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*J Immunol* 2006; 176:2272-2278; doi: 10.4049/jimmunol.176.4.2272

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Human Mast Cells Undergo TRAIL-Induced Apoptosis

Beata Berent-Maoz,2* Adrian M. Piliponsky,2* Isabelle Daigle,† Hans-Uwe Simon,‡ and Francesca Levi-Schaffer3*

Mast cells (MC)4 play a key role in allergy and are important contributors to other inflammatory conditions in which they undergo hyperplasia. In humans, stem cell factor (SCF) is the main regulator of MC growth, differentiation, and survival. Although human MC numbers may also be regulated by apoptotic cell death, there have been no reports concerning the role of the extrinsic apoptotic pathway mediated by death receptors in these cells. We examined expression and function of death receptors for Fas ligand and TRAIL in human MC. Although the MC leukemia cell line HMC-1 and human lung-derived MC expressed both Fas and TRAIL-R, MC lines derived from cord blood (CBMC) expressed only TRAIL-R. Activation of TRAIL-R resulted in caspase 3-dependent apoptosis of CBMC and HMC-1. IgE-dependent activation of CBMC increased their susceptibility to TRAIL-mediated apoptosis. Results suggest that TRAIL-mediated apoptosis may be a mechanism of regulating MC survival in vivo and, potentially, for down-regulating MC hyperplasia in pathologic conditions. The Journal of Immunology, 2006, 176: 2272–2278.

Mast cells (MC) play a key role in allergy and are involved in several chronic inflammatory diseases. Furthermore, they are involved in innate immunity and in tissue repair (1). Although MC are believed to be long-lived cells whose numbers are relatively constant (2), MC hyperplasia is observed in certain disease states (3).

The regulation of MC numbers, as of any other normal cells, depends on both their generation rate and survival time within tissues. Many factors regulate MC viability. The main survival factor for human MC is stem cell factor (SCF), whereas survival of murine MC is regulated by both SCF and IL-3 (4). In the absence of these factors, MC undergo apoptosis (5, 6). Activation status also influences MC survival. FceR1 cross-linking as well as monomeric IgE prevent apoptosis of murine bone marrow-derived cultured MC (BMCMC) (7, 8). MC survival is also regulated by anti-apoptotic proteins of the Bcl-2 family such as Bcl-2 and Bcl-xL (7, 9–11). In the “extrinsic pathway,” apoptosis is mediated by death receptors that are members of the TNF-R superfamily and share a characteristic cytoplasmic death domain. Death receptors such as Fas (CD95/APO-1) and TRAIL-R1 (DR4) or TRAIL-R2 (DR5) are activated by their natural ligands, which belong to the TNF family (12).

Fas is expressed on the murine MC cell lines C57 and MCP-5, on BMCMC, and on rat peritoneal MC (13). However, only C57 cells undergo Fas-mediated apoptosis, whereas the other cell lines appear to be resistant (13).

Currently, little is known about expression and functionality of death receptors on human MC and their potential role in MC biology. Here we investigated whether human MC (cord blood-derived MC (CBMC), human lung-derived MC (HLMC), and HMC-1) express functional death receptors, and the influence of IgE-dependent and -independent activation on death receptor-mediated apoptosis.

Materials and Methods

Cells

CBMC and HLMC were obtained and cultured as described (14). For the experiments, CBMC were resuspended in supplemented MEM-a (10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM l-glutamine, 10 μg/ml ribonucleases; all obtained from Biological Industries) containing SCF at a concentration of 100 ng/ml (a generous gift from Amgen) (MEMa). The MC leukemia line HMC-1 was provided by Dr. J. H. Butterfield (Mayo Clinic, Rochester, MN). The cells were maintained in IMDM (Biological Industries) as previously described (15). The human T cell lymphoma Jurkat line was maintained in RPMI 1640 (Biological Industries) as described (16).

