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CD86 (B7-2) on Human B Cells

A FUNCTIONAL ROLE IN PROLIFERATION AND SELECTIVE DIFFERENTIATION INTO IgE- AND IgG4-PRODUCING CELLS*

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Immunoglobulin (Ig) E production by B cells requires two primary signals provided by T cells, interleukin (IL)-4 or IL-13 and CD40 ligand (CD40L). In addition, costimulatory signals, such as CD23-CD21 interaction, contribute further ensuring a selective control over this production. Recently, CD28, expressed on T cells, has been reported to be involved in this process. The CD28 ligands, CD80 (B7-1) and CD86 (B7-2), are expressed on human tonsillar B cells, and their expression is up-regulated by IL-4, IL-13, and/or an anti-CD40 monoclonal antibody (mAb). We have investigated whether signaling via the B7 molecules affects IgE synthesis. Human B cells were stimulated by IL-4 plus anti-CD40 mAb in the presence of different anti-B7 mAbs. Cross-linking of CD86 with IT2.2 potentiated IgE and IgG4 production and ϵ transcripts expression. The production of the other isotypes was not modulated. Conversely, the anti-CD80 and the other anti-CD86 mAbs tested had no effect. The increase of IgE and IgG4 production induced by IT2.2 was accompanied by an increase in proliferation, in cell surface density of CD23, and in CD23 binding to CD21-expressing B cells. In contrast, the expression of other B cell surface molecules such as CD11a, CD30, and CD58 remained unaffected. Since IT2.2 favors CD23-CD21 pairing, we tested whether blocking this interaction affected IT2.2-increased IgE production. The neutralizing anti-CD23 mAb, Mab 25, caused a dose-dependent inhibition of the effect of IT2.2 on IgE synthesis. Finally, IT2.2 potentiation on B cell proliferation and IgE production required the two primary signals, IL-4 and anti-CD40 mAb, since IT2.2 alone or in combination with only one of these stimuli did not show any effect on B cells. This study is the first demonstration of a signaling role for CD86. Together with IL-4 or IL-13 and CD40L, CD86 favors CD23-CD21 pairing and consequently functions as a selective and potent costimulus for human IgE and IgG4 synthesis.

An efficient activation of T cells requires the engagement of the T cell receptor with the antigen-major histocompatibility complex and the engagement of appropriate costimulatory signals. A potent pathway of costimulation is the one involving the interaction of CD28 and CTLA-4 on T cells with their ligands CD80 (B7-1) and CD86 (B7-2) expressed by numerous antigen-presenting cells, including B cells (1–7). The existence of a third ligand for CTLA-4 has been postulated (B7-3), but the gene for this ligand has not been cloned yet (6). CD28 costimulation, in conjunction with T cell receptor activation, increases the proliferation and the production of interleukin (IL)[1]-2 by T cells (4, 6–9); however, the role of CTLA-4 in T cell activation remains unclear (6, 7, 10–13). Reciprocally, whether the engagement of the B7 molecules affects the function of the antigen-presenting cells is poorly documented. Previous studies have suggested that CD28-CTLA-4/B7 signaling pathways may affect B cell responses and the regulation of immunoglobulin (Ig) synthesis. Indeed, an abnormal *in vivo* Ig production has been observed in CD28 knockout mice (14) and in mice transgenic or deficient for CTLA-4 (15, 16). Moreover, it has been suggested recently that CD28 may modulate IgE synthesis since a neutralizing anti-CD28 monoclonal antibody (mAb) decreased IgE production by B cells cocultured with T cells (17).

IgE and IgG4 production by B cells requires two signals provided by activated T cells, mast cells, or basophils (18–23). The first signal, given by IL-4 or IL-13, induces the expression of the ϵ germline transcript (18–20) and of some cell surface molecules involved in IgE and IgG4 synthesis such as CD23 (24–29) or CD40L (30). The second signal, provided by the engagement of CD40 by CD40L or by an anti-CD40 mAb, induces the expression of the productive ϵ transcript encoding for IgE (20–23). IgE synthesis is also tightly controlled by costimulatory signals provided by T cells, including the interaction between cell surface molecules. Among them, the ligation of CD21 (26, 27, 29), intercellular adhesion molecule 1 (ICAM-1; CD54) (31), and LFA-3 (CD58) (32) on B cells with their specific ligands up-regulates IgE synthesis. CD28 expressed on T cells could also control IgE synthesis (17). However, the nature of the CD28 ligand(s) that may regulate IgE production remains unknown.

Thus, the aim of this study was to evaluate the role of the B7 molecules on IgE synthesis. The results show that ligation of CD86 on IL-4 or IL-13 plus anti-CD40 mAb-stimulated human tonsillar B cells transduces a positive costimulatory signal since it favors CD23-CD21 pairing and potentiates selectively IgE and IgG4 production.

EXPERIMENTAL PROCEDURES

Materials—The anti-CD86 mAbs FUN-1 and IT2.2 (2) were purchased from Pharmingen (San Diego, CA), and BU63 was from Ancell (Bayport, MN). The anti-CD80 mAbs MAB104 and L307.4 were from Immunotech (Marseille Luminy, France) and Becton Dickinson (Mountain View, CA), respectively. Based on previous data, the molecule

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[1] The abbreviations used are: IL, interleukin; Ig, immunoglobulin; mAb, monoclonal antibody; MFI, mean fluorescence intensity; FACS, fluorescence-activated cell sorter.

