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The Epstein–Barr virus-binding site on CD21 is involved in CD23 binding and interleukin-4-induced IgE and IgG4 production by human B cells

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SUMMARY

Human CD21 has previously been described as a receptor for the C3d,g and iC3b proteins of complement, as a receptor for the gp350/220 envelope glycoprotein of the Epstein–Barr virus (EBV) and also as a receptor for interferon-α (IFN-α). Structurally, CD21 consists of 15 to 16 short consensus repeats (SCR) of 60 to 75 amino acids followed by a transmembrane domain and an intracytoplasmic region. We reported that CD23, a low-affinity receptor for IgE (FcεR2), is a new functional ligand for CD21. We recently found that the sites of interaction of CD23 on CD21 are on SCR 5 to 8 and 1–2. The first site is a lectin–sugar type of interaction and the second site is a protein–protein interaction. We report here that amongst the other ligands for CD21 (EBV, C3d,g and IFN-α), only EBV is able to inhibit the binding of CD23 to CD21. Furthermore, even a peptide from gp350/220 of EBV known to bind to CD21 is able to decrease CD23 binding to CD21. Since CD23/CD21 pairing is important in the control of IgE production, we tested the effect of the EBV-derived peptide on immunoglobulin production from peripheral blood mononuclear cells and purified tonsillar B cells. Interestingly, the EBV-peptide inhibited IgE and IgG4 production induced by interleukin-4, in a dose-dependent manner. The same results were obtained using either peripheral blood mononuclear cells or purified tonsillar B cells. Another CD21 ligand, C3, did not affect binding of CD23 to CD21 nor the production of IgE and IgG4. This study indicates that blocking CD23 binding to CD21 SCR 2 on human B cells selectively modulates immunoglobulin production.

INTRODUCTION

Human CD21 is a 140 000 MW membrane glycoprotein found on B lymphocytes,[1] a subpopulation of T lymphocytes,[2] follicular dendritic cells[3] and pharyngeal epithelial cells.[4] CD21 has been described as a receptor for the C3d,g and iC3b proteins of the complement system (CR2),[5] as well as a receptor for the gp 350/220 envelope glycoprotein of the Epstein–Barr virus (EBV)[6,7] and it is also a receptor for interferon-α (IFN-α).[8] We found that CD23, a low-affinity receptor for human IgE (FcεR2),[9,10] is also a ligand for CD21.[11] CD21 and CD23 can be considered as a pair of adhesion molecules.[12,13] CD23–CD21 pairing plays a role in the regulation of IgE synthesis *in vitro*[11] and *in vivo*.[14] In addition, soluble CD23 (sCD23) and anti-CD21 monoclonal antibodies (mAb) promote the survival of germinal centre B cells[19,20] and increase histamine release by basophils.[21] Structurally, the CD21 protein is composed of an extracellular domain of 15[22] or 16[23] repetitive units of 60–75 amino acids, named short consensus repeats (SCR), followed by a transmembrane domain (24 amino acids) and an intracytoplasmic region of 34 amino acids. Using CD21 mutants bearing deletions of extracytoplasmic SCR,[24] we recently found that CD23 binds to SCR 5–8 and 1–2 on CD21. The binding of CD23 to SCR 5–8 is a lectin-like interaction, involving carbohydrates on Asn 295 and 370. In contrast, CD23 binding to SCR 1–2 is a protein–protein interaction.[25] In this study, we tested the effect of the other ligands of CD21 (EBV, C3d,g and IFN-α) on CD23 binding to CD21 and on the regulation of immunoglobulin production in the presence of interleukin-4 (IL-4). Only EBV particles and an EBV-derived peptide were able to inhibit CD23 binding to CD21. Moreover, the EBV-peptide decreased IgE and IgG4 production. These data indicate that CD23 binding to the EBV-binding site on CD21 selectively regulates human immunoglobulin production in the presence of IL-4.

