Differential T Cell-Mediated Regulation of CD23 (FcεRII) in B Cells and Follicular Dendritic Cells[1]

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Differences in murine follicular dendritic cells (FDC)-CD23 expression under Th1 vs Th2 conditions prompted the hypothesis that T cells help regulate the phenotype of FDCs. FDCs express CD40, suggesting that T cell-CD40L and lymphokines may be involved in regulating FDC-CD23. To test this, highly enriched FDCs were incubated with CD40L trimer or anti-CD40 to mimic T cell signaling in the presence of IFN-γ or IL-4. Surface expression of CD23 was determined by flow cytometry, whereas mRNA levels of CD23 and its isoforms CD23a and CD23b were independently measured by quantitative PCR. When FDCs were incubated with either CD40L trimer or agonistic anti-CD40 Ab, the expression of FDC-CD23 was increased both at the mRNA and protein levels. Moreover, engagement of FDC-CD40 enhanced mRNA levels for both CD23a and CD23b isoforms. In addition, IFN-γ substantially enhanced CD23a and CD23b mRNA levels in CD40-stimulated FDCs. Curiously, IL-4 could also up-regulate FDC-CD23a but not -CD23b. Anti-IFN-γ dramatically inhibited FDC-CD23 in mice immunized with CFA, whereas anti-IL-4 had only a modest inhibitory effect. In contrast with FDCs, IFN-γ inhibited surface expression of murine B cell-CD23 as well as mRNA for B cell CD23a and -CD23b, whereas IL-4 dramatically enhanced message for both isoforms as well as protein expression. In short, CD23 was regulated very differently in FDCs and B cells. Previous studies suggest that high levels of FDC-CD23 inhibit IgE production, and this IFN-γ and CD40L-mediated up-regulation of FDC-CD23 may explain, at least in part, why Th1 responses are associated with low IgE responses in vivo. ***The Journal of Immunology,*** **2006, 176: 4811–4817.**

CD23 (FcεRII) is the low-affinity receptor for IgE, and it is thought to play an important role in regulating IgE production (1, 2). CD23 is a type II membrane protein with a C-type lectin domain that trimerizes on the cell surface, and this trimer binds to IgE-Fc through the lectin homology region in a calcium-dependent reaction (3). In the murine immune system, CD23 is expressed exclusively on B cells, follicular dendritic cells (FDCs)[3] (1), and enterocytes (4).

Transgenic mice overexpressing CD23 have low levels of serum IgE, suggesting that IgE production is inhibited by high CD23 levels (5). Irradiated wild-type mice reconstituted with CD23 transgenic B cells have normal serum IgE levels, whereas transgenic mice reconstituted with wild-type B cells have low serum IgE levels, suggesting that CD23 on non-B cells has a role in IgE regulation (6). Additionally, transgenic B cells cocultured in vitro with wild-type FDCs secrete normal levels of IgE, whereas wild-type B cells cocultured with transgenic FDCs secrete low amounts of IgE, suggesting that FDC-CD23 levels might be important in regulating B cell IgE synthesis (6).

Several studies have described a role for CD40 and cytokines in regulating CD23 expression in normal and tumor-derived human B cells, but less is known in the murine system (7, 8). CD23 exists in two isoforms, CD23a and CD23b, and the same gene with alternative transcription initiation sites is used in murine B cells, and CD40 stimulation leads to up-regulation of both isoforms (9). Studies of isoform regulation under Th2 condition indicates that IL-4 up-regulates both CD23a and CD23b in murine B cells (9), but the effects of Th1 cytokine IFN-γ has not been studied, and no information is available for FDCs.

Mice immunized with OVA and CFA, a Th1-polarizing condition, express abundant CD23 on FDCs but very little on B cells (10). In contrast, mice infected with *Nippostrongulus braziliensis*, which induces a strong Th2 bias, express high levels of CD23 on B cells but very little on FDCs (10). These results prompted the hypothesis that, like B cell-CD23, FDC-CD23 is regulated by T cells and associated lymphokines. However, in contrast with B cells, we reasoned that FDC-CD23 would be best up-regulated under Th1 rather than Th2 conditions.

We sought to confirm and extend studies of CD23 isoform regulation in the murine system and to highlight differences in CD23 regulation in B cells and FDCs by ligating CD40 and culturing the B cells in the presence of Th1 (IFN-γ) vs Th2 (IL-4) cytokines. The expression of CD23a and CD23b at the mRNA level and expression of CD23 on the B cell surface were analyzed. Similarly, FDCs were isolated in high purity and were stimulated through CD40 ligation in the presence of IFN-γ vs IL-4, and the expression levels of CD23a and CD23b at the mRNA and CD23 at the protein levels were analyzed.

Our data confirmed that B cells express CD23a and CD23b and that CD40 stimulation up-regulated expression of both isoforms. Furthermore, IL-4 enhanced expression of CD23a and CD23b over the levels seen by CD40 ligation alone, whereas IFN-γ markedly reduced expression of both isoforms. We also confirmed that FDCs express CD40, which when ligated caused an increase in the expression of both CD23a and CD23b. Combining IFN-γ with CD40 stimulation significantly enhanced FDC-CD23a and -CD23b. Furthermore, anti-IFN-γ inhibited FDC-CD23 in vivo, and FDC-CD23b levels were not altered when cocultured with anti-CD40

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[3] Abbreviations used in this paper: FDC, follicular dendritic cell; CD40LT, CD40 ligand trimer; qRT-PCR, quantitative RT-PCR; CGG, chicken γ globulin; GC, germinal center; mIgE, membrane IgE; IC, immune complex.

