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CD23/FcεRII Expression on Phytohemagglutinin-A- or Phorbol-¹²Myristate-¹³Acetate- Ca²⁺-Activated Human Tonsil T Cells

Abstract

The low-affinity Fc receptor for IgE, CD23/FcεRII, has been expressed in T cell lines and pathologic T cells, but its presence on normal human T cells is still debated. We studied the expression of CD23/FcεRII on purified T cells from normal peripheral blood mononuclear cells (PBMC) or tonsil T cells stimulated with 10 μg/ml phytohemagglutinin A (PHA) or phorbol-¹²myristate-¹³acetate (PMA) Ca²⁺. Using two-dimensional flow cytometry, the tonsil T-cell-enriched population showed > 10% of CD23/FcεRII expression when coexpressed with the CD3 antigen. CD4+ T cells appear to be principally involved in the expression of CD23/FcεRII, although we were unable to detect a clear expression of CD23/FcεRII in PBMC that were activated with either PHA or PMA Ca²⁺. PHA stimulation resulted in the release of IgE binding factor (IgEBF). The induction of CD23/FcεRII expression in PHA- and PMA-Ca²⁺-activated T cells was enhanced by IL-4, but not by IgE or IL-6. IL-4 also augmented the PHA- and PMA-Ca²⁺-induced release of IgEBF. The addition of supernatant from the Epstein-Barr virus (EBV)-infected cell line to PHA- or PMA-Ca²⁺-stimulated tonsil T cells did not increase CD23/FcεRII expression. The expression of CD23/FcεRII mRNA was detected in RNA prepared from a tonsil T-cell-enriched population by Northern blot analysis.

Key Words

CD23
T cells
IgE
Low-affinity receptor
FcεRII

Introduction

T cells that express the low-affinity IgE receptor, FcεRII, have been identified in the regulation of IgE synthesis in vitro both in rodents [1], as well as humans [2, 3]. Recently, CD23 has been cloned [4–6] and found to be identical to FcεRII [7, 8]. FcεRII/CD23 is a 45-kD surface

antigen that was originally described as a B-cell-restricted activation antigen that is present at high levels on Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines [9–12]. The presence of FcεRII on normal human T cells is somewhat controversial [4, 13–16]. In initial studies, rosette analysis demonstrated low FcεRII/CD23 expression on T cells [11, 13]. A study by Nagai et al. [17] also found

that T cells from patients with Kimura disease reacted with anti-FcεRII monoclonal antibodies (MAbs). Prinz et al. [18] observed that T cells stimulated with phytohemagglutinin A (PHA) or antigen express FcεRII. A detectable level of FcεRII/CD23 expression has also been reported in HTLV-I- and HIV-1-transformed T cells [19, 20]. These cells clearly react with anti-FcεRII MAb [21]. Recently, a study by Kawabe et al. [22] showed that cells stimulated with PHA in the presence of IL-4, express FcεRII/CD23. Thus, it is clear that in certain conditions human T cells can synthesize and express FcεRII/CD23. In studies where lower levels of FcεRII/CD23 expression were observed on normal and activated T cells, it remains unclear whether FcεRII/CD23 was produced by T cells or acquired by the absorption of a soluble FcεRII/CD23 fragment.

In addition, studies have described FcεRII/CD23 T cell lymphoma [23] and T cell lines [7], and a surface CD23 T cell hybridoma which secretes soluble FcεRII/CD23 [24]. In this study, we examined whether normal tonsil and peripheral blood (PB) T cells express FcεRII/CD23 in response to mitogen stimulation, and we also examined the nature and mechanism of FcεRII/CD23 expression.

Patients and Methods

IgE myeloma protein (provided by Dr. K. Ishizaka, La Jolla, Calif., USA) was isolated from the serum of an E myeloma patient by methods previously described [25]. The purity of the preparation was > 99.7%. MHM6, a mouse MAb anti-human FcεRII/CD23 purchased from Immunotech (San Francisco, Calif., USA), was used at a working dilution of 1:20. An EBV B cell lymphoblastoid, RPMI-8866, which expresses nearly 95% of the FcεRII/CD23 receptor on its surface, served as a positive control for FcεRII/CD23 expression. Another anti-human FcεRII/CD23 MAb, termed H-107, (provided by Dr. J. Yodoi, Kyoto, Japan), was also used to check FcεRII/CD23 expression.

Fluorescent isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin was used for indirect immunofluorescence (Tago, Burlingame, Calif., USA). MAbs termed Leu 2 (CD8), Leu 3 (CD4), Leu 4, and Leu 12 (CD19) were purchased from Becton Dickinson (Mountain View, Calif., USA). The OKT3 preparation utilized was an IgG1 fraction of ascetic fluid (Ortho Diagnostic, Raritan, N.J., USA). Specifically purified goat anti-mouse IgG antiserum (anti-MGG) was purchased from Ortho Diagnostic.