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MATERIALS AND METHODS

Cell lines

Tonsil or blood mononuclear cells were isolated by centrifugation on a Ficoll–Paque density gradient 20 min at 4°. Tonsillar B cells were purified by rosetting with sheep red blood cells, 1 hr

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at 4°. Purity of B cells was > 98% as determined by anti-CD20 mAb staining followed by fluorescence-activated cell sorter (FACS) analysis (Becton Dickinson, Erembodeggem, Belgium).

The B-cell line RPMI-8226 was obtained from the American Type Culture Collection (ATCC, Rockville, MD) and cultured in RPMI-1640 complete medium.

Peptides and CD21 ligands

Two peptides, from gp350 of EBV and C3, known to bind to CD21[26] were synthesized. PepEBV (TGEDPGFFNVEIC-NH_2) was produced on an ABI 431A synthesizer using FastMoc chemistry and PepC3 (GKQLYNVEATSYAC-NH_2) was obtained from Neosystem (Strasbourg, France). Aggregated C3d,g was prepared as described previously.[24] Sucrose gradient-purified EBV was obtained from Advanced Biotechnologies (Columbia, ML) and IFN-α was obtained from Sigma (St Louis, MO). The anti-CD21 mAb was obtained from Prof. I. MacLennan (Birmingham, UK).

Fluorescein isothiocyanate (FITC)-labelled C3 peptide was prepared as follows: 3·25 μM of the peptide were suspended in 1 ml of a pre-prepared mixture of 200 mM aqueous $NaHCO_3$/dimethylformamide (DMF) (1 : 1 by volume) flushed with N_2 and stirred at room temperature for 1 hr after addition of 6·5 μM dithiothreitol (DTT) in 50 μl H_2O. Iodoacetamidomethylfluoresceine (IAMF; 13 μmole) was then added in 50 μl DMF and the reaction was stirred in the dark at room temperature for 1 hr. The reaction was stopped by the addition of 20 μl mercaptoethanol and 250 μl of 1 M acetic acid and the labelled peptide was recovered by small-scale preparative reversed-phase high-performance liquid chromatography (HPLC; 0·1% trifluoroacetic acid with a gradient from 100% aqueous to 60% acetonitrile/40% water) followed by lyophilization of relevant collected fractions. The product was authenticated by electrospray mass.

Liposome preparation

CD23-liposomes were made as previously described[27] using 10 μmol of the synthetic phospholipids POPC (Avanti Polarlipids Inc. Alabaster, AL) mixed with 50 nmol of fluorescent dye DiO18 (Molecular Probes, Eugene, OR) and then dialysed against HEPES buffer together with purified recombinant CD23 or with glycophorin A (0·2 μmol each) referred to as control protein.

Flow cytometry

Liposome binding assay. Cells (10^5) were resuspended in 50 μl of the liposome suspension, diluted 10 times in 0·5% bovine serum ablumin (BSA), 0·1% NaN_3, 2 mM $CaCl_2$, 140 mM NaCl, 20 mM HEPES, pH 7·0 and incubated for 2 hr at 4°. Cells were washed twice before analysis on a FACStar plus (Becton Dickinson, Erembodeggen, Belgium).

Competition of CD23-liposomes with EBV, EBV peptide, IFN-α, C3 peptide, C3d,g and BU-33. RPMI-8226 cells were co-incubated with glycophorin-liposomes or CD23-liposomes and EBV (1×10^5 to 1×10^9 particles/ml), EBV peptide and C3 peptide (50 nM to 50 μM), aggregated C3d,g (4 ng/ml to 1 μg/ml), IFN-α (1000 U/ml) and BU-33 (10 μg/ml from ascites) for 2 hr at 4°. Cells were analysed as described above.