FACS analysis

For FACS analysis, cells (1 × 10⁶ per milliliter) were processed as described (17). Primary Ab and working concentrations were as follows: FITC-conjugated monoclonal mouse anti-human Fas (20 μg/ml, clone DX2; R&D Systems); anti-human TRAIL-R1, -R2, -R3, and -R4 (10 μg/ml) or a mix containing recombinant human soluble TRAIL tagged with FLAG (TRAIL-FLAG, 1.2 μg/ml) and mouse anti-human FLAG (used as an enhancer at 12 μg/ml) (all from Alexis); and mouse IgG1 isotype control (diluted to 1/10; from DakoCytomation). Secondary Ab and working concentrations were: PE/FITC-conjugated goat anti-mouse IgG1 Ab (50 μg/ml; BioSource International) or FITC-conjugated mouse IgG1 Abs (1/200; purchased as an isotype control from Ancell). Stained cells were analyzed on a FACScalibur flow cytometer (BD Biosciences). For each staining, data from at least 10,000 events were collected. Data analysis was performed using CellQuest software (BD Biosciences).

RT-PCR

RNA was isolated from 5 × 10⁶ cells/sample using a commercial kit (RNaseasy Mini Kit; Qiagen). First-strand cDNA synthesis was catalyzed by Super Script II RNA-reverse transcriptase (Invitrogen Life Technologies).
using oligo(dT)_{2-12}, primer, according to the manufacturer’s instructions. PCR amplification for TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4, and for β-actin was performed using published primers and cycles (17–19). Expected sizes of the amplification products were as follows: TRAIL-R1 (506 bp), TRAIL-R2 (502 bp), TRAIL-R3 (546 bp), TRAIL-R4 (418 bp), and β-actin (200 bp). For semiquantitative PCR, cDNA was diluted to 1/5, 1/20, and 1/100 before PCR amplification. Amplified products were electrophoresed on 1.5% agarose gels and stained with ethidium bromide.

**Determination of cell viability, apoptosis, and caspase-3 activity**

CBMC (1 × 10^6 per milliliter) were cultured in MEM+ in the presence of TRAIL (10–500 ng/ml) and enhancer (2 μg/ml) (TRAIL-FLAG) for the indicated times. HMC-1 were cultured with TRAIL-FLAG or mouse anti-human Fas Ab (2 μg/ml) (clone CH-11; Immunotech) with or without ionomycin (1 × 10^-6 M) for the indicated times. MC death was assessed by uptake of propidium iodide (PI, 1 μg/ml; Sigma-Aldrich) and FACS analysis. Viability and apoptosis of CBMC were evaluated by PI (1 μg/ml) and annexin V (10 μg/ml) staining, respectively, and measured cojunctly by FACS. To evaluate apoptosis in HMC-1 cells, oligonucleosomal DNA fragmentation was assessed by FACS (20). Jurkat cells were always included in these experiments as positive controls.

To analyze caspase-3 involvement in MC apoptosis, SDS-PAGE immunoblot, ELISA, and caspase inhibitors were used. For SDS-PAGE analysis, lysates (3 × 10^6 cells per sample) (21) were run on 10% SDS-PAGE, transferred to PVDF membranes (Pierce), and blotted with mouse anti-human procaspase-3 (1 μg/ml) (Oncogene). Proteins were detected with HRP-conjugated goat anti-mouse Ab (1/5000; Jackson Immunoresearch Laboratories) followed by ECL substrate (Amersham Pharmacia Biotech). To normalize the data for protein loading, the membranes were stripped and subsequently incubated with mouse anti-human actin (2 μg/ml; Santa Cruz Biotechnology) and processed for protein detection as described in SDS-PAGE analysis. Concentrations of active caspase-3 were determined in CBMC and Jurkat lysates with a human active caspase-3 Quantikine ELISA kit (R&D Systems).

HMC-1 (2.5 × 10^6) were pretreated (30 min, 37°C) with the specific caspase-3 inhibitor, Z-Asp (Ome)-Glu (Ome)-Val (Asp)-OMe-CH2F (Z-DEVD-FMK, 2 μM; Calbiochem) or with the nonspecific inhibitors: Z-Val-Ala-Asp-fluoromethylketone (Z-VAD-FMK) at a concentration of 50 μM or 6-dichlorobenzoyloxymethylketone (Z-DAKMK) at a concentration of 20 mM (from Bachem). HMC-1 viability was assessed by PI staining (PI, 1 μg/ml) and FACS analysis. The cell death inhibition was calculated as follows: cell death inhibition (percent) = (percentage of dead cells treated with TRAIL + inhibitors)/percentage of dead cells treated with TRAIL.

