CCR3 Expression Induced by IL-2 and IL-4 Functioning as a Death Receptor for B Cells[1]

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We report that CCR3 is not expressed on freshly isolated peripheral and germinal B cells, but is up-regulated after stimulation with IL-2 and IL-4 (~98% CCR3$^+$). Ligation of CCR3 by eotaxin/chemokine ligand (CCL) 11 induces apoptosis in IL-2- and IL-4-stimulated primary CD19$^+$ (~40% apoptotic cells) B cell cultures as well as B cell lines, but has no effect on chemotaxis or cell adhesion. Freshly isolated B cells express low levels of CD95 and CD95 ligand (CD95L) (19 and 21%, respectively). Expression is up-regulated on culture in the presence of a combination of IL-2, IL-4, and eotaxin/CCL11 (88% CD95 and 84% CD95L). We therefore propose that ligation of such newly induced CCR3 on peripheral and germinal B cells by eotaxin/CCL11 leads to the enhanced levels of CD95 and CD95L expression. Ligation of CD95 by its CD95L expressed on neigboring B cells triggers relevant death signaling pathways, which include an increase in levels of Bcl-2 expression, its functional activity, and the release of cytochrome *c* from the mitochondria into the cytosol. These events initiate a cascade of enzymatic processes of the caspase family, culminating in programmed cell death. Interaction between CCR3 and eotaxin/CCL11 may, besides promoting allergic reactions, drive activated B cells to apoptosis, thereby reducing levels of Ig production, including IgE, and consequently limit the development of the humoral immune response. The apoptotic action of eotaxin/CCL11 suggests a therapeutic modality in the treatment of B cell lymphoma. *The Journal of Immunology,* **2003, 171: 1722–1731.**

Chemokine receptors are differently expressed on naive and activated inflammatory cells (1). Although many chemokine receptors can be found on human B cells, such as CCR1 (2), CCR2 (3), CCR6 (4), CCR7 (5), CXCR3 (6), CXCR4 (7), and CXCR5 (8), in general less is known about the functions of these receptors and their ligands on B cells. Nonetheless, important insights have been gained from several studies. For instance, stromal cell-derived factor-1/CXCL12, a ligand for CXCR4, is a potent chemoattractant for both normal and malignant human B cells (9–11). Mice lacking the stromal cell-derived factor-1/CXCL12 gene or the CXCR4 gene show gross defects in B cell development (10, 12, 13). CCR7 is important for retaining B cells in the splenic periarteriolar lymphatic sheath (14). Mice deficient for CCR7 display a severely impaired migration of B cells to lymph nodes or Peyer's patches (14). CXCR5 is necessary for B cell homeostatic trafficking into follicles (15). Disruption of CXCR5 gene leads to loss of B cell follicles and germinal center (GC)[3] in lymph nodes or Peyer's patches (15). Thymus-expressed chemokine attracts mouse pre-B cells and pro-B colonies, but not in later stages of B cell development (16).

The CCR3 is expressed in humans on eosinophils and basophils (17, 18), on a subset of CD4$^+$ T cells (19), and on dendritic cells (20). CCR3 expression on T cells requires stimulation with both the Th1 cytokine IL-2 and Th2 cytokine IL-4 (21). Cellular sources of the CCR3 ligand eotaxin/chemokine ligand (CCL) 11 are bronchial epithelial cells, T cells, macrophages, and eosinophils (22). The importance of this chemokine in allergic diseases is widely recognized due to its activation and attraction of eosinophils, basophils, and T cells (19, 21). To date, CCR3 expression on B cells and the biological functions of CCR3 ligand eotaxin/CCL11 for human B cells have not been characterized.

A considerable amount of data was derived from genomic, structural, and functional studies on the role of chemokine receptors and their ligands in both normal physiology (e.g., organogenesis, hemopoiesis, angiogenesis, and physiological lymphocyte trafficking) and pathophysiology, such as coreceptors for HIV entry and the pathological distribution of leukocytes in chronic inflammation (23). A major role of chemokine receptors and their ligand playing is leukocyte traffic and recruitment to sites of inflammation (24). It was reported that activation of cell surface CXCR4 induced programmed cell death of human peripheral CD4$^+$ T cells, malignant T cells, and CD4/CXCR4 transfectants (25). The interaction between HIV R5 Env and CCR5 activated the Fas pathway and caspase-8 as well as triggering Fas ligand production, ultimately causing CD4$^+$ T cell death (26). However, no

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[3] Abbreviations used in this paper: GC, germinal center, CCL, chemokine ligand; CD95L, CD95 ligand; C.I., chemotactic index; MCNC, migrating cell on negative control; MCP, monocyte chemotactic protein; MIP, macrophage-inflammatory protein; PI, propidium iodide.

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study has looked at the function of chemokine receptors in the programmed B cell death.

In this study, we report that CCR3, expressed on IL-2- and IL-4-stimulated peripheral and germinal B cells and on several B cell lines, is a death-promoting receptor when triggered by its ligand eotaxin/CCL11. Eotaxin/CCL11 via CCR3 promotes the apoptosis of these cells by increasing levels of CD95 and CD95 ligand (CD95L) expression. Ligation of CD95 and CD95L on neighboring cells consequently triggers relevant death signaling pathways, which include either an increase in the level of Bcl-2 expression or in its functional activity. This in turn triggers the release of cytochrome *c* from the mitochondria into the cytosol. These events initiate the cascade of enzymatic events of the caspase family, which lead to a programmed suicide cell death.

Materials and Methods

Cells and reagents

B cells were purified from peripheral blood obtained from healthy individuals by positive selection using Dynabeads (Dynal, Oslo, Norway), according to the manufacturer's instruction. The purity of $CD19^+$ B cells reached 98% demonstrated by flow cytometry. GC B cells ($CD19^+CD10^+$) were isolated from single cell suspensions of human tonsils using double-positive selection, as Chen et al. described with modification (27). The human tonsils were obtained from children undergoing routine tonsillectomy in the clinic, according to institutional guidelines. The presence of GC in the tonsils was confirmed by histological evaluation to be "no diagnostic pathology." The $CD19^+$ tonsil cells were first isolated using anti-CD19 mAb Dynabeads, according to the manufacturer's instructions. Cells were then stained with 10 μg/ml of mouse anti-human CD10 mAb (HI10a; BD PharMingen), followed by MACS rat anti-mouse IgG1 MicroBeads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). $CD10^+$ cells were selected by passing cell suspension through a magnetic separator, according to the manufacturer's instructions. The purity of each $CD19^+CD10^+$ B cell preparation was evaluated by flow cytometry (97%). B cell lines were purchased from commercial biotech companies. All recombinant human cytokines and chemokines IL-2, IL-4, macrophage-inflammatory protein-1α (MIP-1α)/CCL3, RANTES/CCL5, and eotaxin/CCL11 were purchased from R&D Systems Europe (Abingdon, U.K.).

Flow cytometry

To determine levels of surface marker expression, cells were first incubated with CCR3 mAb (clone 61828.111; R&D Systems) at 5 μg/ml or 5 μg/ml isotype-matched mAb (DAKO, Glostrup, Denmark) in staining buffer, as previously described (21, 28). After 20 min, the cells were washed twice with staining buffer and resuspended in 50 μl FITC-conjugated secondary mAb (DAKO) for 20 min. For CD95 and CD95L detection, cells were first incubated with CD95 mAb (clone 13) or CD95L mAb (clone 33) (BD Biosciences Europe, Erembodegem, Belgium) at 5 μg/ml or 5 μg/ml isotype-matched mAb (DAKO) in staining buffer (28). After 20 min, the cells were washed twice with staining buffer and resuspended in 50 μl FITC-conjugated secondary mAb (DAKO) for 20 min. For detection of apoptosis, cells were stained in staining medium (RPMI 1640, 2% FBS, and 0.1% sodium azide) with 1 μg/ml propidium iodide (PI) for 30 min at 4°C, then stained with FITC-conjugated annexin V with binding buffer (BD PharMingen, San Diego, CA), as previously described (29). COULTER XL (Coulter, Miami, FL) was used for analyses.

