The high-affinity IgE receptor (FcεRI) blocks apoptosis in normal human monocytes

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Monocytes have a limited life span, and their homeostasis is regulated by apoptosis in vivo. When cultured in the absence of appropriate exogenous stimuli, they undergo apoptosis, but under the influence of survival signals, these cells differentiate into macrophages or dendritic cells. Here we show that ligation of the high-affinity IgE receptor (FcεRI) on human monocytes from nonatopic individuals markedly reduces apoptosis induced by serum deprivation or by CD95/Fas ligation. Aggregation of FcεRI reduces its own expression but fails to modulate CD95/Fas expression. In contrast, FcεRI ligation enhances the expression of the antiapoptotic molecules Bcl-2 and Bcl-xL, but not Mcl-1, in monocytes. Incubation of unstimulated cells with culture supernatants of FcεRI-activated monocytes prolongs their life span, whereas CD95/Fas expression remains unaffected. The incidence of apoptosis is restored considerably when the supernatant is depleted of TNF-α, whereas elimination of IL-1β, GM-CSF, or IL-12 has no effect. These results indicate that FcεRI mediates signals preventing monocyte apoptosis directly by increasing the levels of Bcl-2 and Bcl-xL, and indirectly by means of TNF-α in an autocrine and paracrine fashion. This process may contribute to the establishment of chronic allergic disorders such as atopic dermatitis.

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Introduction

Monocytes have a limited life span, and their homeostasis is regulated by programmed cell death in vivo (1, 2). Human monocytes cultured in the absence of appropriate exogenous stimuli undergo apoptosis that is enhanced by serum removal (3). The onset of apoptosis can be prevented by adding activating factors such as LPS, TNF-α, IL-1β, and CD40 ligand; and monocytes receiving such signals differentiate into macrophages or dendritic cells (DC) (1–5). These findings suggest that monocytes/macrophages activated in inflamed tissue have a prolonged survival and contribute to the establishment of chronic inflammation (1, 6). Indeed, monocytes from patients with chronic atopic dermatitis (AD) show a significantly lower apoptosis rate in vitro than those from normal individuals (7).

The high-affinity IgE receptor (FcεRI) has recently been identified on monocytes from both atopic donors and healthy individuals, and it is considered to play a pivotal role in atopic disorders and host defense by mediating antigen presentation to T cells (8–11). FcεRI expressed on monocytes and other antigen-presenting cells consists of an IgE-binding α-chain (FcεRIα) and two γ-chains (FcεRIγ) (8, 12–14). The cytoplasmic domain of FcεRIγ exhibits a consensus motif, the immunoreceptor tyrosine activation motif, which is crucial for the initiation of the activation cascade triggered by receptor ligation (15). Aggregation of FcεRI on monocytes induces Ca^{2+} mobilization and production of proinflammatory cytokines by monocytes (16–18). The signals mediated by FcεRI may therefore prolong the survival of monocytes, thereby contributing to the establishment of chronic allergic inflammation. In the present study, we show that FcεRI initiates signals, including Bcl-2 and Bcl-xL expression, which prevent apoptosis of normal monocytes. In addition to these direct effects of FcεRI ligation, cytokines like TNFα, produced upon activation by monocytes, prolonged their survival in an autocrine and paracrine fashion.

Methods

Reagents. Rabbit anti-human IgE (RahIgE), anti-human IgG, anti-human IgM, and anti-CD23 mAb (MHM6, mIgG1) were from DAKO A/S (Glostrup, Denmark). Monomeric human myeloma IgE (hIgE) was obtained from Calbiochem-Novabiochem Corp. (San Diego, California, USA) and was filtered to remove material over 300 kDa (Ultrafree-MC filter unit; Millipore Corp., Bedford, Massachusetts, USA). Anti-human FcεRIα mAb 22E7 (IgG1) was a kind gift from J. Kochan (Hoffman-LaRoche Inc., Nutley, New Jersey, USA). FITC- or PE-labeled anti-CD14 and mouse IgG1 were from Becton Dickinson Immunocytometry Systems (San Jose, California, USA). F(ab)2 fragment goat anti-mouse IgG was from Jackson ImmunoResearch Laboratories Inc. (West Grove, Pennsylvania, USA). Neutralizing mAb's against TNFα (mIgG1), GM-CSF (rat IgG2a), IL-12 p40/70 (mIgG1), and isotype-matched controls were obtained

from PharMingen (Hamburg, Germany), mAb against IL-1β (mIgG1) was from R&D Systems GmbH (Wiesbaden, Germany). Rabbit anti-human Bcl-2, Bcl-x, and Mcl-1 Ab were also obtained from PharMingen. FITC-labeled anti–annexin V, RNase A, and proteinase K were from Roche Molecular Biochemicals (Mannheim, Germany). Anti-CD95/Fas mAb CH11 (mIgM) and the FITC-labeled anti-CD95/Fas mAb UB2 (mIgG1) were from Immunotech (Marseilles, France). All other reagents were obtained from Sigma Chemical Co. (St. Louis, Missouri, USA) unless otherwise indicated.

