, indicating a short lag time between internalization of Lol p 1 and generation of immunogenic peptide. Similar conclusions have been obtained by using fixed and unfixed B cells, used as control, except that the overall antigen-presenting capacity was slightly lower than with mast cells. This can be explained by the cell size of mast cells, which are much larger than B cells. These data suggest

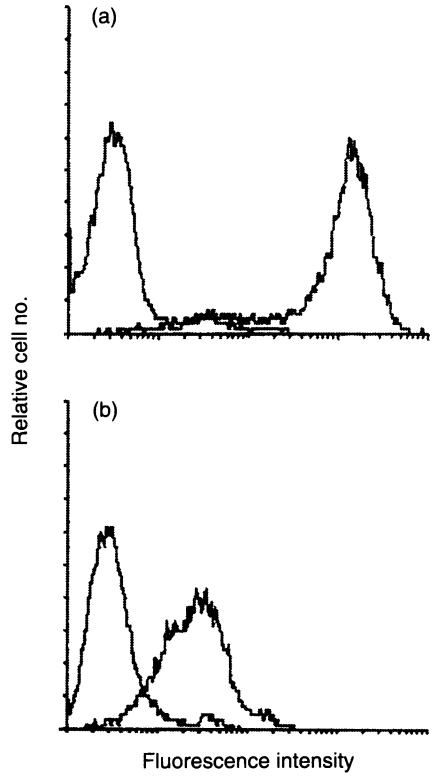

Figure 3. Expression of FcγR and induction of high-affinity receptor for IgE, FcεRI, on mast cells. Induction of FcεRI on mast cells was carried out by incubating cells with 3 mg/ml of anti-DNP monoclonal IgE antibody for 3 days and then FcεRI expression was analysed by flow cytometry using biotinylated rat anti-mouse IgE (RAM-IgE) followed by phycoerythrin-streptavidin (a). The background fluorescence intensity was obtained with mast cells stained with biotinylated-RAM/IgE alone followed by streptavidin–PE. To determine the level of FcγR expression, mast cells were stained with rat anti-mouse FcγRII/RIII 2.4G2 followed by FITC-labelled goat anti-rat IgG (b). Negative controls consisted in incubating cells with an isotype-matched non-specific mAb.

that mast cells as well as B cells operate through an efficient fluid-phase pinocytosis-based mechanism.

Increased efficiency of mast cell presenting function with IgG- and IgE-targeted antigen

Antibody-mediated internalization of antigen and enhancement of specific T-cell responsiveness has been extensively documented using a number of APC. Here, GM-CSF-treated BMMC were analysed for their ability to lower the threshold of T-cell activation by using suboptimal concentrations of antigen the internalization of which was facilitated by specific IgG or IgE antibodies. The expression by BMMC of FcγR was demonstrated by incubating the cells with 2.4G2 mAb (Fig. 3b). Detection of FcεRI required a preincubation of BMMC with anti-DNP IgE mAb for at least 3 days. As previously reported, this procedure is necessary for an optimal induction of FcεRI.[22] As shown in Fig. 3,(a) strong labelling with anti-IgE antibodies indicated that BMMC do express FcεRI. Activation by BMMC of the T-cell hybridoma G8A5.2 by Lol p 1 presented alone or in the presence of three different

anti-Lol p 1 IgG mAb is shown in Fig. 4(a). Lol p 1–anti-Lol p 1 immune complexes activated G8A5.2 T cells to secrete IL-2 at concentrations more than 100-fold less than that required for antigen alone. The three mAb 4F7, 1H1 and 8H5 which recognize distinct epitopes on the Lol p 1 molecule are equally efficient in enhancing the APC function, especially for the low antigen concentrations. Similar to BMMC, B lymphocytes used as control also increased their antigen-presenting capacity when antigen uptake occurred through the three specific mAb (Fig. 4b).