Real-time quantitative RT-PCR assay

Total RNA from cells (1×10^6) was prepared by using Quick Prep total RNA extraction kit (Pharmacia Biotech, Hillerød, Denmark), reverse transcribed by oligo$(dT)_{12-18}$ and Superscript II reverse transcriptase (Life Technologies, Grand Island, NY), as described (30). The real-time quantitative PCR was performed in an ABI PRISM 7700 Sequence Detector Systems (PerkinElmer Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. By SYBR Green PCR Core Reagents Kit (P/N 4304886; PerkinElmer Applied Biosystems), fluorescence signals were generated during each PCR cycle via the 5′ to 3′ endonuclease activity of AmpliTaq Gold (30) to provide real-time quantitative PCR information. CCR3 primers used as follows (28): sense, 5′-TCCTTCTCTC TTCCTATCAATC-3′; antisense, 5′-GGCAATTTTCTGCATCTG-3′.

PCR conditions were 2 min at 50°C, 10 min at 95°C, 40 cycles with 15 s at 95°C, and 60 s at 60°C for each amplification. In some cases, semiquantitative RT-PCR analysis of CCR3 mRNA was performed, as previously described (31). To semiquantitatively estimate CCR3 mRNA, visualized bands were scanned using Gene Tools densitometry (SYNGENE, Cambridge, U.K.).

Blot assays

For mRNA detection (Northern blot), 5 μg of total RNA obtained from each sample was electrophoresed under denaturing conditions, followed by blotting onto Nytran membranes, and cross-linked by UV irradiation, as previously described (32). CCR3 cDNA probe, labeled by α-[^{32}P]dCTP, was obtained by PCR amplification of the sequence mentioned above from total RNA from $CD3^+$ T cells. The membranes were hybridized overnight with 1×10^6 cpm/ml of ^{32}P-labeled probe, followed by intensively washing before being autoradiographed. For protein detection (Western blot), the cells were lysed in lysis buffer (0.4% Nonidet P-40, 150 mM NaCl, 50 mM Tris-HCl, 1 mM sodium vanadate, 0.1 mM PMSF, and 2 μg/ml of leupeptin and apoprotein, pH 8.0), as previously described (27, 33). Lysates were centrifuged at 10,000 rpm for 5 min at 4°C. Protein concentration was measured by Bio-Rad (Hercules, CA) protein assay. Protein (~40 μg) was loaded onto 16% SDS-PAGE, transferred onto polyvinylidene difluoride membranes after electrophoresis, and incubated with the appropriate Abs (a-Bcl-2, clone BCL-2/100; a-cytochrome *c*, clone 7H8.2C12; a-caspase-3, clone CPP32–19; all at 0.5 μg/ml; BD PharMingen). Analyses were conducted using ECL detection (Amersham Pharmacia Biotech, Little Chalfont, U.K.).

Chemotaxis and adhesion assays

For determination of chemotaxis, chemokine suspensions (25 μl RPMI 1640, 0.5% pooled human serum) were placed in the lower wells. The cells were either freshly isolated or $CD19^+$ B cells were stimulated with IL-2 and IL-4 together for 24 h, $CD10^+CD19^+$ GC B cells for 96 h, and B cell line Ramos I for 24 h before the procedure. Cell suspensions (50 μl, 5×10^6 cells/ml) were added to the upper wells of the chamber, which was separated from the lower well by mouse collagen IV-coated membrane (5 μm pore size; Nucleopore, Pleasanton, CA). The chamber was incubated for 120 min at 37°C. The cells that migrated and adhered to the lower surface of the membrane were counted by using a light microscope. The results were expressed as a chemotactic index (C.I.), which is the ratio between the numbers of migrating cells in the sample and migrating cells in the negative control (MCNC) (34). In the adhesion assay, microtiter plates (96-well) were coated with laminin (20 μg/ml; Sigma). The cells either freshly isolated or $CD19^+$ B cells were stimulated with IL-2 and IL-4 together for 24 h, $CD10^+CD19^+$ GC B cells for 96 h, and B cell line Ramos I for 24 h before the procedure. The cell suspensions (100 μl RPMI 1640, 0.2% BSA, appropriate chemokine, 4×10^5 cells/ml) were added in triplicate to plates, and incubated for 60 min at 37°C. Levels of adhesion were determined by staining with 1% crystal violet (21). Spontaneous adhesion (PBS control) is ~7% of cells in our assay.

Immunocytochemistry and confocal microscopy

Cells (10×10^3) were spun down onto a glass slide using a cytocentrifuge and fixed in with 1% paraformaldehyde in PBS, and nonspecific binding was blocked by preincubating with PBS/1% BSA for 10 min. The appropriate primary Abs (a-CCR3, 5 μg/ml; a-Bcl-2, a-cytochrome *c*, a-caspase-3, 0.5 μg/ml) were added; cells were incubated overnight at 4°C and washed; and appropriate secondary FITC-labeled Ab (DAKO) was added. Cells were analyzed by confocal microscopy using a confocal laser scanning system and an inverted microscope (model LSMSIO; Zeiss, Oberkochen, Germany). Images of serial cellular section were acquired, as described (35).

Results

CCR3 is expressed on IL-2- and IL-4-stimulated B cells

We examined CCR3 expression on human peripheral B cells. The results from flow cytometric analyses (Fig. 1*A*) show that there was no detectable expression of CCR3 on freshly isolated B cells (*b*) or following 24-h culture in medium alone (1%; *c*). B cells cultured in the presence of the cytokines IL-2 (*d*) or IL-4 (*e*) showed low levels of CCR3 expression (9 and 10%, respectively), with high levels of expression (up to 98%) induced on culture in the presence of a combination of IL-2 and IL-4 (*f*). Kinetic studies (Fig. 1*B*) revealed that a combination of IL-2 and IL-4 significantly up-regulates CCR3 expression on B cells from 0% (0 h), 9% (1 h), 18% (2 h), 34% (4 h), 67% (8 h), 84% (16 h), to 98% (24 h). This