Cell preparations and cultures. Whole blood was obtained from informed healthy, nonatopic volunteers in accordance with the institutional ethics committee. These individuals lacked a history of atopic diseases. They also had normal serum-IgE levels (<100 kU/L). PBMCs were isolated by density gradient centrifugation and were resuspended in culture medium containing RPMI 1640 (Biochrom KG, Berlin, Germany), 100 mM L-glutamine (GIBCO BRL, Gaithersburg, Maryland, USA), 1% antibiotic/antimycotic (GIBCO BRL), and 100 μg/mL polymyxin B. The cells were seeded out on tissue culture flasks (Falcon; BD Labware, San Jose, California, USA) coated with autologous serum at 37°C with 5% CO_2. Monocytes were enriched by 30 minutes adherence to plates, which resulted in more than 80% CD14-positive cells, whereas the viability was over 95%. 2×10^6 cells/mL were resuspended in culture medium in 12 × 75 mm polypropylene tubes (Falcon). All plasticware and culture reagents were tested for LPS with the Limulus amebocyte lysate "E-Toxate" Multiple test (Sigma Chemical Co.). The endotoxin level in all cases was less than 10 pg/mL LPS, a level at which inhibition of monocyte apoptosis or activation is not occurring (3, 19).

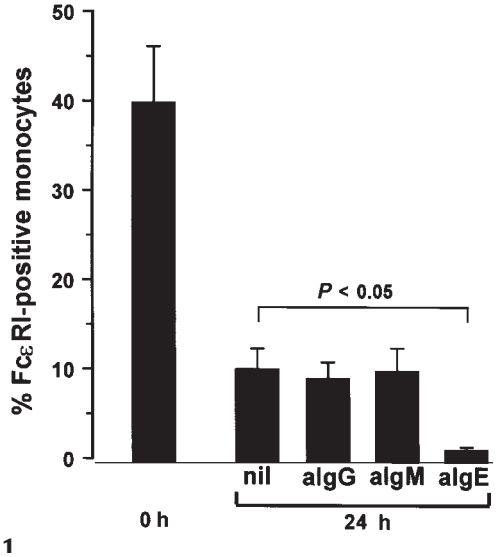

Figure 1
Ligation of FcεRI leads to its downregulation. Monocytes were either untreated (nil) or incubated for 1 hour with either anti-IgG (aIgG), anti-IgM (aIgM), or with IgE followed by anti-IgE (aIgE), washed, and then cultured in the presence of serum. After 24 hours, the receptor expression was monitored by flow cytometry using an anti-FcεRI–specific mAb that does not interact with the IgE-binding site. Note the spontaneous decrease of the FcεRI expression during the culture time further accelerated by receptor ligation. Similar results were obtained in the absence of serum. Data are expressed as the mean ± SD of data from 3 experiments.

Flow cytometric analysis. Labeling for FcεRI and CD95/Fas was performed as reported previously (20). Bcl-2 family protein staining was performed according to a modified method published in Dibbert et al. (21). Briefly, after staining with anti–CD14-PE, the cells were fixed in 4% paraformaldehyde and then washed twice with PBS containing 1% FBS, 0.1% sodium azide, and 0.1% saponin (permeabilization buffer). After incubation with the first Ab or rabbit IgG and then two washes with permeabilization buffer, staining with goat anti-rabbit IgG-FITC (Sigma Chemical Co.) was performed. Then the cells were washed once with permeabilization buffer and again with PBS, and then they were analyzed on a FACSCalibur (Becton Dickinson Immunocytometry Systems). All steps were performed at 4°C.

Receptor ligation. Cross-linking of FcεRI was done as described previously (14). Briefly, the cells were incubated for 30 minutes with 5 μg/mL hIgE or 10 μg/mL anti-FcεRI mAb [F(ab)2] 22E7 or isotype-matched control mIgG1 at 37°C. After washing with culture medium, 20 μg/mL RahIgE or 5 μg/mL GaM-IgG were added and incubated for an adequate period of time. Anti-human IgG and IgM antibodies were used for control purposes. Ligation of CD95/Fas was performed using the agonistic mAb CH11 according to Kiener et al. (22).

Generation of culture supernatants from monocytes and cytokine ELISA. Monocytes were cultured without serum for 24 hours with or without ligation of FcεRI. The supernatants were collected and centrifuged twice at 4°C, followed by immediate storage at –80°C. In blocking experiments, 500 μL of supernatant was first treated with anti-TNFα, IL-1β, GM-CSF, or IL-12-neutralizing mAb (10 μg/mL) for 30 minutes at 37°C. Then 20 μL of protein G–Sepharose (Amersham Pharmacia Biotech, Uppsala, Sweden) were added at 4°C for 2 hours to precipitate bound cytokines. After centrifugation, the supernatant was collected, and then monocytes were cultured in the supernatants.

