THE LOW-AFFINITY RECEPTOR FOR IgE (CD23) ON
B LYMPHOCYTES IS SPATIALLY ASSOCIATED WITH
HLA-DR ANTIGENS

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Receptors for the Fc region of IgE (FcER) have been found on a variety of
cell types of hemopoietic origin. High-affinity receptors are expressed on mast
cells and basophils, whereas low-affinity receptors (FcER,) are expressed on T
and B lymphocytes, monocytes, and platelets (1–4). FcER,-bearing lymphocytes
play a major role in the regulation of IgE production (5–7). Recently, we reported
the generation of a hybridoma secreting mAb 25, which specifically recognizes
the 42,000 M, FcER, expressed on human EBV-transformed B cell lines and on
a fraction of normal human B cells (8). We and others (9) have demonstrated
that the CD23 antigen is an FcER, since the CD23 mAbs inhibited the binding
of IgE.

After fusion of splenocytes from mice immunized with either RPMI 8866 cells
or an enriched FcER,./CD23 preparation obtained from RPMI 8866 cells, we
obtained two mAbs, mAb 135 and 449114, respectively, which inhibited the
binding of IgE to FcER,./CD23-bearing cells. Interestingly, these antibodies were
found to bind to cell lines that do not express the FcER,./CD23. In this report, it
is shown that mAb 135 and 449114 bind to HLA-DR antigens and that the
FcER,./CD23 is spatially associated with the HLA-DR antigens on the B cell
surface.

Materials and Methods

Production of mAbs

mAb 135. BALB/c mice (Iffa Credo, Les Oncins, France) were injected intraperito-
neally three times with 5 × 10^7 RPMI 8866 cells in PBS at 4-wk intervals. 2 d before the
fusion, mouse serum samples were collected and were demonstrated to have anti-FcER,
activity. The binding of IgE to RPMI 8866 cells was inhibited at 1/50,000 mouse antisera
dilutions using the assay described below.

Mouse spleen cells were fused with NS-1 myeloma cells (ratio 5:1) using polyethylene
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Abbreviations used in this paper: DSF, dithio-bis-(succinimidyl propionate); FcER, receptors for
the Fc region of IgE; FcER, low-affinity receptors for the Fc region of IgE.

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glycol 1000 (Merck-Schuchardt, Darmstadt, Federal Republic of Germany). After incubation overnight at 37°C in a 50-ml flask in complete RPMI 1640 medium as described below, the cell suspension was distributed in 24-well plates in medium containing hypoxanthine-azaserine. 1 wk later, hybridoma supernatants were screened for their ability to inhibit binding of IgE to RPMI 8866 cells. Hybridomas that produced antibodies inhibiting the binding of IgE were cloned and subcloned by limiting dilution.

mAb 449 B4. mAb 449 B4 was obtained from a mouse immunized with an enriched preparation of FcER, CD23 derived from RPMI 8866 cells. Briefly, RPMI 8866 cells were solubilized in extraction buffer (see below) containing 0.5% NP-40. The cell extract was passed on an IgE-Sepharose immunoabsorbent. The bound fraction was eluted with glycine-HCl buffer, pH 3.0. BALB/c mice were repeatedly immunized by intraperitoneal injections of the fraction included in CFA, and their spleen cells were fused with SP2/O-Ag14 cells (10).

For ascites production, BALB/c mice were injected intraperitoneally with 0.5 ml of pristane (Aldrich Chemical Co., Strasbourg, France). 10 d later, 10⁵ hybridoma cells were injected intraperitoneally and ascitic fluid was collected 2–3 wk later. The ascitic fluid was centrifuged at 30,000 g for 30 min at 4°C to remove lipids and cellular debris.

mAbs were isolated from ascitic fluid by affinity chromatography on protein A columns according to manufacturers instructions (Bio-Rad Laboratories, Richmond, CA). In some cases, mAbs were purified from culture supernatants by affinity chromatography on rabbit anti–mouse Ig coupled to Affigel 10 (Bio-Rad Laboratories). The mAbs were eluted with 0.2 M glycine-HCl buffer, pH 2.5, and the pH was further adjusted to 7.2 with 1 M phosphate buffer. The isotype of the mAb was determined by Ouchterlony analysis using polyclonal antibodies specific for mouse Ig classes and subclasses (Zymed Laboratories, San Francisco, CA). Preparation of mAb F(ab')₂, and Fab' fragments, and biotinylation of mAbs were performed according to standard procedures as described previously (8).

Cell Lines

The Burkitt lymphoma lines (Daudi and BJAB) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). These cell lines were cultured in RPMI 1640 (Flow Laboratories, Irvine, Scotland) supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine; all were purchased from Flow Laboratories.