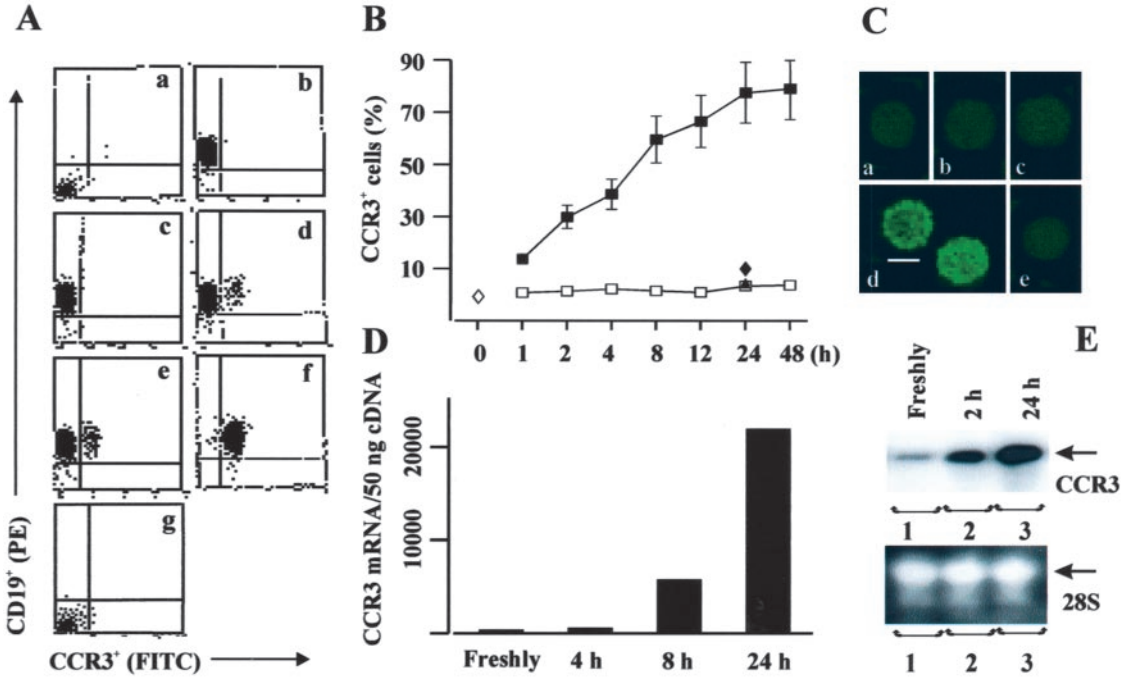

FIGURE 1. CCR3 expression on B cells examined by flow cytometry (*A* and *B*), confocal microscopy (*C*), real-time quantitative RT-PCR (*D*), and Northern blot (*E*). *A*, The cells were either freshly isolated (*b*) or stimulated for 24 h with cytokine-free medium (*c*), IL-2 (*d*) (10 ng/ml), IL-4 (*e*) (10 ng/ml), and IL-2 and IL-4 (*f*), respectively. The isotype Ab controls are for freshly isolated cells, and the cells were stimulated with IL-2 and IL-4 together for 24 h, respectively (*a*, *g*). The cells were then stained with anti-CCR3 mAb, as described in *Materials and Methods*. The gating in the forward scatter and side scatter histograms was adhered to the lymphocyte region. The percentages of $CCR3^+$ cells are indicated in *Results*. The data are from a single experiment, which is representative of six similar experiments performed. Flow cytometric analysis of the kinetics of CCR3 expressions on B cells (*B*). The cells were freshly isolated (◇); cultured with cytokine-free medium (□); and stimulated with IL-2 (10 ng/ml) (▲), IL-4 (10 ng/ml) (◆), and IL-2 and IL-4 (■) at different time intervals indicated, respectively. The percentages of $CCR3^+$ cells are indicated in *Results*. The data are from a single representative experiment of three similar experiments performed for B cells. For confocal microscopy, cells were either freshly isolated (*a*) or stimulated for 24 h with IL-2 (*b*) (10 ng/ml), IL-4 (*b*) (10 ng/ml), and IL-2 and IL-4 (*d*, *e*), respectively. The isotype Ab controls are for the cells stimulated with IL-2 and IL-4 for 24 h (*e*). The cells were then stained with anti-CCR3 mAb, as described in *Materials and Methods*. The cells were photographed under epifluorescent conditions. Original magnification, ×1200. Bar, 11 μm. The detection of mRNA of CCR3 (*D*). The bars are for the detection of mRNA of CCR3 in freshly isolated B cells, and for the detection of CCR3 mRNA of IL-2- and IL-4-stimulated B cells for 4, 8, and 24 h, as indicated. These data are representative of three similar experiments conducted. CCR3 mRNA Northern blot of freshly isolated, or IL-2- and IL-4-stimulated B cells at the indicated time intervals (*E*). Total RNA from different cells, as indicated, was isolated, electrophoresed, and blotted, as described in *Materials and Methods*. The hybridization signals for CCR3 mRNA from different cells are shown in *upper panel*. The 28S rRNAs in *lower panel* confirm that comparable amounts of total RNA were used.

level of expression (99%) was maintained for up to 48 h. The observation was also confirmed by confocal microscopy (Fig. 1*C*). There was no detectable CCR3 expression on freshly isolated B cells in the representative field (*a*). B cells cultured in the presence of IL-2 (*b*) or IL-4 (*c*) showed no significant induction of CCR3 expression on the cells in the representative field. A high level of CCR3 expression was induced on the cells in culture in the presence of IL-2 and IL-4 together (*d*). CCR3 mRNA was detected at very low levels in freshly isolated B cells (Fig. 1*D*) (5.2×10^1 copies, $C_T = 31.1$), but increased on stimulation with IL-2 and IL-4 for 4 h (6.1×10^2 copies, $C_T = 21.5$), 8 h (5.8×10^3 copies, $C_T = 18.0$), and 24 h (2.2×10^4 copies, $C_T = 15.9$). This observation was confirmed by Northern blot analysis (Fig. 1*E*), which showed that freshly isolated B cells expressed CCR3 mRNA at very low level, whereas a substantial increase occurred in IL-2- and IL-4-stimulated B cells. The cytokines IL-2 and IL-4 had no effect on the viability of B cells within 48 h of incubation, as demonstrated by trypan blue exclusion test (data not shown). The CCR3 expression could not be seen on B cells in the GC in the tonsils detected in biopsy specimen of tonsillectomy using immunohistochemistry (data not shown). There was undetectable expression of CCR3 on freshly isolated $CD19^+CD10^+$ GC B cells. CCR3 expression on GC B cells in the presence of the IL-2 and IL-4 was detected, but in longer time than peripheral B cells (within 96 h) by flow cytometry and confirmed by mRNA real-time detection and Northern blot analysis (data not shown).

Eotaxin/CCL11 induces neither chemotaxis nor adhesion in B cells

Most chemokines have been demonstrated to induce chemotaxis and adhesion of inflammatory cells by up-regulating chemokine receptor expression. To investigate the effect of CCR3 in B cells, we studied the functional activity of CCR3 on these cells. The CCR3 ligand, eotaxin/CCL11, failed to induce chemotaxis in freshly isolated B cells (C.I. = 1.09 ± 0.34, MCNC = 6 560 ± 1070) (Fig. 2*A*), whereas MIP-1α/CCL3, a ligand for CCR5 that was reported to be expressed on B cells, had a significant chemotactic effect on these cells (C.I. = 2.95 ± 0.69) (Fig. 2*A*). Interestingly, eotaxin/CCL11 failed to induce chemotaxis in B cells stimulated with IL-2 and IL-4 for 24 h (C.I. = 1.13 ± 0.25, MCNC = 6888 ± 1280) (Fig. 2*B*), even though CCR3 are abundantly expressed on the cells (Fig. 1*A*), whereas MIP-1α/CCL3 had a significant chemotactic effect on these cells (C.I. = 3.13 ± 0.43) (Fig. 2*B*). Moreover, eotaxin/CCL11 also failed to induce adhesion either in freshly isolated B cells or in B cells stimulated with IL-2 and IL-4 for 24 h (all <8%; Fig. 2, *C* and *D*), even though CCR3 are abundantly expressed on the cells (Fig. 1*A*). In contrast, MIP-1α/CCL3 induced significant ($p < 0.01$) adhesion of freshly isolated cells and following culture in the presence of