For IL-12 (p40/p70) and IL-18 determination, supernatants were subject to ELISA according to the manufacturers' protocols using kits from Genzyme Pharmaceuticals (Cambridge, Massachusetts, USA) and Beckman Coulter (Fullerton, California, USA), respectively.

Detection of apoptosis. DNA extraction and electrophoresis were performed as described elsewhere (23). In brief, 5×10^6 monocytes were lysed in a hypotonic buffer (10 mM Tris, 10 mM EDTA, and 0.5% Triton X-100). After centrifugation, supernatants were treated with RNase A and proteinase K, DNA was extracted by phenol/chloroform and separated on a 2% agarose gel. The percentage of cells displaying DNA fragmentation was quantified using propidium iodide (PI) as previously described (23). Briefly, 10^6 cells were fixed in 70% ethanol for 1 hour at 4°C, washed once in PBS, and then incubated with RNase A. The cells were

then incubated with 50 μg/mL PI for 10 minutes at room temperature and analyzed on a FACScalibur. Because of DNA fragmentation, apoptotic cells appeared as hypoploid or "sub-G1 phase" in the DNA histogram. Alternatively, the expression of annexin V was determined to detect early apoptotic cells according to the manufacturer's instructions (22).

Amplification of mRNA transcripts. RT-PCR was done as described previously (20). Specific primer sequences for each gene were as follows: human GAPDH: reverse 5′-CCA CCC ATG GCA AAT TCC ATG GCA-3′ and direct 5′-TCT AGA CGG CAG GTC AGG TCC ACC-3′ (598 bp); human Bcl-2 based on Ando et al. (24): reverse 5′-CGG GAG ATA GTG ATG AAG TA-3′and direct 5′-CAG AGA CAG CCA GGA GAA AT-3′ (618 bp); human Bcl-x based on Sanz et al. (25): reverse 5′-CGG GCA TTC AGT GAC CTG AC-3′ and direct 5′-TCA GGA ACC AGC GGT TGA AG-3′ (340 bp for Bcl-xL and 151 bp for Bcl-xS); human Mcl-1 based on Ando et al. (24): reverse 5′-CGG CAG TCG CTG GAG ATT AT-3′ and direct 5′-GTG GTG GTG GTG GTT GGT TA-3′ (575 bp). Amplification was done on a Perkin-Elmer GeneAmp PCR system 9600 (Perkin Elmer Applied Biosystems, Foster City, California, USA). The cycle numbers were 30 for GAPDH and 35 for Bcl-2, Bcl-x, and Mcl-1. PCR products were separated on 1.5% agarose gels and visualized with ethidium bromide.

Statistical analysis. Statistical differences were determined by paired Student's *t*-test. A *P* value of more than 0.05 was considered statistically significant. Results are expressed as mean ± SD. Pearson's correlation coefficient was used for evaluating putative correlations between the degree of apoptosis and the expression of FcεRI on monocytes.

Results

Cross-linking of FcεRI, but not FcεRII/CD23, prevents apoptosis induced by serum starvation in normal human monocytes. First, flow cytometric analysis of FcεRI expression on freshly isolated monocytes (>90% CD14-positive) from nonatopic donors revealed a variable expression, ranging from 2.2% to 40.0% with a mean of 11.6% ± 11% (n = 12). Interestingly, whereas a spontaneous downregulation of FcεRI expression was observed, this phenomenon was further accelerated upon receptor ligation (Figure 1).

In a first approach to induce apoptosis, monocytes were cultured for various time periods without serum, and the proportion of apoptotic cells was assessed by flow cytometry using annexin V– and PI-stainings (Figure 2a). The results were confirmed by agarose gel electrophoresis of fragmented DNA (Figure 2b). Immediately after isolation, apoptotic cells were hardly detectable by either of these techniques, whereas apoptotic monocytes could be observed after 4 hours of culture with increasing proportions over time (data not shown). After 24–48 hours without serum, most of the monocytes underwent apoptosis (Figure 2a, top panels). There was no statistically significant correlation between FcεRI expression and the number of apoptotic cells at 24 or 48 hours (P = 0.2325; r = 0.299). In contrast, pretreatment by FcεRI ligation with anti–FcεRIα 22E7 and goat anti-mouse IgG F(ab)2 (GaM-IgG) markedly reduced spontaneous apoptosis induced by serum deprivation (Figure 2, a and b [lanes 6 and 7]). This rescue effect was dose dependent and sig-