Monoclonal Antibodies

The mAb 25 is specific for the FcER, on B lymphocytes (8) and precipitates a 42,000 Mᵣ polypeptide from EBV-transformed lymphoblastoid cell lines. The mAbs L243 (anti-HLA-DR) (11) and B7/21 (anti-HLA-DP) were obtained from Becton Dickinson & Co. (Mountain View, CA). The mAb IOT2 (anti-HLA-ABC) was obtained from Immunotech (Luminy, France). The SPV-L3 mAb (anti-HLA-DQ) has been described previously (12). The mAb IF5 (CD20) was a kind gift of Dr. E. Clark (University of Washington, Seattle, WA).

Analysis with a FACS

Fluorescence analysis was performed with a FACS 440 (Becton Dickinson & Co., Sunnyvale, CA) equipped with a 5 W argon laser running at 488 nm, 0.5 W. Fluorescence parameters were collected using a built-in logarithmic amplifier after gating on the combination of forward light scatter (FLS) and perpendicular light scatter (PLS), which was used to discriminate viable from nonviable cells.

Inhibition of the Binding of IgE to RPMI 8866 Cells. Hybridoma supernatants were screened for their ability to inhibit the binding of IgE to RPMI 8866 cells as described previously (13) or to inhibit rosette formation of RPMI 8866 cells with IgE-coated erythrocytes (10). Briefly, 5 × 10⁵ cells were incubated 30 min at 4°C under gentle agitation with hybridoma supernatants (100 μl), in a 0.2-ml microtiter plate well. After two washes (150 μl, 7 min) with PBS, 1% BSA, 0.1% NaN₃, soluble monomeric (IgE PS) was added at a concentration of 10 μg/ml. The plate was then incubated 30 min at 4°C.
Subsequently, cells were washed and incubated with a specific rabbit anti-human IgE antiserum (1/1,000 diluted) for 30 min at 4°C. Cells were then washed and incubated with goat anti-rabbit Ig coupled to fluorescent microspheres (Fluoresbrite Carboxylate, diameter 0.57μm, ref. 15700; Polysciences, Inc., Warrington, PA) for 30 min at 4°C. Finally, the cell suspension was layered on 3 ml heat-inactivated FCS and centrifuged 15 min at 100 g to remove nonbound beads. The samples were resuspended in 200 μl PBS, BSA, NaNO₃, and analyzed by flow cytometry as described above. Under these conditions, 45-70% of the cells were fluorescent. mAb 25 (anti FcER₁/CD23) stains a higher percentage of cells (80-95%) because it has a 1,000-fold higher affinity for the FcER₁/CD23 than the IgE.

Single-Color Indirect Staining. Single- and double-fluorescence stainings were performed in microtiter plates. 5 X 10⁵ cells (in 50 μl) were incubated with 100 μl of hybridoma supernatant or 10 μl of mAb appropriately diluted. After two washes, cells were incubated with fluoresceinated F(ab’)₂ fragments of goat anti-mouse Ig (Grub, Vienna, Austria) for 30 min at 4°C at a dilution of 1/100. The stained cells were analyzed with the FACS.

Two-Color Immunofluorescence. For double-fluorescence analysis, mononuclear cells were incubated simultaneously with commercially available FITC-conjugated mAbs appropriately diluted and biotinylated mAb 135. The binding of biotinylated antibody was assessed using phycoerythrin-conjugated streptavidin (Becton Dickinson & Co).

Inhibition of the Binding of mAbs. Inhibition of binding was carried out as follows. The cell suspension was incubated with an excess of the first mAb for 30 min at 4°C. After three washes with PBS/BSA, cells were incubated with a second biotinylated mAb (see below). After three washes, the binding of the second antibody was revealed by incubation of the cells with phycoerythrin-conjugated streptavidin. After three washes, cells were analyzed with the FACS.

Human B Lymphocyte Cultures with Human IL-4

B cells were isolated from tonsils obtained from children with chronic tonsillitis. Mononuclear cells were separated on Ficoll/Hypaque. T cells were removed from the mononuclear cells by twice rosetting with 2-aminoethylisothiouronium bromide (AET, Sigma Chemical Co., St. Louis, MO)-treated sheep erythrocytes. Monocytes were depleted by adhering 250 X 10⁶ T cell-depleted cells to plastic flasks (Corning Glass Works, Corning, NY) containing 25 ml RPMI 1640 with 10% FCS for 1 h at 37°C. The tonsillar B cell preparations contained: >95% sIg⁺ cells, as determined by staining with a fluorescein-conjugated F(ab’)₂ fragment of goat anti-human Ig (Behring werke, Marburg, Federal Republic of Germany); >95% CD20 antigen-positive cells, as determined by staining cells with a mouse anti-human B cell-specific mAb antibody, B1 (Coulter Immunology, Hialeah, FL) and fluorescein-conjugated F(ab’)₂ goat anti-mouse Ig (Grub); <1% T cells, as determined by the Leu-1 mAb (Becton Dickinson & Co.); <1% monocytes, as determined by the Leu-M3 mAb (Becton Dickinson & Co).