**CBMC activation**

For IgE-dependent activation CBMC (1 × 10^6 per milliliter, in MEM+) were incubated for 5 days with chimeric IgE anti-NP (5 μg/ml; Serotec) or with myeloma IgE (2 μg/ml; Calbiochem). For IgE-independent activation and baseline measurements, CBMC were cultured in MEM+ alone. On day 0, cells were resuspended in Tyrode buffer containing 0.1% gelatin (TG), 1.8 mM CaCl2, 0.9 mM MgCl2 (TG+), and sensitized CBMC were cultured with goat anti-mouse IgE (λ-chain specific) Ab (2 μg/ml; Southern Biotechnology), rabbit anti-mouse IgE (5 μg/ml; DakoCytomation), or with anti-FceRI mAb (clone 22E7, 2 μg/ml; provided by Dr. J. P. Kochan (Hoffman-La Roche, Nutley, NJ)). For nonimmunologic activation, MEM+-cultured CBMC were treated with compound 48/80 (5 μg/ml; Sigma-Aldrich) or with cosinophil major basic protein (MBP, 1 μM; a gift from Dr. G. J. Gleich (University of Utah, Salt Lake City, UT)) for 30 min, at 37°C. Nonactivated controls were treated with TG+ buffer only. An aliquot (1 × 10^6 cells) from each treatment well was withdrawn to assess MC activation by measuring β-hexosaminidase release into supernatants relative to intracellular β-hexosaminidase, which was measured in cell lysates as described (22). The percentage of β-hexosaminidase release was calculated as follows: β-hexosaminidase in supernatant/(β-hexosaminidase in supernatant + β-hexosaminidase in lysate) × 100%. Remaining aliquots were either incubated with TRAIL and evaluated for apoptosis, or used for RT-PCR analysis of TRAIL-R and actin expression.

**Results**

**Human MC express Fas and TRAIL-R**

Initially, protein expression levels for Fas and TRAIL-R were assessed in human MC derived from three different sources. Flow cytometry analysis revealed that TRAIL-R were expressed on the surface of HMC-1 cells, HLMC (in three of three donors examined), CBMC (eight of nine donors examined), and Jurkat cells that were used as a positive control. Fas surface protein was also detected in HMC-1 and HLMC (three of three donors examined) but not in CBMC (from all four donors) (Fig. 1). Fas expression was not induced by incubating CBMC with either anti-FceRI mAb (2 μg/ml) or with MBP (1 μM). Fas and TRAIL-R protein expression correlated with that of mRNA. Fas mRNA was detected in HLMC and HMC-1 but not in CBMC, whereas the mRNA for TRAIL-R1 and -R2 were found in all three types of MC (data not shown). When CBMC (from three donors) were analyzed (Fig. 2A), they were all found to express mainly TRAIL-R2, whereas TRAIL-R1 expression was low. TRAIL-R3 expression was variable and TRAIL-R4 was not detected in these cells. However, the mRNAs of all four receptors were detected by RT-PCR (Fig. 2B).

**Fas is not functional in human MC**

Activation of Fas by incubation with anti-Fas Ab (2 μg/ml) for up to 72 h induced HMC-1 death in only 6.0% of the cell population. Also, Fas activation did not result in HLMC death (data not shown). In contrast, 91.3 ± 1.3% Jurkat cells died when incubated with anti-Fas for 48 h, whereas only 5.1 ± 2.6% cell death was detected in untreated Jurkat cells (p < 0.001, n = 4). It has been observed that T cells treated with high doses of Ag or repetitive stimulation, die of apoptosis mediated by death receptors (23). Even a strong and prolonged stimulation of the HMC-1 with ionomycin (1 × 10^-6 M, 18 h) before Fas activation did not influence their Fas-mediated death in comparison with nonstimulated cells (data not shown). Because HLMC were limited in number and Fas was not expressed on CBMC and was found to be nonfunctional in HMC-1 and in HLMC, we decided to focus our investigations on TRAIL-R expression and function, mainly in CBMC.