Antigens and Interleukins

PHA and phorbol-¹²myristate-¹³acetate (PMA) were obtained from Sigma (St. Louis, USA). Human interleukin-2 (IL-2) was used to stimulate T cells (Electronucleonics, Silver Spring, Md., USA). There was no detectable interferon-γ or PHA in the IL-2 preparation. Recombinant IL-4 and IL-6 (Amigen, San Diego, Calif., USA) were used in the cell cultures at a concentration of 10 U/ml of IL-4 and 100 U/ml of IL-6, respectively.

Cell Preparation

Peripheral blood mononuclear cells (PBMC) were obtained from the heparinized venous blood of ten normal subjects and from tonsil tissue that was gently teased. The PBMC were isolated using a Ficoll-Hypaque gradient (Pharmacia, Uppsala, Sweden).

The PBMC were separated into a T-cell and a B-cell-enriched population by rosette formation with aminoethylisothiuronium-treated sheep erythrocytes [26]. The erythrocytes were lysed with buffered 0.87% NH₄Cl, and the T cells were recovered from pellets containing rosette-linked cells. There was no detectable B cell contamination of the depleted fractions as determined by Leu 12 (CD19). CD3 positivity was > 95% in the population. Leu 3 (CD4) and Leu 2 (CD8) MAbs were used to differentiate the T cell subpopulation. All samples, including the tonsil T cells, were examined immediately after purification.

Culture Conditions

We used an RPMI-1640 culture medium that was supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 50 mM 2-mercaptoethanol, 100 U/ml penicillin and 100 mg/ml streptomycin at 37°C with 5% CO₂. One milliliter each of the purified T cell fractions was suspended in the culture medium at 0.5 × 10⁶/ml. The samples were then activated with 10 μg/ml of PHA, or PMA (1 ng/ml) plus ionomycin (0.125 mg/ml) for 3 days, and cultured in well plastic plates. The cells were recovered, resuspended in fresh culture medium and cultured for 4 days with purified IL-2 (60 U/ml). In several of the samples, T cells propagated by IL-2 were also stimulated by the MAb OKT3. Limbro plates were coated with 10 μg/ml of purified anti-MGG and incubated overnight at 4°C. After washing, 10 μg/ml of OKT3 was placed in the wells and incubated for 6 h at 4°C. The wells were washed and a 2-ml suspension of each sample of the lymphoblastoid cells (10⁶/ml) was cultivated for 24 h. The cells were then recovered and examined by cytofluorometric analysis.

Immunofluorescence

The proportion of B cells, T cells and their subsets was determined by flow cytometry. 5 × 10⁵ cells were incubated with 10 μl of the appropriate MAb for 30 min at 4°C. For two-color analysis, 5 × 10⁵ cells were incubated first with FITC MHM6 or H-107 MAbs for 40 min at 4°C and washed with PBS + 2% FCS. They were then incubated with either anti-Leu 2 or Leu 3 phycoerythrin. The cells were reacted with an irrelevant antibody to demonstrate the specificity of the staining shown by MHM6 or H-107. Cytofluorometric analysis was performed with an EPICS flow cytometer coulter equipped with software for two-color analysis.

Detection of IgE Binding Factor (IgEBF)

Human T cell hybridoma cells 166A2 [27] were incubated for 24 h in the presence of a dimer of human IgE (50 ng/ml), and were used as a standard for the formation of IgEBF. IgEBF was purified using an IgE sepharose column [28]. The culture supernatants were filtered through YM-100 Diaflo membranes, and the filtrates were concentrated 10-fold by ultrafiltration. One milliliter of the concentrated filtrate was combined with 0.25 ml of IgE-coated Sepharose and incubated overnight at 4°C. The IgE Sepharose column was packed and washed with 5 ml of Dulbecco's phosphate-buffered saline (DPBS). The bound material was eluted with 1 ml of glycine/HCl buffer (pH 3.0). The elutes were immediately neutralized with Tris/HCl buffer and dialyzed against DPBS.

The presence of IgEBF in the acid elute fractions was confirmed by the inhibition of rosette formation between the RPMI-8866 cells and the fixed ox erythrocytes coated with 0.02 µg/ml of human IgE (E'IgE). First, the E'IgE were preincubated with a test sample and then RPMI-8866 cells were added to the suspension for rosette analysis. The percentage of rosette inhibition was determined in duplicate slides and was expressed as an average. Under these experimental conditions, 25–45% of the RPMI-8866 cells formed rosettes. There was <8–10% inhibition which was statistically insignificant when considering experimental error.

The radioimmunoassay (RIA) for IgEBF has been previously established [20]. Briefly, wells of microtest flexible plates were coated with 10 µg/ml of MAb anti-CD23 (H-107) in 100 µl of 0.1 M carbonate/bicarbonate buffer (pH 9.6), and incubated overnight at 4 °C. The wells were then washed with DPBS and blocked with 2% bovine serum albumin. The wells were washed again with DPBS containing 0.05% Tween 20, and 50 µl of the samples were then added to duplicate wells and stored overnight at 4 °C. The wells were washed several times with DPBS containing Tween 20 and 100 µl of ¹²⁵I-labeled dimeric IgE in DPBS containing 0.1% bovine serum albumin. Next, 0.05% Tween 20 was added to each well. The plates were incubated overnight at 4 °C and washed thoroughly with DPBS/Tween 20. The radioactivity of each well was determined with an automatic γ counter and the background count with DPBS instead of sample was subtracted.