For activation, the purified B cells were cultured at a density of 10⁶ cells/ml in Iscove’s medium enriched with 50 μg/ml human transferrin, 0.5% BSA, and oleic, linoleic, and palmitic acid (all from Sigma Chemical Co.) as described by Yssel et al. (14). 2% of FCS was added to the medium. Rabbit anti-IgM antibody coupled to beads (Bio-Rad Laboratories) was added at a final concentration of 5 μg/ml. Human rIL-4 (15) was added as a COS-7 transfection supernatant at a final concentration of 1% (80 U/ml). 1 U of IL-4 is defined as the amount resulting in a half maximal [³H]TdR uptake in activated PHA blasts (15).

This lymphokine is able to induce FcER₁/CD23 on normal human B lymphocytes (16, 17). After 48 h of culture, viable B lymphocytes were separated from the nonviable cells by centrifugation on a Ficoll/Hypaque gradient and were surface labeled as described below.

Cytotoxicity Inhibition Assay

The cytotoxicity inhibition assay was carried out as described elsewhere (18). Briefly, ⁵¹Cr-labeled JY cells were incubated with diluted samples of different mAbs. Then effector
FcER\textsubscript{I}/CD23 IS ASSOCIATED WITH B CELL HLA-DR ANTIGENS

cells were added and after 4 h of incubation, supernatants were harvested, counted, and
lysis was calculated with the formula: Percent specific lysis = \( \frac{100 \times [(\text{cpm of sample}) - (\text{cpm of medium control})]/(\text{cpm in presence of Triton} \times 100) - (\text{cpm of medium control})]}{100} \).

The alloreactive cytotoxic IL-2-dependent human T cell clones HY640 and JR-2-10
(19) were used as effector cells. The clones HY640 and JR-2-10 specifically recognized
HLA-DQ-1 and HLA-DRW6 antigens, respectively, on JY cells.

**Immunoprecipitation and SDS-PAGE**

5 \times 10^7 RPMI 8866 cells or rIL-4-treated B lymphocytes were washed twice with PBS
containing 1 mM PMSF (Sigma Chemical Co.) and then resuspended in 1 ml PBS/0.5
mCi \(^{125}\)I-Na (Oris, Saclay, France); 100 \( \mu \)l lactoperoxidase at 5 mg/ml (Calbiochem-
Behring Corp., La Jolla, CA), and 50 \( \mu \)l H\textsubscript{2}O\textsubscript{2} (0.03% in water) were added. After 3 min
of gentle agitation at room temperature, another 100 \( \mu \)l of lactoperoxidase and 50 \( \mu \)l of
H\textsubscript{2}O\textsubscript{2} were added. The suspension was again agitated for 3 min at room temperature.
This step was repeated once again and the cells were finally washed with PBS containing
5 mM K\textsubscript{l}.

Cells were resuspended at 0°C for 15 min in 300 \( \mu \)l extraction buffer composed of
PBS, pH 7.8, with the protease inhibitors: 20 mM iodoacetamide, 1 mM PMSF, 0.01 M
benzamidine hydrochloride, 1 \( \mu \)g/ml each of leupeptin, pepstatin, and 100 \( \mu \)g/ml soybean
trypsin inhibitor (all from Sigma Chemical Co.) containing either 0.5% NP-40 or 1%
digitonin (Aldrich Chemical Co.). Digitonin was prepared as a 2% stock solution: the solid
detergent was added to boiling water, which was stirred for 2 min, cooled, allowed to
stand at room temperature for 1 wk, and then filtered. The lysates were then centrifuged
at 10,000 g for 15 min and the supernatant at 100,000 g for 30 min before preclearing
overnight with a 50-\( \mu \)l packed volume of protein A–Sepharose beads (Pharmacia Fine
Chemicals, Uppsala, Sweden) precoated with a nonrelated mAb (100 \( \mu \)l of ascites).
Immunoprecipitation was performed by incubating the extract for 4 h at room tempera-
ture with 50 \( \mu \)l protein A–Sepharose beads precoated with the different mAbs. Immu-
noprecipitates were washed five times with the extraction buffer and subjected to SDS-
PAGE using the method of Laemmli (20). The gels were then fixed, dried, and autoradiographed using X-OMAT AR-65 film (Eastman Kodak, Paris, France) at -70°C.

**Protein Elution from SDS-PAGE Gel**

The SDS-PAGE gel containing the 42,000 M\textsubscript{r} polypeptide isolated by immunoprecipi-
tation with mAb 135 from \(^{125}\)I-labeled and dithio-bis-(succinimidylpropionate) (DSP)-
crosslinked RPMI 8866 cell lysates, was sliced into small pieces and a carrier protein,
BSA, (Sigma Chemical Co.) was added. The polypeptide was eluted in extraction buffer
mentioned above for 20 h at 37°C. After centrifugation, 12,000 rpm for 30 min, the
supernatant was then immunoprecipitated with mAb 25 or with normal mouse Ig as
control.