IL-4-induced immunoglobulin production assays

Cells were incubated at 10^6/ml for 14 days in Iscove's medium enriched with transferrin, bovine insulin, oleic acid, linoleic acid, palmitic acid, BSA (all from Sigma) and 10% fetal calf serum (FCS; Flow Laboratories, Irvine, UK) as described by Claassen *et al.*[28] Assays were performed using total peripheral blood mononuclear cells with IL-4 alone (200 U/ml) or IL4 plus anti-CD40 (1 μg/ml) (Serotec Ltd, Oxford, UK), or using purified tonsillar B cells with IL-4 and anti-CD40. IgE, G, A and M were quantified by specific enzyme-linked immunosorbent assay (ELISA) as previously described.[29] IgG4 was measured by ELISA as follows. A mouse anti-human IgG4 antibody (Southern Biotechnology, Birmingham, UK) diluted at 10 μg/ml in bicarbonate buffer, pH 9·6 was coated overnight in 96-well plates (100 μl/well). Saturation was then performed with phosphate-buffered saline (PBS) plus 1% BSA (200 μl/well) for 2 hr at room temperature. Samples to be tested were diluted in PBS plus 0·5 % BSA and 0·1% Tween (100 μl/well) and incubated overnight at 4°. After washes with PBS plus Tween, a peroxidase-labelled sheep anti-human IgG4 antibody (Vital Products, St Louis, MO) diluted 1/5000 in PBS/BSA plus Tween was added for 1 hr at room temperature. After washes with PBS plus Tween, *o*-phenylene diamine (Sigma) was added and the colorimetric reaction was stopped with 2 M H_2SO_4. Plates were finally read at 492 nm.

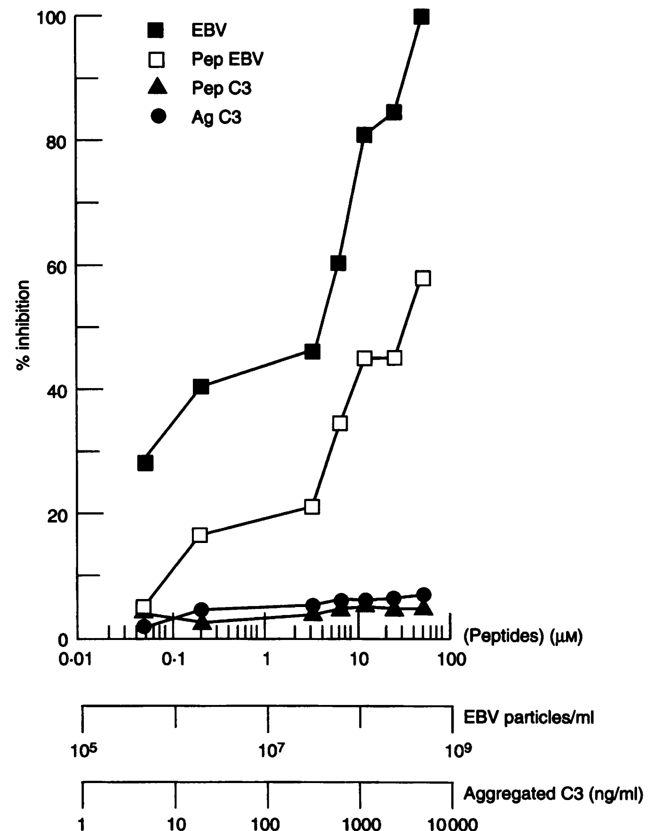

Figure 1. Inhibition of CD23-liposome binding to RPMI-8226 cells by some CD21 ligands. RPMI-8226 cells were co-incubated for 2 hr with CD23-liposomes or glycophorin-liposomes and various concentrations of EBV (particles/ml), PepEBV, PepC3 (μM) and aggregated C3 (ng/ml). Percentage of inhibition is calculated as follows: [(MFI CD23-L) − (MFI CD23-L + ligands)]/(MFI CD23-L) × 100. The MFI of glycophorin-liposomes was subtracted from the MFI of CD23-liposomes. Results are taken from a representative experiment.