To analyse the influence of DNP-specific IgE antibodies on the ability of mast cells to induce IL-2 production by G8A5.2 T cells in response to Lol p 1, the antigen was coupled to DNP. The coupling of Lol p 1 to DNP and the preservation of its antigenicity were assessed by the recognition of the conjugate by the mAb 1H5 and by the anti-DNP IgE mAb (data not shown). Mast cells expressing, or not expressing, anti-DNP IgE mAb were pulsed with DNP–Lol p 1 for various

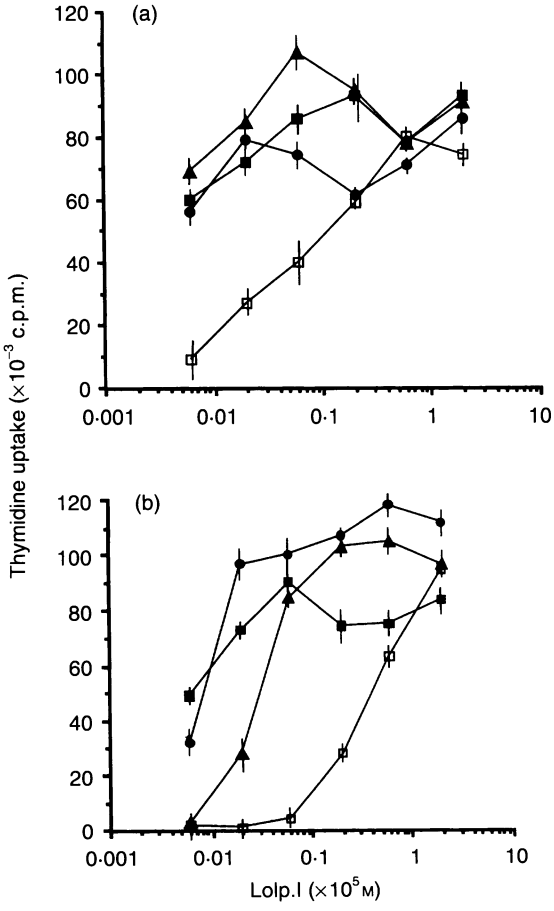

Figure 4. Presentation of Lol p 1 or Lol p 1–IgG complexes to T cells by mast cells and B cells. The T-cell hybridoma G8A5.2 (5×10^4/well) was cultured with Lol p 1 alone (□) at the indicated concentrations in the absence or in the presence of 3 μg/ml of anti-Lol p 1 mAb 4F7 (■), 1H5 (●), or 8H5 (▲). Antigen presentation was carried out using 10^5 GM-CSF-treated mast cells/well (a) or 3×10^5 purified LPS-stimulated B cells/well (b). IL-2 content in culture supernatants (50 μl) was measured by CTL-L2 proliferation assay. Background values of mast cells and B cells cultured alone with T cells were 500 ± 150 c.p.m. (a) and 2500 ± 170 c.p.m. (b), respectively. The data represent the mean ± SD of three different experiments.

times and tested for their ability to stimulate G8A5.2 T cells to secrete IL-2. Results in Fig. 5 show that FcεRI-mediated uptake, as compared to fluid-phase pinocytosis, led to a more efficient presentation of Lol p 1 to T cells. The difference between IgE-bearing mast cells and mast cells without IgE in stimulating the T-cell hybridomas increased over time and was maximal after 3 hr of antigen uptake. These findings raised the question whether the mere cross-linking of IgE mAb by an irrelevant antigen or aggregation followed by IgE-mediated endocytosis of the specific antigen is necessary to the observed IgE-enhanced antigen presentation. Mast cells expressing anti-DNP IgE mAb were cocultured with T-cell hybridoma and various concentrations of Lol p 1 in the absence or in the presence of DNP–HSA. As shown in Fig. 6, cross-linking of anti-DNP IgE mAb by DNP–HSA only slightly altered the antigen presentation of Lol p 1 to the T-cell hybridoma, suggesting that the IgE-enhanced antigen presentation was the result of a combined contribution of uptake, FcεRI aggregation and endocytosis of the antigen by membrane-bound IgE mAb. It should also be emphasized that aggregation of IgE could not be dissociated from IgE-dependent endocytosis of antigen for efficient antigen presentation.

Altogether these data suggest that mast cells are capable of inducing substantial increase in T helper responses when antigens are targeted to these cells via FcγR and FcεRI.