Purification of Soluble FcεRII/CD23

Culture supernatants of EBV lymphoblastoid cell line (EBVLCL) 8866 were collected and filtered through YM-100 Diaflo membranes. The filtrates were concentrated 10-fold by ultrafiltration. One milliliter of the concentrated filtrate was combined with 0.25 ml of IgE-coated Sepharose and incubated overnight at 4 °C. The IgE Sepharose was packed into a small column and washed with 5 ml DPBS. The bound material was eluted with 1 ml of glycine HCl buffer (pH 3.0), and the elutes were immediately neutralized with Tris/HCl buffer and dialyzed against DPBS. The presence of soluble FcεRII/CD23 in the acid elute fractions was assessed by its ability to inhibit rosette formation between RPMI-8866 cells and fixed ox erythrocytes coated with IgE [28]. Parallel culture supernatants from EBVLCL were precleared by two cycles of rotation for 1 h at 4 °C with MHM6 coupled to cyanogen-bromide-activated Sepharose beads.

Northern Blot Analysis

Purified tonsil T cells (10⁶) were cultured with PMA in the presence or absence of IL-4 for 48 h. Half of the cells were incubated with MAbs against CD20 (100 µg) and CD14 (100 µg) for 30 min at 4 °C. The other half were incubated without any antibodies. All of the cells were then incubated with 100 µl of rabbit complement for 1 h at 37 °C and washed with PBS. Total cellular RNA was isolated by the guanidine isothiocyanate method [29] using cesium chloride [30]. Aliquots of 20 µg of total RNA were electrophoresed on a 1% agarose gel and transferred to a nitrocellulose filter. A ³²P-labeled RNA probe for FcεRII/CD23 cDNA (kindly provided by Dr. R. Eisenback) was prepared with an SP6/T7 RNA polymerase labeling system [31]. The pSP65 vector containing an FcεRII/CD23 cDNA inserted was used. To determine the amount of RNA on the membranes, the nitrocellulose filter was rehybridized with a ³²P-labeled pseudoactin [32] cDNA fragment at 42 °C, and the density of the hybridized band was analyzed by a laser densitometer. The pseudoactin cDNA fragment was kindly provided by Dr. R. Eisenback.

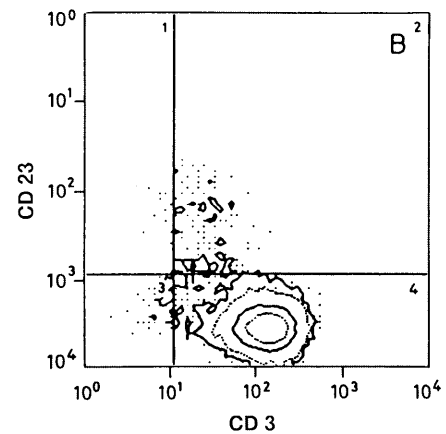


Fig. 1. Two-color fluorescence histogram showing FcεRII/CD23, CD3+ T cells. The purified T cell population of a subject was incubated with FITC MAb MHM6 on the vertical axis and then phycoerythrin coupled with anti-Leu 4 on the horizontal axis. Virtually 10% of the Leu 4+ cells were also positive for FcεRII/CD23 (upper right quadrant)

Results

Presence of CD23 Expression on the Cell Membrane of Antigen-Stimulated T Cells

Using MHM6 and H-107 MAbs, a detectable level of FcεRII/CD23 was found on the purified tonsil T cells stimulated with PHA. MHM6 and H-107 are likely to recognize two distinct epitopes on the CD23 molecule. Two-color FACS analysis revealed that only 10% of the CD3+ cells coexpress the FcεRII/CD23 receptor on their cell membranes (fig. 1). The B cell concentration of the depleted fractions was not detectable by CD19 (data not shown).

Cytofluorometric analysis of tonsil T cells and PB T cells stimulated with PHA and IL-2, and those that were stimulated further with OKT3, showed a significant increase in CD23 expression (approximately 10%) only in the tonsil T cell population, particularly when the cells were cultured for 9 days (i.e., PHA+IL-2+OKT3) and further stimulated for 24 h with OKT3 (table 1). In contrast, PB T cells consistently failed to exhibit CD23 expression before or after mitogenic stimulation (table 1). Comparable levels of staining were observed with the two MAbs used, i.e., MHM6 and H-107.