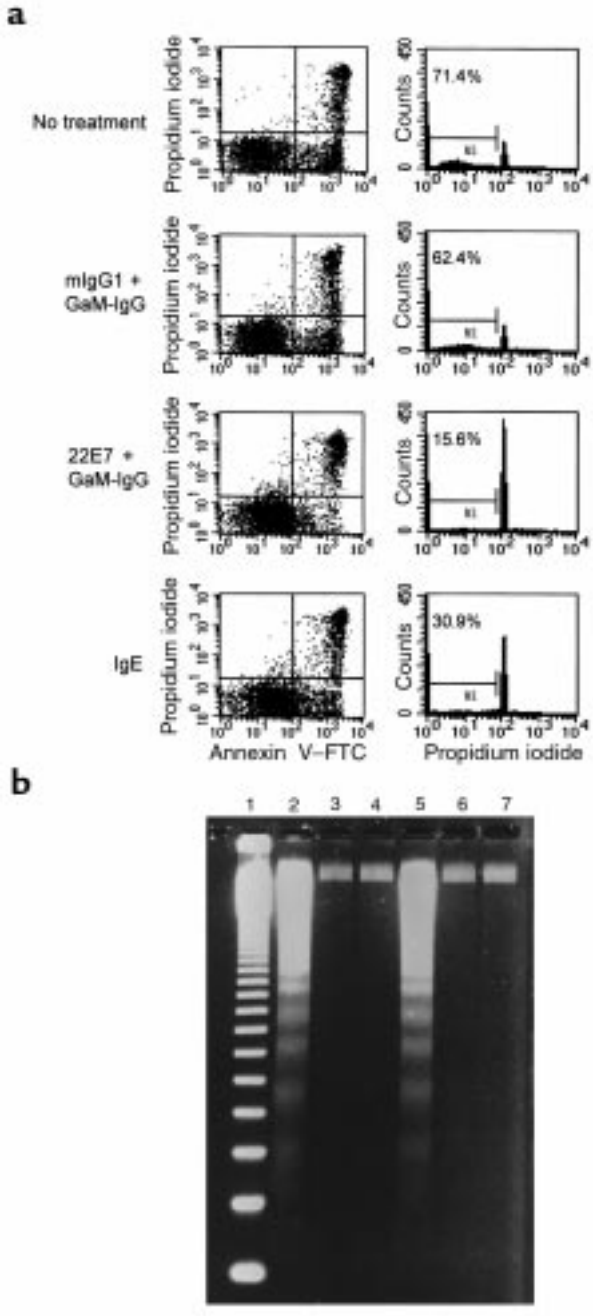

Figure 2
FcεRI mediates survival signals in human monocytes. Monocytes cultured for 24 hours were analyzed for the degree of apoptosis. (**a**) Flow cytometric analyses of annexin V expression (left) and PI staining (right) reveal that treatment with 22E7 mAb and GaM-IgG or hIgE prevents monocytes from apoptosis. Each graph represents the results of 1 of 6 separate experiments that produced similar results. (**b**) Electrophoresis of low–mol wt DNA isolated from monocytes without any treatment (lane 2), hIgE and RahIgE Ab (lanes 3 and 4), mIgG1, GaM-IgG (lane 5), and 22E7 and GaM-IgG (lanes 6 and 7); lane 1 shows the 123-bp size marker.

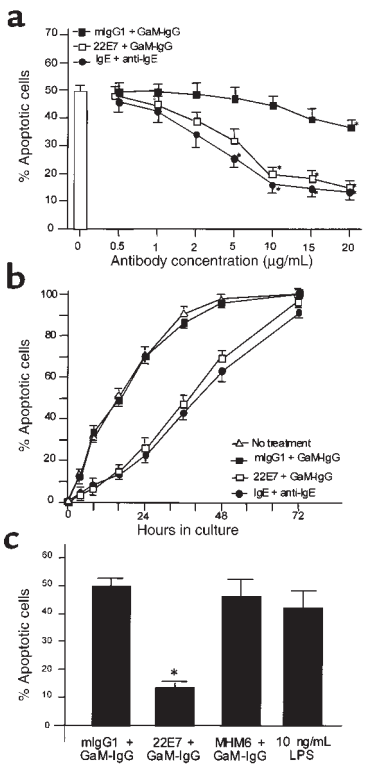

Figure 3
Dose dependency (**a**), kinetics (**b**), and specificity (**c**) of prevention of monocyte apoptosis by cross-linking of FcεRI. Percentage of apoptotic cells (*y*-axis) was evaluated by PI-staining and flow cytometric analysis. (**a**) Monocytes (2×10^6/mL) were cultured for 15 hours after activation with the indicated concentration of Abs. (**b**) Monocytes (2×10^6/mL) were treated with mIgG1 or 22E7 followed by incubation with GaM-IgG, or hIgE and RahIgE Ab as described in Methods for the indicated period of time. (**c**) Monocytes (2×10^6/mL) were treated with mIgG1, mAb 22E7, or mAb MHM6 for 30 minutes, washed, and then incubated with GaM-IgG for 15 hours (*$P < 0.05$). Alternatively, monocytes were cultured in the presence of 10 ng/mL LPS for 15 hours. Data are expressed as the mean ± SD of data from four experiments in triplicate.

nificant up to 48 hours (Figure 3, a and b). More importantly, the number of rescued cells was always higher than that of FcεRI-expressing cells. Furthermore, this phenomenon was independent of the monocyte isolation method, i.e., whether cells were isolated by adhesion or density gradient centrifugation.

