TGFbeta1 induces mast cell apoptosis

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Norozian F, Kashyap M, Ramirez CD, Patel N, Kepley, CL, Barnstein BO, et al. TGFbeta1 induces mast cell apoptosis. *Experimental Hematology* 2006; 34:579-87.

Made available courtesy of Elsevier: https://doi.org/10.1016/j.exphem.2006.02.003

Abstract:

Mast cells are potent effectors of the inflammatory response, playing an important role in atopy, bacterial immunity, and animal models of arthritis, multiple sclerosis, and heart disease. Hence controlling mast cell numbers and responsiveness is essential for preventing inflammatory disease. We demonstrate that the cytokine transforming growth factor (TGF) β1 is a potent inducer of mast cell apoptosis, a finding that was consistent in cultured mouse bone marrow–derived mast cells, peritoneal mast cells, and human mast cells. Cell death appeared to be caused by TGF-mediated repression of interleukin-3 (IL-3) receptor expression and function, leading to mitochondrial damage and activation of an apoptotic cascade acting via p53 and caspases. Although IL-3 receptor expression was reduced within 1 day of TGFβ1 stimulation, apoptosis required at least 3 days to occur. This delay in onset is postulated to allow protective mast cell effector functions, protecting the host from infection while preventing the establishment of chronic inflammation. Our data support the theory that TGFβ1 is an inhibitor of mast cell survival. The widespread expression of TGFβ1 offers this cytokine as an ideal candidate for control of mast cell homeostasis.

Keywords: transforming growth factor | mast cells | TGFβ1 | IL-3

Article:

The transforming growth factor (TGF) β superfamily is a group of structurally related cytokines that includes TGFβ1, TGFβ2, TGFβ3, activins, and bone morphogenetic proteins. TGFβ1, the prototypic family member, is synthesized as a precursor polypeptide and secreted in a latent form by most tissues and cell types [1, 2, 3]. TGFβ family members have tissue-specific stimulatory or inhibitory effects on a variety of cellular functions that include differentiation, proliferation, and apoptosis. A number of studies have implicated TGFβ proteins in physiologic processes including inflammation, fibrosis, and angiogenesis. Importantly, TGFβ proteins have been linked to autoimmune disease, antherosclerosis, fibrotic disease, and cancer in humans [4, 5]. Studies

showing the inhibitory effects of TGFβ1 on immune cell function support the role of TGFβ1 as a suppressor of immunity and inflammation (reviewed by Blobe et al. [5]). Underscoring the importance of TGF-mediated immunosuppression is the observation that TGFβ1-null mice develop severe inflammation, wasting syndrome, and organ failure leading to death by 3 weeks of age [6, 7].

Mast cell activation results in the release of preformed vasoactive amines and de novo synthesized cytokines, chemokines, and prostaglandins that collectively induce a local or systemic inflammatory response [8]. Although mast cells are responsible for immediate hypersensitivity responses, their widespread distribution in the skin and respiratory tract suggests a role as for these cells as a first-line defense against invading pathogens. In fact, mast cells are now known to be critical for resistance to some bacterial infections [9, 10]. Despite this protective role, unregulated mast cell activation can result in deleterious effects. In fact, mast cells are often associated with human allergic disease and are implicated in mouse models of the autoimmune diseases multiple sclerosis and recently rheumatoid arthritis [11, 12]. Thus the mast cell may significantly contribute to immunity by shifting the balance of protective and pathologic responses.

We recently showed that TGFβ1 inhibited mast cell development and dampened expression and function of mast cell immunoglobulin E (IgE) receptors [13, 14]. These studies raised the possibility that TGFβ1 is a paracrine or autocrine inhibitor of mast cell function. This theory is bolstered by the widespread presence of TGFβ1, which circulates in the blood at concentrations exceeding 30 ng/mL [15]. In support of this, TGFβ1 has been shown to inhibit interleukin (IL)-3-dependent proliferation of peritoneal and bone marrow–derived mast cells (BMMCs) [16, 17]. In addition, TGFβ1 inhibited stem cell factor–mediated rescue of BMMCs from apoptosis induced by IL-3 deprivation [18]. To further our understanding of mast cell homeostasis, we directly measured the effects of TGFβ1 on mast cell survival. Our results show that TGFβ1 is a potent inducer of apoptosis in both mouse and human mast cells. These data support the supposition that TGFβ1 can dampen mast cell activity, a finding that has implications for the treatment of allergic and autoimmune disorders.