**TRAIL induces death of human MC**

Incubation of either CBMC (Fig. 3A) or HMC-1 (Fig. 3B) with TRAIL-FLAG (500 and 250 ng/ml, respectively) for 48 h, reduced their viability (as determined by PI staining) (as measured by PI staining) in CBMC from 75.8 ± 2.8% to 56.7 ± 6.0% (p < 0.05, n = 5) and in HMC-1 from 83.0 ± 3.4% to 46.9 ± 12.8% (p < 0.05, n = 4). A significant decrease in HMC-1 viability was also observed after 24 h

![FIGURE 1. Expression of Fas and TRAIL-R on human MC. HMC-1, CBMC, HLMC, and Jurkat were labeled with anti-Fas-FITC or incubated with TRAIL-FLAG followed by goat anti-mouse F(ab')2-PE. Cells were analyzed by FACS. Data are representative of three independent experiments performed with CBMC and HLMC from at least three different donors.](image-url)
Although HMC-1 were more sensitive to TRAIL-FLAG than CBMC, both MC sources were relatively resistant to TRAIL-induced death in comparison to Jurkat cells (Fig. 3C) the viability of which was reduced to 5.4 ± 2.6% (p < 0.01; n = 2) after treatment with TRAIL-FLAG (100 ng/ml) for 48 h.

TRAIL induces human MC apoptosis

TRAIL-induced death of CBMC and HMC-1 was due to apoptosis. In fact, a significant percentage of CBMC underwent apoptosis (as detected by annexin V/PI staining) within 6 h of incubation with the highest concentration of the ligand (500 ng/ml; 28.7 ± 8.79% vs control 9.2 ± 0.3% (p < 0.05, n = 3)), and after 24 h with a lower concentration of the ligand (100 ng/ml; 32.5 ± 7.5% vs control 14.0 ± 4.5 (p < 0.05, n = 4)) (Fig. 4). A slight, although insignificant increase (p > 0.05) in spontaneous death of untreated CBMC was observed at 12 and 24 h in comparison with 6 h (Fig. 4).

DNA fragmentation, a characteristic feature of apoptosis in its later stages, was observed in HMC-1 treated for 24 h with TRAIL-FLAG (250 ng/ml; 17.8 ± 0.9% sub-G1 phase cells) but not in cells incubated with medium alone (3.9 ± 1.2% sub-G1 phase cells, p < 0.05, n = 3).

Role of caspase-3 in TRAIL-mediated human MC apoptosis

The role of caspase-3 in TRAIL-induced apoptosis of CBMC and HMC-1 was evaluated. Caspase-3 activation was assessed by using specific Abs against the inactive procaspase-3 in SDS-PAGE/immunoblot analysis. Reduced amounts of the procaspase-3 protein were found in CBMC and HMC-1 cells treated with TRAIL-FLAG (500 and 250 ng/ml, respectively) in comparison with the untreated cells (Fig. 5A). By densitometry analysis, a 3- and 21-fold decrease in the level of procaspase-3 was detected by ELISA in lysates of CBMC treated with TRAIL-FLAG (500 ng/ml) for 24 h in comparison to untreated cells (0.1 ± 0.1 ng/ml, n = 3; Fig. 5B). To confirm that caspase-3 was involved in TRAIL-induced death of MC, HMC-1 were preincubated with specific and nonspecific caspase-3 inhibitors before TRAIL treatment (Table I). Z-DEVD-FMK, a specific inhibitor for caspase-3, partially inhibited HMC-1 death induced by TRAIL. The effect of this inhibitor was maximal after 24 h of incubation with TRAIL (83.0 ± 8.0% death inhibition (p < 0.05; n = 3)). However, the nonspecific caspase inhibitors Z-VAD-FMK and Z-D-AMK decreased HMC-1 death with similar efficacy.
IgE-dependent activation increases TRAIL-induced MC apoptosis