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and IL-4. In short, the effects of IFN-γ and IL-4 on FDC contrast with those seen in B cells, suggesting a major difference in CD23 regulation in B cells and FDCs.

Materials and Methods

Animals

BALB/c mice (6–8 wk) were purchased from the National Cancer Institute. Mice were housed in standard plastic cages, and food and water were given ad libitum. Mice were irradiated with 1000 rads in a Cesium irradiator 24 h before FDC isolation and housed in protected hoods. On the day of isolation, mice were killed by cervical dislocation. All animals were handled humanely and according to protocols approved by Virginia Commonwealth University institutional animal care and use committee.

Abs and reagents

The FDC-specific mAb FDC-M1 was a gift from Dr. M. Kosco-Vilbois (Novimmune, Geneva, Switzerland). Additionally, FDC-M1 was later bought from BD Biosciences. Biotin-conjugated anti-rat Ab (clone MRK-1), FITC-anti-CD45R/B220 (clone RA3-6B2), hamster anti-mouse CD40 (clone HM40-3, for labeling), FITC-anti-hamster IgM (clone G188-9), PE-streptavidin, and isotype controls FITC-rat IgG2a and PE-rat IgG2b were purchased from BD Biosciences. Rat anti-mouse CD40 (clone 1C10, for activation) and isotype controls PE-rat IgG2a and FITC-rat IgG2b were purchased from Southern Biotechnology Associates. CD40-Ligand trimer (CD40LT) was a gift from Amgen, and its preparation has been described previously (11).

Isolation of FDCs and B cells

FDCs were isolated as per previously published techniques (12). In short, lymph nodes from irradiated mice were isolated, digested with an enzyme mixture of liberase blendzyme 2 (Roche) and DNase (Sigma-Aldrich) diluted in DMEM. The cell suspension was then incubated serially with FDC-M1, biotin-anti-rat κ L chain, and anti-biotin microbeads (Miltenyi Biotec) after sufficient washing to remove unbound Ab. The cells were then loaded on to an LS column (Miltenyi Biotec) placed in a magnetic field (varioMACS; Miltenyi Biotec) and washed thoroughly with buffer (0.5% BSA, 2 mM EDTA in PBS (pH 7.2), sterile filtered, and degassed). The column was then removed from the magnet, and FDCs were eluted. The FDC preparations thus obtained were routinely >90% pure.

To isolate B cells, lymph nodes from normal BALB/c mice were dissected out and crushed between two slides. The cell suspension thus obtained was washed in buffer and incubated with anti-B220 microbeads (Miltenyi Biotec) and isolated as described for FDCs. The B cells thus isolated were routinely >95% pure and were used to isolate RNA after further culturing with various cytokines. Total lymph node cells were used in flow cytometric analysis of B cell CD23 protein expression.

Cell culture and flow cytometry

Cell cultures contained 2×10^5 FDCs or lymphocytes in complete DMEM (supplemented with 0.02 M HEPES buffer, 0.2 mM MEM nonessential amino acids, 2 mM L-glutamine, and 1 μg/ml gentamicin). FDCs or lymphocytes were incubated with 0.1 μg/ml CD40L trimer (CD40LT) (Amgen) trimerized by a leuzine zipper and then cross-linked by an anti-leucine zipper Ab. In other experiments, FDCs or lymphocytes were incubated with 5 μg/ml rat anti-mouse CD40 (clone 1C10), which was then cross-linked with mouse anti-rat κ Ab in the presence or absence of 100 ng/ml IFN-γ or 100 ng/ml IL-4. FDCs and B cells labeled as detailed in *Results* were analyzed using FC500 flow cytometer, and results were analyzed with Cytomics RXP analysis software (Beckman Coulter). In some figures, the data was analyzed and plotted using WinMDI software (Scripps Research Institute).

Quantitative RT-PCR (qRT-PCR)

After 24 h in culture, cells were lysed in TRIzol (Invitrogen Life Technologies), and total RNA was isolated by strictly following the protocol recommended by the manufacturer. The RNA thus isolated was dissolved in DNase RNase free water (Sigma-Aldrich) and stored at −20°C before use. The mRNA expression levels of CD23 or its isoforms CD23a and CD23b were analyzed by qRT-PCR (icycler; Bio-Rad) using the TaqMan one-step RT-PCR master mix reagents kit (Applied Biosystems). PCRs were performed in 96-well thin-wall PCR plates covered with transparent optical-quality sealing tapes (Bio-Rad). Amplifications were performed using the One-step RT-PCR kit (Applied Biosystems) under the following conditions: 48°C for 30 min (cDNA synthesis), initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, and a combined annealing/extension step at 60°C for 1 min. Data analysis was performed using the iCycler iQ software (Bio-Rad). Finally, differences in mRNA expression levels were calculated using the $\Delta\Delta C_T$ method. The relative expression of CD23 mRNA was then normalized using 18s rRNA as an internal control. The $2^{-\Delta\Delta C_T}$ method as described by Livak and Schmittgen (13) was used to analyze qRT-PCR results for the analysis of relative gene expression data. The probes and primers used for qRT-PCR are listed in Table I and were ordered from Integrated DNA Technologies.