Materials and Methods

Cytokines and reagents

Murine IL-3, stem cell factor (SCF), and TGFβ1 were purchased from R&D Systems (Minneapolis, MN, USA). Human SCF was the kind gift of Amgen Corp (Thousand Oaks, CA, USA). Mouse IgE, fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse Kit, phycoerythrin (PE)-conjugated anti-mouse IL-3Rβ, and PE-conjugated anti-human Kit were purchased from BD PharMingen (San Diego, CA, USA). FITC-conjugated rat anti-mouse IgE was purchased from Southern Biotechnology Associates (Birmingham, AL, USA). Stat5 antibodies were purchased from Upstate Biotechnology (Charlottesville, VA, USA). Brefeldin A was purchased from Sigma (St. Louis, MO, USA).

Mast cell cultures

BMMCs were derived from C57BL/6 or BL6x129 mice by culture of bone marrow cells in complete RPMI medium (cRPMI), supplemented with either 25% WEHI-3 cell-conditioned medium or with IL-3 and SCF (30 ng/mL each). After 3 to 4 weeks in culture, >95% of these cells were mast cells, as judged by morphology and surface expression of FcεRI and Kit (data not shown). These cells were used within 3 months of their maturation. Peritoneal cells were harvested from euthanized animals by lavage of the peritoneum using 5 mL of cRPMI injected into the peritoneal cavity. These cells were cultured in cRPMI/IL-3 + SCF. Human skin–derived mast cell populations, obtained after informed consent and institutional approval, were derived as described previously [19, 20].

Cell viability and apoptosis

Cells were assessed for ≥ diploid (viable) or < diploid (apoptotic) DNA content by propidium iodide (PI) staining following cell fixation and permeabilization (PI-DNA staining) as described [21]. Briefly, 200 μL of cells was removed from cultures and centrifuged in a 96-well v-bottom plate for 5 minutes, then washed in phosphate-buffered saline (PBS) and fixed in 150 μL of PI fixation buffer (70% EtOH/10% fetal calf serum (FCS) in 1× PBS) for 4 hours to 7 days at 4°C. After fixation, cells were washed with PBS and incubated with PI-DNA staining buffer containing 100 μg/mL RNase A and 50 μg/mL PI for 2 to 3 hours in the dark at room temperature. Samples were analyzed by flow cytometry to determine the percentage of the population within the subdiploid DNA marker. To measure changes in mitochondrial membrane potential (ΔΨm), cells were incubated with 1 nM $Di(OC_6)_3$, purchased from Molecular Probes (Eugene, OR, USA) for 30 minutes prior to analysis by flow cytometry. Caspase-3/7 activity was measured by flow cytometry using the caspase-3/7 fluorochrome inhibitor of caspases (FLICA) assay from Immunochemistry Technologies, LLC (Bloomington, MN, USA).

Tissue culture conditions for cell viability and surface receptor expression

Cells were washed and replated at 3×10^5 cells/mL 200 μL cRPMI/well using 96-well flat-bottom plates. IL-3 was added to 5 ng/mL, followed by the indicated concentrations of TGFβ1. Cultures were incubated for the indicated times. Every 4 days half the media and cytokines were replaced. Mast cell surface antigen expression was assessed by flow cytometry as described previously [22]. The mean fluorescence intensity of IL-3Rβ or Kit staining was used to determine the percent inhibition of expression caused by the addition of TGFβ1.

RNase protection assay

RNase protection assay were performed using the RiboQuant system (BD Pharmingen) as per the manufacturer's instructions. Pixel intensities of individual bands were obtained using a Typhoon phosphorimager (Molecular Dynamics, Sunnyvale, CA, USA). The ratios of the pixel intensity for each band of interest to the sum of the pixel intensities for the housekeeping genes (L32 + GAPDH) in that lane were determined. Calculations of percent change in expression relative to control conditions were determined by comparing these ratios.

Western blot analysis

Tyrosine phosphorylated Stat5, total Stat5, and actin were detected by Western blotting of total cell lysates (approximately 50 μg/lane) as described [14]. To determine the percent change in expression, band intensity was first measured by densitometry. The ratio of tyrosine phosphorylated Stat5 to total Stat5 was determined, and ratios were compared between lanes to determine percent decrease in the presence of TGFβ1.