**Crosslinking**

Radiolabeled cells were resuspended at 3 \times 10^7/ml in PBS pH 7-4 containing 1 mM
MgCl\textsubscript{2}, 0.02% sodium azide. The cleavable crosslinker DSP (Pierce Chemical Co., Rock-
ford, IL) was dissolved in DMSO at a concentration of 10 mM and added to the cell
suspension at a final concentration of 1 mM. Reaction was allowed to occur for 30 min at
4°C under gentle agitation. After two washes with PBS to remove free DSP, the cell
suspension was lysed and immunoprecipitations were carried out as described above.

**Results**

*Certain mAbs Specific for HLA-DR Antigens Partially Inhibit the Binding of
IgE to FcER\textsubscript{I}/CD23*

To obtain mAbs specific for the FcER\textsubscript{I}, the supernatants of hybridomas
obtained from a mouse immunized with the FcER\textsubscript{I} + RPMI 8866 cell line were
Figure 1. mAb 135 inhibits the binding of IgE to RPMI 8866 cells. (A) RPMI 8866 cells were incubated with 10 μg/ml of IgE (open) or without IgE as negative control (shaded), then with polyclonal anti-IgE antiserum and goat anti-rabbit Ig coupled to fluorescent microspheres. (B) RPMI 8866 cells were preincubated with 50 μg/ml of mAb 135 (shaded) or without (open), then reacted subsequently with IgE, polyclonal anti-IgE antiserum and goat anti-rabbit Ig coupled to fluorescent microspheres. (C) RPMI 8866 cells were preincubated with 50 μg/ml of mAb IOT2 (anti HLA-ABC) (shaded) or without (open), then treated as in A and B. Fluorescence was analyzed by flow cytometry. Abscissa, log fluorescence intensity; ordinate, relative number of cells.

screened for their ability to inhibit the binding of IgE to the same cells. The supernatant of one hybridoma, 9P135, inhibited the binding of IgE as determined in an indirect fluorescent microspheres assay. One subclone of this hybridoma was selected that secreted mAb 135 (IgG1) (Fig. 1). Purified mAb 135 and its derived F(ab')2 and Fab' fragments inhibited the binding of IgE to RPMI 8866 cells (Table I), and several other EBV-transformed cell lines (data not shown), in a dose-dependent manner. In contrast with mAb 25 (anti-FcεR1/CD23), which totally inhibits the binding of IgE to its receptor, mAb 135, even at high concentrations, only partially inhibits IgE binding to the FcεR1. MAb 135 decreases both the percentage of cells binding IgE and the fluorescence intensity of cells that still bind IgE (Fig. 1). RPMI 8866-reactive nonrelated mAbs such as mAb IOT2 (anti-HLA-ABC) and the CD20 mAbs 1F5 and B1 failed to inhibit IgE binding to RPMI 8866 cells (Table I, and data not shown). MAb 135 was, however, found to bind to FcεR1/CD23− cells (e.g., Daudi, BJAB). Immunoprecipitation analysis demonstrated that mAb 135 precipitated two polypeptides of 36,000 and 28,000 M, (Fig. 2), suggesting that it was specific for the HLA class II antigens. Binding of mAb 135 analyzed by flow cytometry to a large panel of cell lines differentially expressing HLA-DP, -DQ, and -DR antigens suggested that it was binding to HLA-DR antigens (not shown here). The specificity of mAb 135 for HLA-DR antigens was further substantiated by the finding that mAb 135 was able to specifically inhibit the cytotoxicity of the T cell clone JR-2-10 recognizing HLA-DR antigens on the JY target cells, whereas the cytotoxicity of the T cell clone HY640 recognizing HLA-DQ antigens was not affected by mAb 135 (Table II). Another mAb, 449B4, obtained from a mouse immunized with enriched FcεR1/CD23 preparations, was found to inhibit the binding of soluble IgE to EBV cell lines and inhibit rosette formation of RPMI 8866 cells with IgE-coated ox erythrocytes. Furthermore, mAb 449B4 was found to inhibit the binding of mAb 135 to a variety of cell lines (not shown) and to precipitate two polypeptides with 36,000 and 28,000 M, demonstrating that it had the same specificity as mAb 135. The sequence of the 36,000 M, polypeptide recognized by mAb 449B4 proved to be identical to the sequence of HLA-DRα, and the sequence of the 28,000 M, polypeptide, also identified by
mAb 135 Inhibits IgE Binding to its Receptor on RPMI 8866 Cells via its Fab Fragments