Because it is known that MC activation is associated with MC survival, experiments were performed to determine whether activation influences CBMC susceptibility to TRAIL-induced apoptosis. As shown in Fig. 6, there was no significant difference in the levels of TRAIL-induced apoptosis in the activated CBMC in comparison to nonactivated CBMC within 6 h of exposure to activating agents (Fig. 6A). However, IgE-dependent (anti-IgE Ab), but not IgE-independent activation (compound 48/80), augmented CBMC sensitivity to TRAIL and enhanced apoptosis after 12 h of incubation with TRAIL-FLAG (500 ng/ml) from 27.3 ± 0.8% to 38.86 ± 5.45% (p < 0.05, n = 4) (Fig. 6B) and after 24 h from 48.0 ± 7.2% (p < 0.05, n = 4) to 58.9 ± 7.7% (Fig. 6C). At the lower concentration of TRAIL-FLAG (100 ng/ml) after 24 h, there was an increase from 32.5 ± 8.4% to 45.4 ± 10.8% (p < 0.05, n = 4) (Fig. 6C). It is important to note that MC activation, as measured by β-hexosaminidase release, was similar in both cases (18.5 ± 2.7%; p > 0.05 for IgE-dependent activation and 20.4 ± 4.8%; p > 0.05 for compound 48/80).

We hypothesized that the increase in susceptibility to TRAIL-induced apoptosis following IgE-dependent activation could be due to an up-regulation of TRAIL death receptors. Semiquantitative RT-PCR analysis of CBMC that were activated either with anti-IgE Ab or with compound 48/80 revealed that immunological activation but not compound 48/80 activation increased TRAIL-R2 mRNA expression, whereas TRAIL-R1 was not influenced (Fig. 7A). However surface TRAIL-R2 expression levels did not change as a result of activation (Fig. 7B).

Discussion

We are reporting for the first time on the expression of the death receptors for TRAIL in three different human MC sources, namely: HMC-1, HLMC, and CBMC.

Four different types of surface TRAIL-R have been described: TRAIL-R1 and TRAIL-R2 that transmit death signals, and the decoy receptors, TRAIL-R3 and TRAIL-R4 (12).

We have shown that human MC express both mRNA and protein for TRAIL-R1 and TRAIL-R2. However, we found no correlation between expression of mRNA and surface protein for TRAIL-R4 on MC in all the donors that were examined, whereas TRAIL-R3 was variably expressed. Such a discrepancy between mRNA and surface protein expression level of TRAIL receptors had been previously observed in primary and transformed myeloid cells, in resting NK and CD8+ T cells, and in eosinophils and neutrophils (24–26).

FIGURE 5. A, Role of caspase-3 in TRAIL-mediated human MC apoptosis. a, Immunoblot analysis of procaspase-3 (32 kDa) in CBMC and HMC-1 treated for 18 h with TRAIL-FLAG (500 and 250 ng/ml, respectively). b, The level of procaspase-3 was quantified by densitometry and represents fold decrease in comparison to TRAIL-FLAG-untreated cells, normalized to actin. The figure is representative of three independent experiments performed with CBMC from three different donors. B, Active caspase-3 was quantified in lysates of CBMC from at least three different donors treated with TRAIL (500 ng/ml) for 24 h, by a specific ELISA kit (see Materials and Methods).

There was no significant variability in expression levels of TRAIL-R1, -R2, and -R4 among the donors; however, expression levels of TRAIL-R3 were variable. Nevertheless, this did not result in a difference of sensitivity to TRAIL in CBMC from three different donors (data not shown). TRAIL-R3 was shown to be inefficient in human neutrophils (27). Moreover, other studies also suggest that decoy receptors might not be involved in the protection of tumor or normal cells from TRAIL-induced apoptosis (28).