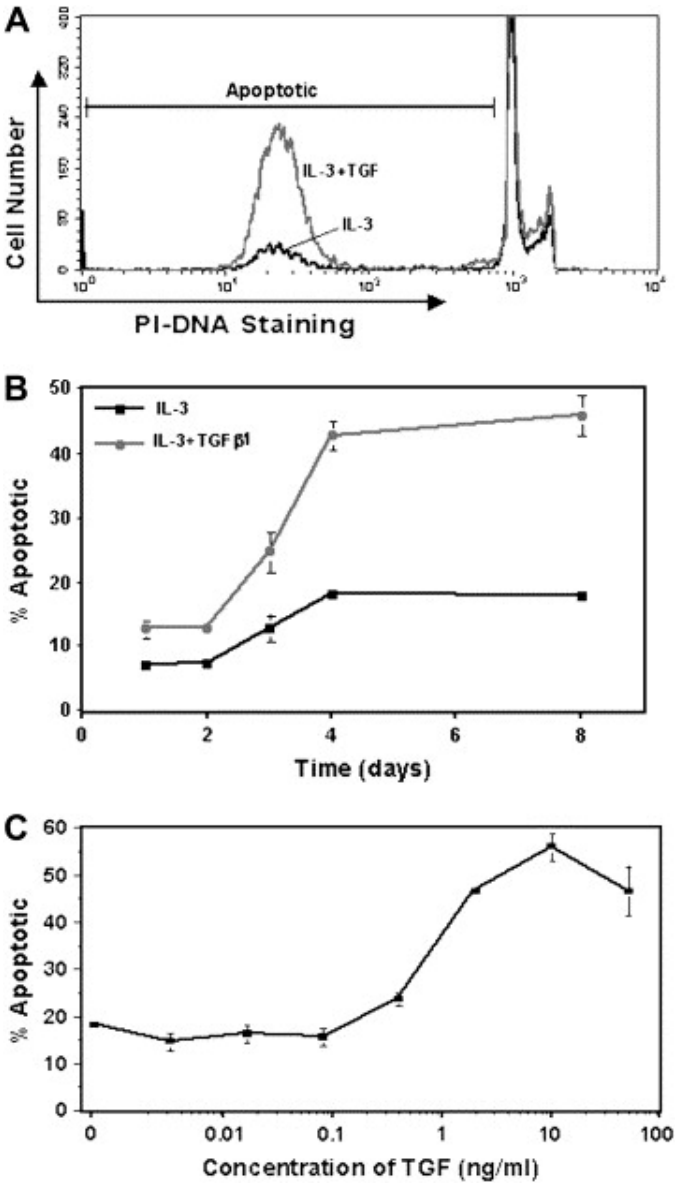

Figure 1. TGFβ1 induces mast cell apoptosis. **(A):** Mouse BMMCs were cultured for 6 days in IL-3 ± 10 ng/mL TGFβ1. Apoptosis was measured by the presence of subdiploid DNA content after PI-DNA staining, as indicated by marked region in histogram. **(B):** Time course of apoptosis. BMMCs were cultured as in part **(A)** for the indicated days, and apoptosis was measured by PI-DNA staining. Data shown are means and standard errors from at least six samples/point. **(C):** Concentration response for TGFβ1-induced apoptosis. BMMCs were cultured in IL-3 ± the indicated concentrations of TGFβ1 for 7 days. Apoptosis was measured by PI-DNA staining. Data shown are means and standard errors of six samples/point.

Results

TGFβ induces mast cell apoptosis

To determine the effect of TGF on mast cell survival, we cultured mouse BMMCs with IL-3 ± TGFβ1. BMMCs are primary, IL-3-dependent mast cells that function as a reliable model system for mucosal mast cells [23], hence the effects of TGF on this population are likely to be representative of tissue mast cells. As shown in Figure 1A, BMMCs cultured with TGFβ1 for 6 days exhibited an overt increase in subdiploid DNA content, indicating DNA fragmentation that is consistent with apoptosis. Apoptosis occurred consistently after 3 days of culture, with a peak of 40 to 50% cell death after 4 days of culture (Fig. 1B). This effect was dose-dependent, requiring approximately 2 ng/mL TGFβ1, with maximum apoptosis observed using 10 ng/mL. As stated, this is well within the range of TGFβ1 available in normal human serum [15].