FcεRII/CD23 was barely detectable on PMA-Ca²⁺-activated T lymphocytes (data not shown). In contrast, T cells stimulated with PHA plus IL-2 showed that CD23 was al-

Fig. 2. Two-color fluorescence histogram showing FcεRII/CD23, CD4+ (**A**) or CD8+ (**B**) T cells. The cell-enriched population of an individual was stimulated with PMA plus IL-2, cultured for 7 days and then incubated with FITC MAb MHM6 (vertical axis), and phycoerythrin-coupled anti-leu 3 or anti-Leu 2 (horizontal axis). Several of the Leu 3+ cells were positive for FcεRII/CD23 (upper right quadrant) but none of the Leu 2 were positive for FcεRII/CD23.

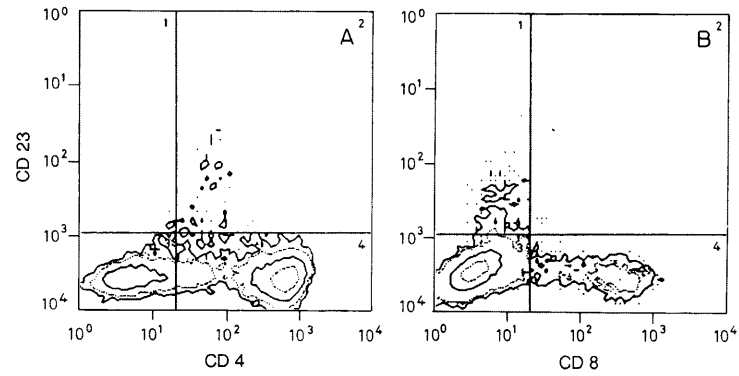


Table 1. Cytofluorometric analysis of CD23 expression on tonsil and PB T cells

	Tonsil T cells		PB T cells	
	MHM6	H-107	MHM6	H-107
Day 0	0	0	0	0
Day 3 + PHA	1 ± 1	2 ± 1	0	0
Day 7 + PHA + IL-2	5 ± 1	6 ± 1	1 ± 1	1 ± 1
Day 7 + OKT3	8 ± 1	9 ± 2	0	0
Day 9 + OKT3 + IL-2 + OKT3	11 ± 3	10 ± 2	ND	ND

Cells were studied at day 0, or at day 3 of culture with PHA alone, or after culture for 7 days with PHA plus IL-2. Alternatively, cells were subcultured for 7 days and stimulated for a further 24 h with OKT3 on a coated plate. Another batch of cells from the same experiment was activated with PHA plus IL-2 plus OKT3 for 8 days, then washed and further stimulated with OKT3 on a coated plate for a total of 9 days of culture.

most exclusively expressed on Leu 3+ (CD4) T cells (fig. 2A), while CD23 was not detectable on Leu 2+ (CD8) T cells (fig. 2B).

Soluble CD23 Does Not Increase CD23 Expression

EBV lymphoblastoid cell line 8866 supernatant (EBVLCL SN) was added to PHA- or PMA-stimulated tonsil T cell cultures. This did not increase the expression of CD23 on these T cells. Negative results were also seen when soluble FcεRII/CD23 was removed from EBV SN precleared with MHM6-coupled Sepharose beads (data not shown).

Taken together, these results strongly suggest that the presence of CD23+ in the culture supernatants (i.e., soluble FcεRII/CD23), cannot increase the amount of CD23 receptor expressed by mitogen-stimulated tonsil T cells.

FcεRII/CD23 Expression on T Cells with IL-2, IL-4, IL-6 or IgE and Release of IgEBF

Figure 3 shows cytofluorometric analysis of FcεRII/CD23 expression on human tonsil T cells after addition of IgE, IL-4, IL-6 and OKT3 in the last 24 h of culture. Prior to the addition of different stimulants, cells were activated with PMA or PHA and expanded with IL-2 for 7 days. FcεRII/CD23 expression on activated T cells was enhanced by the addition of IL-4. However, there was no augmentation when IL-4 was used in combination with IgE or IL-6. IL-4 increased CD23 expression in a dose-dependent manner between 5 and 60 U/ml (data not shown). The best FcεRII/CD23 expression was seen at a concentration of 30 U/ml IL-4 (fig. 3).

Soluble FcεRII (IgEBF) was detectable after 24 h of cell culture by RIA in the supernatants of tonsil PHA-stimulated cells (table 2). Comparable results were obtained when the same samples were tested by rosette inhi-

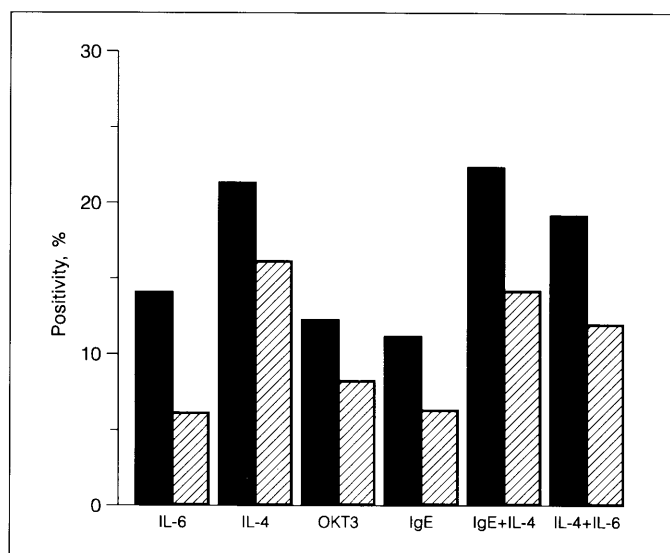


Fig. 3. FcεRII/CD23 expression after the addition of IgE, IL-2, IL-4 or IL-6. IL-4 was the only interleukin able to increase FcεRII/CD23 expression more than PHA or PMA. Results were considered positive when they were 2 SD above the control represented by day 0 of culture.