<table>
<thead>
<tr>
<th>mAb</th>
<th>FcεR1⁺ cells*</th>
<th>Inhibition IgE binding²</th>
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</thead>
<tbody>
<tr>
<td>mAb 135</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 µg/ml</td>
<td>26</td>
<td>44</td>
</tr>
<tr>
<td>20 µg/ml</td>
<td>34.6</td>
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<tr>
<td>10 µg/ml</td>
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<td>9</td>
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<tr>
<td>F(ab')² mAb 135</td>
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<td>36</td>
</tr>
<tr>
<td>20 µg/ml</td>
<td>36.5</td>
<td>17</td>
</tr>
<tr>
<td>Fab' mAb 135</td>
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<td></td>
</tr>
<tr>
<td>50 µg/ml</td>
<td>34</td>
<td>24</td>
</tr>
<tr>
<td>20 µg/ml</td>
<td>37.7</td>
<td>14</td>
</tr>
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</tr>
<tr>
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<td>91</td>
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</tr>
<tr>
<td>C⁻¹</td>
<td></td>
<td>4.5</td>
</tr>
</tbody>
</table>

* Cells were incubated with intact mAb 135, F(ab')², Fab' or Fc fragments and nonrelated mAb (as control). Then 10 µg/ml of IgE, polyclonal anti IgE antiserum and goat antirabbit Ig coupled to microspheres were successively added.

* Calculated according to the formula: Percent inhibition = 100 × ([assay - C] / (C⁺ - C⁻)) (normalized values of 3 assays).

§ mAb 25 was used at 10 µg/ml and stained 90% of cells.

† mAb IOT2 (anti HLA ABC) was used at 50 µg/ml and stained 98% of cells.

IgE was omitted in the microsphere assay procedure.

mAb 449B4, was shown to be homologous to reported sequences of HLA-DRβ (Dr. M. Bond, DNAX Research Institute, personal communication). This demonstrated that certain anti-HLA-DR antibodies are able to partially block the binding of IgE to FcεR1/CD23, suggesting a possible association of HLA-DR antigens with FcεR1/CD23. Not all the anti-HLA-DR antibodies were found to inhibit the binding of IgE. The well-characterized mAb L243 (11) was unable to inhibit the binding of IgE to FcεR1/CD23⁺ cell lines and was also unable to inhibit the binding of mAb 135 (Fig. 3) to the same cell lines, demonstrating that these antibodies are binding to different epitopes of the HLA-DR molecule. An mAb specific for HLA-DQ antigens (12) and an mAb specific for HLA-DP antigens were unable to inhibit the binding of IgE to RPMI 8866 cells.

Anti-HLA-DR antigen mAbs may therefore be divided into two well-defined groups: one group inhibiting the binding of both IgE and mAb 135 and a second group that does not affect the binding of IgE or mAb 135. Nevertheless, both groups of antibodies bound 36,000 and 28,000 M, proteins expressed on the cell
TABLE II

The Effect of Antibodies against Class II MHC Antigens on the Lysis of JY Cells by Two CTL Clones

<table>
<thead>
<tr>
<th>CTL clone</th>
<th>Lysis of the control</th>
<th>Percent lysis in the presence of antibody*</th>
<th>100*</th>
<th>500</th>
<th>100</th>
<th>500</th>
<th>100</th>
<th>500</th>
<th>100</th>
<th>500</th>
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<tr>
<td>HY-640</td>
<td></td>
<td></td>
<td>44%</td>
<td>97</td>
<td>44</td>
<td>97</td>
<td>44</td>
<td>97</td>
<td>44</td>
<td>97</td>
</tr>
<tr>
<td>JR-2-10</td>
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<td></td>
<td>50%</td>
<td>97</td>
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<td>97</td>
<td>50</td>
<td>97</td>
<td>50</td>
<td>97</td>
</tr>
</tbody>
</table>

* Cr-labeled JY cells were incubated with different mAbs and used as targets in a cytotoxicity assay with class II MHC antigen-specific CTL clone.
* Cytotoxicity was measured at an E/T ratio of 5:1.
* Reciprocal dilution of ascites fluid.

Taken together, these data demonstrate that certain mAbs directed against HLA-DR antigens have the ability to inhibit partially the binding of IgE to FcεRI/CD23 and suggest a physical interaction between FcεRI/CD23 and HLA-DR antigens.

Spatial Association between FcεRI/CD23 and HLA-DR Antigens

Crosslinking Studies. One approach to studying the possible physical associa-
Staining of RPMI 8866 cells with the mAbs L243 and mAb 135. RPMI 8866 cells were stained with normal mouse Ig (NMlg) and goat anti-mouse coupled to FITC (A), with biotinylated mAb 135, revealed with phycoerythrin-streptavidin (B), with FITC-conjugated L243 (C), or cells were double-stained with biotinylated mAb 135 and FITC-conjugated L243. Biotinylated mAb 135 was developed with phycoerythrin-streptavidin (D). Samples were analyzed with a FACS 440 with logarithmic amplification.