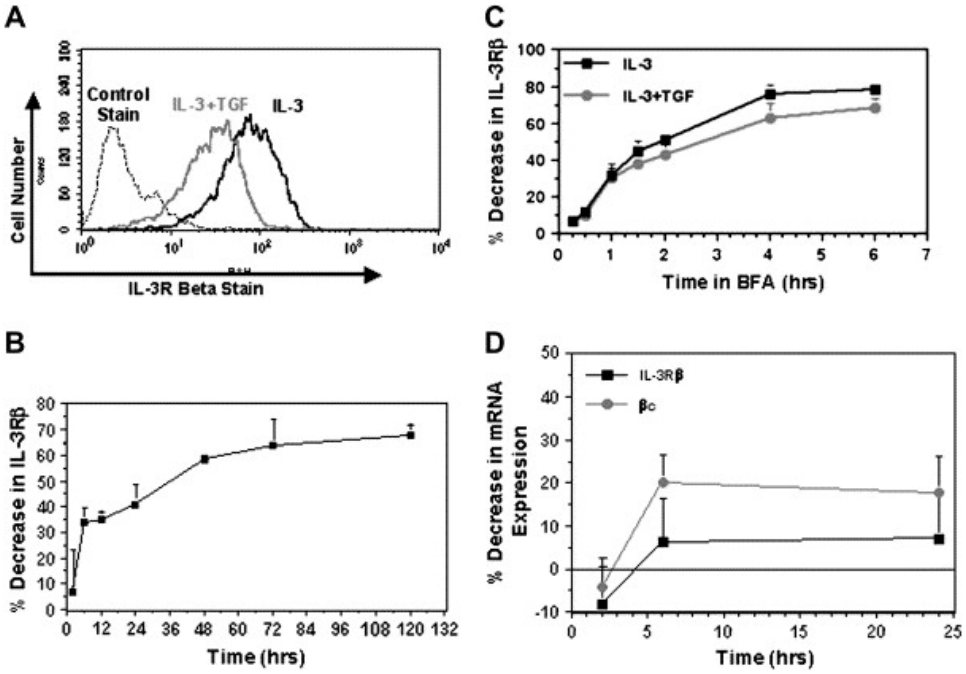

Figure 2. TGFβ1 inhibits mast cell IL-3Rβ expression. **(A):** Mouse BMMCs were cultured for 3 days with IL-3 ± TGFβ1 (10 ng/mL). Cells were analyzed for surface IL-3Rβ expression via flow cytometry. Control stain was performed with PE-coupled IgG. **(B):** Time course of TGFβ1-mediated inhibitory effects on IL-3Rβ expression. Cells were cultured for the indicated times, and percent decrease in IL-3Rβ expression was calculated by comparing mean fluorescence intensity of IL-3Rβ staining from cells cultured ± TGFβ1, as measured by flow cytometry. Data shown are means and standard deviation of at least three samples/point. **(C):** Effect of TGFβ1 on IL-3Rβ surface stability. BMMCs were cultured for 24 to 48 hours in IL-3 ± TGFβ1, after which BFA (20 μ g/mL) was added for the indicated times. The percent decrease in IL-3Rβ expression was calculated by comparing the change in IL-3Rβ expression between samples cultured with and without BFA, using flow cytometry. **(D):** Effect of TGFβ1 on IL-3Rβ mRNA expression. RNAse protection assay was used to measure IL-3Rβ mRNAs from BMMCs cultured for the indicated times in IL-3 ± TGFβ1. After normalizing to L32 + GAPDH loading controls, percent decrease in mRNA expression was calculated as by phosphorimager as described in Materials and Methods. Data shown are means and standard deviation of three samples/point.

TGFβ1-mediated apoptosis correlates with reduced IL-3 receptor expression

TGFβ1 is known to block expression of the mast cell survival receptor Kit and also inhibits Kit-mediated rescue from factor withdrawal-induced apoptosis [18, 24, 25]. Because IL-3 is the

survival factor employed in these assays, loss of its survival function seemed a plausible means by which TGFβ1 could elicit programmed cell death. To address this possibility, we measured the effects of TGFβ1 on BMMC IL-3 receptor expression by flow cytometry. The IL-3R is composed of an alpha chain paired with either an IL-3-specific β chain or a βc chain shared with IL-5 and granulocyte-macrophage colony-stimulating factor [26]. We found expression of IL-3Rα in viable cells to be below the level of detection (data not shown). In contrast, an antibody that recognizes both IL-3Rβ chains demonstrated robust expression that was significantly inhibited by TGFβ1 (Fig. 2A). Importantly, the reduction in IL-3Rβ expression preceded the onset of cell death, with 30% inhibition after 8 hours of TGFβ1 stimulation, peaking at 65% inhibition by day 3 of culture, when apoptosis was first detected (Fig. 2B). Because BMMCs die approximately 3 days after IL-3 withdrawal (our unpublished findings), the timing of TGFβ1-mediated inhibition of IL-3R expression fits well with the onset of apoptosis.

We recently demonstrated that TGFβ1 blocked mast cell FcεRI expression by reducing translational efficiency, with little effect on mRNA expression or protein stability [14]. Like FcεRI, we found that TGFβ1 had no effect on the rate of surface IL-3Rβ turnover. The addition of the vesicular transport inhibitor Brefeldin A (BFA) reduced IL-3Rβ expression at similar rates in cells cultured in IL-3 ± TGFβ1 (Fig. 2C). Also like FcεRI, TGFβ1 did not significantly alter IL-3Rβ mRNA expression, reducing βc message by only 20% at time points preceding or after the onset of IL-3R repression (Fig. 2D). These data support the theory that TGFβ1 inhibits IL-3R expression through posttranscriptional effects that, like FcεRI, alter translation efficiency. This inhibitory effect occurs with the appropriate timing to explain the onset of mast cell apoptosis.