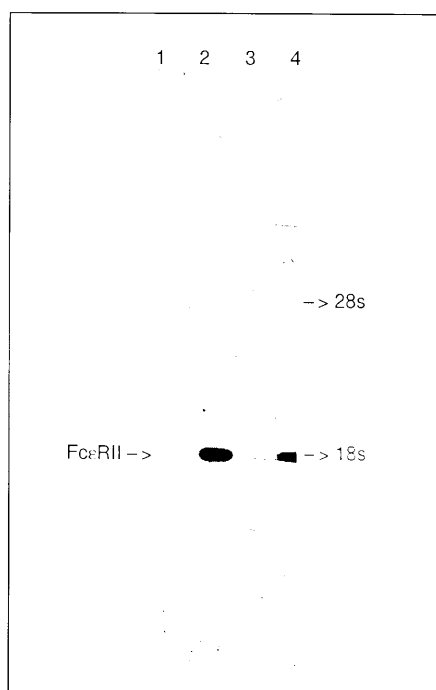


Fig. 4. Expression of FcεRII/CD23 mRNA on human tonsil T cells. Tonsil T cells were stimulated with PMA, cultured for 7 days and analyzed by Northern blot using a ³²P-labeled probe specific for FcεRII/CD23; 20 μg of total RNA were applied to each lane. Lane 1 = Jurkatt cell line; lane 2 = RPMI-8866 cell line; lane 3 = tonsil T cells; lane 4 = tonsil T cells stimulated with IL-4.

Table 2. Formation of IgE BF by T cells

Tonsil cells	Binding of IgE dimer ¹ , c.p.m.			
	IL-4	IgE	IL-4 + IgE	medium
PHA	1,653	931	1,715	229
PMA	1,818	979	1,795	293
166 A2	—	2,600	—	0

¹ Data represent ¹²⁵I-labeled IgE dimer bound to wells in a RIA; values < 350 c.p.m. are not significant; background counts were subtracted.

bition (data not shown). When the cells were cultured in the presence of IL-4, the culture supernatants showed a 2-fold increase of IgEBF formation. Similar results were obtained when the cells were stimulated with PMA (table 2). These results concur with the CD23 expression where the receptor expression was increased primarily in the presence of IL-4. There was no time-dependent increase in the amount of IgEBF when the cells were cultured in the presence of IL-4 for 48 or 96 h (data not shown). The concentration of IgEBF increased in the tonsil PHA-stimulated cells in the presence of IL-4, but not in the presence of IgE (table 2).

Northern Blot Analysis of FcεRII Expression on Purified T Cells

We noticed a significant expression of the 1.7-kb mRNA for FcεRII/CD23 (fig. 4) in the purified tonsil T cell population where most of the cells were CD3+. This result suggests that the expression of FcεRII/CD23 on T cells is due to the synthesis of FcεRII by T lymphocytes.

Discussion

The FcεRII/CD23 receptor was originally described as a B cell activation antigen [10, 32, 33]. Many reports have shown that this receptor is not present on resting or activated T cells [4, 14, 15], although T cells expressing the IgE receptor have been described [13, 16, 22, 34]. Studies concluding that normal T lymphocytes do not express FcεRII/CD23 have almost exclusively utilized PB T cells. The data presented here confirm the lack of CD23 expression on PB T cells, whether examined immediately after isolation or following activation with various stimuli. In contrast, a minority of T cells, although CD23-negative when tested directly ex vivo, consistently expressed CD23, as con-

firmed by staining with MHM6 and H-107 MAbs after activation with PHA or PMA Ca^{2+} . Additionally, the proportion of CD23+ cells increased when IL-4 was added to the activated cells.

The results presented here indicate that high levels of CD23 expression seem to be unique to activated tonsil T cells. Our data support the findings of Kawabe et al. [22] that around 10% of the cells expressing CD23 are CD3+ T cells. The high level of Fc ϵ RII/CD23 expression observed seems to be characteristic of PMA- Ca^{2+} -activated T cells because stimulation with OKT3 had no effect. Moreover, PHA or PMA Ca^{2+} plus IL-4 induced Fc ϵ RII/CD23 expression on CD3+ cells (fig. 3), whereas IL-4 alone did not. A similar pattern was observed when IgEBF was measured in the culture supernatants (table 2). These findings clearly indicate that activated PB or tonsil T cells express Fc ϵ RII/CD23 on their surface. This is in accordance with the observation that many HTLV-1+ T cell lines [7, 21, 31], HIV-1-infected T cells [20], and allergen- or mitogen-stimulated T cells [18, 22], also express Fc ϵ RII/CD23.