As monocytes may also express the low-affinity IgE receptor FcεRII/CD23, the cells were similarly incubated with the anti-CD23 mAb MHM6 and GaM-IgG, but no rescue was observed (Figure 3c). This confirms that the observed effect is restricted to FcεRI. The use of a control antibody had no effect on excluding the possibility of a rescue resulting from exogenous protein (Figures 2, a and b [lane 5], and 3). Most monocytes underwent apoptosis 72 hours after receptor ligation.

Similarly, rescue from apoptosis occurred when monomeric human myeloma IgE (hIgE) and anti-IgE Ab were used for aggregation of FcεRI (Figures 2b [lanes 3 and 4], and 3, a and b). However, incubation with 10 μg/mL filtered (cut off 300 kDa) monomeric hIgE alone also partly, but significantly, reduced monocyte apoptosis (Figure 2a, bottom panels) suggesting that the binding of monomeric IgE by itself may rescue monocytes from cell death.

As a control, and to further exclude the possibility of contaminating LPS having caused the observed rescue from apoptosis (3), the cells were incubated with 10 ng/mL LPS (approximately 1,000-fold higher than found in our reagents) in our system containing 100 μg/mL polymyxin B. This did not inhibit the spontaneous apoptosis (Figure 3c), therefore implying that the effect observed herein was not due to LPS contamination.

Protective effect of FcεRI ligation on the CD95/Fas-induced apoptosis on monocytes. In a second approach, we asked whether FcεRI ligation may affect the anti-CD95/Fas–induced apoptosis. For this purpose, CD95/Fas expression was monitored after FcεRI ligation. In contrast to FcεRI (see above), CD95/Fas expression (which spontaneously disappeared in culture) was not significantly affected by the receptor cross-linking (Figure 4a). This excludes putative indirect effects resulting from modulation of CD95/Fas expression. Then, the putative protective effect of FcεRI ligation on CD95/Fas-induced apoptosis was investigated. Preliminary experiments showed an increased apoptotic rate using the anti-CD95/Fas mAb DX2 or Fas-Ligand in the absence of serum. However, these reagents failed to exhibit significant and reproducible pro-apoptotic effects on monocytes in the presence of serum (data not shown). Therefore, apoptosis was induced on freshly isolated monocytes using the anti-CD95/Fas mAb CH11. Under these conditions, an increased apoptosis rate was measurable upon CD95/Fas ligation. This phenomenon was almost completely neutralized by pretreatment for 1 hour with IgE/anti-IgE. When both CD95/Fas- and FcεRI ligations were performed simultaneously, the protective effect was less pronounced but still significant ($P < 0.05$) (Figure 4b). Thus, our findings strongly suggest that signals initiated by FcεRI ligation are able to induce antiapoptotic mechanisms in monocytes.

Upregulation of Bcl-2 family expression on FcεRI ligation on monocytes. Bcl-2 is known as a cell death antagonist; and recently proteins with homology to Bcl-2, such as the Bcl-xL isoform and Mcl-1, have been shown to

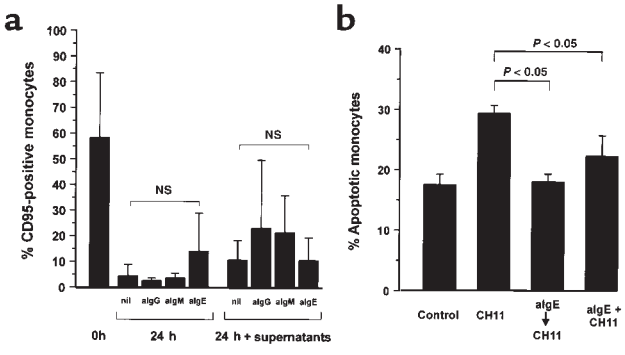

Figure 4
Protective effect of FcεRI ligation on CD95/Fas-induced apoptosis on monocytes. (**a**) Expression of CD95/Fas is not affected by FcεRI ligation. Monocytes were prepared and cultured for 24 hours as described in Figure 1. The expression of CD95/Fas was monitored by flow cytometry. (**b**) Apoptosis was induced in monocytes by the agonistic anti-CD95/Fas mAb CH11 for 18 hours in the presence of serum. Pretreatment for 1 hour with IgE and anti-IgE before anti-CD95/Fas addition (aIgE → CH11) lead to an almost complete inhibition of the apoptotic rate, whereas the simultaneous addition of both anti-IgE and CH11 (aIgE + CH11) resulted only in a partial but significant reduction ($P < 0.05$; $n = 3$) of apoptosis in monocytes.