TGFβ1-mediated IL-3R repression inhibits Stat5 activation and maintenance of mitochondrial membrane potential

If the reduction in IL-3R expression is functionally significant, it should prevent proper activation of the Stat5 pathway, which we have shown to be essential for mast cell survival [27]. To test the effects of TGFβ1 on IL-3-mediated Stat5 signaling, BMMCs were cultured in IL-3 ± TGFβ1 for 3 days, the point at which IL-3R expression reached its nadir and apoptosis was initiated. After a starvation period to remove any residual IL-3 signaling, these cells were restimulated with IL-3, and Stat5 phosphorylation was measured by Western blotting. As shown in Figure 3A, TGFβ1 reduced Stat5 phosphorylation by 45 to 50%. As we have found that Stat5 expression is necessary for maintaining mitochondrial membrane potential (ΔΨm) [27], we determined the effect of TGFβ1 on ΔΨm via $Di(OC_6)_3$ staining. As shown in Figure 3B, TGF-stimulated cells exhibited reduced $Di(OC_6)_3$ staining, shifting toward the spectrum displayed by BMMCs cultured without IL-3, a condition known to induce mitochondrial damage [28]. These results argue that TGFβ1-mediated repression of the IL-3 receptor is biologically relevant, reducing IL-3 signaling to an extent that induces mitochondrial damage.

TGFβ1-induced apoptosis requires p53 expression

Loss of IL-3 signaling has been shown to induce a p53-dependent apoptotic cascade that occurs with mitochondrial damage [29]. The effects of TGFβ1 mirror IL-3 deprivation and hence may employ the p53 pathway for programmed cell death. In fact, we found that p53-deficient (KO) BMMCs exhibited little apoptosis after culture with TGFβ1 (Fig. 4A). This p53 dependency was

confirmed by a substantial reduction in TGFβ1-mediated activation of the effector caspases-3 and -7 (Fig. 4B). These data support the theory that TGFβ1 induces mast cell apoptosis by sufficiently repressing IL-3 receptor expression to mimic IL-3 withdrawal and the p53-dependent/mitochondrial pathway that ensues from this deprivation.

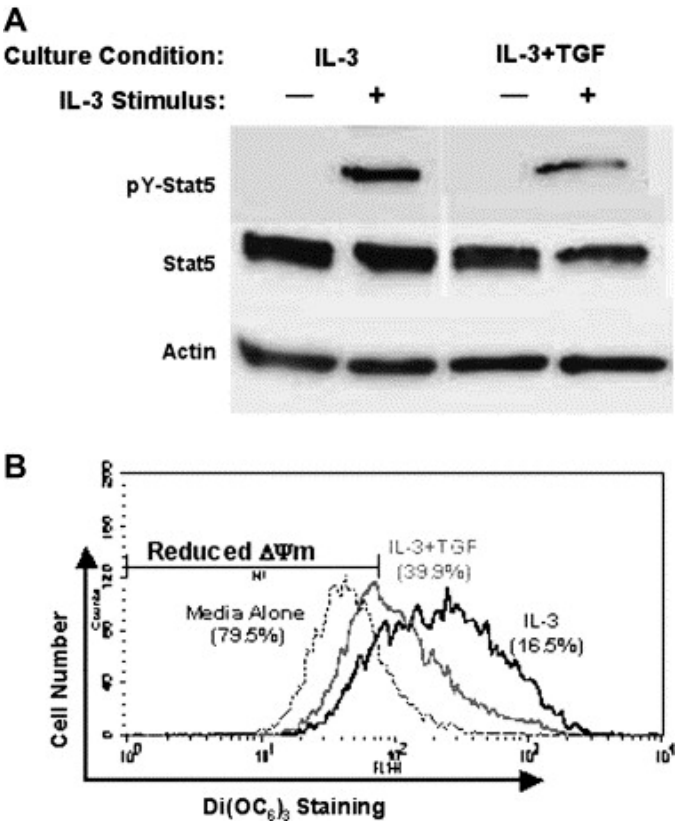

Figure 3. TGFβ1 inhibits IL-3R signaling. **(A):** BMMCs were cultured for 3 days in IL-3 ± TGFβ1, washed and incubated overnight in the same medium lacking IL-3, then restimulated with IL-3 (100 ng/mL) for 15 minutes. Total cell lysates were subjected to Western blot analysis with phosphotyrosine-specific (pY) anti-Stat5. Membrane was stripped and reprobed with anti-Stat5 and anti-actin. After normalizing to Stat5 expression via densitometry, pY-Stat5 expression was found to be reduced by 44.6% in samples receiving TGFβ1. Similar results were found in two experiments. **(B):** BMMCs were cultured for 4 days in IL-3 ± TGFβ1 or in media lacking cytokines. $Di(OC_6)_3$ staining was used to measure changes in ΔΨm, as detected by flow cytometry. Numbers in parentheses indicate the percentage of each population demonstrating reduced ΔΨm.