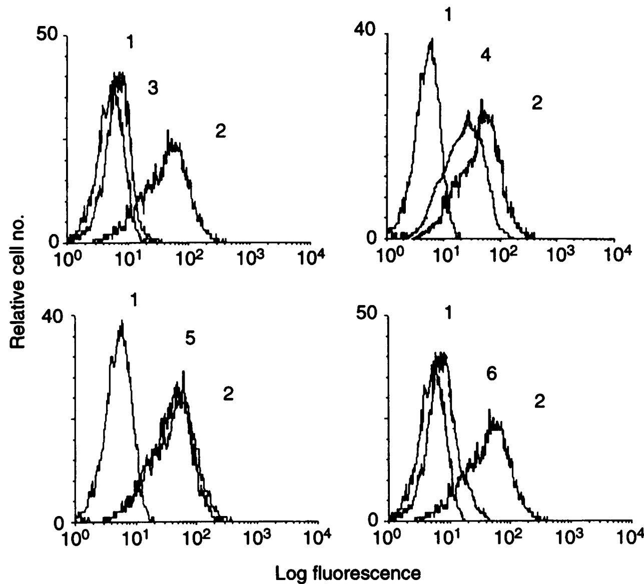

Figure 2. FACS profiles of RPMI-8226 cells stained with glycophorin-liposomes (histograms 1) or CD23-liposomes (histograms 2) alone or in presence of optimal concentrations of EBV particles (histogram 3), PepEBV (histogram 4), PepC3 (histogram 5) and BU-33 (histogram 6). Results are taken from a representative experiment.

RESULTS AND DISCUSSION

Human CD21 has been previously described to be a receptor for the C3d,g and iC3b proteins of the complement system,[5] for the gp350/220 envelope glycoprotein of EBV[6,7] and for IFN-α.[8] We have therefore tested all these CD21 ligands for their ability to inhibit CD23-liposome binding to the CD21-expressing cells, RPMI-8226 cells.[27] Intact particles of EBV were able to inhibit CD23 binding to CD21 in a dose-dependent manner (Fig. 1). Of the other CD21 ligands tested for inhibition of CD23 liposome binding, only EBV decreased the binding of CD23. A complete inhibition of CD23 binding was observed with EBV intact particles, although EBV is reported to bind to SCR 2 of CD21[26] and not to SCR 5–8 where CD23 binds to sugars in this latter region.[25] This complete inhibition of CD23 binding could be due to the size of the virus particles or to the fact that EBV may modify the conformation of the CD21 molecule. In order to exclude that inhibition was due to steric hindrance by the virus particles, we then tested the effect of a peptide of gp350/220, known to bind to CD21.[26] This EBV peptide was able to inhibit CD23 binding to CD21 in a dose-dependent manner, with a maximum of 55% inhibition (Fig. 1 and 2). These experiments suggest that the EBV peptide binding is close to the CD23-binding site and partially blocks CD23 binding. These data confirm our previous finding that CD23 does bind to CD21[11] and extend them by showing that SCR 2 is probably a region interacting with CD23. In contrast, a C3 peptide corresponding to the CD21 binding site on C3d,[26] aggregated C3d,g (Fig. 1 and 2) and IFN-α (data not shown) were unable to inhibit the binding of CD23 to RPMI-8226 cells. The absence of effect observed with the C3 peptide was not due to absence of binding to CD21. FITC-labelled C3 peptide was shown to bind to RPMI-8226 by FACS and the binding was specifically inhibited by 'cold' C3 peptide and aggregated C3 but not by EBV peptide (Fig. 3). This suggests that SCR 1 and 3–4, where C3 and IFN-α bind respectively, may not be involved in CD23 binding. As reported previously,[11] BU-33 anti-CD21 mAb was used as an internal positive control of inhibition (Fig. 2).