exhibit a similar function, whereas the Bcl-xS isoform has the opposite effect (26–28). In transgenic mice expressing human Bcl-2 in monocytes, these do not undergo apoptosis in the absence of serum and M-CSF (2). Thus, we examined whether the expression of Bcl-2 family proteins was affected by FcεRI aggregation. After FcεRI ligation, monocytes were cultured for 24 hours in the absence of serum and then stained for intracellular Bcl-2 family proteins for flow cytometric analysis. Cross-linking of FcεRI significantly enhanced the expression of Bcl-2 and Bcl-x in monocytes compared with treatment with the isotype control (Figure 5). Furthermore, because brefeldin A (a cytokine exocytosis blocker) did not alter this phenomenon, we assume that the upregulation of Bcl-2 molecules is an event directly linked to receptor ligation (data not shown). RT-PCR studies (data not shown) were performed to determine whether either Bcl-xL or Bcl-xS was responsible for anti–Bcl-x staining (28). This revealed that the antiapoptotic Bcl-xL was the predominant isoform over the apoptotic Bcl-xS isoform. These experiments also could confirm Bcl-2 and Mcl-1 expression at the transcriptional level. Mcl-1 levels were not affected by FcεRI ligation. Incubation of monocytes with culture supernatant of FcεRI-activated monocytes (Sup-Fc) showed an increase of Bcl-2 and Bcl-x expression, but the degree of upregulation was much weaker than the one obtained by FcεRI aggregation (data not shown). These data suggest that FcεRI aggregation directly induces upregulation of Bcl-2 and Bcl-xL, which contributes to an intracellular antiapoptotic pathway.

Role of soluble factors produced after cross-linking of FcεRI on monocytes. In our experiments, the percentage of FcεRI-expressing monocytes was always lower than the percentage of monocytes rescued from apoptosis by receptor ligation. However, we could find no correlation between the percentages of FcεRI expression and of rescued cells. This result suggests that some soluble factors produced by monocytes after receptor aggregation might also rescue them from apoptosis. To test this hypothesis, monocytes were cultured with supernatant of FcεRI-activated monocytes (Sup-Fc). Under these conditions, the CD95/Fas expression was not significantly affected (Figure 4a). A significant decrease in apoptotic cells was observed in monocytes cultured with Sup-Fc compared with control antibody–treated supernatant (Figure 6, Sup-iso). The effect of Sup-Fc on monocyte survival was dose dependent (data not shown) and blocked when monocytes were pretreated with brefeldin A (data not shown).

Proinflammatory cytokines such as TNFα, IL-1β, and GM-CSF inhibit monocyte apoptosis (3, 7), and aggregation of FcεRI induces production of these cytokines by monocytes (18). We also suggested that FcεRI ligation could induce production of IL-12 and/or IL-18. These cytokines are known to upregulate IFNγ in T and/or B cells, which in turn could mediate prevention of apoptosis in monocytes (29–31). Using the ELISA technique, we found that FcεRI ligation induced moderate amounts of IL-12 (88 ± 22 pg/mL in unstimulated vs. 1,236 ± 353 pg/mL in stimulated cells). However, production of IL-18 was much lower (24 ± 12 pg/mL in unstimulated vs. 82 ± 24 pg/mL in stimulated cells) than the concentrations reported to induce IFNγ in target cells (28, 30), so only IL-12 was included in the experiments. To elucidate whether they contributed to the effect of Sup-Fc on monocyte survival, we precipitated IL-1β, TNFα, GM-CSF, and IL-12 with specific antibodies or isotype-matched control Ab followed by protein G–Sepharose incubation before adding them to the monocyte cultures. The incidence of apoptosis was restored to a degree comparable with control cultures when TNFα was eliminated from Sup-Fc, whereas treatment with anti–IL-1β,