Factor-independent mastocytoma cells are resistant to TGFβ1-induced apoptosis

If the effects of TGFβ1 are mediated via its blockade of the IL-3 receptor, cells not requiring this growth factor should be resistant to TGFβ1. To test this, we cultured factor-independent P815 mastocytoma cells in IL-3 ± TGFβ1. These cells possess a mutant, constitutively Kit receptor that drives their continual proliferation. As shown in Figure 5, TGFβ1 stimulation for 6 days had little effect on the viability of P815 cells. We detected no significant change in subdiploid DNA content and no increase in caspase-3/7 activation. Thus these IL-3-independent mast cell tumors are completely resistant to TGFβ1-induced cell death, supporting our hypothesis that TGFβ1 acts through repression of IL-3R expression and signaling.

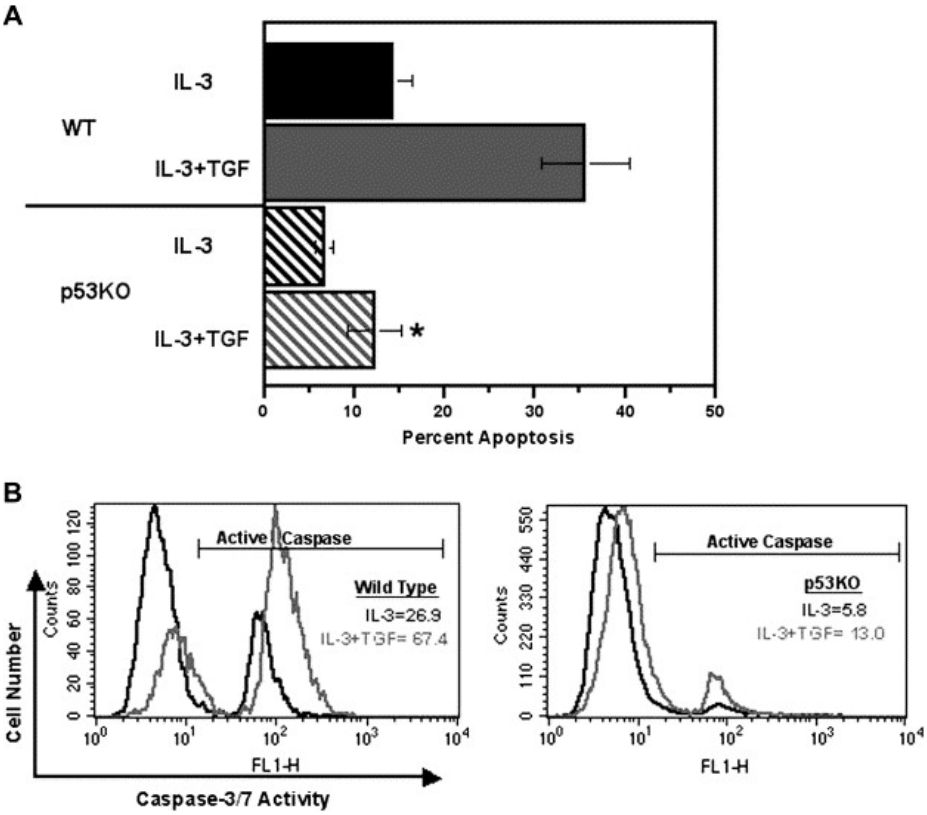

Figure 4. TGFβ1-mediated apoptosis proceeds via the mitochondrion and p53. **(A):**Wild-type (WT) and p53-deficient (KO) BMMCs were cultured in IL-3 ± TGFβ1 for 6 days, and apoptosis was measured by PI-DNA staining. Data shown are means and standard errors of at least nine samples/point. $^{*}p < 0.001$ by Student's *t*-test. **(B):**Effect of TGFβ1 on caspase-3/7 activation in WT and p53KO BMMCs. Cells were cultured for 6 days in IL-3 ± TGFβ1, and caspase activation was measured by flow cytometry as described in Materials and Methods.

