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Rapid Communication

Human mast cells express intracellular TRAIL

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ABSTRACT

Recently we demonstrated that human mast cells (MC) express functional TRAIL death receptors. Here we assessed the expression of TRAIL on both mRNA and protein level in cord blood derived MC (CBMC) and HMC-1. The TRAIL release either spontaneous or induced by LPS, IFN- γ and IgE-dependent activation, was evaluated as well. The protein location was restricted to the intracellular compartment in CBMC, but not in HMC-1. The intracellular TRAIL was not localized inside the granules. The treatment with IFN- γ and LPS up-regulated intracellular TRAIL expression in CBMC, but did not induce its release. These *in vitro* data show that human MC can produce and express intracellular TRAIL whose location could not be altered by different stimuli.

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1. Introduction

TRAIL is a type-II membrane protein belonging to the TNF family. TRAIL can be found as a trans-membrane or soluble protein. The soluble form is generated through enzymatic shedding of the membrane-bound form or through its release in association with microvesicles [1]. Most of the studies of TRAIL biological functions have concentrated mainly on its effects on tumor growth and survival [2]. However, there is increasing evidence that TRAIL is involved in the regulation of immune responses associated with allergic and autoimmune diseases [3,4]. MC are the key effector cells of allergic inflammation but are also regulators of several kinds of other inflammatory disorders [5]. We have recently reported that human MC express TRAIL receptors and undergo TRAIL-induced apoptosis [6]. To elucidate further the significance of the TRAIL/TRAIL receptors pathway in MC and to explore the possibility of a potential autocrine regulation of TRAIL-induced apoptosis, we investigated the expression and function of TRAIL in human CBMC.

2. Materials and methods

2.1. Cells

CBMC were obtained and cultured as described in medium containing SCF at a concentration of 100 ng/ml (a generous gift

from Amgen, Thousand Oaks, CA, US) [7]. The MC leukemia cell line HMC-1 kindly provided by Dr. J.H. Butterfield (Mayo Clinic), and Jurkat T cell line [8,9] were maintained as previously described.

2.2. CBMC sensitization and activation

CBMC (1×10^6) cells/ml were sensitized for 3 days with human myeloma IgE (1 μ g/ml; Calbiochem) and challenged with mouse anti-human IgE (5 μ g/ml; Southern Biotech). MC activation was assessed by measuring β -hexosaminidase levels in both supernatants and cell lysates as described [10]. For some experiments CBMC (2.5×10^6 /ml) were cultured in the absence or in the presence of either IFN- γ (100 ng/ml, Cytolab) or LPS (100 ng/ml; Sigma-Aldrich) for 24 h. MC cultures and activation were performed at 37 °C, 5% CO₂.

2.3. Reverse transcription-polymerase chain reaction

First-strand cDNA synthesis reaction was catalyzed by Super Script II RNA-reverse transcriptase (Gibco BRL) and oligo(dT) 12–18 primer, according to the manufacturer's instructions. PCR amplification was carried out using the following primers: TRAIL: 5'-TTATTTTCCGGCCCCAGACG-3' and 5'-AGACCTGCGTGCGTGCTGATCGTG-3'; β -actin: 5'-GTACAGGGATAGCACAGCCT-3' and 5'-TCACCACTGGGACGACATG-3'. The cycling parameters were as follows: 30 cycles of 94 °C for 60 s, 58 °C for 60 s and 72 °C for 90 s followed by a primer extension period at 72 °C, 10 min.

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2.4. Flow cytometry

For surface staining MC (1×10^6 cells/ml) were incubated for 30 min with polyclonal rabbit anti-human TRAIL (5 μ g/ml) or rabbit IgG as an isotype control, followed by goat anti-rabbit FITC Ab (30 min, 4 °C). For intracellular staining, MC were fixed in 4% paraformaldehyde for 15 min, then permeabilized and blocked in PBS containing saponin (0.1%), BSA (1 mg/ml), and human serum (10%) for 30 min on ice. Cells were then incubated with rabbit anti-human TRAIL or rabbit IgG Ab (5 μ g/ml) followed by FITC anti-rabbit Ab. Cells were analyzed on a FACS Calibur system (BD Biosciences).