Immunizations and immunohistochemistry

BALB/c mice received injections i.p. with 100 μg of anti-IFN-γ (clone XMG6) or anti-IL-4 (clone 11B11) or rat IgG as isotype-matched control Ab 1 day before immunization with 100 μg/100 μl of chicken γ globulin (CGG) emulsified in 100 μl of CFA in the nape of the neck. Ten days postimmunization, draining lymph nodes (axillary and deep cervical) were dissected out and snap frozen immediately in Tissue-Tek OCT compound in plastic molds. These lymph nodes were sectioned using a microtome into 5-μm thin sections, and serial sections were stained for CD32 and CD23, respectively. After rehydration, sections were incubated with 1% phenyl hydrazine to block endogenous peroxidase for 30 min and then blocked with 10 μg/ml mouse IgG in 10% BSA for CD32 labeling or 10 μg/ml anti-CD32 Ab (Fc block) (clone 2.4G2) for CD23 for 30 min. The sections were then incubated with either biotinylated anti-CD32 (clone 2.4G2) or biotinylated anti-CD23 (clone B3B4) overnight followed by 2-h incubation with streptavidin HRP. The sections were then developed with a Diamino benzidine kit (Biomedia), and images were captured using optronics digital camera and analyzed using Bioquant Nova software.

Statistical analysis

For analysis of qRT-PCR results a *t* test (two-tailed-distribution) was used. In some experiments, up to three different comparisons were done, and a *p* value of <0.01 was required to account for multiple comparisons.

Table I. *Primer and probe sequences used in real-time RT-PCR analysis*

CD23 (hybridizes to both a and b isoforms)	
Sense primer	GTGGCAAAGCTGTGGATAGAGA
Antisense primer	TAGTAGCACTTCTGTTGGAAATGGA
Probe	5′FAM-ATGTTGCATGCAGTTCCCTTTGAAATCA-3′ TAMRA
CD23a	
Sense primer	AGGACAGACTTCAAGTTCAAATCA
Antisense primer	TTGTTTGGATCCTTTCAGTGATGA
Probe	5′FAM-TGCACTACCTGCCAAGGCACTTGT-3′ BHQ-1[a]
CD23b	
Sense primer	AAAGCCAATTTGAACGGGAACTTG
Antisense primer	CTCAGCAGCCCCACCAACAT
Probe	5′FAM-TGTCCCACGTCTTGCACAGCAGCA-3′ BHQ-1[a]
18s rRNA	
Sense primer	AAAATTAGAGTGTTCAAAGCAGGC
Antisense primer	CCTCAGTTCCGAAAACCAACAA
Probe	5′Cy5-CGAGCCGCCTGGATACCGCAGC-3′ BHQ-2[a]

[a] Black hole quencher.

Results

CD40 expression on FDCs

FDCs in mice immunized using Th1-polarizing CFA exhibit high levels of CD23, whereas little CD23 is detectable on FDCs from mice infected with the Th2-polarizing *Nippostrongylus brasiliensis* (10). These results prompted the hypothesis that T cells and associated lymphokines regulate FDC-CD23. Histochemical studies indicate that FDCs can express CD40 (14), and we reasoned that CD40L from activated T cells could interact with FDC-CD40 and thus participate in the up-regulation of FDC-CD23. We first sought to confirm that FDCs express CD40, by isolating FDCs from lymph nodes of naive mice and analyzing for surface expression of CD40 using flow cytometry. More than 95% of the purified FDCs expressed significant levels of CD40 (Fig. 1*A*).

Effects of CD40 stimulation on FDC-CD23 expression

To mimic CD40-mediated T cell signaling, FDCs were stimulated with either CD40LT or agonistic anti-CD40 for 2 days, and the surface expression of CD23 was analyzed by flow cytometry. Expression of FDC-CD23 was up-regulated when cultured in the presence of CD40LT (Fig. 1*B*) or anti-CD40 (Fig. 1*C*). Furthermore, when cocultured with activated D1.6 cells, a Th1 cell line that expresses CD40L when stimulated with rabbit γ globulin (data not shown), FDCs up-regulated expression of CD23 (Fig. 1*D*). To ascertain whether FDC-CD40 stimulation leads to increased gene expression, CD23 mRNA levels were analyzed by qRT-PCR. As expected, FDCs significantly up-regulated expression of CD23 mRNA when cultured in the presence of CD40LT for 24 h (Fig. 2).

Regulation of CD23a and CD23b in B cells

CD23 exists in two isoforms on B cells, and to determine the effects of Th1 vs Th2 conditions on isoform expression, purified B cells from naive mice were stimulated with anti-CD40 in the presence or absence of IFN-γ or IL-4. Messenger RNA levels for CD23a and CD23b were analyzed by qRT-PCR. As expected, both isoforms were up-regulated when stimulated with anti-CD40 and further enhanced on addition of IL-4 (Fig. 3, *A* and *B*). In marked contrast, when IFN-γ was used with anti-CD40 the levels of both CD23a and CD23b were inhibited, and the levels were below those obtained using anti-CD40 alone (Fig. 3, *A* and *B*).

To quantify surface expression of B cell-CD23, lymphocytes from naive mice were stimulated with anti-CD40 in the presence or absence of IL-4 or IFN-γ. After 2 days of culture, the cells were double labeled using anti-B220-FITC and CD23-PE and analyzed by flow cytometry. Approximately 64% of unstimulated B cells expressed CD23 (Fig. 4*A*), which increased to 87% when stimulated with anti-CD40 (Fig. 4*B*). When anti-CD40 and IL-4 were used in combination, >97% of B cells expressed surface CD23 (Fig. 4*C*), and the intensity of labeling increased correlating with the increase in mRNA levels. However, when IFN-γ was used in place of IL-4, the effect of anti-CD40 was blocked (Fig. 4*D*), and CD23 expression reverted back to the level in the unstimulated control in Fig. 4*A*.