There has been some controversy as to whether the Fc ϵ RII/CD23 expressed on activated T cells is a synthesized product or whether it is derived from other cells such as B cells. Kinetic studies of IgEBF in culture supernatants (table 2) revealed that the release of IgEBF was markedly enhanced by PHA plus IL-2 and IL-4 compared to other conditions. Because of the high levels of IgEBF accumulating in the culture supernatants, it is highly unlikely that any IgEBF derived from other cells was trapped by the T cells. This is supported by the fact that the presence of soluble CD23 did not increase the number of CD23 receptors expressed by the stimulated T cells. Northern blot analysis detected mRNA for Fc ϵ RII/CD23 in enriched fractions of tonsil T cells stimulated with PHA and IL-4. These results confirm that the receptor is synthesized by T cells and not acquired via passive binding to the membrane surface.

It is clear that IL-4 can augment Fc ϵ RII/CD23 expression on B cells [32, 35–44]. This IL-4 enhancement may be the result of a reduction in the spontaneously occurring degradation of Fc ϵ RII/CD23. Moreover, the relation between Fc ϵ RII/CD23-enhanced expression and IL-4 activity is supported by its suppression by anti-IL-4 MAb (data not shown).

In conclusion, we have shown that activated T cells alone were able to express CD23 on their surface. Our findings not only confirm that the antigen defined by the MAb with Fc ϵ RII/CD23 activity is expressed on activated T cells, but also indicate that Fc ϵ RII/CD23 expression is caused by the activation of antigen-specific T cells through the T cell receptor. Our results also show that the addition

of IgE to the cell cultures had an abating effect on the decrease of Fc ϵ RII/CD23, similar to that seen on normal B cells [45]. One explanation for this phenomenon may be that IgE reduces the spontaneously occurring degradation of Fc ϵ RII/CD23, and probably stimulates the resynthesis of the receptor [34, 45]. Previous reports by Almerigogna et al. [46] showed that a protein synthesis inhibitor abolished the protective effect of IgE on the loss of Fc ϵ RII/CD23 on normal B cells. A similar explanation can be suggested for T cells, where IgE protein stimulates the de novo synthesis of the receptor [34, 45]. It is interesting that the expression of Fc ϵ RII/CD23 on T cells was not affected by the addition of EBVLCL SN. This differs from a previous report by Armitage et al. [47] which showed that EBV supernatant augmented Fc ϵ RII/CD23 expression.

Whatever the mechanism, this data provides additional information on the kinetics of CD23. The meaning of these findings with regard to the biological function of CD23 on either normal or pathological T cells remains to be determined. It is clear, however, that T cell Fc ϵ RII/CD23 seems to be synthesized by T cells and is not acquired via passive binding of soluble Fc ϵ RII/CD23 to the membrane surface. Prompted by the discovery of a steric relationship between CD23 and the human leukocyte antigen HLA-DR [48], we speculate that the Fc ϵ RII/CD23 receptor may be involved in antigen presentation. This observation combined with evidence that allergens can induce specific expression of CD23 on T cells [18] indicates that T cell Fc ϵ RII/CD23 has an important role in the immunological response.