TABLE I

CD80, CD86, and B7–3 expression on human tonsillar B cells

B cells were either unstimulated or stimulated with 200 units/ml IL-4, 20 ng/ml IL-13, 0.1 μg/ml anti-CD40 mAb, IL-4 plus anti-CD40 mAb, or IL-13 plus anti-CD40 mAb. CD80, CD86, and B7–3 expression was evaluated by FACS analysis using FITC-labeled mAbs. Results are expressed in MFI values − background (mean ± S.D., $n = 6$); ND means not done.

Stimulus	CD80		CD86		B7–3	
	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3
None	13 ± 4	15 ± 4	14 ± 5	18 ± 3	31 ± 11	32 ± 8
IL-4	12 ± 3	31 ± 9	28 ± 5	43 ± 9	35 ± 12	67 ± 15
IL-13	ND	ND	30 ± 8	45 ± 11	ND	ND
Anti-CD40	22 ± 8	45 ± 16	43 ± 13	52 ± 13	35 ± 15	35 ± 11
IL-4 + anti-CD40	35 ± 9	52 ± 10	59 ± 15	65 ± 15	62 ± 16	73 ± 18
IL-13 + anti-CD40	ND	ND	58 ± 6	66 ± 12	ND	ND

recognized by the BB1 mAb (purchased from Ancell) was defined as B7-3 (3). The anti-CD40 mAb B-B20 was from Serotec (Oxford, U. K.). All of these mAbs were azide-free except L307.4 and FUN-1, which were dialyzed before use. Fluorescein isothiocyanate-labeled IT2.2, MAB104, and BB1 mAbs were from the same companies. Fluorescein isothiocyanate-labeled anti-CD11a, -CD11c, -CD49d, -CD54, and -CD58 mAbs were from Serotec. Anti-CD30 mAb was from Dako (Glostrup, Denmark). Anti-CD11b, -CD21, -CD23, and -CD25 were from Immunotech. Control IgG1 and IgG2a were from Becton Dickinson. Fluorescent CD23- and glycophorin A-liposomes (used as a negative control) were made as described (27).

Epitope Mapping Experiments—Expression plasmids encoding the full-length extracellular portions of human CD80 (CD80wt) and CD86 (CD86wt) and single domain variants of these, fused to rat Fcγ2b at the hinge, have already been described (33). These were transfected into COS cells using Transfectam (Promega, Madison, WI) and supernatants containing fusion protein harvested after 4 days. The Ig fusion protein concentration was determined by enzyme-linked immunosorbent assay as described (33). Anti-CD86 mAbs, control IgG1, IgG2b, and anti-rat IgG antiserum (all from Sigma, St. Louis, MO, U. S. A.) were coated onto 96-well plates (Nunc, Roskilde, Denmark) at a concentration of 2 μg/ml in 0.1 M carbonate-bicarbonate buffer, pH 9.6, and then saturated with 3% (w/v) bovine serum albumin in phosphate-buffered saline. COS supernatants (100 μl/well) were applied for 1 h. After washing, bound fusion proteins were detected with peroxidase-conjugated anti-rat IgG2b (Binding Site, Birmingham, U. K.) and revealed using *o*-phenylenediamine. The colorimetric reaction was stopped with 2 M H_2SO_4. Optical density values were determined at 492 nm.

Immunoglobulin Assays—Human tonsillar B cells were purified as described and cultured in enriched Iscove's medium (29). B cells (2×10^5/200 μl/well) were added to 96-well plates (Nunc) precoated with 1 μg/well of the mAb tested and stimulated, in quintuplicate, with 200 units/ml IL-4 (Geneva Biomedical Research Institute), 20 ng/ml IL-13 (R&D systems, Minneapolis, MN), 0.1 μg/ml anti-CD40 mAb, or combinations of IL-4 or IL-13 plus anti-CD40 mAb. In some sets of experiments, B cells stimulated with IL-4 plus anti-CD40 mAb in the presence or absence of IT2.2 were incubated with 0.2–20 μg/ml of the neutralizing anti-CD23 mAb, Mab 25 (25) (Immunotech) or of control mouse IgG1 (Sigma). In others, these stimulated B cells were incubated with CD80wt or CD86wt. In all cases, supernatants were collected at day 12 to quantify Ig.