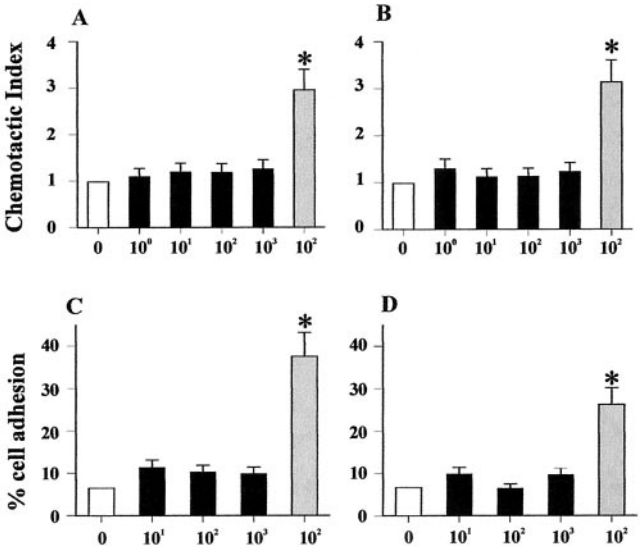

FIGURE 2. The chemotaxis and adhesion of B cells. The chemotaxis (*A*, *B*) and adhesion (*C*, *D*) of freshly isolated B cells (*A*, *C*) and IL-2- and IL-4-stimulated B cells for 24 h before the procedure (*B*, *D*) toward eotaxin/CCL11 (filled bars) or MIP-1α/CCL3 (gray bars) (100 ng/ml) or PBS control (open bars). All results were determined, as described in *Materials and Methods*, and were expressed as C.I. or percentage of adhesive cells with SD ($\pm$SD), and based on triplicate determination of chemotaxis and adhesion on each concentration of chemokine indicated as ng/ml. Statistical significant differences as compared with controls are indicated: $*$, $p < 0.001$. Values of $p > 0.05$ are considered nonsignificant.

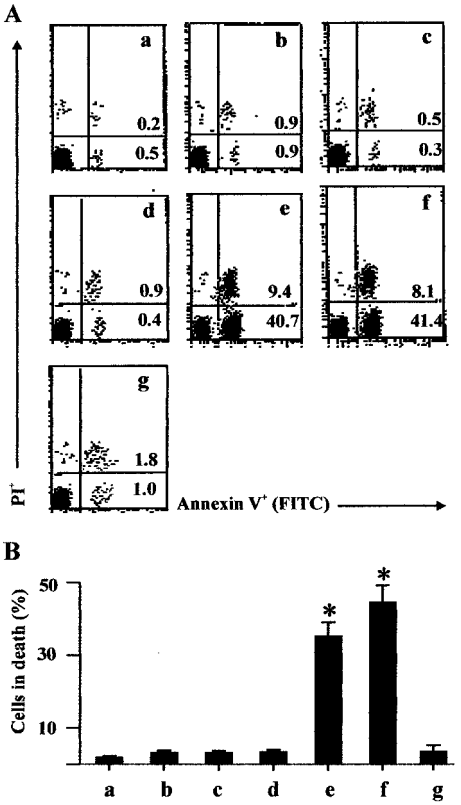

FIGURE 3. Flow cytometric analysis of apoptotic and total dead (necrotic and apoptotic) B cells. Flow cytometric analysis of apoptotic (*A*) and total dead (*B*) B cells following stimulation of cytokines and chemokines with different combinations. Cells were cultured in medium alone for 48 h (*a*), or in the presence of IL-2 (10 ng/ml) and IL-4 (10 ng/ml) for 48 h (*b*); eotaxin/CCL11 (100 ng/ml) for 48 h (*c*); in the presence of IL-2 and IL-4 with MIP-1α/CCL3 (100 ng/ml) added for the last 24 h of culture (*d*); or alternatively with eotaxin/CCL11 (100 ng/ml) for the last 24 h (*e*). As a control, cells were cultured with campotothecin (5 μM) for 6 h (*f*). Cells were cultured in the presence or absence of IL-2 and IL-4 with consequently eotaxin/CCL11 (100 ng/ml) and a-CCR3 mAb (5 μg/ml) for the last 24 h (*g*). The cells were analyzed by flow cytometry for PI (*y*-axis) and FITC-conjugated annexin V (*x*-axis), as described in *Materials and Methods*. The gating in the forward scatter and side scatter histograms was adhered to the lymphocyte region. The percentages of PI^- annexin V^+ cells and PI^+ annexin V^+ cells were indicated in the figure. The data (*A*) are from a single experiment, which is representative of six experiments performed. The data for total dead cells (PI^- annexin V^+ + PI^+ annexin V^+) (*B*) are mean values $\pm$ SD of six experiments performed. Statistically significant differences as compared with controls are indicated ($*$, $p < 0.001$).

IL-2 and IL-4 (37 and 27%, respectively; Fig. 2, *C* and *D*). We also investigated the effect of RANTES/CCL5, another CCR3 ligand, in terms of induction of B cell chemotaxis and adhesion. RANTES/CCL5 induced no or insignificant levels of chemotaxis and adhesion of IL-2- and IL-4-stimulated $CCR3^+$ B cells (data not shown). Eotaxin/CCL11 induced neither chemotaxis nor adhesion in the $CD19^+CD10^+$ GC B cells freshly isolated or stimulated with IL-2 and IL-4 together for 96 h (data not shown).

Death-promoting effect of eotaxin/CCL11 in B cells

CXCR4 is the only known chemokine receptor (25), which has been found to mediate a rapid CD95-independent cell death in $CD4^+$ T cells. We examined the death-promoting effect of signals transmitted through CCR3 on IL-2- and IL-4-stimulated B cells following ligation by eotaxin/CCL11. Flow cytometric analysis revealed that only a small percentage of cells was apoptotic (PI^- annexin V^+ fractions) or necrotic (PI^+ annexin V^+ fractions), if cultured in either medium alone (*a*), or in the presence of IL-2 and IL-4 (*b*), eotaxin/CCL11 (*c*), or a combination of IL-2, IL-4, and MIP-1α/CCL3 (*d*) (Fig. 3*A*). However, the number of apoptotic and necrotic cells was significantly ($p < 0.001$) increased on culture in the presence of a combination of IL-2, IL-4, and eotaxin/CCL11 (*e*). As a positive control, cells were stimulated with campotothecin, which induces apoptosis (*f*). To confirm that the induction of apoptosis of $CD19^+$ B cells is indeed via signaling through CCR3, we added anti-CCR3 mAb to the culture system together with IL-2 and IL-4 and eotaxin/CCL11; the mAb could significantly block the induction of apoptosis of eotaxin/CCL11 in B cells (*g*), whereas isotype Ab did not have such blocking effect (data not shown). As shown in Fig. 3*B*, the total fraction of dead cells in eotaxin/CCL11-stimulated B cell cultures (35.4 $\pm$ 8.5%) was significantly increased as compared with cells cultured with other stimuli, but comparable to levels observed in cells stimulated with campotothecin. The total fraction of dead cells could be completely blocked by stimulating B cells with IL-2 and IL-4 and eotaxin/CCL11 in the presence of a specific anti-CCR3 mAb (*g* in Fig. 3*B*), whereas isotype Ab did not have such blocking effect (data not shown). Stimulation with IL-2, IL-4, and eotaxin/CCL11 led to an increase in levels of CD95 expression on B cells (data shown below). We also investigated RANTES/CCL5 (another CCR3 ligand) and MIP-1α/CCL3 (ligand for CCR5) in terms of induction of B cell apoptosis. Neither chemokine induced detectable levels of apoptosis in IL-2- and IL-4-stimulated B cells (CCR3-bearing cells; data not shown). This raises the question as to why RANTES/CCL5, which like eotaxin/CCL11 also binds CCR3, fails to induce B cell apoptosis. The data suggest that IL-2 and IL-4 induce CCR3 expression on B cells and that subsequent stimulation with the ligand eotaxin/CCL11 promotes the apoptosis of these cells via signals transmitted through CCR3.