Interestingly, HMC-1 and HLMC, but not CBMC, expressed Fas. The expression of Fas in HMC-1 and also in murine MC has been previously reported (13, 29). However, we were unable to induce apoptosis of either HMC-1 or HLMC by Fas. This finding is compatible with that of another study that showed that the majority of Fas-positive murine MC are not susceptible to Fas stimulation (13). In contrast, both CBMC and the leukemia-derived line HMC-1 displayed functional TRAIL receptors and their activation by TRAIL-FLAG significantly decreased the viability of these lines. As expected, the reduction in human MC viability by TRAIL was due to apoptosis that involved caspase-3 activation. This observation is very intriguing as TRAIL-R are broadly expressed on the surface of normal cells such as keratinocytes, fibroblasts, melanocytes, HUVECs, human hepatocytes, activated CD8 T cells, and NK cells (25, 30–33); yet, most of these cells are resistant to TRAIL-induced apoptosis. Although we observed a significant decrease in MC viability only after 48 h of treatment with TRAIL, a significant increase in MC apoptosis was detected as early as 6 h. Phosphatidyl serine exposure (detected by annexin V staining) on the outer membrane of cells is believed to be an
treated with TRAIL and were calculated as follows: (percentage of death cells with caspase inhibitors as evaluated by PI incorporation. Data are expressed as per-cent of inhibition of death and were calculated as follows: (percentage of death cells treated with TRAIL – percentage of death cells treated with TRAIL and inhibitors) / percentage of death cells treated with TRAIL. Data are mean ± SEM, (n = 3); *, p < 0.05 in comparison with cells that were treated only with TRAIL.

Table I. Inhibition of TRAIL-induced HMC-1 death by caspase inhibitors

<table>
<thead>
<tr>
<th>Incubation Time</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z-DEVD-FMK*</td>
<td>83 ± 11.1*</td>
<td>66.2 ± 5.3*</td>
<td>41.2 ± 7.3*</td>
</tr>
<tr>
<td>Z-VAD-FMK*</td>
<td>100 ± 0.0*</td>
<td>100 ± 0.0*</td>
<td>100 ± 0.0*</td>
</tr>
<tr>
<td>Z-D-AMK*</td>
<td>83.9 ± 11.1*</td>
<td>94.7 ± 3.0</td>
<td>56.9 ± 0.4</td>
</tr>
</tbody>
</table>

* Specific caspase-3 inhibitor.

Early event in apoptosis, whereas the loss of the membrane integrity in vitro (detected by uptake of PI), occurs much later (34).

It is noteworthy that CBMC were found to be more resistant to TRAIL than HMC-1, and that both MC types are relatively resistant compared with Jurkat cells. This is in accordance with several reports showing that human cancer-derived cell lines are more susceptible to TRAIL-induced death than normal cells (35). Indeed, in most apoptosis studies with normal cells, treatment with TRAIL has shown effectiveness at relatively high concentrations such as those we used in this study for MC. The observed relative insensitivity of human MC to TRAIL-induced apoptosis could be explained by the presence in the culture medium of an optimal concentration of SCF, which has been shown to reduce sensitivity of erythroid progenitors to TRAIL (36). In any case, the level of TRAIL-induced MC apoptosis observed in this study was comparable to that reported for other TRAIL-sensitive normal human cells such as neutrophils, in which treatment with 1 μg/ml LZTRAIL (another stable cross-linked TRAIL construct similar to TRAIL-FLAG) for 6 h induced apoptosis in 25% of the cells (27).

We assume that the main source of TRAIL in the tissues is tissue-infiltrating inflammatory cells such as T lymphocytes (37), neutrophils (38), and macrophages (39) that either express the surface form or release soluble TRAIL. However, it is difficult to estimate the physiological concentrations of TRAIL required for MC apoptosis, because to the best of our knowledge, only qualitative and not quantitative analyses of TRAIL in tissues have been performed (40, 41).

The critical event in allergic reactions is allergen-induced cross-linking of specific IgE molecules bound to FcεRI receptors on the MC surface, which triggers MC degranulation and release of inflammatory mediators. Non-IgE-mediated activation may also contribute to continued degranulation of MC during the late phase of allergic reactions (14). Little is known about the effects of MC activation on their survival, and the link between activation and human MC survival has not yet been established. To better understand the role of TRAIL-R on MC survival in an in vitro setting mimicking allergy, we examined the influence of IgE-dependent (anti-IgE Ab) and independent (compound 48/80) activation on TRAIL-induced apoptosis. We found that IgE-dependent activation, but not activation with compound 48/80, significantly increased the susceptibility of human MC to TRAIL-mediated apoptosis. However, these findings are in contrast with what was described for murine MC. IgE-dependent activation of the IL-3-dependent murine MC line MC9 increased their resistance to Fas-induced apoptosis (42), whereas activation of murine BMCMC induced the expression of the prosurvival molecule, Bcl-2 homologue A1 (10).