CD23 isoform expression by FDCs under Th1 vs Th2 conditions

Purified FDCs were cultured under the same conditions as the B cells, and CD23 isoform expression was determined. FDCs expressed CD23a mRNA (Fig. 5*A*), and this expression increased when stimulated with agonistic anti-CD40. The expression induced by anti-CD40 was further increased by addition of IFN-γ (Fig. 5*A*) or IL-4 (Fig. 5*B*). Stimulation with anti-CD40 also increased expression of CD23b mRNA (Fig. 6*B*), and this expression was markedly enhanced by the addition of IFN-γ (Fig. 5*C*), whereas IL-4 had no effect (Fig. 5*C*).

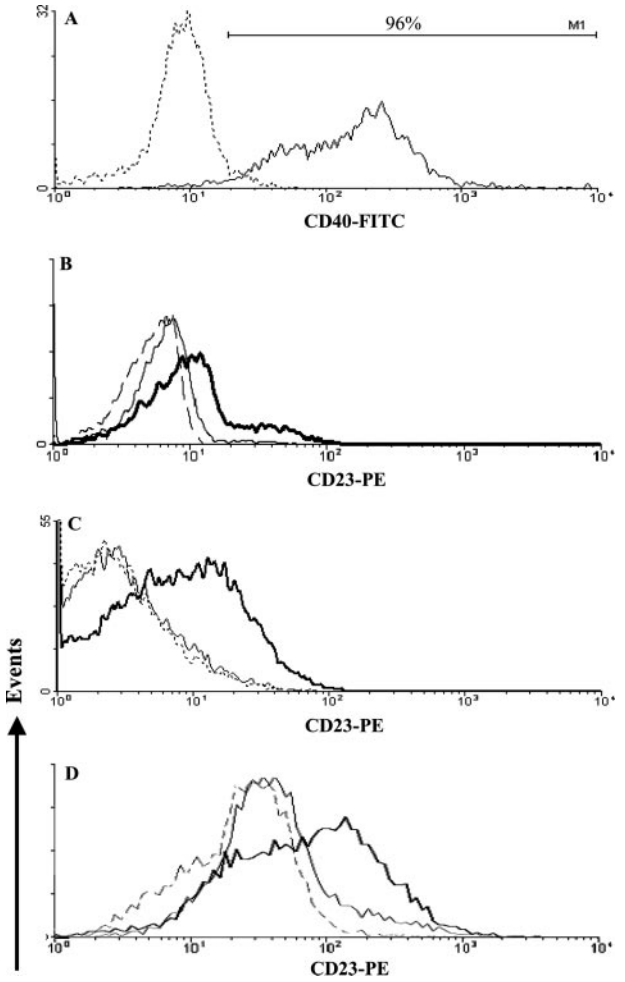

FIGURE 1. FDCs express CD40 and increased surface expression of CD23 when stimulated through CD40. Highly enriched preparations of FDCs were labeled with IgM hamster anti-mouse CD40 followed by FITC anti-hamster IgM, and surface expression of CD40 was analyzed by flow cytometry (*A*). After 2 days of culture in the presence or absence of CD40LT (*B*) or agonistic anti-CD40 (clone 1C10) (*C*), FDCs were analyzed for the surface expression of CD23. In addition, FDCs were cocultured in the presence or absence of activated D1.6 cells for 2 days and analyzed for CD23 expression by flow cytometry (*D*). The histogram in *D* was gated for FDCs based on their forward and side scatter properties, which was independently established with FDC phenotypic markers including FDC-M1, CD21/CD35, and CD32 (data not shown). In *A–C*, histograms were not gated. Thin lines represent untreated FDCs, thick lines represent appropriately treated FDCs, and dotted lines represent isotype-matched Ab controls. The figures illustrate data from a single experiment, but the results are representative of three independent experiments.

When FDCs were cultured with IL-4 alone, an increase in CD23a mRNA was apparent, but the level was less than that obtained using anti-CD40. In contrast, levels of CD23b were not altered (Fig. 6*A*). When stimulated with IFN-γ alone, a change in FDC-CD23a or -CD23b mRNA was not detected (Fig. 6, *A* and *B*). In short, the mRNA data suggest that T cells help regulate expression of CD23 in both B cells and FDCs, but that the impact of Th1 vs Th2 conditions are quite different as summarized in Table II.

Effects of anti-IFN-γ and anti-IL-4 on CD23 expression in vivo

If FDC-CD23 expression is largely attributable to IFN-γ, as suggested by the observation that IFN-γ can markedly up-regulate both CD23a and CD23b mRNA in CD40-stimulated FDCs (Table

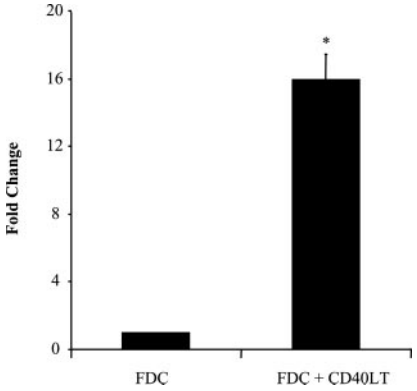

FIGURE 2. CD40LT increases expression of FDC-CD23 mRNA. FDCs were cultured for 24 h with or without CD40LT, and the expression of CD23 mRNA was measured by qRT-PCR. The fold change in the expression of CD23 mRNA from that of untreated FDCs is plotted. Error bars represent SDs from the mean of three replicates. *, $p < 0.01$. The figure illustrates data from a single experiment, and the error bars represent the SD between replicates in the experiment, but the results are representative of three independent experiments.