Western blots (Fig. 4*A*, *left*) revealed that cytochrome *c* protein levels were increased in lysates obtained from B cells cultured with eotaxin/CCL11. Data obtained by immunocytochemistry (Fig. 4*A*, *right*) showed that cytochrome *c* was randomly distributed within cells after treatment. Mitochondria and mitochondrial factors are intimately connected with the process of apoptosis. The mechanism of action of certain apoptotic stimuli, such as Fas receptor ligation, involves opening of the mitochondrial permeability transition pore, dissipation of mitochondrial membrane potential, and release of mitochondrial factors such as cytochrome *c* from the mitochondria into the cytosol (36). Western blot showed (Fig. 4*B*, *left*) that the protein level of precaspase-3 was reduced in cell lysates obtained from B cells following treatment with eotaxin/CCL11. Caspase-3, thought to be a key apoptotic executioner enzyme in mammalian cells (37), appears following culture in the presence of eotaxin/CCL11. The immunocytochemistry data (Fig. 4*B*, *right*) showed that the expression pattern of caspase-3 was condensed in the cellular plasma following stimulation with eotaxin. Western blot analysis (Fig. 4*C*, *left*) showed that Bcl-2 protein levels were reduced B cell lysates obtained from cells cultured with eotaxin/CCL11. This was confirmed by data obtained by confocal microscopy (Fig. 4*C*, *right*), which demonstrated a decrease in levels of BCL-2. The antiapoptotic effect of the Bcl-2 family members has been shown to be mediated through a direct protein-protein interaction with voltage-dependent anionic channel, a component of the mitochondrial pore, thus stabilizing the pore (38). One possible mechanism by which eotaxin/CCL11, signaling through CCR3, causes B cell apoptosis may be through reduction in the level or functional activity of Bcl-2, thus promoting programmed B cell death. Thus, eotaxin/CCL11 by means of its receptor may induce the release of cytochrome *c* from the mitochondria into the cytosol, which will trigger the cascade of enzymatic events that culminates in cell death.

To examine further the mechanisms involved in the induction of B cell apoptosis by means of CCR3 ligation, we investigated levels of CD95 and CD95L expression on B cells stimulated with different combinations of IL-2, IL-4, and eotaxin/CCL11. Data obtained from flow cytometric analyses (Fig. 5*A*) show that freshly isolated $CD19^+$ B cells expressed low levels of CD95 (19%) (*b*). After 24-h culture with different combinations of IL-2, IL-4, and eotaxin/CCL11, only IL-2, IL-4, and eotaxin/CCL11 together

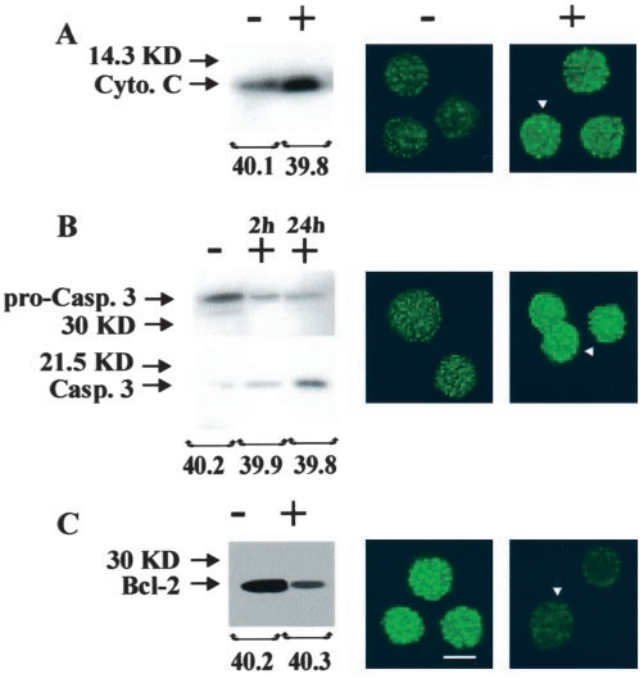

FIGURE 4. Occurrence of cytochrome *c*, caspase-3, and Bcl-2 in B cells. The cytochrome *c* (*A*), caspase-3 (*B*), and Bcl-2 (*C*) were examined using Western blot analyses (*left panels*), and confocal microscopy detection (*right panels*) for B cells was cultured in the presence of IL-2 (10 ng/ml) and IL-4 (10 ng/ml) alone for 48 h (−), or with eotaxin/CCL11 (100 ng/ml) added for the last 24 h (+; *A*, *B*, *C*) or 2 h (*B*) of culture. For Western blots, cells were lysed and total protein content was electrophoresed and blotted, as described in *Materials and Methods.* Numbers indicate the quantity of total cellular protein from the tested samples loaded in each lane (μg) (27, 41). Arrows indicate markers used to verify equivalent m.w. of appropriate proteins in each lane. For confocal microscopy, cells were collected after stimulation and stained, as described in *Materials and Methods*. The cells were photographed under epifluorescent conditions. Original magnification, ×1200. Bar, 11 μm.

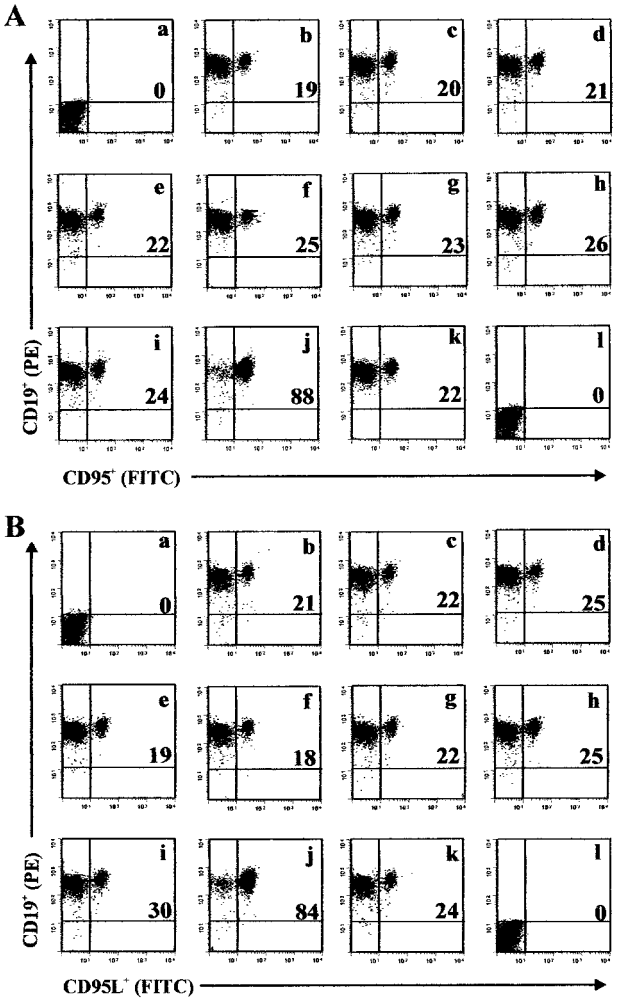

FIGURE 5. CD95 (*A*) and CD95L (*B*) expression on $CD19^+$ B cells examined by flow cytometry. The cells were either freshly isolated (*b*) or stimulated for 24 h in medium (*c*), or in the presence of 10 ng/ml IL-2 (*d*), 10 ng/ml IL-4 (*e*), 100 ng/ml eotaxin/CCL11 (*f*), IL-2 and IL-4 (*g*), IL-2 and eotaxin/CCL11 (*h*), IL-4 and eotaxin/CCL11 (*i*), IL-2, IL-4, and eotaxin/CCL11 together (*j*), and IL-2, IL-4, eotaxin/CCL11, and a-CCR3 mAb (5 μg/ml) together (*k*), respectively. Isotype Ab controls are for freshly isolated cells (*a*) and the cells stimulated with IL-2, IL-4, and eotaxin/CCL11 together for 24 h (*l*). The cells were then stained with PE-labeled CD19 mAb, as described in *Materials and Methods*. The gating in the forward scatter and side scatter histograms was adhered to the lymphocyte region. The numbers represent percentage of $CD95^+$ and $CD95L^+$ cells, as shown in *Results*. The data are from a single experiment, which is representative of five similar experiments performed.