The association of two cell surface molecules on the cell membrane surface is chemical crosslinking. This approach was used to show the association between the T cell receptor and the CD3 complex (21) and the association between the insulin receptor and the HLA class I antigens (22). To study the possible physical association between FcαR/CD23 and HLA-DR antigens, 125I-labeled RPMI 8866 cells were crosslinked with DSP and the NP-40 cell lysate was then precipitated with either mAb 135 or mAb 25 (anti-FcαR/CD23). SDS-gel analysis shows that mAb 135 precipitated, in addition to the expected 36,000 and 28,000 M₉, class II MHC polypeptides, a 42,000 M₉, polypeptide and, weakly, a 90,000 smear (Fig. 4). The anti-HLA-DR mAb L243 also precipitated these three major polypeptides from the crosslinked cell extract (not shown). To demonstrate that the 42,000 M₉, polypeptide was the FcαR/CD23, the SDS-PAGE gel was sliced in the 40,000–45,000 M₉, area and the protein was eluted and subjected to
FIGURE 4. FcεRI/CD23 and HLA-DR antigens are spatially associated on RPMI 8866 cells. RPMI 8866 cells were labeled by surface iodination with 125I-lactoperoxidase and solubilized with digitonin lysis buffer or treated with DSP and then solubilized with NP-40 lysis buffer. After preclearing, the cell lysates were immunoprecipitated with mAb 25 (A), mAb 135 (B), or normal mouse Ig (C). The precipitates were analyzed in the same conditions as in Fig. 2. Arrows indicate the migration of FcεRI and HLA-DR antigens.

precipitation with the FcεRI/CD23-specific mAb 25. Fig. 5 shows that the eluted polypeptide could specifically be precipitated by mAb 25, thus demonstrating that the 42,000 M, polypeptide coprecipitated with the anti-HLA-DR antibody mAb 135 was indeed FcεRI/CD23. The smear around 90,000 M, was also present and most probably represents an aggregated form of the FcεRI/CD23. Conversely, mAb 25 precipitated in addition to the expected 42,000 M, FcεRI/CD23 polypeptide and 90,000 M, smear, two polypeptides with 36,000 and 28,000 M, when cell surface components on RPMI 8866 cells were cross-linked (Fig. 4).

Digitonin Cell Extract. The use of the detergent digitonin made it possible to prepare cell extracts in which complex receptors composed of noncovalently linked proteins could be isolated (23). Therefore 125I-labeled RPMI 8866 cells extracts were prepared using digitonin and immunoprecipitated with either mAb 25 or mAb 135. Under these conditions, mAb 25 was able to precipitate, in addition to the expected 42,000 M, FcεRI/CD23, the polypeptides with 36,000 and 28,000 M, (Fig. 4). Conversely, mAb 135 coprecipitated with the expected 36,000 and 28,000 M, polypeptides, the 42,000 M, molecule (Fig. 4). The 90,000 M, smear was also found under these conditions.

Taken together, these biochemical data demonstrate that the FcεRI/CD23 and HLA-DR antigens are spatially associated on the cell surface of EBV-transformed B cell lines.

The IL-4-induced FcεRI/CD23 on Normal B Cells Is Associated with HLA-DR Antigens

It has recently been shown that human IL-4 (B cell stimulatory factor 1 [BSF1]) (15) was able to induce a strong expression of FcεRI/CD23 on normal B
lymphocytes (16, 17). We therefore investigated whether the IL-4-induced FcR\textsubscript{R1}/CD23 on normal B lymphocytes was spatially associated with HLA-DR antigens as demonstrated above on the EBV-transformed cell lines. Purified tonsil B cells were cultured for 48 h with rIL-4 (80 U/ml) in the presence of anti-IgM antibody coupled to beads, conditions that were found to be optimal for the induction of FcR\textsubscript{R1}/CD23. As a control, cells were cultured with anti-IgM antibody coupled to beads, which does not induce a significant expression of FcR\textsubscript{R1}/CD23. Viable cells, obtained after centrifugation over Ficoll/Hypaque, were labeled with \textsuperscript{125}I-lactoperoxidase and crosslinked with DSP. The NP-40 extract was precipitated with mAb 135 or mAb 25. In Fig. 6 it is demonstrated that mAb 135 is able to precipitate the three bands with 42,000, 36,000, and 28,000 \(M_r\) from the rIL-4-cultured B cells. Conversely, mAb 25 coprecipitated with the expected 42,000 \(M_r\) FcR\textsubscript{R1}/CD23, the 36,000 and 28,000 \(M_r\) polypeptides from the rIL-4-cultured B cells. The 90,000 \(M_r\) smear was also observed in the IL-4-treated cells. From control cells, which are FcR\textsubscript{R1}/CD23\textsuperscript{−} negative and DR\textsuperscript{+}, mAb 135 could only precipitate the 36,000 and 28,000 \(M_r\) polypeptides, and mAb 25, as expected, no polypeptide. These results demonstrate that the IL-4-induced FcR\textsubscript{R1}/CD23 on normal B cells is spatially associated with HLA-DR antigens.
Discussion

We have previously described the generation of mAb 25, which specifically recognized the FcεR₁/CD23 (8), and blocked the binding of IgE to human B cells. We found two other mAbs (mAbs 135 and 449 B4) that partially inhibited the binding of IgE to the low-affinity receptor for IgE on the EBV-transformed lymphoblastoid cell line RPMI 8866. These antibodies decreased both the percentage of cells binding IgE and the fluorescence intensity of cells that still bind IgE. mAb 135 was obtained from fused splenocytes of a mouse immunized with RPMI 8866 cells, whereas 449 B4 was obtained from fused splenocytes of a mouse immunized with an enriched preparation of FcεR₁ derived from RPMI 8866 cells.