2.5. ELISA

Concentrations of TRAIL in supernatants and cells lysates were measured by using commercial enzyme-linked immunosorbent assay (ELISA) Duo-Set (R&D systems) according to the manufacturer's instructions. The sensitivity of the assay is 5.4 pg/ml.

2.6. Immunofluorescence

The immunofluorescent staining of HMC-1 cytopins was performed as described [11]. Anti-human tryptase mAb (Dako, 1:100), polyclonal anti-TRAIL Ab (Alexis, 1:100) or mouse IgG1 and rabbit IgG isotype controls were used as primary Abs. Goat anti-rabbit Alexa Fluor 488 (1:500, Invitrogen) together with goat anti-mouse Alexa Fluor 647 (1:500, Invitrogen) were used as secondary Abs (1 h, 4 °C).

2.7. Statistical analysis

Data are expressed as mean $\pm$ SEM of at least three independent experiments. Parametric analysis (ANOVA, followed by Tukey–Kramer post hoc test) was used to compare the effects. In both cases, a probability of <0.05 was considered statistically significant.

3. Results

3.1. TRAIL is expressed by human MC

As can be seen in Fig. 1A, resting HMC-1 and CBMC from three different donors all expressed 413-bp TRAIL PCR product as did the Jurkat T cell lymphoma line, which was used as a positive control [12]. TRAIL content in the CBMC lysates from six different donors as quantified by ELISA was found to be 28.8 ± 14.5 pg/ 1×10^6 cells.

3.2. TRAIL is not located inside the granules

The location of TRAIL in human MC was evaluated by flow cytometric analysis. TRAIL was differently expressed in HMC-1 and in CBMC. In fact the expression of surface protein was detected on HMC-1, but not on CBMC (Fig. 1B). However, both types of human MC expressed intracellular protein (Fig. 1B). To evaluate whether intracellular TRAIL is located inside the granules, confocal microscopy analysis was performed. The granules were visualized by anti-tryptase staining. No overlapping between tryptase and TRAIL staining was observed, indicating that TRAIL is located in the cytoplasm of the cells and not in the granules (Fig. 1C). This observation is in accordance with our observation that short time (30 min) IgE-dependent activation of CBMC brings the release of preformed granular mediators such as tryptase and β -hexosaminidase but does not cause TRAIL release. In fact in the ELISA assay for TRAIL the supernatants of control MC incubated with medium alone and of MC incubated with anti-IgE, following IgE sensitization, provided similar values, i.e. 0.0873 ± 0.0012 OD and 0.0898 ± 0.011 OD, respectively, and well below the sensitivity of the ELISA assay.

3.3. IFN- γ and LPS upregulate intracellular TRAIL expression in CBMC

IFN- γ and LPS have been shown to upregulate TRAIL expression in different cells [13]. We evaluated whether these agonists would influence the TRAIL expression in CBMC following 24-h culture. An increase in intracellular expression of TRAIL as evaluated by ELISA was observed in CBMC cultured in the presence of IFN- γ or LPS