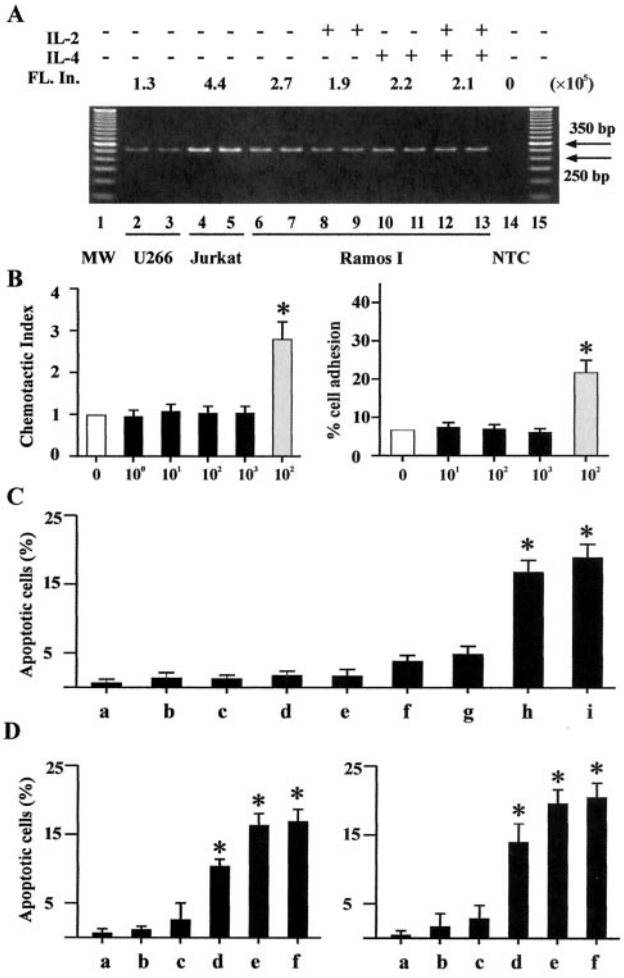

FIGURE 6. CCR3 expression and functional activity on B cell lines. This was determined by RT-PCR analysis (*A*), chemotaxis and adhesion (*B*), flow cytometry of apoptosis (*C*), and flow cytometry of apoptosis in time kinetics and dose response (*D*). Cells were cultured as indicated, and the PCR products were electrophoresed and visualized with bromide, as described in *Materials and Methods*. NTC, no template control. Samples were set up in duplicate and fluorescence intensity (Flu. In.). *A*, β-actin was used as housekeeping gene to control for loading identical amounts of cDNAs (data not shown). The extent of chemotaxis (*right graph*) and adhesion (*left graph*) of the B cell line Ramos I in response to a range of concentrations (ng/ml) of eotaxin/CCL11 (filled bars) or MIP-1α/CCL3 (100 ng/ml; gray bars) or PBS control (open bars) was performed, as described in *Materials and Methods*, and expressed as described in the legend for Fig. 2. Statistical significant differences as compared with controls are indicated: $*$, $p < 0.001$. Values of $p > 0.05$ are considered nonsignificant (*B*). The extent of apoptosis of the B cell line Ramos I was determined by flow cytometric analysis (*C*), performed as described in *Materials and Methods*, and expressed as described in the legend for Fig. 3. The following culture conditions were used: cells were cultured for 24 h in medium alone (*a*), or in the presence of the following cytokines: IL-2 (*b*), IL-4 (*c*), eotaxin/CCL11 (*d*), IL-2 and IL-4 (*e*), IL-2 and eotaxin/CCL11 (*f*), IL-4 and eotaxin/CCL11 (*g*), IL-2, IL-4, and eotaxin/CCL11 (*h*), or campothecin (*i*). The extent of apoptosis of the B cell line Ramos I was determined by flow cytometric analysis (*D*) by eotaxin/CCL11 in dose response (*right graph*) and in time kinetics (*left graph*), performed as described in *Materials and Methods*, and expressed as described in the legend for Fig. 3. In the *right graph*, the following culture conditions were used: cells were cultured for 48 h in the presence of IL-2 and IL-4 and absence of eotaxin/CCL11 (*a*) or in the presence of IL-2, IL-4, and eotaxin/CCL11 (0.1 ng/ml, *b*; 1 ng/ml, *c*; 10 ng/ml, *d*; 100 ng/ml, *e*; or 1000 ng/ml, *f*). The data are from a single experiment, which is representative of six similar experiments performed. In the *left graph*, the following culture conditions were used: cells were cultured in the presence of IL-2, IL-4, and eotaxin/CCL11 (100 ng/ml) for 0 h (*a*), 8 h (*b*), 16 h (*c*), 24 h (*d*), 48 h (*e*), or 72 h (*f*).

could change levels of CD95 expression. Under different culture conditions, CD95$^+$ B cells are as follows: 20% in cytokine-free medium (*c*), 21% in IL-2 alone (*d*), 21% in IL-4 alone (*e*), 25% in eotaxin/CCL11 alone (*f*), 23% in IL-2 and IL-4 (*g*), 26% in IL-2 and eotaxin/CCL11 (*h*), 24% in IL-4 and eotaxin/CCL11 (*i*), and 88% in IL-2 and IL-4 and eotaxin/CCL11 together (*j*), respectively. Furthermore, the results from flow cytometric analyses (Fig. 5*B*) reveal a similar expression pattern of CD95L as CD95 on B cells were stimulated with different combinations of IL-2, IL-4, and eotaxin/CCL11. There were low levels of CD95L expression on freshly isolated CD19$^+$ cells (21%) (*b*). Twenty-four hours after culture with different combinations of IL-2, IL-4, and eotaxin/CCL11, we found that only a combination of IL-2, IL-4, and eotaxin/CCL11 increased levels of CD95L expression. Under different culture conditions, CD95L$^+$ B cells are as follows: 22% in cytokine-free medium (*c*), 25% in IL-2 alone (*d*), 19% in IL-4 alone (*e*), 18% in eotaxin/CCL11 alone (*f*), 22% in IL-2 and IL-4 (*g*), 25% in IL-2 and eotaxin/CCL11 (*h*), 30% in IL-4 and eotaxin/CCL11 (*i*), and 84% in IL-2 and IL-4 and eotaxin/CCL11 together (*j*), respectively. To confirm that CD95 and CD95L induction is indeed via signaling through CCR3, we added anti-CCR3 mAb to the culture system together with IL-2 and IL-4 and eotaxin/CCL11; the mAb could block the up-regulatory effect of eotaxin/CCL11 on CD95 and CD95L expressions (22% CD95, Fig. 5*Ak*; 24% CD95L, Fig. 5*Bk*), whereas isotype Ab did not have such blocking effect (data not shown).