DISCUSSION

Because mast cells have been previously shown to express MHC class II molecules, CD32 (FcγRIIa and FcγRIIb1) and FcεRI and to present various antigens to T cells, an interesting issue is to investigate the antigen-processing capacity of mast cells after antigen-specific capture by IgG or IgE antibodies and its implications on the APC function of mast cells.

As the antigen model, Lol p 1 the major allergen from *Lolium perenne* was selected for its immunogenicity and induction of high specific IgG and IgE titres in mice. The availability of recombinant Lol p 1 and a collection of some of its fragments led to the production of specific T-cell hybridoma. Among a collection of hybrids, one hybridoma G8A5.2, which was found to be particularly sensitive to low antigen concentrations, was extensively used throughout this work.

Using a panel of Lol p 1 fragments, the antigenic determinant recognized by this T-cell hybridoma was located at the N terminus of the molecule within amino acid sequence 1–26. The reactivity of G8A5.2 toward recombinant Lol p 1 and its fragments was analysed. Results showed that IL-2 responses induced with mast cells used as APC were consistently higher than those obtained with the A20 B-cell line.

A characteristic feature of APC such as B cells is that they express surface immunoglobulins directed to particular antigens and become considerably more efficient at presenting their specific antigen.[2,23,24] Antigen presentation by specific B-cell clones can be mimicked by using antibody–antigen complexes with non-specific B cells. Expression of specific antibodies can directly influence the expressed T-cell repertoire resulting from immunoglobulin-directed proteolytic processing within the B cell[25–27]. In other words, specific T and B cells can influence each other's repertoire in a reciprocal manner. It has been shown that specific antibodies complexed to antigens could suppress the generation of certain T-cell

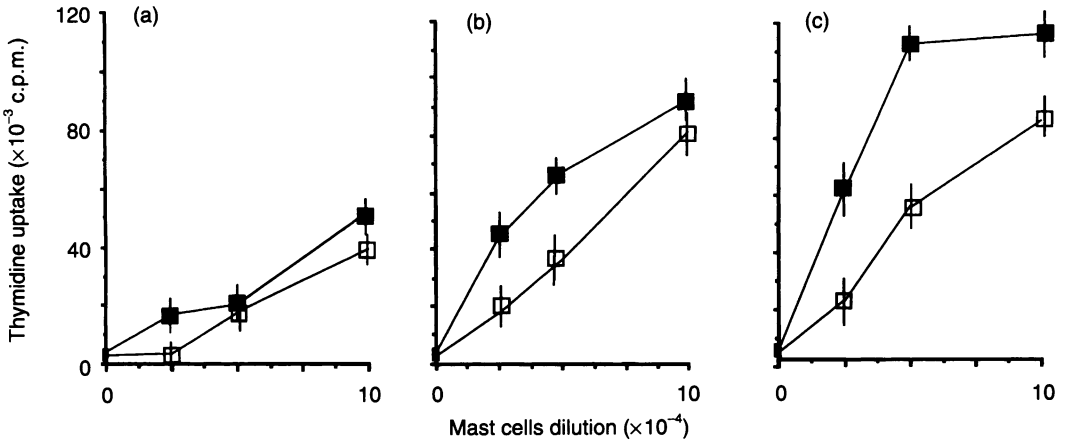

Figure 5. Enhancement of antigen-presenting capacity of mast cells when antigen is captured by membrane-bound specific IgE. Mast cells cultured in the presence of IL-3/IL-4/GM-CSF were sensitized (■) or not (□) for 48 hr with 5 μg/ml of anti-DNP IgE mAb. Mast cells were incubated for 30 min (a), 1 hr (b), or 3 hr (c) with 50 μg/ml of Lol p 1–DNP, washed and incubated at different concentrations in the presence of 5×10^4/well T-cell hybridoma G8A5.2. T-cell response was measured by IL-2 content in supernatants by using the CTL-L2 proliferation assay. Each value represents the mean ± SD of three separate determinations.