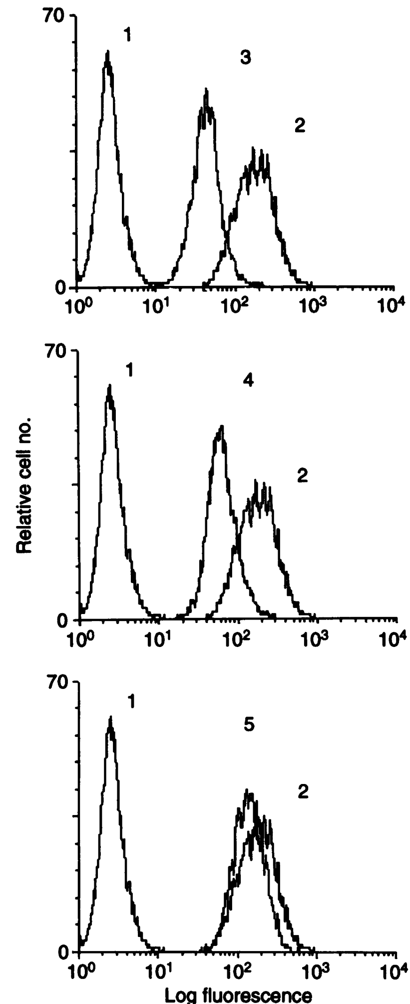

Figure 3. FACS profiles of RPMI-8226 cells stained with FITC–IgG1 as control (histograms 1) or FITC-labelled C3 peptide at 80 μM (histograms 2) alone or in presence of C3 peptide at 500 μM (histogram 3), aggregated C3 at 1 μg/ml (histogram 4) or EBV peptide at 500 μM (histogram 5). Results are taken from a representative experiment.

The EBV binding to CD21 does not require glycosylation of SCR 2 of CD21.[30] Likewise, CD23 binding to SCR 2 region is independent of sugars.[25] This is in line with our observation that a non-glycosylated synthetic peptide is able to decrease CD23 binding to CD21.

Therefore, CD23 binds to a binding site in SCR 2 on CD21 that is close or identical to the EBV binding site which differs from the binding sites previously described for C3d,g and IFN-α.

CD23 was previously shown to regulate positively IgE production by binding to CD21 on B cells.[11] Based on the observation that an EBV peptide blocked CD23 binding to CD21, we investigated the effect of this EBV peptide on IgE production. The EBV peptide was able to inhibit IL-4-induced IgE production in a dose-dependent manner (Fig. 4). This effect was observed in not only T-cell-dependent but also in T-cell-independent IgE production systems, in which T-cell help is

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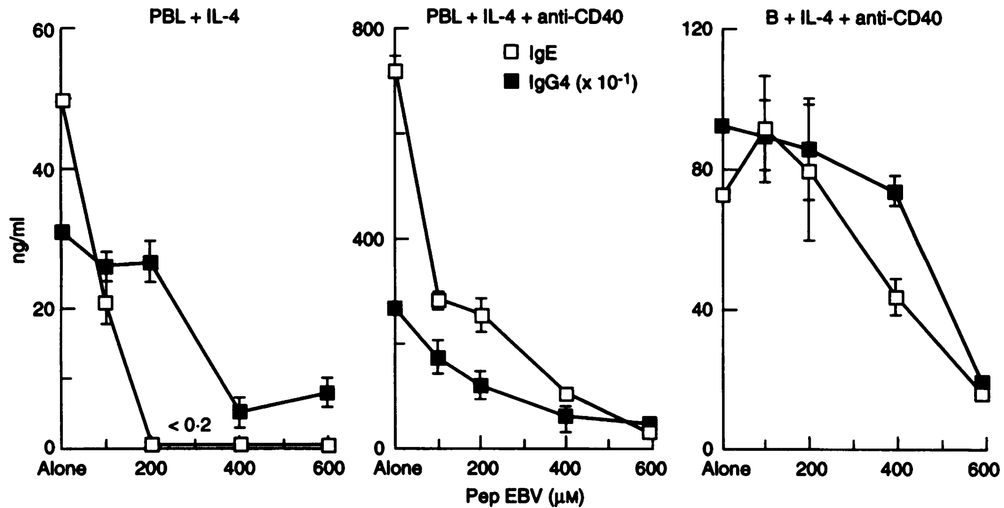