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References

- Ishizaka K: IgE binding factor and regulations of IgE antibody response. *Annu Rev Immunol* 1988;6:513.
- Conrad DH: Low affinity IgE receptors. *Clin Rev Allergy* 1989;7:165.
- Delespesse G, Sarfati M, Hofstetter H, Suter U, Nakajima T, Peleman R, Letellier M, Kilchherr E, Frost H: Structure, function and clinical relevance of the low affinity receptor for IgE. *Immunol Invest* 1988;17:363.
- Kikutani H, Suemura M, Owaki H, Nakamura H, Sato R, Yamasaki K, Barsumian EL, Hardy RR, Kishimoto T: Fcε receptor, a specific differentiation marker transiently expressed on mature B cells before isotype switching. *J Exp Med* 1986;164:1455.
- Ludin C, Hofstetter H, Sarfati M, Levy CA, Suter U, Alaimo D, Kilchherr E, Frost H, Delespesse G: Cloning and expression of the cDNA coding for a human lymphocyte IgE receptor. *EMBO J* 1987;6:109.
- Ikuta K, Takami M, Kim CW, Honjo T, Miyoshi T, Tagaya Y, Kawabe T, Yodoi J: Human lymphocyte Fc receptor for IgE: Sequence homology of its cloned cDNA with animal lectins. *Proc Natl Acad Sci USA* 1987;84:819.
- Kikutani H, Inui S, Sato R, Barsumian EL, Owaki H, Yamasaki K, Kaisho T, Uchibayashi N, Hardy RR, Hirano T, Tsunasawa S, Sakiyama F, Suemura M, Kishimoto T: Molecular structure of human lymphocyte receptor for immunoglobulin E. *Cell* 1987;47:657.
- Yukawa K, Kikutani H, Owaki H, Yamasaki K, Yokata A, Nakamura H, Barsumian EL, Hardy RR, Suemura M, Kishimoto T: A B cell-specific differentiation antigen, CD23, is a receptor for IgE (FcεR) on lymphocytes. *J Immunol* 1987;138:2576.
- Slovin SF, Frisman DM, Tsoukas CD, Royston I, Baird SM, Wormsley SB, Carson DA, Vaughan JH: Membrane antigen on Epstein-Barr virus infected human B cells recognized by a monoclonal antibody. *Proc Natl Acad Sci USA* 1982;79:2649.
- Thorley-Lawson DA, Nadler LM, Bhan AK, Schooley RT: BLAST-2 [EBVCS], an early cell surface marker of human B cell activation, is superinduced by Epstein-Barr virus. *J Immunol* 1985;134:3007.
- Spiegelberg HL, Thompson LF, McNeil D, Buckley RH: IgE Fc receptor positive T, B and NK cells in patients with the hyper-IgE syndrome. *Int Arch Allergy Appl Immunol* 1985;77:277.
- Nadler LM, Reinherz EL: *Leucocyte Typing II*. New York, Springer, 1985, vol 2, p 3.
- Yodoi J, Ishizaka K: Lymphocytes bearing Fc receptors for IgE. I. Presence of human and rat T lymphocytes with Fcε receptors. *J Immunol* 1987;138:1257.
- Suemura M, Kikutani H, Barsumian EL, Hattori Y, Kishimoto S, Sato R, Maeda A, Nakamura H, Owaki H, Hardy RR, Kishimoto T, et al: Monoclonal anti Fcε receptor antibodies with different specificities and studies on the expression of Fcε receptors on human B and T cells. *J Immunol* 1986;137:1214.
- Rao M, Lee WT, Conrad DH: Characterization of a monoclonal antibody directed against the murine B lymphocyte receptor for IgE. *J Immunol* 1987;138:1845.
- Nakajima T, Sarfati M, Delespesse G: IgE receptors on human lymphocytes I. Identification of the molecules binding to monoclonal anti-Fcε receptor antibodies. *Eur J Immunol* 1986;16:809.
- Nagai T, Adachi M, Noro N, Yodoi J, Uchino H: T and B lymphocytes with immunoglobulin in E Fc receptors (FcεR) in patients with nonallergic hyperimmunoglobulinemia E: Demonstration using a monoclonal antibody against FcεR associated antigen. *Clin Immunol Immunopathol* 1985;35:261.
- Prinz JC, Endres N, Rank G, Ring J, Rieber EP: Expression of Fcε receptors on activated human T lymphocytes. *Eur J Immunol* 1987;17:757.
- Sarfati M, Nutman TB, Suter U, Hofstetter H, Delespesse G: T-cell-derived IgE binding factors. II. Purification and characterization of IgE binding factors produced by human T cell leukemia lymphoma virus-1-transformed T lymphocytes. *J Immunol* 1987;139:4055.
- Carini C, Margolick J, Yodoi J, Ishizaka K: Formation of IgE-binding factors by T cells of patients infected with human immunodeficiency virus type 1. *Proc Natl Acad Sci USA* 1988;85:9214.
- Nutman TB, Delespesse G, Sarfati M, Volkman DJ: T-cell-derived IgE-binding factors. I. Cloned and transformed T cells producing IgE-binding factors. *J Immunol* 1987;139:4049.
- Kawabe T, Maekawa N, Maeda Y, Hosada M, Yodoi J: Induction of FcεRII/DC23 on phytohemagglutinin-activated human peripheral blood T lymphocytes. I. Enhancement by IL-2 and IL-4. *J Immunol* 1991;147:548.
- Pallensen G: *Leucocyte Typing II*. New York, Springer, 1985, vol 2, p 277.
- Kisaki T, Huff TF, Conrad DH, Yodoi J, Ishizaka K: Monoclonal antibody specific for T cell-derived human IgE binding factors. *J Immunol* 1987;138:3345.
- Ishizaka K: Immunoglobulin E. *Methods Enzymol* 1985;116:76.
- Noro N, Yoshioka A, Adachi M, Yasuda K, Masuda T, Yodoi J: Monoclonal antibody (H107) inhibiting IgE binding to FcεR(+) human lymphocytes. *J Immunol* 1986;137:1258.
- Falkoff RJ, Zhu LP, Fauci AS: Separate signals for human B cell proliferation and differentiation in response to *Staphylococcus aureus*: Evidence for a two-signal model of B cell activation. *J Immunol* 1982;129:97.
- Huff TF, Jardieu P, Ishizaka K: Regulatory effects of human IgE-binding factors on the IgE response of rat lymphocytes. *J Immunol* 1986;136:955.
- Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 1974;13:2633.
- Gilisin V, Crkvenjakov R, Byus C: Ribonucleic acid isolated by cesium chloride centrifugation. *Biochemistry* 1974;13:2633.
- Kawabe T, Takami M, Hosoda M, Maeda Y, Sato S, Mayumi H, Mikawa H, Arai K, Yodoi J: Regulation of FcεR2/CD23 gene expression by cytokines and specific ligands (IgE and anti-FcεR2 monoclonal antibody): Variable regulation depending on the cell types. *J Immunol* 1988;141:1376.
- Bonnefoy JY, Aubry JP, Peronne C, Wijdenes J, Banchereau J: Production and characterization of a monoclonal antibody specific for the human lymphocyte low affinity receptor for IgE: CD23 is a low affinity receptor for IgE. *J Immunol* 1987;138:2970.
- Rowe M, Heldreth JE, Rickinson AB, Epstein MA: Monoclonal antibodies to Epstein-Barr virus-induced transformation associated cell surface antigens: Binding patterns and effect upon virus-specific T-cell cytotoxicity. *Int J Cancer* 1982;29:373.
- Delespesse G, Sarfati M, Rubio-Trujillo M, Wolowicz T: IgE receptors on human lymphocytes. II. Detection of cells bearing IgE receptors in unstimulated mononuclear cells by means of a monoclonal antibody. *Eur J Immunol* 1986;16:815.
- Bonnefoy JY, Defrance T, Peronne C, Menetrier C, Rousset E, Pène J, DeVries JE, Banchereau J: Human recombinant interleukin 4 induces normal B cells to produce soluble CD23/IgE binding factor analogous to that spontaneously released by lymphoblastoid B cell lines. *Eur J Immunol* 1988;18:117.
- Bonnefoy JY, Guillot O, Spits H, Blanchard D, Ishizaka K, Banchereau J: The low-affinity receptor for IgE (CD23) on B lymphocytes is spatially associated with HLA-DR antigens. *J Exp Med* 1988;167:57.
- Capron A, Dessaint JP, Capron M, Bazin H: Specific IgE antibodies in immune adherence of normal macrophages to *Schistosoma mansoni* schistosomules. *Nature* 1975;253:474.
- Capron A, Dessaint JP, Capron M, Joseph M, Ameisen JC, Tonnel AB: From parasites to allergy: A second receptor for IgE. *Immunol Today* 1986;7:15.
- Capron M, Jouault T, Prin L, Joseph M, Ameisen JC, Butterworth AE, Papin JP, Kusnier JP, Capron A: Functional study of a monoclonal antibody to IgE Fc receptor (FcεR2) of eosinophils, platelets, and macrophages. *J Exp Med* 1986;164:72.