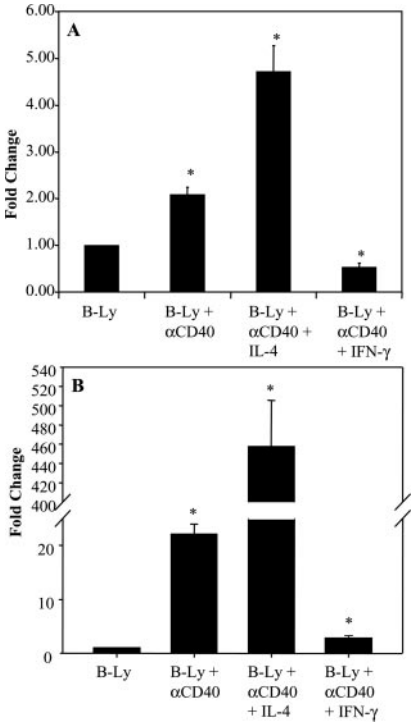

FIGURE 3. B cells increase expression of both CD23a and CD23b when stimulated with anti-CD40, and this expression was further enhanced by IL-4 but suppressed by IFN-γ. B lymphocytes isolated from naive mice were stimulated with anti-CD40, which was then cross-linked with a secondary Ab, in the presence or absence of either IL-4 or IFN-γ for 24 h, and the mRNA expression of CD23a (*A*) or CD23b (*B*) was quantified using qRT-PCR. Error bars represent SDs of the mean calculated with the three replicates in the same experiment. *, $p < 0.01$. *A* (CD23a), The results illustrated are representative of five independent experiments, and analysis of cumulative data from all five experiments confirmed relationships. To normalize the variable data a value of 100 was assigned for the highest response in each experiment, wherein B cells were treated with anti-CD40 + IL-4. Compared with this result, untreated B cells expressed 7.8%, anti-CD40-treated B cells expressed 31%, and B cells treated with anti-CD40 and IFN-γ expressed 6.5% ($p < 0.01$) of the maximum response. *B* (CD23b), The results illustrated are representative of four independent experiments, and analysis of cumulative data from all four experiments confirmed relationships. To normalize the variable data, a value of 100 was assigned for the highest response in each experiment, wherein B cells were treated with anti-CD40 and IL-4. Compared with this result, untreated B cells expressed 0.16%, anti-CD40-treated B cells expressed 4.0%, and B cells treated with anti-CD40 and IFN-γ expressed 0.43% ($p < 0.01$) of the maximum response.

II), then anti-IFN-γ should have a major inhibitory effect on FDC-CD23 expression in vivo. To examine this theory, mice received injections with 100 μg of rat IgG, anti-IFN-γ, or anti-IL-4 1 day before immunization in the nape of the neck with CGG emulsified in CFA. Ten days later, the draining lymph nodes were collected and sectioned, and serial sections were labeled with mAbs for CD32 (FcγRII) and CD23. FDC networks in germinal center (GC) light zones are readily outlined with anti-CD32(10), and this area on the adjacent section can be examined for the expression of CD23 on FDCs. Isotype controls exhibited well-developed GCs, and convoluted FDC networks were obvious as a consequence of intense labeling with both anti-CD32 and anti-CD23 (Fig. 7, *upper panels*). Mantle zone B cells express both CD32 and CD23, but labeling was very light when compared with the intense labeling of FDCs in the networks. B cells in the GC dark zone do not express significant amounts of CD23(10) and did not label. Mice treated with anti-IFN-γ also developed GCs, and convoluted FDC networks could be identified by the intense CD32 labeling (Fig. 7, *left middle panel*). However, FDCs in these mice showed little expression of CD23, and the typical convoluted FDC networks were not apparent (Fig. 7, *right middle panel*). In contrast, CD23 expression on GC B cells increased when IFN-γ was neutralized. This increase was especially apparent in the GC dark zone where B cells typically don't label (Fig. 7, *right middle panel*). This suggests that the IFN-γ levels in these CFA-immunized mice inhibited CD23 expression on GC B cells in vivo, and this coincides with the in vitro data showing IFN-γ-mediated suppression of CD23 in B cells in Figs. 3 and 4. Treatment with anti-IL-4 had no apparent influence on the expression of CD32 in FDC networks. However, whereas FDC-CD23 networks were readily apparent in the anti-IL-4-treated mice (Fig. 7, *right bottom panel*), they were consistently reduced when compared with CD32 networks in the same mice or with CD23 networks in the isotype controls, suggesting that whereas IFN-γ appeared to have a major influence on FDC-CD23 expression, IL-4 was necessary for optimal FDC-CD23. This result is consistent with the observation that IFN-γ up-regulated both FDC-CD23a and -CD23b mRNA in the presence of a CD40 agonist, but that FDC-CD23a was also up-regulated by IL-4.

Discussion

Elevated FDC-CD23 levels under Th1 conditions contrasted with low levels under Th2 conditions, suggesting that T cell factors may regulate FDC-CD23 levels (10). Activated T cells express high levels of CD40L, and CD40L is involved in T cell-mediated activation and/or maturation of B cells, macrophages, and dendritic cells, all of which express CD40 (15–17). FDCs also express significant amounts of CD40, and the results presented in this study indicate that, as in other cell types expressing CD40, FDCs are activated through CD40 signaling as evidenced by an increase in CD23 levels. T cells are activated early in an immune response (18, 19), and the high levels of CD40L on activated T cells may help activate FDCs during the initial phases of GC formation. Although it is well known that B cells are needed for FDC development (20–22), these results are the first reported data to document a direct T cell-mediated activation of FDCs through CD40 and T cell lymphokines.