To distinguish differences in apoptosis induced by cross-linking of the CD19 (39, 40) or by signaling through CCR3, we performed experiments in which purified B cells were cultured with immobilized anti-CD19 mAb. Only 12% of B cells were apoptotic on cross-linking of anti-CD19 as compared with 38% induced on signaling through CCR3, following culture in the presence of IL-2, IL-4, and eotaxin/CCL11. The data imply that B cell apoptosis induced either following cross-linking of the CD19 or following CCR3 ligation operates by means of different signaling pathways. Because CD40 signaling mediates rescue B cells from BCR-mediated apoptosis (41, 42), we added the anti-CD40 mAb (10 μg/ml, 5C3; BD PharMingen) (41, 42) in the culture system. The a-CD40 mAb did not significantly inhibit the apoptosis of B cells induced by IL-2, IL-4, and eotaxin/CCL11 (data not shown), implying CCR3 ligation induces B cell apoptosis by means of unique signaling pathways. No cellular proliferation was seen in B cells stimulated with different combinations of IL-2 (10 ng/ml), IL-4 (10 ng/ml), and eotaxin (100 ng/ml) within 24 h, as detected by [^{3}H]thymidine incorporation into DNA assay.

Thus, IL-2 and IL-4 induce CCR3 expression on B cells, and subsequent ligation of CCR3 by eotaxin/CCL11 promotes apoptosis by increasing levels of CD95 and CD95L expression, and ultimately binding of CD95 to CD95L expressed on adjacent cells, trigging relevant death signaling pathways, which initiate programmed death process in these cells.

An apoptotic effect of eotaxin/CCL11 in CD19$^+$CD10$^+$ GC B cells was observed by flow cytometric analyses (data not shown). CD95 and CD95L expression could be significantly up-regulated by stimulation with combination of IL-2, IL-4, and eotaxin/CCL11 within 96 h (data not shown).

CCR3 expression and functional activity on different B cell lines

We examined further the expression and functional activity of CCR3 on a number of B cell lines. As shown in Fig. 6*A*, the unstimulated B cell lines U266 and Ramos I (Ramos.2G6.4C10) clearly expressed CCR3 transcripts (fluorescence intensities 1.3 $\times$

10^5 and 2.7×10^5, respectively), and the signal of CCR3 transcripts was also detected in specimens of Jurkat T cell line (fluorescence intensity 4.4×10^5). The stimulation with IL-2 and/or IL-4 did not change the pattern of expression of CCR3 mRNA in Ramos I (fluorescence intensities 1.9×10^5, 2.2×10^5, and 2.1×10^5, respectively). In other B cell lines, Ramos II (Ramos RA1), Daudi, and Raji, a consistent expression of CCR3 mRNA was found (data not shown). Eotaxin/CCL11 induced neither chemotaxis (Fig. 6*B*, *right graph*) nor adhesion (Fig. 6*B*, *left graph*) in the B cell line Ramos I was stimulated with IL-2 and IL-4 for 24 h (C.I. = 0.9 ± 0.25, MCNC = 5660 ± 907; <5% of cell adherent), whereas MIP-1α/CCL3 showed significant chemotactic and adherent effects in these cells (C.I. = 2.76 ± 0.56; 21% of cell adherent). Similar phenomena were observed in other B cell lines, Ramos II and Daudi, but not in Raji (data not shown). An apoptotic effect of eotaxin/CCL11 in B cell line Ramos I was observed by flow cytometric analyses (Fig. 6*C*). The presence of eotaxin/CCL11 together with IL-2 and IL-4 induced up to ~15% of apoptotic cells, which is similar to that of the cells stimulated with campotothecin, whereas only 2% of apoptotic cells were seen in absence of eotaxin/CCL11. The anti-CCR3 mAb can block the apoptotic effect of eotaxin/CCL11 in B cell line Ramos I (data not shown). The addition of a-CD40 mAb (10 μg/ml) did not significantly inhibit the apoptosis of B cell line Ramos I induced by IL-2, IL-4, and eotaxin/CCL11 (data not shown). In the study of dose response of apoptotic induction of eotaxin/CCL11 in B cell line Ramos I (Fig. 6*D*, *right graph*), we found that eotaxin/CCL11 at concentration of 10 ng/ml induced significant apoptosis of B cell line Ramos I (12% of apoptotic cells). The concentration of 100 ng/ml of eotaxin/CCL11 seems to be optimal (16% of apoptotic cells). In the study of time kinetics of apoptotic induction of eotaxin/CCL11 in B cell line Ramos I (Fig. 6*D*, *left graph*), we found that eotaxin/CCL11 at optimal concentration (100 ng/ml) for 24 h induced significant apoptosis of B cell line Ramos I (14% of apoptotic cells). The 48-h incubation with eotaxin/CCL11 seems to be optimal (17% of apoptotic cells).

Discussion

B cells take part in a defense against invading pathogens. When nonself Ags are detected, B cells undergo a complex developmental program to generate large quantities of high-affinity neutralizing Abs that help in clearing the pathogen from the host. To encounter Ag and to obtain help in generating a potent humoral response, B cells need to migrate into specific areas at specific times during their development. Naive B cells migrate from the bone marrow to the peripheral lymphoid organs, where, following recirculation through blood, they encounter specific Ag, undergo clonal expansion, and colonize the primary lymphoid follicles (43). In this study, naive B cells differentiate into GC cells that first proliferate, then somatically mutate Ig V region genes, and finally are positively selected according to the affinity of surface Ig for Ag presented by follicular dendritic cells (44). GC B cells are a resident cell subset with poor propensity to migrate. Following positive selection, GC B cells differentiate into memory or effector plasma cells outside the lymphoid follicles (44). Memory B cells home to specific sites, such as the splenic marginal zone or the tonsil subepithelial criptae (45), where they settle until they interact with specific Ags, followed by recirculation of the majority of Ag-activated memory B cell (44). Chemokine receptors and their ligands mobilize subsets of effector leukocytes during inflammatory reactions or regulate the constitutive homing of B and T cells to peripheral lymphoid organs (46, 47). To understand the migration and selection of B cells during their life span, we focused our study on CCR3 expression and functions of its ligands on $CD19^+$ B cells and $CD19^+CD10^+$ GC B cells from tonsils. We have demonstrated that CCR3 is high frequently expressed in B cells stimulated with IL-2 and IL-4. Interestingly, eotaxin/CCL11 induced neither chemotaxis nor adhesion, but significant apoptosis in both $CD19^+$ B cells and $CD19^+CD10^+$ GC B cells. To our knowledge, this is the first report on up-regulated expression of CCR3 on B cells in humans, and is the first direct evidence of biological activity (apoptosis) of eotaxin/CCL11 on B cells. A rather complex picture is now beginning to take shape of how B cells selectively transmigrate through lymphoid tissue to contact with Ags and to be selected and maturized at the presence of chemokine receptors and their ligands to carry out physiological and pathophysiological events during humoral response.

What is the meaning of the found function of CCR3 on B cells? From our results, clear showed that only 40–50% of B cells in fact become apoptotic (Fig. 3*A*), despite an almost 100% up-regulation of CCR3 (Fig. 1*B*). This finding may be related to an as yet unresolved differentiation of B cells into responsive and nonresponsive subpopulations. The eotaxin/CCL11-responsive subpopulation of B cells could be selected out to process into death. The eotaxin/CCL11-nonresponsive subpopulation could be selected out to process into maturation. Because combination of Th1 cytokine IL-2 and Th2 cytokine IL-4 together can induce the expression of CCR3 on B cells, the balance of Th1 and Th2 cytokines could be pivotal for the selection of B cells during their development. In contrast, it is shown that the Ab production requires a series of signals to be delivered to the B cells. These signals are classified as either Th1 dependent when Th1 cells interact with B cells and secrete IFN-γ to induce B cells to produce IgG2a, or Th2 dependent when Th2 cells interact with B cells and secrete IL-4 to induce B cells to produce IgG1 and IgE in mouse system (48). The Th2 pathway is thought to be crucially important for initiation and development of allergic inflammation. The outcome of imbalance of Th1 and Th2 cytokines leads to the suppression of some B cell subpopulations via apoptosis induced by CCR3 receptor-ligand pair. This in turn may result in enhancing the allergic inflammation and other Th-polarized diseases.