Unlike mAb 25, mAbs 135 and 449 B4 were able to stain FcεR₁/CD23⁻ cell lines (Daudi, BJAB). This suggested that these antibodies were binding to either an epitope common to FcεR₁/CD23 and to another cell surface molecule or to an antigen not antigenically related to the FcεR₁/CD23 but linked to it in such a fashion that the binding of the mAb 135 or mAb 449 B4 would affect the binding of IgE to the FcεR₁/CD23.

The partial inhibition of binding of IgE by mAbs 135 and 449B4 and the finding that some FcεR₁/CD23 could still be immunoprecipitated by mAb 25 from NP-40 extracts of DSP crosslinked cells precleared with mAb 135 (data not shown) suggest that not all the FcεR₁/CD23 were associated with the HLA-DR antigens. Immunofluorescence analysis performed with a panel of cell lines differentially expressing HLA-DR antigens (as well as DP and DQ, not shown here), biochemical analysis (immunoprecipitation of 36,000 and 28,000 M,
polypeptides), inhibition of cytotoxicity of HLA-DR-specific T cell clones, and protein sequencing of mAb 449B4–recognized antigen (Dr. M. Bond, DNAX Research Institute, personal communication) demonstrated that mAb 135 and 449B4 were specific for HLA-DR antigens. This suggested that the FcER1/CD23 was somehow associated with HLA-DR antigens. It could be excluded that the mAb 135 and 449B4 were binding to an epitope common to FcER1/CD23 and HLA-DR since they only precipitated the HLA-DR polypeptides under standard conditions. Interestingly, not all the anti-HLA-DR mAbs were able to inhibit the binding of IgE. For instance, the well-characterized anti-HLA-DR mAb L243 (11) was unable to inhibit the binding of either IgE or mAb 135 to RPMI 8866 cells, suggesting that mAb L243 binds to an epitope of the HLA-DR molecule distant from the site of interaction of HLA-DR molecules with the FceR1/CD23.

To study the possible association of HLA-DR antigens and FceR1/CD23, 125I-Na-lactoperoxidase-labeled cells were crosslinked with DSP and solubilized with NP-40. The anti-FceR1/CD23 mAb 25 could precipitate the expected 42,000 M, polypeptide, a 90,000 M, smear, and two polypeptides of 36,000 and 28,000 M,.

Conversely, mAb 135 and 449 B4 precipitated the 42,000 M, polypeptide and the 90,000 M, smear, in addition to the expected 36,000 M, and 28,000 M, polypeptides. The 42,000 M, polypeptide precipitated by anti-HLA-DR antibodies was found to be the FceR1/CD23 since it could be precipitated by mAb 25 after solubilization from the SDS-PAGE gel. The smear around 90,000 M, has been previously suggested to represent an aggregated dimer of the 42,000 M, band (24). Moreover this 90,000 M, band, inconsistently found, appeared when the 42,000 M, eluted material was stored at −20°C for a few days before use (data not shown). When intact noncrosslinked labeled cells were solubilized with Digitonin instead of NP-40, a procedure that maintains the noncovalent association between the different polypeptides of complex receptors (23), mAb 25 could immunoprecipitate the 28,000 and 36,000 M, polypeptides together with the 42,000 M, polypeptide. Conversely, the mAb 135 was able to precipitate the 42,000 M, polypeptide in addition to the 28,000 and 36,000 M, ones. This suggests that the FcER1/CD23 is spatially associated with the HLA-DR antigens on the B lymphocyte cell surface. Finally the association of FcER1/CD23 with HLA DR antigens is further confirmed by the fact that the anti-HLA-DR mAb 449 B4 was obtained after immunizing mice with the partially purified FcER1/CD23 eluted from an IgE immunoabsorbent. The demonstration of a physical association between FcER1/CD23 and HLA-DR antigens on the surface of cell lines raises the question as to whether this phenomenon is linked to the transformation induced by EBV. This is particularly relevant since only a fraction of normal B cells express at very low levels the FcER1/CD23. Recently, it has been demonstrated that human IL-4 (15) is able to induce the FcER1/CD23 on normal B cells (16, 17). We therefore investigated whether the IL-4-induced FcER1/CD23 antigen was associated to HLA-DR antigens. Crosslinking experiments demonstrated that the IL-4-induced FcER1/CD23 on normal B cells is also associated with HLA-DR antigens. This demonstrated that the association of FcER1/CD23 with HLA-DR antigens is not a consequence of the transformation of B cells by EBV.