Immunoglobulin Quantification—IgA, IgE, IgG4, and IgM were quantified by enzyme-linked immunosorbent assay as described (25, 29). IgG1, IgG2, and IgG3 were quantified as follows. 96-well plates were coated overnight at 4 °C with 1 μg/well anti-IgG1 mAb (clone NL16) (Oxoid Unipath, Hampshire, U. K.), 0.2 μg/well anti-IgG2 mAb (clone HP6002), or 0.2 μg/well anti-IgG3 mAb (clone HP6050) (both from Calbiochem, La Jolla, CA, U. S. A.) in 0.1 M carbonate-bicarbonate buffer and then saturated with 1% bovine serum albumin in phosphate-buffered saline. Cell culture supernatants diluted in phosphate-buffered saline/bovine serum albumin containing 0.1% (v/v) Tween 20 were incubated for 4 h at room temperature. After washing, peroxidase-conjugated anti-human γ-chain (Sigma) in phosphate-buffered saline/bovine serum albumin/Tween was incubated at room temperature for 3 h, and bound Abs were detected using *o*-phenylenediamine. The colorimetric reaction was stopped with 2 M H_2SO_4. Optical density values were determined at 492 nm. Standard curves were realized with purified IgG1, IgG2, and IgG3 (Sigma). Results are expressed in ng/ml or in percent of increase defined as $A - O \div O \times 100$, where A and O were the concentrations of Ig produced by B cells stimulated with or without IT2.2, respectively.

Proliferation Assays—B cells (2×10^5/200 μl/well) were stimulated in triplicate in 96-well plates precoated or not with the mAb tested, as described above. After a 48-h incubation, cells were pulsed with 0.25 μCi/well [^{3}H]thymidine (Amersham International, Amersham, U. K.). Radioactive incorporation was measured by standard liquid scintillation counting. Results are given as stimulation index calculated as follows: $A \div O$, where A and O are the counts/min values obtained when B cells were stimulated or not with the mAb tested, respectively.

Immunostaining—Surface phenotyping of B cells and liposome binding assays were performed by flow cytometry, as described previously (27). Results were expressed either as the mean fluorescence intensity after subtraction of the MFI obtained with the control Ab or as a percentage of MFI increase defined as follows: $A - O \div O \times 100$ where A and O are the MFI obtained with B cells activated or not with the mAbs tested, respectively.

Analysis of IgE mRNA Transcription by Northern Blotting—B cells were either cultured for 2 days with or without IL-4 or for 10 days with IL-4 plus anti-CD40 mAb in the presence or absence of immobilized IT2.2. Total RNA extraction and hybridizations with ^{32}P-labeled cRNA probes complementary to Cε and to β-actin mRNA were performed as described (20). In the same experiment, IgE was quantified in the 10-day cell-free supernatants.

Statistical Analysis—Statistical analysis was performed using Student's *t* test.

RESULTS

Factors Inducing IgE and IgG4 Synthesis Increase CD80, CD86, and B7-3 Expression on Human Tonsillar B Cells—CD80 and CD86 were expressed on freshly isolated tonsillar B cells but at a lower extent than B7-3 (Table I). A significant increase of CD80 and B7-3 expression occurred after a 3-day stimulation with IL-4 (increase of 106 and 109%, respectively) (Table I). A 3-day stimulation with anti-CD40 mAb also enhanced CD80 expression (increase of 200%) but did not significantly affect B7-3 expression (Table I). Concerning CD86 expression, a significant increase occurred earlier, after a 24-h stimulation with IL-4 or with anti-CD40 mAb alone (increase of 100 and 207%, respectively) (Table I). Finally, a stimulation with both IL-4 plus anti-CD40 mAb induced a potent and early increase of CD80, CD86, and B7-3 expression (increase of 169, 321, and 100% at day 1, respectively). Stimulation with IL-13 plus anti-CD40 mAb also up-regulated CD86 expression to the same extent (increase of 314% at day 1). The different anti-CD80 and anti-CD86 mAbs tested gave identical results (data not shown).

Thus, the expression of the B7 molecules is detectable on fresh human tonsillar B cells and is strongly up-regulated by stimulation with IL-4 or IL-13 plus anti-CD40 mAb.

CD86 Cross-linking Potentiates IgE and IgG4 Production by B Cells Stimulated with IL-4 or IL-13 Plus Anti-CD40 mAb—To determine whether the B7 molecules are involved in the regulation of IgE synthesis, B cells were stimulated by IL-4, IL-13, and/or anti-CD40 mAb in the presence of immobilized anti-B7 mAbs. When B cells were either unstimulated or stimulated with IL-4, IL-13, or anti-CD40 mAb alone, the ligation of CD80 (with MAB104 or L307.4 mAbs), CD86 (with IT2.2, FUN-1, or BU63 mAbs), or B7-3 (with BB1 mAb) did not induce