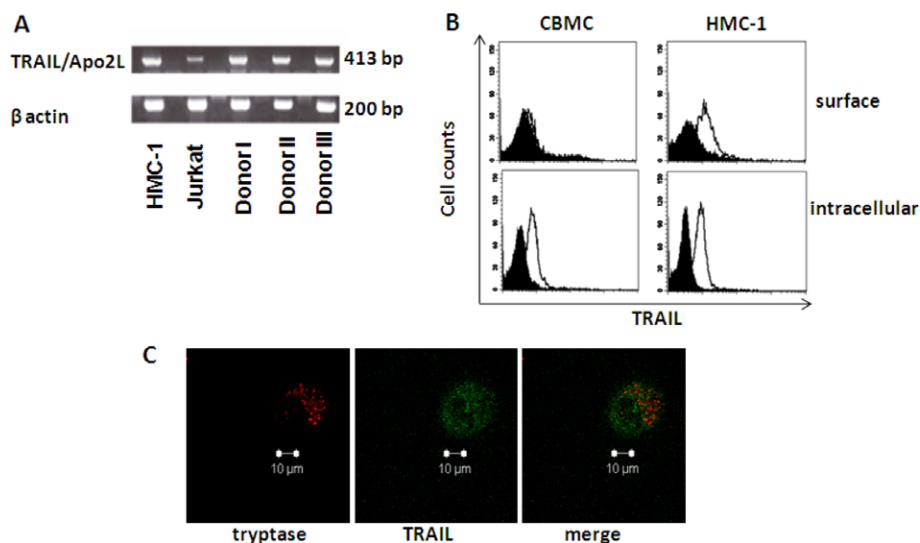


Fig. 1. Human MC express TRAIL which is not located in the cytoplasmic granules. (A) RNA from JK, HMC-1 and CBMC was isolated and first-strand cDNA synthesis was performed. The cDNA of TRAIL was amplified by PCR using specific primers. The RT-PCR products were electrophoresed in 1.5% agarose gel and visualized by ethidium bromide staining. (B) Surface and intracellular TRAIL expression was examined by FACS analysis in intact and permeabilized CBMC and HMC-1. The filled histograms represent staining with rabbit IgG and the open histograms indicate staining with rabbit anti-human TRAIL pAb. (C) TRAIL colocalization with tryptase, as a marker of human MC granules, was checked in resting HMC-1. Cells were stained using anti-human TRAIL and anti-human tryptase antibodies. Images were acquired at 1000 $\times$ original magnification.