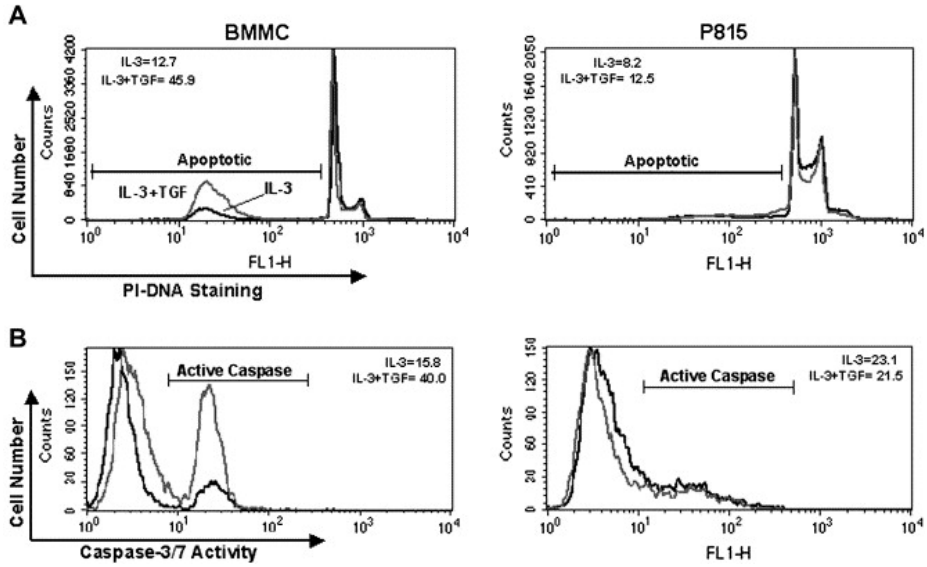

Figure 5. Effect of TGFβ1 on mastocytoma cells. BMMCs and P815 mastocytoma cells were cultured in IL-3 ± TGFβ1 for 6 days. **(A):** Apoptosis was measured by PI-DNA staining. Percentage of the population demonstrating subdiploid DNA content is indicated. **(B):** Caspase-3/7 activation was measured by flow cytometry using a cleavable substrate, as described in Materials and Methods. Percentage of each population with active caspase is indicated.

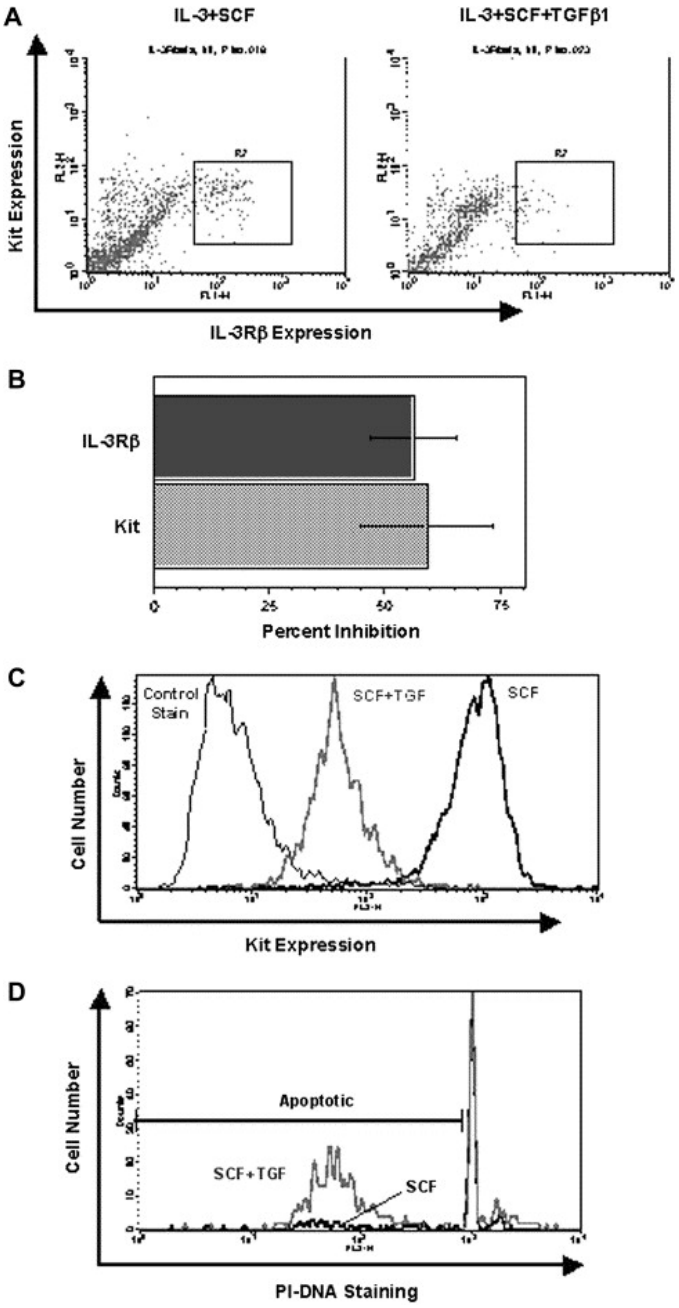

Figure 6. Effect of TGFβ1 on mouse peritoneal and human mast cells. **(A,B):** Mouse peritoneal cells were cultured in IL-3 + SCF ± TGFβ1 for 6 days, followed by flow cytometric analysis with anti-Kit and anti-IL-3Rβ. **(A):** Representative dot plot from 1 of six sample sets. **(B):** Average decrease in Kit and IL-3Rβ expression ± SD. **(C):**Skin-derived human mast cells were cultured in SCF ± TGFβ1. On day 3, surface Kit expression was measured by flow cytometry. **(D):** Apoptosis was measured in cultures described in part **(C)** on day 7, by PI-DNA staining. Data shown are representative of three independent mast cell cultures that yielded similar results.