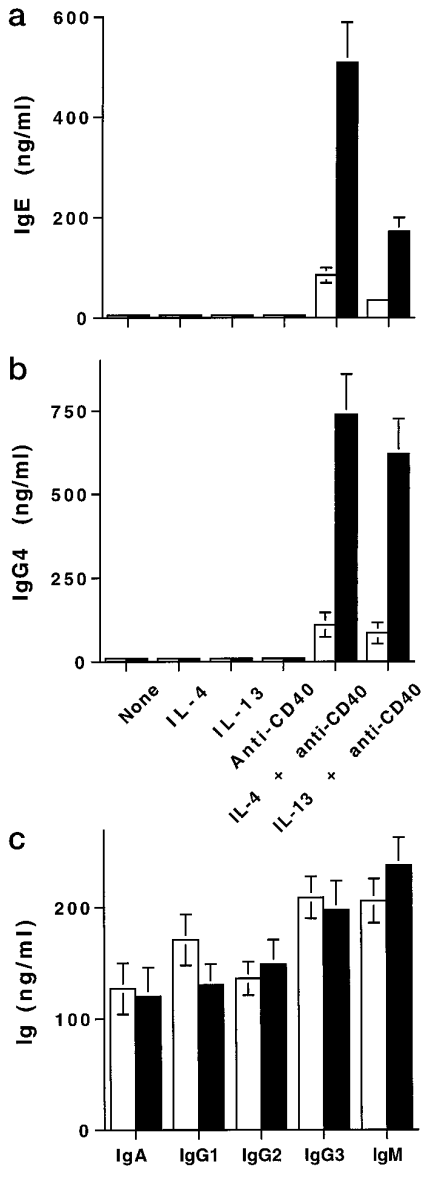

FIG. 1. **CD86 ligation enhances IL-4/IL-13 plus anti-CD40 mAb-induced IgE and IgG4 synthesis.** *Panels a* and *b*, human tonsillar B cells were either unstimulated or stimulated with IL-4, IL-13, or anti-CD40 mAb alone or with IL-4 or IL-13 plus anti-CD40 mAb, in the presence (■) or absence (□) of IT2.2. IgE (*panel a*) and IgG4 (*panel b*) were quantified in the 12-day supernatants by enzyme-linked immunosorbent assay. *Panel c*, B cells were stimulated with IL-4 plus anti-CD40 mAb in the presence or absence of IT2.2. IgA, IgG1–3, and IgM were quantified in the 12-day supernatants. *Panels a–c*, results are expressed in ng/ml (as mean ± S.D. of quintuplicate values) and are representative of one of six separate experiments.

IgE nor IgG4 production. Representative results obtained with the IT2.2 mAb are presented in Fig. 1, *a* and *b*.

Interestingly, IgE and IgG4 synthesis induced by a stimulation with IL-4 plus anti-CD40 mAb was potentiated by IT2.2 (Fig. 1, *a* and *b*, and Table II). In contrast, IgA, IgG1, IgG2, IgG3, and IgM production were unaffected (Fig. 1*c*). According to the subject tested ($n = 6$), the increase of IgE and IgG4 production induced by IT2.2 was from 4- to 10-fold and from 5- to 12-fold, respectively. This effect of IT2.2 was not enhanced when suboptimal concentrations of IL-4 (from 5 to 200 units/ml) and of anti-CD40 mAb (from 5 to 100 ng/ml) were used in combination (data not shown). Moreover, IT2.2 also potentiated the production of IgE and IgG4 induced by IL-13 plus anti-CD40 mAb (Fig. 1, *a* and *b*). Neutralizing experiments showed

TABLE II
Effect of CD80, CD86, and B7–3 triggering on IgE and IgG4 production

Human tonsillar B cells were stimulated with IL-4 plus anti-CD40 mAb in the absence or presence of different anti-B7 mAbs. At day 12, IgE and IgG4 were quantified in the supernatants. Results are expressed in ng/ml, mean ± S.D., $n = 6$.

mAb and clone	Immunoglobulins	
	IgE	IgG4
	ng/ml	
None	89 ± 13	99 ± 19
CD80		
MAB104	85 ± 10	105 ± 28
L307.4	82 ± 13	96 ± 15
CD86		
IT2.2	467 ± 51	940 ± 256
BU63	88 ± 15	110 ± 32
FUN-1	76 ± 12	98 ± 18
B7–3		
BB1	91 ± 16	119 ± 29

that IT2.2 acted on B cells by triggering specifically CD86 since its effect on IgE production was strongly inhibited (80% inhibition) by the addition of CD86wt but not of CD80wt fusion proteins (data not shown).

In contrast, the anti-CD80 mAbs, the BB1 mAb, and the other anti-CD86 mAbs tested (FUN-1 and BU63) did not affect IgE or IgG4 production by stimulated-B cells (Table II).

The different effects of the anti-CD86 mAbs could result from a binding to different epitopes. CD86 is a member of the Ig superfamilly with an extracellular region containing both a V (Ig-V-like) domain, which is known to contain the CTLA-4 binding site, and a C (C2-like) domain (2, 4, 33). Mapping experiments showed that the three anti-CD86 mAbs bound to the CD86 V-region (Fig. 2). However, in agreement with other (34), we have observed by FACS analysis that FUN-1 and BU63 did not block the binding of IT2.2 to CD86-expressing B cells (data not shown). These results suggest that IT2.2 binds to an epitope located in the CD86-V domain different from that identified by FUN-1 or BU63.

Thus, the epitope of CD86 recognized by IT2.2 has a unique signaling role in human B cells since it potentiates IgE and IgG4 production induced by a costimulation with both IL-4 or IL-13 plus anti-CD40 mAb.

CD86 Cross-linking Enhances the Expression of the ϵ mRNA Transcripts Induced by IL-4 Plus Anti-CD40 mAb—Whereas IL-4 induces the expression of the germline ϵ transcript in tonsillar B cells (18–20), both IL-4 and anti-CD40 mAb are required to induce the expression of the productive ϵ transcript encoding for IgE (Fig. 3) (20–23). Thus, we have evaluated the effect of CD86 triggering on ϵ transcript expression. The expression of both the germline and productive ϵ mRNA transcripts was increased by IT2.2 in B cells stimulated with IL-4 plus anti-CD40 mAb (Fig. 3).