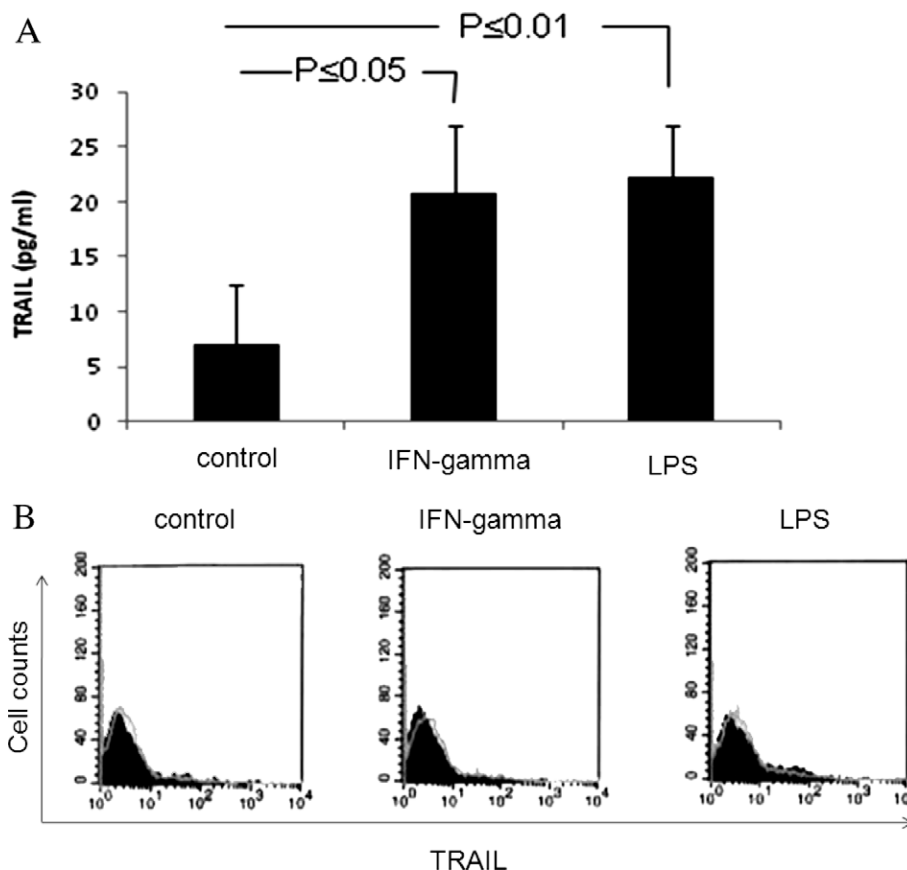


Fig. 2. IFN- γ and LPS increase intracellular TRAIL content in CBMC. CBMC (5×10^5 cells) were incubated with either medium (control), or mediator containing IFN- γ (100 ng/ml) or LPS (100 ng/ml) for 24 h. (A) Cells were lysed and intracellular TRAIL content was analyzed by ELISA. The data is the mean $\pm$ SEM of three independent experiments performed in duplicates. (B) Surface TRAIL expression in CBMC was examined by Flow Cytometry. The filled histograms represent staining with rabbit IgG and the open histograms indicate staining with rabbit anti-human TRAIL pAb. The data are representative of three independent experiments.

(Fig. 2A). However, no change in the surface protein (Fig. 2B) expression was found. Similarly, the release of soluble TRAIL was undetectable or below the sensitivity of the specific ELISA assay. The OD in the supernatants of control MC incubated in media alone and of MC cultured for 18 h with either IFN or LPS were very similar: 0.088 ± 0.001 (control), 0.093 ± 0.005 (LPS) and 0.099 ± 0.005 (IFN- γ). These OD values of control and LPS correspond to no release according to the TRAIL standard curve. The release of TRAIL after treatment with IFN- γ was 1.5 ± 1.7 pg/ml, a value that is below the sensitivity of the specific ELISA assay, which is 5.4 pg/ml.

4. Discussion

TRAIL is expressed in various human cells of the immune system such as monocytes, dendritic cells, NK cells, T lymphocytes [14], eosinophils [3], and neutrophils [15] mostly in an activation-dependent manner. We have previously reported that human MC express functional TRAIL death receptors [6]. In this study, we wanted to evaluate the expression of TRAIL by human MC. The TRAIL expression can cause apoptosis in an autocrine fashion [16] or paracrine manner, by which stimulated NK, dendritic cells, and cytotoxic T cells induce apoptosis of tumor cells [17]. We hypothesized that the expression of TRAIL by human MC can be involved in autocrine regulation of MC homeostasis and/or their role as immune effector cells by inducing death in cells of other lineages. We found that both transformed and primary MC constitutively express TRAIL. The constitutive expression of TRAIL was also previously reported in neutrophils [18]. However, in contrast to neutrophils, TRAIL location in MC was cytoplasmic rather than

granular. Therefore, it was not released upon degranulation stimuli in CBMC. Similarly to TRAIL, FasL, whose expression was reported in murine MC, was also shown to be exclusively located within the cells and was not released upon activation [19].

Various inflammatory stimuli increase TRAIL expression [13,15]. In our study we observed that IFN- γ and LPS significantly increased the level of total TRAIL in CBMC. However, these stimuli did not cause the release of intracellular TRAIL or its surface expression upregulation.

Cells of the immune system not only express TRAIL, as mentioned above, but are also susceptible to TRAIL-induced apoptosis [20]. Moreover the role of TRAIL has been demonstrated in allergy as well. TRAIL deficient mice exhibit attenuation in TH2 cytokine release, inflammation and in airway hyperreactivity in a model of allergic airways disease [4]. Similarly, TRAIL might play an anti-inflammatory role in atopic eczema [3]. The fact that only intracellular TRAIL expression was observed does not point by itself to a pathophysiological pro-apoptotic role of TRAIL in normal human MC. Nevertheless, we suppose that TRAIL release or appearance on the MC surface may be possible and probably would require a specific stimulus or perhaps a series of stimuli present in the *in vivo* milieu whose nature needs to be clarified.

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