The results in the present study correlate well with in vivo data. Mice infected with *Nippostrongylus braziliensis* express high levels of IL-4 (23) and have high levels of CD23 on B cells, while

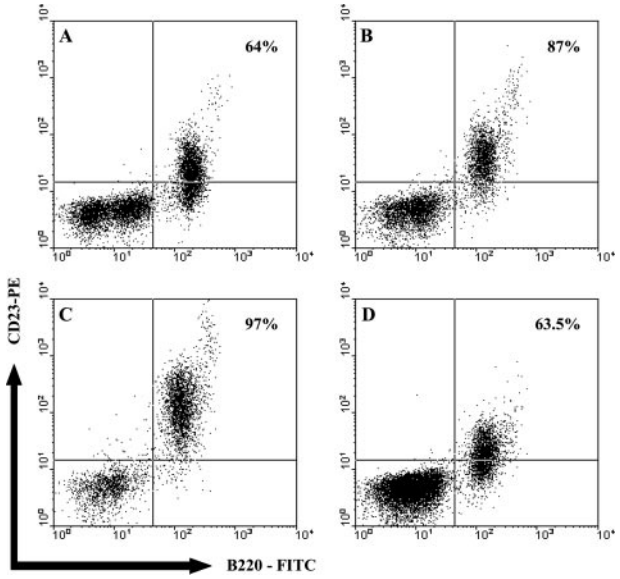

FIGURE 4. B cells increase surface expression of CD23 upon CD40 stimulation, and this expression was augmented by IL-4 but suppressed by IFN-γ. Total lymphocytes isolated from naive mice were cultured for 2 days with (*B*) or without (*A*) anti-CD40. Lymphocytes were also stimulated with anti-CD40 in the presence of either IL-4 (*C*) or IFN-γ (*D*). The cells were then double labeled with anti-B220-FITC and anti-CD23-PE and analyzed by flow cytometry. The percentages of B cells ($B220^{+ve}$) expressing CD23 are indicated in the *upper right quadrant*. The figure illustrates data from a single experiment, but the results are representative of three independent experiments.

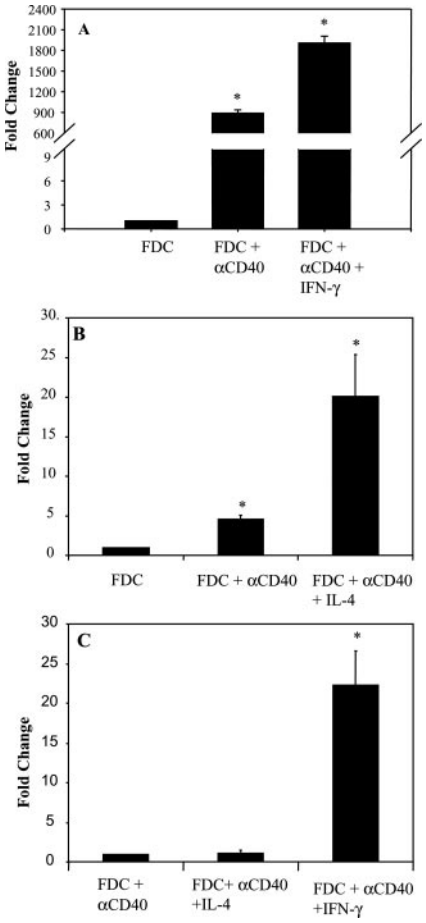

FIGURE 5. FDCs expressed both CD23a and CD23b, and anti-CD40 stimulation increased expression of both the isoforms. Highly enriched FDCs were either left unstimulated or stimulated with anti-CD40 in the presence or absence of IFN-γ (*A*) or IL-4 (*B*), and the mRNA expression of CD23a (*A* and *B*) was determined by qRT-PCR. Expression of FDC-CD23b mRNA was analyzed using qRT-PCR after 24 h in culture with IL-4 or IFN-γ in presence of anti-CD40 (*C*). Untreated FDCs in this particular experiment (*C*) did not express measurable levels of CD23b, and hence FDCs stimulated with anti-CD40 was used as a control to estimate the fold change in mRNA expression. Error bars represent SDs from the mean of three replicates. *, $p < 0.01$. *A*, The results illustrated are representative of three independent experiments, and analysis of cumulative data from all three experiments confirmed relationships. To normalize the variable data, a value of 100 was assigned for the highest response in each experiment, wherein FDCs were treated with anti-CD40 and IFN-γ. Untreated FDCs expressed 1.5%, and anti-CD40 treated FDCs expressed 26% ($p < 0.01$) of the maximum response. *B*, The results illustrated are representative of three independent experiments, and analysis of cumulative data from all three experiments confirmed relationships. To normalize the variable data, a value of 100 was assigned for the highest response in each experiment wherein FDCs were treated with anti-CD40 + IL-4. Untreated FDCs averaged 2.1%, and anti-CD40-treated FDCs averaged 22% ($p < 0.01$) of the maximum response. *C*, The results illustrated are representative of three independent experiments, and analysis of cumulative data from all three experiments confirmed relationships. To normalize the variable data, a value of 100 was assigned for the highest response in each experiment, wherein FDCs were treated with anti-CD40 and IFN-γ. Compared with this result, untreated FDCs expressed 5.7% and FDCs treated with anti-CD40 + IL-4 expressed 2.7% ($p < 0.01$) of the maximum response.