This result shows that B7-2 triggering increases IgE synthesis by enhancing the expression of ϵ mRNA transcripts.

CD86 Cross-linking Potentiates IL-4 Plus Anti-CD40 mAb-induced B Cell Proliferation—In addition to inducing IgE and IgG4 production, a stimulation of B cells with IL-4 plus anti-CD40 mAb induces proliferation (35). Thus, we have evaluated the effect of CD86 cross-linking on B cell proliferation. The IT2.2 mAb but not the other anti-CD86 mAbs tested significantly increased B cell proliferation induced by IL-4 plus anti-CD40 mAb (stimulation index = 3 ± 0.8, mean ± S.D., $n = 5$) or by IL-13 plus anti-CD40 mAb (stimulation index = 2.7 ± 0.4). This effect was not enhanced when suboptimal concentrations of IL-4 and of anti-CD40 mAb were used in combination (data not shown). Moreover, IT2.2 did not induce or modulate

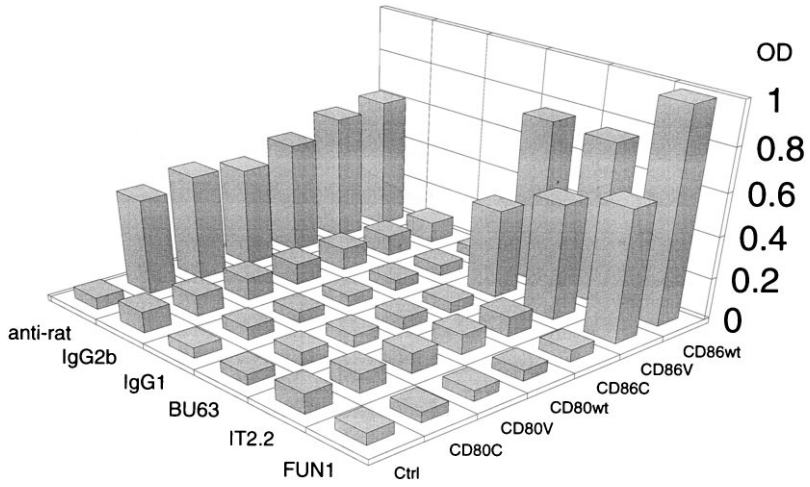

FIG. 2. **The anti-CD86 mAbs, FUN-1, BU63, and IT2.2, recognize the V-like domain of CD86.** The anti-CD86 mAbs were screened for their ability to capture rat Ig2b fusion proteins containing the C-like domain of human CD80 (CD80C) and CD86 (CD86C), the V-like domain of human CD80 (CD80V) and CD86 (CD86V), or the full-length extracellular portion of human CD80 (CD80wt) and CD86 (CD86wt). Control Abs and anti-rat Ig were used as negative and positive capturing agents, respectively. Control supernatants were obtained from mock-transfected COS cells. Captured Ig fusion protein was detected using a peroxidase-conjugated Ab against the Fc region. Results are expressed in optical density values and are representative of one out of three experiments.

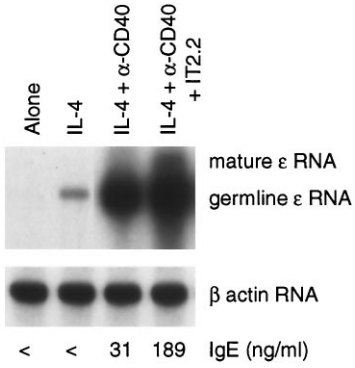

FIG. 3. **CD86 ligation enhances ϵ transcripts expression.** Tonsillar B cells were either unstimulated or were stimulated for 3 days with IL-4 or for 10 days with IL-4 plus anti-CD40 mAb without or with IT2.2. RNA was isolated and used for Northern blot analysis using probes complementary to Cϵ. The *upper left panel* shows an autoradiograph from a 12-h exposure (alone ± IL-4). The *upper right panel* shows a 1-h exposure (IL-4 + anti-CD40 mAb ± IT2.2). As a control, probe specific for actin was used (*bottom*). In parallel, IgE was quantified in the 10-day supernatants.

the proliferation of B cells either unstimulated or stimulated with anti-IgM Ab plus IL-2 or with IL-4, IL-13, or anti-CD40 mAb used alone (data not shown).

Thus, IT2.2 potentiates IL-4 plus anti-CD40 mAb-induced B cell proliferation.