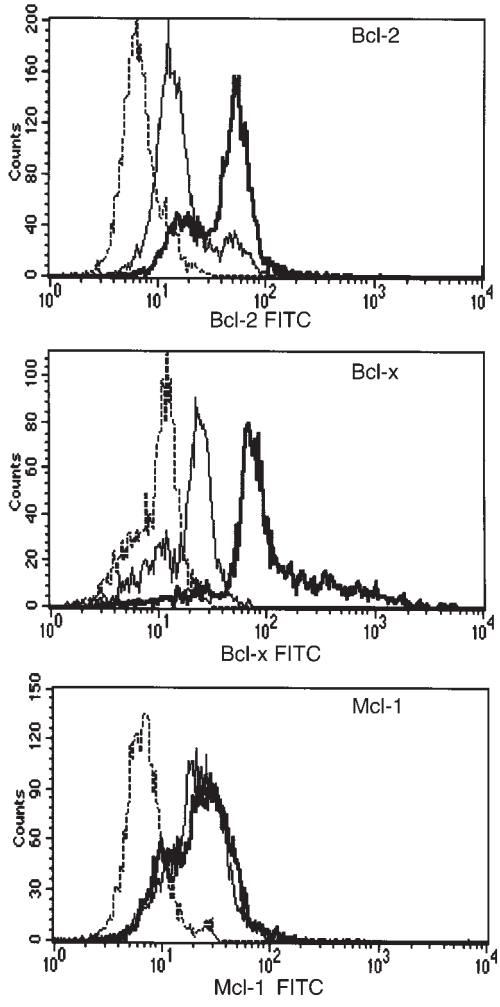

Figure 5
FcεRI ligation leads to upregulation of Bcl-2 proteins in monocytes. Monocytes were incubated with mIgG1 (thin line) or 22E7 (thick line) at 37°C for 30 minutes, washed, and then incubated with GaM-IgG for 24 hours at 37°C and 5% CO_2. Staining for Bcl-2, Bcl-x, Mcl-1, or control antibody (dotted line) was performed as described in Methods. Each panel represents 1 representative of 4 separate experiments that produced similar results.

anti–GM-CSF mAb, or anti–IL-12 did not significantly alter the effect of Sup-Fc on monocyte survival (Figure 6).

Discussion

AD is a chronic inflammatory skin disease resulting from complex pathophysiological events mainly dominated by elevated serum IgE levels; and it can be associated with rhinoconjunctivitis and/or asthma (32, 33). Thus, IgE and its receptors are thought to play a key role in this condition. Because AD is clinically and immunohistologically an eczematous reaction, i.e., a cell-mediated hypersensitivity reaction, it has been proposed that FcεRI-expressing antigen-presenting cells (APC), e.g., DC and monocytes/macrophages, may be involved in the initiation and/or perpetuation of this disease (9, 11, 34). In the present report, we demonstrate: (i) FcεRI ligation on monocytes leads to rescue from apoptosis; (ii) this effect can be achieved partly by binding of monomeric IgE; (iii) cross-linking of FcεRI increases the expression of antiapoptotic Bcl-2 family genes; and (iv) FcεRI-induced TNF-α secretion contributes to the rescue in a paracrine fashion.

A putative intrinsic defect in monocytes of AD patients has been suspected to play a role in its pathogenesis. Whereas increased activity of phosphodiesterase IV (35) leads to increased prostaglandin E2 production by monocytes, more recently a prolonged survival of monocytes in response to autocrine GM-CSF secretion was documented (7); both phenomena represent crucial aspects in the immunobiology of these cells in AD. The finding that receptor ligation on monocytes from nonatopic individuals leads to induction of antiapoptotic mechanisms may explain why monocytes from patients with severe AD who express high levels of FcεRI may have been activated in vivo. They therefore display this apparent apoptosis dysregulation either by the mechanisms described herein or by other yet-to-be-defined priming events.

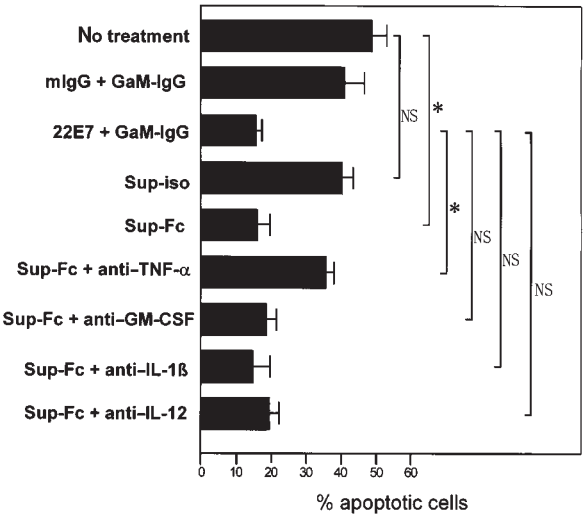

Figure 6

Culture supernatant of FcεRI-aggregated monocytes (Sup-Fc) protects monocytes from apoptosis. The preventing effect of Sup-Fc is blocked by precipitation of TNFα from Sup-Fc before addition to the cultures, but not by precipitation of GM-CSF, IL-1β, and IL-12. The percentage of apoptotic cells was evaluated by PI staining and flow cytometric analysis (*$P < 0.05$; NS, not significant). Data are shown as the mean ± SD from 4 experiments in triplicate.