Association between Fc receptors for IgG and MHC class II molecules on B
lymphocytes has been reported earlier in rodents, since anti-Ia antibodies inhibited the binding of IgG complexes (25–28). It was further proposed that this inhibition was indeed due to an alteration of the Ia antigens mediated by the binding of the anti-Ia antibody and resulting in the association of the Ia antigens with the Fc receptors, independent of the anti-Ia antibody Fc fragment (29). Thus, although these observations showing interaction of murine MHC class II antigen with FcγR correlate with our observation that human MHC class II antigens are associated with FcεR₁, conclusions of these studies differ. It therefore seems of interest to reinvestigate the possible physical association of murine MHC class II antigens with FcγR using the biochemical approach described in our study. Actually, soluble IgG binding factors derived from mouse T cells were found to be absorbed by anti-Ia antisera (30, 31), suggesting either a physical association between IgG binding factors and Ia antigens or the presence of common antigenic determinants on both structures. Actually, the presence of antigenic determinants common to rodent MHC class II antigens and IgE binding factors was recently reported since OX3 mAb was able to recognize an epitope containing an N-linked oligosaccharide common to Ia antigens and 60,000 M₇ cell–derived IgE suppressive factors (32, 33).

Association between other cell surface receptors and HLA antigens has been reported recently. First, an anti-HLA class I mAb was found to effectively inhibit the binding of insulin to insulin receptors (34). Upon crosslinking of cell surface antigens the insulin receptor has been shown to coprecipitate with HLA class I antigens (22, 34, 35). Second, a monoclonal as well as a polyclonal anti-HLA class I antibody have been found to inhibit the binding of epidermal growth factor (EGF) to A431 cells (36). This inhibition required crosslinking of HLA antigens since Fab fragments needed to be crosslinked with an anti-mouse Ig antibody to be effective. This suggests that the mechanisms of inhibition of EGF binding by anti-HLA class I antibodies is different from the mechanisms of inhibition of IgE binding by anti HLA-DR antibodies.

It remains to be determined whether the anti HLA-DR antibodies inhibiting the binding of IgE are specific for the HLA-DR α or HLA-DR β chain. Finally, a further investigation of the possible biological function of the FcεR₁/CD23–HLA-DR complex must await an understanding of the role of FcεR₁/CD23 itself.

Summary

Two hybridomas that produce the mAbs 135 and 449 B4 were obtained that inhibited the binding of IgE to the FcεR₁/CD23 on the EBV-transformed B cell line RPMI 8866. mAb 135 was obtained from a mouse immunized with RPMI 8866 cells, whereas mAb 449 B4 was obtained from a mouse immunized with a partially purified preparation of FcεR₁/CD23 obtained as the eluate of an IgE immunoabsorbent loaded with a soluble extract of RPMI 8866 cells. These two mAbs bound to FcεR₁/CD23− cell lines and precipitated two polypeptides with 36,000 M₇ and 28,000 M₇, which were the HLA-DR α and β chains, respectively.

Immunoprecipitation with mAb 135 of NP-40 lysates from dithio-bis(succinimidyldiisopropionate) (DSP) crosslinked 125I-labeled RPMI 8866 or normal B cells incubated with rIL-4 showed three polypeptides with 42,000, 36,000, and 28,000 M₇. The 42,000 M₇ polypeptide is identical to the FcεR₁/CD23 since it could be
FcεRι/CD23 is associated with B cell HLA-DR antigens precipitated by the anti-FcεRι/CD23 mAb 25 after resolubilization from the SDS-PAGE gel. Immunoprecipitations of the crosslinked cell extracts carried out with the anti-FcεRι/CD23 mAb 25 yielded the same three polypeptides. Furthermore, when RPMI 8866 or rIL-4 preincubated normal B cells were solubilized with a digitonin buffer, which prevents the dissociation of noncovalently linked polypeptide complexes, mAb 135 and mAb 25 precipitated complexes composed of three molecules with 42,000, 36,000, and 28,000 M₀. The well-characterized anti-HLA-DR mAb L243 was unable to block the binding of either IgE or mAb 135 to RPMI 8866 cells, although it could immunoprecipitate the complex (HLA-DR–FcεRι/CD23) from crosslinked cell lysates. Since mAb 135 and L243 were able to both bind the RPMI 8866 cells, it demonstrates that they bind to different epitopes of the HLA-DR complex, the mAb 135 epitope of the HLA-DR molecule being close to the IgE binding site of the FcεRι/CD23. These data demonstrated that the FcεRι/CD23 and HLA-DR antigens are spatially associated on the B cell membrane.

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