CD86 Triggering Increases CD23 Expression on Stimulated B Cells—The CD23-CD21 interaction is involved in IgE and IgG4 synthesis induced by IL-4 plus anti-CD40 mAb (25–27, 29). Therefore, following the observation that CD86 triggering potentiated IgE and IgG4 production, we tested in a first set of experiments whether a stimulation with IT2.2 affected CD23 expression on B cells. IT2.2 caused a potent increase of CD23 expression induced by IL-4 plus anti-CD40 mAb, significant at day 1 (increase of 56% ± 8, $n = 6$) and maximal at day 2 (135% ± 18) (Fig. 4, *a* and *c*). This effect was not potentiated by using a combination of suboptimal concentrations of IL-4 and of anti-CD40 mAb (data not shown). IT2.2 also up-regulated IL-13 plus anti-CD40 mAb-induced CD23 expression (increase of 260% ± 35 at day 2) (Fig. 4*b*). In contrast, the expression of other B cell surface molecules potentially involved (CD58) (32) or not (CD11b, CD11c, CD25, CD30, CD49d) in IgE synthesis was unaffected except for CD54 expression, which was increased to a low extent (increase of 30% ± 9, $n = 6$) (data not shown). Whatever the time point, IT2.2 did not affect CD23 expression on B cells either unstimulated or stimulated with IL-4, IL-13, or anti-CD40 mAb alone (data not shown). None of the other anti-B7 mAbs tested affected CD23 expression.

Thus, CD86 triggering with the IT2.2 mAb enhances IL-4 or IL-13 plus anti-CD40 mAb-induced CD23 expression on B cells.

CD86 Triggering Potentiates CD23 Binding to CD21-expressing B Cells—In a second set of experiments, we have evaluated whether signaling through CD86 affected the lectin-sugar interaction occurring between CD23 and CD21 (26, 27, 36). Using the anti-CD21 mAb IOB1a, which recognizes an epitope distinct from the binding site of CD23 (36), it appeared that IT2.2 did not affect the cell surface density of CD21 on IL-4 plus anti-CD40 mAb-stimulated B cells (increase of 30% ± 9 and 28% ± 11 with and without IT2.2, respectively, at day 2). However, a stimulation with IT2.2 increased the binding of CD23 liposomes (26) to stimulated B cells with a maximal effect at day 2 (increase of 57% ± 8, $n = 6$) (Fig. 4*c*). In contrast, the other anti-B7 mAbs were ineffective (data not shown).

Thus, by increasing both the cell surface density of CD23 on B cells and the number of CD23 molecules binding to CD21, CD86 cross-linking favors CD21-CD23 pairing.

CD86 Potentiation of IgE Production Requires CD23-CD21 Pairing—In view of the above results, the effect of blocking CD23-CD21 interaction on IT2.2-increased IgE synthesis was evaluated using a neutralizing anti-CD23 mAb, Mab 25 (25). As expected, Mab 25 decreased in a dose-dependent manner IL-4 plus anti-CD40 mAb-induced IgE production (Fig. 4*d*). Interestingly, Mab 25 also inhibited in a dose-dependent manner the potentiation of IgE production induced by IT2.2. In one representative experiment presented in Fig. 4*d*, IT2.2 induced a 400% increase of IgE production which was of 280, 48, and 0% in the presence of 1, 5, or 20 μg/ml of Mab 25, respectively. A control mouse IgG1 mAb did not affect IgE production (data not shown).

Thus, CD86-potentiated IgE production is mediated mainly through an increase of CD23-CD21 pairing.

DISCUSSION

We demonstrate a functional role of CD86 in human B cells both in proliferation and differentiation. Results showed that CD86 acts as a novel player in the regulation of IgE synthesis together with CD40 and CD21. Indeed, the cross-linking of CD86 provides a potent costimulatory signal to stimulated B cells resulting in a selective increase of IgE and IgG4 synthesis.

Although some studies reported that the B7 molecules were not or poorly expressed on resting human B cells (2, 3, 7, 6), we have observed that CD80, CD86, and B7-3 were detectable on fresh human tonsillar B cells, which are indeed preactivated *in vivo*. In agreement with previous data showing that activated B cells express the B7 molecules (2, 3, 7, 6), we report that CD80 and CD86 expression was up-regulated by a stimulation with IL-4 or anti-CD40 mAb (2, 37–39), the most potent increase of