Among a number of cell surface molecules involved in T-B cell cooperation and subsequent B cell fate, CD40 stimulation activates B cells and promotes various aspects of a functional humoral immune response. It mediates a costimulatory signal and plays a pivotal role in B cell proliferation, memory B cell differentiation, and B cell survival (49–52). Like many TNFR family members, CD40 activates the c-Jun N-terminal kinase/stress-activated protein kinase and NF-κB pathways (52–54), which involve serine/threonine kinases that regulate gene expression through activation of AP1 and Rel transcription factors, respectively. Another stress-responsive pathway activated by CD40 (55) is the p38 kinase pathway, which leads to the phosphorylation and activation of transcription factors such as ATF2 (56). CD40 has also linked to the activation of the extracellular signal-regulated kinase/mitogen-activated protein kinase and phosphatidylinositol-3 kinase pathways (57, 58). In our study, we have observed that CD40 activation could not protect $CD19^+$ B cells and $CD19^+CD10^+$ GC B cells from apoptosis induced by eotaxin/CCL11, strongly indicating that the activation pathway of eotaxin/CCL11 ligation in B cells is different from that of CD40 activation to protect the cells from apoptosis. Other explanation would be that the pathways triggered by eotaxin/CCL11 via CCR3 could not cross talk with that activated by CD40 signaling. Further pinpoint of the pathways of eotaxin/CCL11 ligation in B cells may reveal the mechanism of apoptosis triggered by chemokines.

The redundancy is one of the important features of chemokine ligand and receptor superfamilies. Some of the CC chemokines

bind to a single CCR, whereas others share different receptors. For example, monocyte chemotactic protein-1 (MCP-1)/CCL2 binds exclusively to CCR2; this receptor binds also to MCP-2/CCL8 and MCP-3/CCL7. Both of the latter chemokines interact with CCR1 and CCR3; MCP-2/CCL8 interacts with CCR5 (59). RANTES/CCL5 was demonstrated to bind to four CCRs (CCR1, CCR3, CCR4, and CCR5) (60), whereas eotaxin binds exclusively to CCR3 (59). Furthermore, CCR3 is associated with responsiveness to a number of chemokines, including eotaxin/CCL11, MCP-3/CCL7, MCP-4/CCL13, RANTES/CCL5, as well as eotaxin-2/CCL24 and eotaxin-3/CCL26 (17). In our study, RANTES fails to induce apoptosis even though it also binds to CCR3, and was tested in a reasonable wide concentration range (1–1000 ng/ml). The diversity of RANTES effects in vitro was shown in some studies (60). The RANTES effects can be separated into two groups according to its concentration. At low concentrations, RANTES acts in a monomeric (or dimeric) form directly on its specific chemokine receptors; at high concentrations, it self aggregates and acts through interactions with cell surface glycosaminoglycans. RANTES induces cell killing that is restricted in a rare subpopulation of cytotoxic T cells (HIV-specific $CD8^+$) through Fas interactions with its ligand (61). The explanations for our results could be suggested as that the induction of apoptosis via CCR3 is ligand restricted. In other words, different ligand triggers different pathways via even same chemokine receptor. It could also be that different ligand binds to different sites in the same receptor to induce different function. This interesting point needs to be further investigated.

During the experiments of generating B cell functions, it is necessary to prevent binding of self Ag that is potentially autoreactive (39, 40). Cross-linking of B cell receptor rapidly activates Src family protein tyrosine kinases such as Lyn, Fyn, and Blk and induces phosphorylation of immunoreceptor tyrosine-based activation motifs in the cytoplasmic portion of Igα and Igβ (62–64), which could positively regulate B cell receptor-induced apoptosis. In our study, the cells were cultured in different conditions (medium alone, IL-2 alone, IL-4 alone, eotaxin/CCL11 alone, IL-2 + IL-4 + MIP-1α/CCL3, and IL-2 + IL-4 + eotaxin/CCL11). Only IL-2 + IL-4 + eotaxin/CCL11 induced significant apoptosis. The results are convincible to have demonstrated that apoptosis is indeed triggered by CCL11/CCR3 signaling, but not binding of self Ag in the culture. In other words, the apoptosis in B cells via CCL11/CCR3 signaling is distinctive from that induced by the cross-linking of B cell receptor.

During their life span, developing B cells undergo dramatic changes in expression of some of the surface chemokine receptors and in their responses to chemokines. For example, none of the $CD34^+Lin^-$ hemopoietic stem cell progenitors or the $CD34^+CD19^+$ pro-B or the $CD19^+CD10^+$ pre-B/immature B cell progenitors expressed CCR6. CCR6 is acquired when CD10 is lost and B cell progeny matures, entering into the surface IgD^+ mature B cell pool. CCR6 is expressed by all bone marrow-, umbilical cord blood-, and peripheral blood-derived naive and/or memory B cells, but is absent from GC B cells of secondary lymphoid organs. CCR6 is down-regulated after B cell Ag receptor triggering and remains absent during differentiation into Ig-secreting plasma cells, whereas it is reacquired at the stage of post-GC memory B cells (65). For another example, bone marrow pre-pro-B cells migrate to thymus-expressed chemokine /CCL25, a response lost in later stages of B cell development. B cell-attracting chemokine 1/CXCL13 responses correlate with CXCR5 expression, are first displayed by a pro-B cell subset, are lost in pre-B cells, and then are regained just before and after egress from the marrow (66). Thus, unique patterns of chemokine receptor expression and chemokine responses may help define developing B cell populations and direct their maturation in the marrow and migration to the periphery. Our data that CCR3 is not expressed on peripheral and GC B cells, but appeared on those cells under stimulation with IL-2 and IL-4, and that ligation of CCR3 by eotaxin/CCL11 induces apoptosis in IL-2- and IL-4-stimulated primary $CD19^+$ or $CD10^+CD19^+$ GC B cell after long-term cultures, are indicating that naive B cells are not expressing CCR3, and that this chemokine receptor could be acquired in some of the activated and/or postmature B cells, which could be a small part of the total peripheral B cells in vivo. These cells will finally develop into a programmed cell death after ligation of eotaxin/CCL11.

The fact that CCR3 expression on B cells and its ligand induces apoptosis of B cells raises a new interpretation as to how the chemokines and their receptors participate and modulate the allergic inflammation. On the one hand, CCR3 and eotaxin/CCL11 attract the inflammatory cells such as T cells and eosinophils to particular sites to generate an inflammatory allergic response; in contrast, they trigger apoptosis of B cells to reduce the synthesis of Igs including IgE, and consequently to change the process of the development of allergic inflammation in which IgE plays an important role.

Apoptosis, a programmed cell death, is crucial for animal and human development, organ morphogenesis, tissue homeostasis, aging, and the removal of infected or damaged (malignant) cells (67–70). In response to death stimuli, mitochondrial membranes are permeabilized (71, 72), and cytochrome *c* is released from mitochondria (25, 69, 73) and associates with Apaf1 and procaspase-3 and procaspase-9 to trigger a caspase activation cascade that culminates in cell death characterized by apoptotic morphology (25, 74–77). Failure to invoke appropriate cell death can result in cancer or autoimmunity, whereas increased programmed cell death can lead to degenerative processes such as immunodeficiency and neurodegenerative disease (78). Rather interestingly, CCR3 is constantly expressed on several B cell lines, and receptor-ligand pair of CCR3-eotaxin/CCL11 induces apoptosis of B cell lines. This aspect obviously deserves further studies, in particular if the newly discovered biological activity of eotaxin/CCL11 should be moved into clinical use for treatment of B cell lymphoma.

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