FDCs express very low but detectible levels (10). These relationships are reversed when mice were immunized with the Th1-promoting CFA (10). This in vivo study suggested that T cells may be regulating expression of FDC-CD23 (10), which is supported in the current study. In short, this study extends the observation of increased FDC-CD23 after immunization with CFA and provides a potential mechanism for the CFA effect. CFA promotes a Th1 response, FDC-T cell interactions mediated by CD40-CD40L promote FDC-CD23, and Th1 cytokines further enhance the production of CD23 by FDCs. This observation contrasts with B cells in which IL-4 is the principal cytokine involved in up-regulation. In brief, the combined data suggest that T cells are important in the regulation of CD23 in both B cells and FDCs but that the impact of Th1 and Th2 conditions are quite different (Table II and Fig. 7).

The modest inhibitory effect of anti-IL-4 on FDC-CD23 in vivo was not surprising given the inability of IL-4 to increase the FDC-CD23b isoform and the ability of anti-IL-4 to promote Th1 responses that enhance expression of both FDC-CD23 isoforms. The low intensity of CD23 labeling in flow cytometry was surprising when compared with the intense labeling obtained using immunohistochemistry. Unfortunately, CD23 is easily cleaved from cell surfaces by small quantities of proteases, including those in the enzyme digestion mixture used to isolate FDCs (6). We reason that small amounts of proteases may persist in cell cultures and that protease activity may reduce the CD23 levels on cultured cells.

CD23a and CD23b have significant homology and differ only in the short cytoplasmic tail (9). Interestingly, variants of CD23b have been reported in mice enterocytes (4). The amino acid sequences of CD23a and CD23b in FDCs have not been definitively established, and minor differences in these sequences might be responsible for this apparent differential regulation in B cells and FDCs. Nevertheless, it is clear that CD40-mediated signals in the B cells are inhibited by IFN-γ (Figs. 3 and 4), whereas IFN-γ clearly enhanced CD40-mediated signals in FDCs (Fig. 5). Thus, important cytokine-mediated intervention likely takes place in the CD40 stimulation pathway.

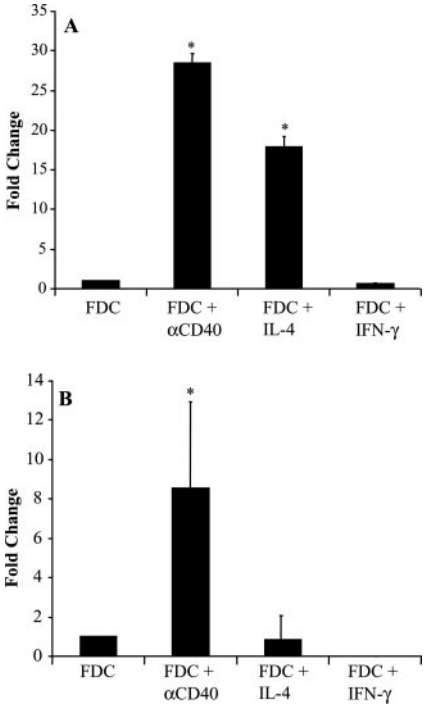

FIGURE 6. IL-4 can act independently of CD40 stimulation, but IFN-γ required anti-CD40. FDCs were cultured for 24 h with either anti-CD40 or IL-4 or IFN-γ, and the expression of CD23a (*A*) or CD23b (*B*) was determined using qRT-PCR. Error bars represent SDs from the mean of three replicates. ∗, $p < 0.01$ (In *B*, logarithmic transformation of data was used.) The figure illustrates data from a single experiment, and the error bars represent the SD between replicates in the experiment, but the results are representative of three independent experiments.

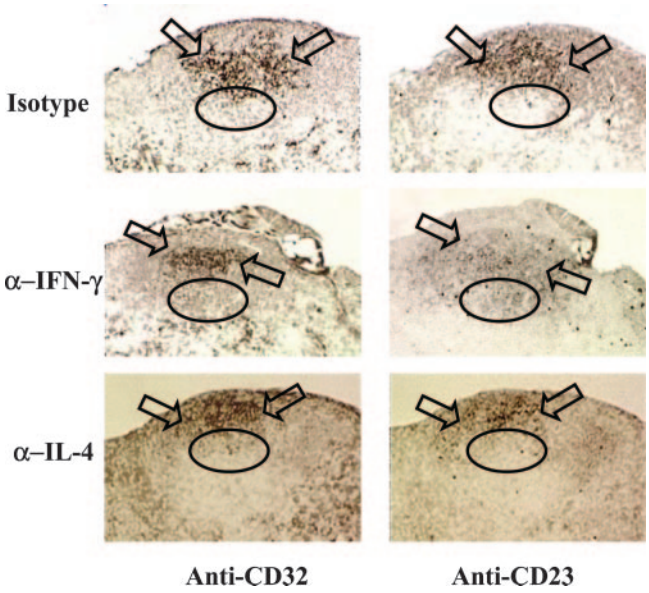

FIGURE 7. Induction of FDC-CD23 under Th1 conditions was inhibited by pretreatment with anti-IFN-γ, whereas anti-IL-4 had a modest effect. Mice were immunized with CGG emulsified in CFA, and draining lymph nodes were harvested after 10 days and sectioned, and serial sections were labeled for CD32 (*left panels*) and CD23 (*right panels*). To neutralize IFN-γ or IL-4, mice received injections with anti-IFN-γ (clone XMG6) or anti-IL-4 (clone 11B11) i.p. 1 day before immunization and analyzed for CD32 and CD23 expression. The arrows indicate the location of the convoluted FDC networks in the light zones, and the circles indicate dark zone areas in the GCs. $n = 5$ mice for each condition, and illustrations are representative of the results for mice in each group.