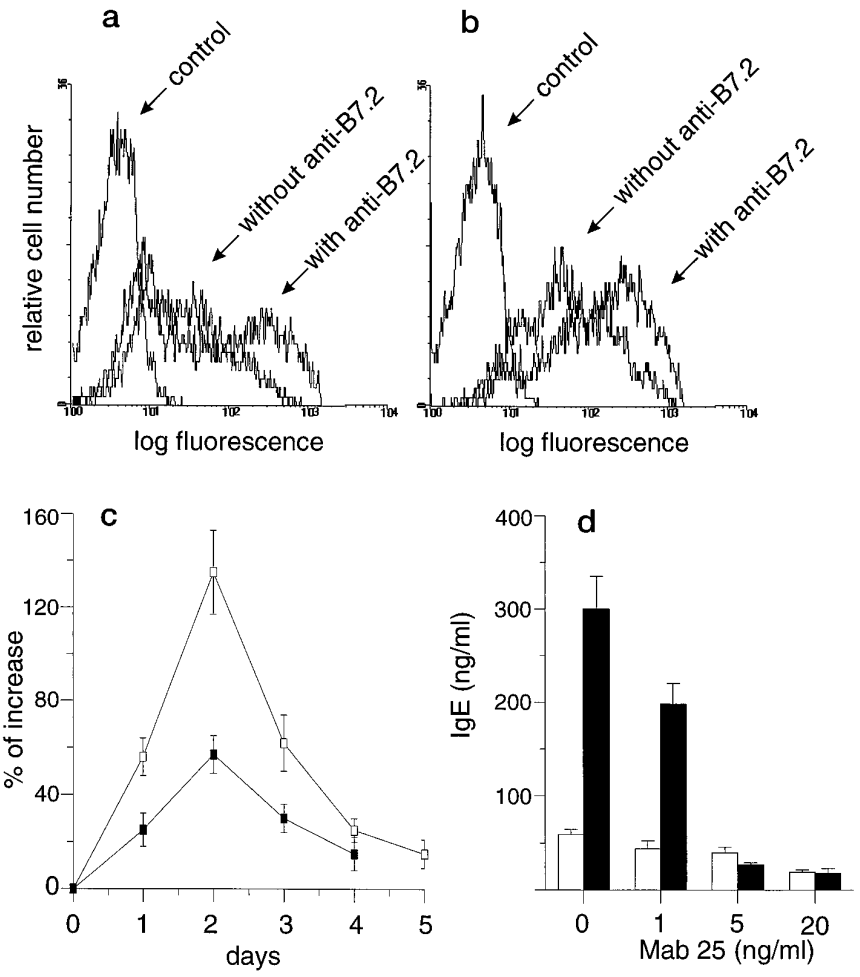

FIG. 4. **CD86-potentiated IgE synthesis is mediated through an increase of CD23-CD21 pairing.** *Panels a* and *b*, CD86 triggering increases CD23 expression. Human tonsillar B cells were stimulated by IL-4 (*panel a*) or IL-13 (*panel b*) plus anti-CD40 mAb without or with IT2.2. CD23 expression was evaluated by FACS analysis at 48 h. Results expressed in MFI values are representative of one of six experiments. *Panel c*, CD86 triggering increases CD23-liposome binding to CD21-expressing B cells. B cells were stimulated with IL-4 plus anti-CD40 mAb without or with IT2.2. The expression of CD23 (□) and the binding of CD23-fluorescent liposomes (■) were evaluated at different time points by FACS analysis. Results are expressed in percent of increase of MFI (mean % ± S.D., $n = 6$). *Panel d*, CD86-potentiated IgE production requires CD23-CD21 interaction. B cells stimulated with IL-4 plus anti-CD40 mAb without (□) or with (■) IT2.2 were incubated with different concentrations of the Mab 25 mAb. IgE was quantified in the 12-day supernatants. Results, expressed in ng/ml (mean ± S.D., $n = 5$), are representative of one of three experiments.

the B7 molecules being achieved by a stimulation with both IL-4 plus anti-CD40 mAb. The existence of B7-3 as a new ligand for CTLA-4 is controversial since BB1 also binds to CD80 (1, 40).[2] Nevertheless, these data show that a stimulation of B cells with IL-4 or/and via the CD40/CD40L pathway controls the expression of the B7 molecules and suggest that in diseases associated with a predominant T helper 2 cell response, activated T cells may contribute to up-regulate their expression.

The three B7 molecules were highly expressed on IL-4 or IL-13 plus anti-CD40 mAb-stimulated B cells. However, only CD86 triggering with IT2.2, the mAb used to clone CD86 from a B cell line (2), potentiated IgE production. This effect of IT2.2 was mediated through a specific binding to CD86, as assessed in neutralizing experiments using CD86-rIg. The inability of the other anti-CD86 mAbs to affect B cell activation has to be related to the fact that they bind to epitope(s) of the CD86-V domain distinct from the binding site of IT2.2 (34).[2] Thus, the ability of an anti-CD86 mAb to affect B cell activation seems to be epitope-restricted. Consequently, although the anti-CD80 mAbs tested and BB1 were ineffective, we cannot exclude a potential role of CD80 and B7-3 in B cell activation. However, an interesting point is that CD86 triggering has a signaling role in B cells. This observation is in agreement with the fact that B7-2 has a cytoplasmic tail containing three potential sites for protein kinase C phosphorylation (6).

CD86 triggering potentiated IgE and IgG4 production, proliferation, and CD21-CD23 interaction induced by IL-4 or IL-13 plus anti-CD40 mAb. However, it was unable to replace IL-4, IL-13, or anti-CD40 mAb signals in inducing IgE and IgG4 synthesis. Thus, CD86 appears as a costimulatory molecule associated with a pathway involving both IL-4 or IL-13 receptors and CD40. Although the intracellular events remain unclear, different mechanisms could explain the effect of CD86 triggering. Different costimuli such as some lymphokines (IL-6 and tumor necrosis factor-α) (21, 41) and the ligation of B cell surface molecules (CD21 and ICAM-1) (26, 29, 31) have been reported to potentiate the effects of IL-4 and CD40 triggering on B cells. Actually, IT2.2 did not modulate tumor necrosis factor-α or IL-6 production by stimulated-B cells (data not shown). It is also unlikely that the small increase of CD54 expression induced by CD86 triggering fully explains its potent effect on IgE synthesis. In fact, CD23-CD21 pairing enhances the ϵ transcript expression and potentiates IgE production induced by IL-4 plus anti-CD40 mAb (26, 29). Since CD86 triggering increased concomitantly CD23 density on B cells and CD21 affinity for CD23, it was tempting to speculate that CD86 up-regulates IgE production by favoring CD21-CD23 pairing. This hypothesis was confirmed by neutralizing experiments showing that blocking CD23-CD21 pairing prevented the effect of IT2.2 on IgE synthesis. Taken together, these data show that the ligation of CD86 favors CD23-CD21 pairing and, consequently, up-regulates selectively IgE/IgG4 production.