We observed an induction of Bcl-2 family genes in monocytes by FcεRI ligation. This effect was not due to indirect pathways because it was not blocked by brefeldin A, and the proportion of cells displaying an increased Bcl-2 expression was lower than the one of rescued monocytes. This is of particular interest because we have recently been able to show that FcεRI ligation activates the transcription factor NF-κB in human APC including monocytes (S. Kraft and T. Bieber, manuscript in preparation). When we take into account recent observations showing a major antiapoptotic role for NF-κB activation (36), it is tempting to speculate about an involvement of this signal transduction pathway in the rescue phenomenon observed above. Recent studies show that the promoter regions of Bcl-2 family genes contain NF-κB sites (37), and, indeed, survival mechanisms mediated by NF-κB and consecutive upregulation of Bcl-2 family proteins have been reported (38, 39). Studies elucidating a putative role of NF-κB in FcεRI-mediated rescue from apoptosis in monocytes are currently in progress. Unlike Bcl-2 and Bcl-x, Mcl-1 is more likely found to be expressed in more differentiated cells (40). This may explain why this particular member of the Bcl-2 family is not increased when ligating FcεRI in monocytes. Whether Mcl-1 plays a role in the survival of monocyte-derived dendritic cells remains to be determined.

In addition to the direct mechanisms, we provide evidence for autocrine and paracrine regulation of monocyte cell death. In previous experiments, we have shown that normal monocytes produce the proinflammatory cytokines IL-1β, IL-6, IL-8, and TNFα upon FcεRI ligation (18). Above all, our results suggest that TNFα produced by monocytes under these conditions contributes to rescue both FcεRI-positive and FcεRI-negative monocytes. This explains our observation that the proportion of surviving cells was higher than the FcεRI-expressing cells. In contrast to our findings, the survival of unstimulated monocytes has been attributed to GM-CSF in patients with severe AD (7). That eliminating GM-CSF from the supernatant of FcεRI-activated monocytes did not abrogate the rescue indicates once more that, in contrast to normal monocytes, monocytes from patients with severe AD may have experienced other priming signals in vivo, signals distinct from those described herein. In addition, other cytokines like IL-1β or IL-12 seem to have no effect. However, this is the first observation to show that FcεRI ligation in APC can induce IL-12 production. It is well established that APC-derived signals can influence T helper cell differentiation, e.g., APC-derived IL-12 is able to induce a Th1 phenotype (41). Therefore, one could hypothesize an involvement of FcεRI-induced signals like IL-12 in the chronification of

AD lesions, which are characterized by a switch from a Th2 to an IFNγ-producing Th1 phenotype sustaining the inflammatory reaction (42).

Monocytes express variable amounts of FcεRI, but it has been suggested that the highest expression is associated with AD (8). Because these cells are considered to be the precursors of tissue macrophages and some subtypes of DC (5), increased survival of monocytes may explain why lesional skin of AD harbors high amounts of IgE-bearing macrophages (43) and DC (44, 45). Our observation strongly suggests that cross-linking of FcεRI-bound IgE with environmental allergens (e.g., dust mites and pollen), or autoantibodies to epidermal proteins (46) may activate monocytes and enhance their survival. However, because these types of antigens are rarely encountered in vivo by monocytes in peripheral blood, other mechanisms implicating FcεRI ligation are more likely to interfere with monocyte apoptosis. In this regard, binding of monomeric IgE or anti-FcεRI mAb F(ab) to FcεRI has been shown to enhance the expression of adhesion molecules or proinflammatory cytokines (17, 18). Borish et al. (17) pointed out that this phenomenon might be caused by contaminating agglutinated IgE, so we applied filtered hIgE to monocytes to reduce agglutinated IgE molecules and to enrich monomeric hIgE (190–200 kDa). Thus, activation of FcεRI resulting from contamination with agglutinated IgE is rather unlikely in our system. Although the mechanisms by which ligation of FcεRI with hIgE activates monocytes are unknown, we can not exclude that the binding of monomeric hIgE may alter the sterical conformation of FcεRI (47), which results in signals activating monocytes and in the rescue from apoptosis. Alternatively, the presence of significant amounts of IgE/anti-IgE complexes has been reported in patients with AD (48). Hence, such complexes may aggregate the receptor on circulating monocytes in vivo and contribute to their activation and subsequent prolonged survival.

On effector cells of anaphylaxis (i.e., mast cells and basophils), FcεRI is mainly responsible for the release of preformed and newly synthesized mediators by these cells. In contrast, the function of this receptor on APC is not yet clear. Aside from the obvious role in antigen capture, we discovered a new and unexpected involvement of this receptor in the regulation of monocyte longevity. Furthermore, because monocytes are the precursors of macrophages and tissue DC, it can not be excluded that FcεRI activation on monocytes from atopic individuals preferentially drives the cells into one or the other direction and may thus contribute to perpetuate the inflammatory reaction. Hence, controlling FcεRI-mediated monocyte survival may represent a future strategy for the resolution of chronic AD lesions.

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