Transgenic mice expressing high amounts of CD23 have reduced serum IgE levels, suggesting an inverse relationship between CD23 and IgE levels (5). Because IL-4 increases both CD23 expression and IgE production in B cells (24, 25), it is hard to explain the inverse relationship observed in these transgenic mice. Interestingly, whereas serum IgE levels are normal in irradiated wild-type mice repopulated with B cells from transgenic mice, they were significantly reduced when irradiated transgenic mice were repopulated with wild-type B cells, suggesting that non-B cells play an important role in regulating serum IgE levels (6). In addition, when transgenic B cells were cultured in the presence of wild-type FDCs, IgE levels in the culture supernatant fluids were comparable to controls (6). In contrast, wild-type B cells produced markedly reduced levels of IgE when cultured in the presence of transgenic FDCs, suggesting that FDC-CD23 might be involved in the regulation of B cell IgE production (6). Furthermore, the data presented in this study are in agreement with results in mice responding to an Ag with CFA as the adjuvant. CFA is known to promote Th1 responses, and CFA immunization results in significantly higher amounts of CD23 on FDCs and low serum levels of IgE (26, 27). These results strongly suggest an inverse relationship between surface expression of CD23 on FDCs and serum IgE levels.

Table II. *Changes in expression of CD23a and CD23b mRNA in FDCs and B cells under Th1 and Th2 conditions*[a]

	FDCs		B Cells	
	CD23a	CD23b	CD23a	CD23b
Untreated	+	+	+	+
IL-4	++	+	++	++
IFN-γ	+	+	+	+
Anti-CD40	++	++	++	++
Anti-CD40 + IL-4	++++	++	++++	++++
Anti-CD40 + IFN-γ	++++	++++	+	+

[a] Changes are based on mRNA expression levels in untreated FDCs or B cells, respectively, as baseline.

The mechanism(s) involved in FDC-CD23-mediated inhibition of IgE is not clear, but we reason that the first step in the inhibitory pathway is likely the binding of FDC-CD23 to membrane IgE (mIgE) on B cells. CD23 interacts with IgE in the Cε2/Cε3 (primarily Cε3) domains (28), and mIgE expressed on the B cell is attached to the cell membrane through the membrane anchor region of the Cε4 domain; thus, the Cε2/Cε3 is free to bind FDC-CD23. In normal mice, FDC-CD23 traps IgE immune complexes (ICs) (10) and most FDC-CD23 may be occupied with IgE-ICs, which appear to be stably trapped via multiple point binding to CD23 molecules (10). However, some free FDC-CD23 likely exists, and we reason that high levels of free FDC-CD23 would exist under Th1 conditions, or in CD23Tg mice that have little IgE to make ICs. We also reason that free FDC-CD23 would bind mIgE on GC B cells attempting to engage Ag on FDCs, and thus B cell signaling could be altered, leading to dampened IgE responses. Membrane expression of IgE appears to be indispensable for IgE secretion, and the cytoplasmic tail of mIgE is important in the process (29). Another potential inhibitory pathway could involve interactions of CD23 with CD21, which is known to occur in the human system (30). The high levels of CD23 on FDCs might signal B cell CD21 in the B cell coreceptor complex, and such B cell costimulation might alter class switching, leading to reduced IgE. However, we have been unable to demonstrate that CD23 and CD21

interact in the murine system (31) or demonstrate increased expression of CD21 on B cells or FDCs when stimulated with anti-CD40 plus IFN-γ or IL-4 (data not shown), prompting doubts about the importance of this potential pathway. Clearly, it is important to understand how overexpression of FDC-CD23 dampens IgE responses, and we plan to address this question in future experimentation.

Serum Ag-specific IgE levels are high in allergy-prone patients, and IgE is implicated in the pathogenesis of most allergies. Multiple therapeutical approaches are aimed at reducing serum IgE levels in general and, more importantly, allergen-specific IgE (32–34). Approaches designed to decrease allergen-specific IgE and increase allergen-specific IgG have met with considerable success and are believed to be vital in treating allergies (35). FDCs have been shown to promote class switching to IgG and IgG recall responses (12, 36, 37), and high levels of CD23 on FDCs suppress IgE production in B cells (6). In this study, we show that CD23 on FDCs can be up-regulated by Th1 cytokines, and we reason that this may, in part, be responsible for the Th1-mediated suppression of IgE levels. Clearly, Th1 cytokines can promote class switching to IgG2a (38) and suppress germline Cε transcription and thus inhibit class switching to IgE (39). However, redundant mechanisms controlling important biological events are not uncommon, and combinations of mechanisms are often powerful. Thus, the effect of Th1 cytokines on Ig class switching in combination with the inhibitory effect of high levels of FDC-CD23 could be most effective in minimizing IgE responses. It is also well known that GC reactions go on for weeks, leading to production of memory B cells and promoting production of high-affinity Ab involved in affinity maturation. FDC-CD23 is maintained for weeks (data not shown) and could play a role in minimizing the production of IgE memory cells and plasma cells producing high-affinity IgE. Therefore, if FDC-CD23 can be up-regulated during an immune response, it might be possible to skew specific responses toward IgG, and thus FDCs may represent a target for immunotherapy.

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Disclosures

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