Different studies have suggested a role of CD28 and CTLA-4 in the regulation of Ig synthesis. Defective production of switched Ig isotypes is seen both in mice treated with soluble CTLA-4 and in mice transgenic for CTLA-4 (15, 43). CD28-deficient mice have reduced basal levels of IgG1 and IgG2a and diminished Ig class switching (14). *In vitro*, activation of T cells by cross-linking CD28 enhances IL-4 production, CD40L ex-

[2] J. Ellis, unpublished data.

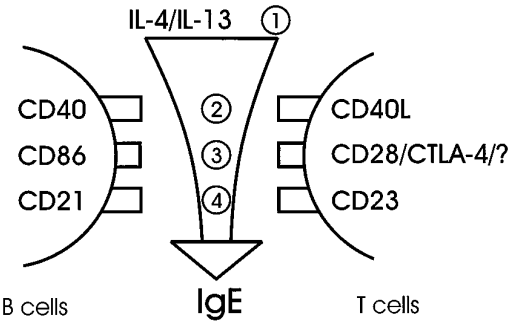

FIG. 5. **Hypothetical model of the sequential interactions involved in human IgE production.** Activated T cells produce IL-4/IL-13 and express CD40L, the two signals required to induce IgE production. IL-4/IL-13 also increase the cell surface expression of CD23 on B and T cells and of CD40L on T cells. Both IL-4/IL-13 and CD40L up-regulate CD86 expression. Triggering of CD86 by specific ligand(s) favors CD23-CD21 pairing and consequently potentiates IL-4/IL-13 plus anti-CD40 mAb-induced IgE synthesis.

pression, and consequently provides an efficient help for IgG and IgM production (44, 45). In addition, CD28 expression by T cells is required for IgE synthesis since a neutralizing anti-CD28 mAb decreased IgE production by B cells cultured with T cells (17). Finally, it has been recently observed that IgE synthesis in mice was modulated by administration of CTLA-4-Ig or anti-CD86 mAb.[3] In line with these observations, we demonstrate that CD86 triggering up-regulated IgE production by human B cells. However, although IT2.2 prevents the binding of CTLA-4 to CD86 (2, 34),[2] additional experiments are required to determine whether CD28 and/or CTLA-4 bind to CD86 and mimic the effect of IT2.2 on B cells.

We have observed that addition of soluble IT2.2 decreases IgE and IgG4 production by IL-4-stimulated peripheral blood mononuclear cells (data not shown). Different mechanisms may explain this observation. Masking CD86 may: (i) prevent the increase of CD23 expression and CD23-CD21 interaction and consequently down-regulate IgE/IgG4 synthesis; (ii) prevent the interaction of CD86 with its ligands on T cells, and recent studies have shown that this interaction potentiates IL-4 production (46–48); or (iii) favor the interaction of CD28 or CTLA-4 with other ligands (*i.e.* CD80 and B7-3), and the role of these interactions on IgE production remains unknown. Nevertheless, the main point is that IT2.2 directly activates B cells but also interferes with T cell-derived costimulatory signals, thereby demonstrating that IgE synthesis is controlled by different signals provided through direct B-T cell contact and including CD86-ligand(s) interaction. However, since CTLA-4 and CD28 are expressed, in some conditions, by activated B cells (49) and by plasma cells (42), CD86 triggering may probably also take place, in some conditions, in B cell-B cell homotypic interaction.

In conclusion, we report that CD86 has a signaling role in B cells since it increases CD23-CD21 pairing and consequently potentiates IgE and IgG4 production induced by IL-4 or IL-13 plus anti-CD40 mAb. Taken together with data from others, these observations allow us to propose that sequential positive feedbacks between B and T cells may control IgE/IgG4 synthesis (Fig. 5). T cells provide the two signals required to induce IgE/IgG4 production, IL-4 and CD40L. IL-4 favors the cell surface expression of CD23 on T and B cells (24, 28) and CD40L (30) on T cells. IL-4, IL-13, and CD40L up-regulate CD86 expression on human B cells. Then, on one hand, the interaction of CD86 with its ligands on T cells may result in a positive feedback since CD86 triggering has been reported to increase IL-4 production (44, 46–48) and CD40L expression by T cells (44, 45). On the other hand, our results show that a stimulation through CD86 transduces a positive signal resulting in an increase of CD23-CD21 pairing and consequently in an increase of IgE/IgG4 synthesis.

Therefore, preventing the interaction of CD86 with its ligands may have a double effect: a decrease of both IL-4 and IgE production by T and B cells, respectively.

[3] A. J. Coyle, submitted for publication.

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CD86 (B7-2) on Human B Cells: A FUNCTIONAL ROLE IN PROLIFERATION AND SELECTIVE DIFFERENTIATION INTO IgE- AND IgG4-PRODUCING CELLS

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