- 40 Chen SS, Bohn JW, Liu ET, Katz DH: Murine lymphocytes expressing Fc receptors for IgE (Fc ϵ R). I. Conditions for including Fc ϵ +R lymphocytes and inhibition of the inductive events by suppressive factor of allergy (SFA). *J Immunol* 1981;127:166.
- 41 Coffman RL, Carty J: A T cell activity that enhances polyclonal IgE production and its inhibition by interferon G. *J Immunol* 1986;136:949.
- 42 Conrad DH, Peterson LH: The murine lymphocyte receptor for IgE. I. Isolation and characterization of murine B cell Fc ϵ receptor and comparison with Fc ϵ receptors from rat and human. *J Immunol* 1984;132:796.
- 43 Defrance T, Aubry JP, Rousset F, Vanbervliet B, Bonnefoy JY, Arai N, Takebe Y, Yokota T, Lee F, Arai K, DeVries JE, Banchereau J: Human recombinant interleukin 4 induces Fc ϵ receptors (CD23) on normal human lymphocytes. *J Exp Med* 1987;165:1459.
- 44 Moller P, Moldenhauer G, Momburg F, Lammler B, Eberlein-Gonska M, Kiesel S, Dorken B: Mediastinal lymphoma of clear cell type is a tumor corresponding to terminal steps of B cell differentiation. *Blood* 1987;69:1087.
- 45 Delespesse G, Sarfati M, Rubio-Trujillo M: In vitro production of IgE-binding factors by human mononuclear cells. *Immunology* 1987;60:103.
- 46 Almerigogna F, Biagiotti R, Giudizi MG, Alessi A, Ricci M, Bosi A, Romagnani S: The Fc ϵ R2/CD23 antigen: A hallmark of chronic lymphocytic leukemia B cells. *Ric Clin Lab* 1989;19:129.
- 47 Armitage RJ, Goff LK, Beverley PC: Expression and functional role of CD23 on T cells. *Eur J Immunol* 1989;19:31.
- 48 Bonnefoy JY, Guillot O, Spits H, Blanchard D, Ishizaka K, Banchereau J: The low-affinity receptor for IgE (CD23) on B lymphocytes is spatially associated with HLA-DR antigen. *J Exp Med* 1988;167:57.