Figure 4. Inhibition of IL-4-induced IgE and IgG4 production by an EBV peptide binding to CD21. Peripheral blood lymphocytes or purified tonsillar B cells (10^6/ml) were incubated for 14 days with 200 U/ml of IL-4 alone or in the presence of anti-CD40 antibody (1 μg/ml) and increasing concentrations of EBV peptide. IgE and IgG4 were measured by specific ELISA and mean values ± SD of one representative experiment are presented ($n = 4$). Control values for PBL alone were: IgE < 0·2 and IgG4 < 2; for B cells alone: IgE < 0·2, IgG4 < 2, IgG = 660, IgM = 610 and IgA = 520; and for B cells + IL-4: IgE < 0·2 and IgG4 < 2 (all in expressed in ng/ml).

replaced by anti-CD40 antibody (Fig. 4). This confirms our previous observation[31] that CD23–CD21 can regulate IgE production even in absence of T cells by a homotypic B–B-cell interaction, since B cells can express both CD23 and CD21 molecules. Intact EBV has been reported to provide the permissive signal for IgE switching,[32] like T cells or CD40L. The CD21 binding site on the virus is presumably multivalent and monovalent on the EBV peptide. In contrast to the EBV particles, the EBV peptide is probably unable to cross-link membrane CD21 and is therefore unable to increase IgE production. The EBV peptide is rather inhibitory, decreasing IgE production by preventing the CD23–CD21 interaction.

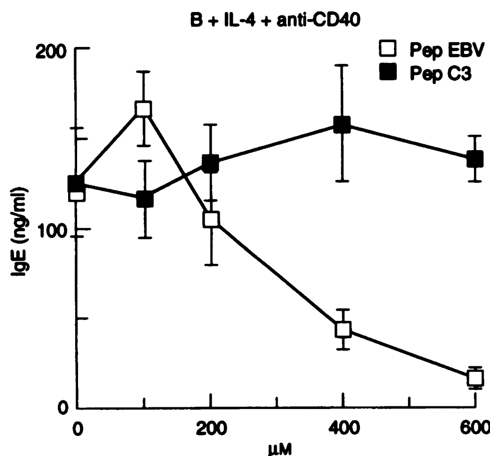

Figure 5. Absence of inhibition of IgE production with a C3 peptide binding to CD21. Purified tonsillar B cells (10^6/ml) were incubated for 14 days with 200 U/ml of IL-4 and anti-CD40 antibody (1 μg/ml) and increasing concentrations of C3 peptide or EBV peptide. IgE was measured by specific ELISA and mean values ± SD of one representative experiment are presented ($n = 4$).

Since IL-4 is known to induce IgG4 as well as IgE,[33] we investigated the effect of the EBV peptide on IL-4-induced IgG4 production. As shown in Fig. 4, the EBV peptide was also able to inhibit IgG4 in a dose-dependent manner. This observation suggests that the CD23–CD21 interaction also controls IgG4 production.

Not all CD21 ligands can regulate IgE/IgG4 production. A C3 peptide binding CD21 did not inhibit IgE (Fig. 5) and IgG4 production (not shown). The C3 peptide did not inhibit CD23 binding (Fig. 1 and 2). These results highlight again the correlation between CD23–CD21 pairing and IgE/IgG4 production. IFN-α was not tested since it is already known that IFN-α inhibits IgE production,[34] although IFN-α had no effect on CD23 binding to CD21 (not shown). Nevertheless, it remains to be proven that this negative effect of IFN-α on IgE production is mediated by binding to CD21 or to some other IFN-α receptor.[35]

In conclusion, this study therefore shows that an EBV peptide decreases CD23 binding to CD21 and decreases IgE and IgG4 production by human B cells.

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