MC activation has been shown to induce the synthesis and release of survival factors. For instance, IgE-dependent activation prevented apoptosis of IL-3-deprived MC/9 cells by an autocrine mechanism that resulted in the synthesis of IL-3, IL-4, and GM-CSF (43).

We hypothesized that increased susceptibility of CBMC to TRAIL-induced apoptosis after immunological activation might be due to increased expression of TRAIL death receptors. It was found that mRNA of TRAIL-R2, but not that of TRAIL-R1, increased as the result of IgE-dependent activation of CBMC. This increase was not observed when CBMC were activated with compound 48/80. Our results are compatible with recent findings that IgE-dependent activation of BMCMC not only induces prosurvival mechanisms but also up-regulates proapoptotic molecules such as Bim (44). Interestingly, Fas was not up-regulated on CBMC either by FcεRI cross-linking or by nonimmunologic activation with MBP. In a separate study, GeneChip expression analysis of CBMC also showed that after IgE-dependent but not independent activation, the expression of TRAIL-R2 mRNA increased (1.2-fold), whereas expression of TRAIL-R1, TRAIL-R3, and TRAIL-R4 were unaffected (I. Bachelet and F. Levi-Schaffer, unpublished results). Moreover, Kashiwakura et al. (45) have also found that in tonsillar MC and in HLMC, IgE-dependent activation increased TRAIL-R2 gene expression level.

Surprisingly, we did not find an up-regulation in surface protein expression of TRAIL-R2 as a result of IgE-dependent activation. This would suggest that other mechanisms are involved in the increased susceptibility of human MC to TRAIL-induced apoptosis after IgE-dependent activation. The explanation for the discrepancy between TRAIL mRNA and protein expression, which was...
also observed for TRAIL decoy receptors, could be that protein expression was restricted to an intracellular location rather than to the surface.

It is noteworthy that, in our experiments, CBMC were cultured with an optimal concentration of SCF. Under physiological conditions, SCF is produced by stromal cells such as fibroblasts, epithelial cells, endothelial cells, and bronchial smooth muscle cells (46). SCF is involved in the pathogenesis of allergic airway inflammation, and its expression is increased in allergic diseases such as allergic rhinitis (46) and asthma (47). Therefore, we speculate that TRAIL-induced MC apoptosis may also occur under pathological conditions where SCF is overproduced and, as a result, could reduce or resolve MC hyperplasia.

Recent studies have shown that, in addition to its role in limiting growth of cancer cells, TRAIL also has a role in the immune regulation. It induces apoptosis of human peripheral blood lymphocytes and Th1 clones (48, 49), of “helpless” CD8+ T cells (50), and immature dendritic cells (51). TRAIL expression has been correlated with allergic inflammation in asthma (52), and TRAIL—R are expressed on eosinophils (24), where they have a prosurvival effect (24, 52). They have also been found on neutrophils (53), where their role is controversial (27, 53). In this study, for the first time, we have reported that human MC express TRAIL-R and are susceptible to TRAIL-induced apoptosis. Our data showing that human MC can undergo efficient apoptosis upon activation of TRAIL-R and FcεRI even in the presence of SCF support the notion that TRAIL-induced apoptosis may have a role in down-regulating or resolving MC hyperplasia in allergic inflammation.

Acknowledgments
We thank Prof. Leah Bellehsen for critically reading the manuscript, Dr. Ilan Bar (Department of Thoracic Surgery, Asaf Harofeh Medical Center, Zerifin, Israel) for supplying lung biopsies, and Prof. Arnon Nagler (Department of Bone Marrow Transplantation, The Chaim Sheba Medical Center, Tel-Hashomer, Israel, and Dr. David Mankuta (Department of Obstetrics and Gynecology, Hadassah University Hospital, Jerusalem, Israel) for supplying cord blood.

Disclosures
The authors have no financial conflict of interest.

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