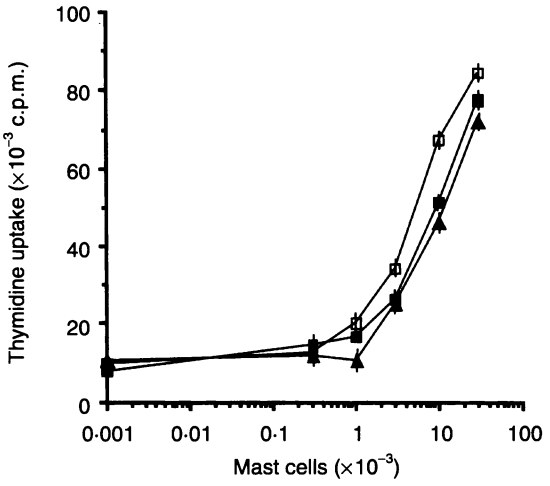

Figure 6. Cross-linking of IgE by irrelevant antigen is not sufficient to increase the antigen-presenting capacity of mast cells. Mast cells (2×10^6/ml) sensitized with anti-DNP IgE mAb were pulsed with 50 μg/ml of Lol p 1 alone (▲) or in the presence of 100 ng/ml (■) or 10 ng/ml (□) of DNP–HSA for 3 hr. After washing, the cells were cocultured at various concentrations in the presence of the T-cell hybridoma G8A5.2 (5×10^4/well). As control, DNP–HSA alone did not induce any T-cell activation. IL-2 response was measured by the CTL-L2 proliferation assay. The data represent the mean ± SD of two different experiments.

epitopes while at the same time enhance the expression of other epitopes by influencing the processing pathway or the antigen uptake, respectively.[28,29] In another study, uptake of antigen–antibody complex through the FcγRIIb1, although 10-fold less effective than FcγRIIb2, resulted in enhanced antigen presentation.[30] In the present work, complexes between Lol p 1 and three different mAb recognizing distinct epitopes on the Lol p 1 molecule were shown to be extremely effective in allowing not only B cells but also mast cells to significantly reduce the threshold of T-cell activation.

IgE-dependent antigen focusing via FcεRI and CD23 (FcεRII) has already been reported in mice and in humans and has been shown to operate in B cells, EBV-transformed B cells and monocytes. In humans, in addition to mast cells and basophils, Langerhans' cells[31,32] as well as monocytes from allergic patients[33] and eosinophils can express FcεRI. Therefore, a role for IgE-mediated antigen presentation in FcεRI-positive cells should be taken into consideration. Our data provide the first direct evidence that mouse mast cells can efficiently internalize low amounts of antigen complexed with IgE via FcεRI. Kinetic studies indicate that IgE-bearing mast cells present immunogenic peptides in a more accelerated and efficient manner than non-sensitized mast cells. We anticipated that a relationship may exist between aggregation and subsequent signalling mediated by FcεRI and the antigen-processing pathway. Our data indicate that aggregation of anti-DNP IgE mAb with an irrelevant antigen (DNP–HSA) did influence, but only slightly, the capacity of mast cells to present Lol p 1 uncoupled to DNP. This does not preclude the possibility that the mere cross-linking of FcεRI could be the essential mechanism underlying IgE-mediated enhanced antigen presentation in IgE-bearing mast cells cultured in alternative cytokine conditions. Indeed, the mast cells used as APC throughout this work were cultured in the presence of IL-3/IL-4/GM-CSF which are the appropriate conditions for optimal antigen presentation.[15] Interestingly, mast cells cultured in IL-3 alone or treated with interferon-γ (IFN-γ), which strongly increases MHC class II expression, are not able to present antigens to T cells.[15] One could argue that depending on mast cell culture conditions, in a particular cytokine environment, signalling via IgE–FcεRI complexes may overcome deficient antigen presentation. This issue is currently under study in a model system using IFN-γ-treated mast cells.

Major implications may result from mast cell–T cell interaction: IgE–antigen complex formation leads to aggregation of FcεRI and causes immediate release of inflammatory mediators and cytokines.[12] Furthermore, cytokine gene activation, especially IL-4, has recently been shown also to occur through aggregation of mast cell MHC class II molecules.[34] This synergy between FcεRI-mediated and MHC class II-mediated IL-4 production may occur during antigen

presentation and could lead to the up-regulation of specific IgE synthesis by inducing T helper cells to differentiate into Th2 phenotype.

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