TGFβ1 represses Kit expression and induces apoptosis in mouse peritoneal mast cells and cultured human mast cells

Although mouse BMMCs are a reliable model system for mast cell biology, their IL-3 dependency is distinct from the importance of SCF in vivo. To confirm that our findings with

BMMC cultures were consistent in other mast cell populations, we measured the effects of TGF1 on freshly isolated mouse peritoneal mast cells and cultured human mast cells, which rely upon SCF for survival and proliferation signals. Unseparated mouse peritoneal cells were used to mimic the effects of TGF in the context of the peritoneal population. These cells were cultured for 6 days in IL-3 + SCF ± TGFβ1. Mast cells were examined by gating on the Kit+ population (Fig. 6A). As shown in Figure 6A and B, TGFβ1 reduced the expression of both IL-3Rβ and the SCF receptor, Kit, nearly 60%. We also noted that the number of mast cells in these cultures was reduced 80.5% (SD = 14.2, n = 6). Human skin–derived mast cell populations (HSMCs) also confirmed the inhibitory effects of TGFβ1. By day 3 of culture in SCF + TGFβ1, HSMCs showed greatly reduced Kit expression (Fig. 6C). This effect mimicked our observations with the mouse IL-3R. Further, TGFβ1 induced human mast cell apoptosis, as judged by the presence of subdiploid DNA (Fig. 6D). These results were consistent in HSMC derived from three individuals, with apoptosis increasing from 18.2 to 36.1% after the addition of TGFβ1 ($p = 0.02$). Hence the apoptotic effects of TGFβ1 are consistent in murine and human mast cells cultured ex vivo.

Discussion

Mast cell activation is a central facet of atopic diseases such as allergic asthma. The incidence of these diseases has risen dramatically in recent years, emphasizing the importance of understanding and controlling mast cell function. Our efforts have focused on mast cell homeostasis, regulating cell numbers and function. As mast cells provide critical resistance to bacterial and parasitic infections but also elicit inflammation related to atopy, arthritis, multiple sclerosis, and heart disease, this cellular homeostasis may be the fulcrum balancing health and disease.

Mast cells are responsive to many cytokines that can provide homeostatic control. We previously found that the Th2 cytokines IL-4 and IL-10 repress mast cell development, activation, and survival [21, 22, 30, 31, 32, 33]. Although Th2 cells are closely tied to mast cell activity, the presence of TGFβ1 in tissues where mast cells reside and the high level of serum TGFβ1 available during inflammation-induced vasodilation drew our attention to this cytokine. In testing the effects of TGFβ1 on mast cells, we found it capable of suppressing mast cell development and inhibiting IgE receptor expression and function [13, 14]. It was during these experiments that we noted the apoptotic effects of TGFβ1.

We show in the current work that TGFβ1 elicits mast cell apoptosis by repressing IL-3R expression and signaling, resulting in a factor-withdrawal response requiring p53 expression and occurring with mitochondrial damage and caspase activation. This means of inducing apoptosis appears to be lacking in P815 mastocytoma cells. However, these data should be interpreted carefully, as transformed tumor cells can have many defects preventing cell death, including alterations in p53 and other critical apoptotic mediators. As such, it may be that reducing growth factor receptor expression alone on mastocytoma cells would do little to slow their growth. Although our data offer mechanistic insight into TGF-induced cell death, there is still much to understand. It is likely that changes in intracellular signaling pathways by growth factors such as IL-3 and Kit are the prime targets of TGFβ1. By reducing IL-3R and Kit expression, TGF would effectively diminish survival signals. We have previously demonstrated that Stat5 activation is

critical to mast cell survival [27], and indeed this pathway is dampened by TGFβ1. However, this may be one of several signal transduction proteins altered by TGF. A systematic dissection of how TGFβ1 alters IL-3 signaling is needed and is the focus of our current work.

The apoptotic effects of TGFβ1 were consistent in cultured mouse mast cells, peritoneal mouse mast cells, and human mast cells. Moreover, Gebhardt and coworkers recently found that TGFβ1 induced apoptosis in human mast cells derived from intestinal tissue [34]. Thus it is unlikely that our data are related to culture artifacts or species differences. This inhibitory signaling was sensitive, occurring at 1 to 2 ng/mL, which is well below the physiologic concentrations of TGFβ1.

Our studies with TGF demonstrate its role as a potent inhibitor of mast cells, supporting the hypothesis that it can contribute to mast cell homeostasis. This theory is bolstered by the work of several other labs. For example, TGFβ1 diminished IgE-mediated histamine release and tumor necrosis factor alpha production in vitro [35] and inhibited in vivo mast cell responses [36]. It has also been shown to inhibit IL-3-, IL-4-, and SCF-mediated signaling in mast cells, decreasing proliferation or rescue from apoptosis [16, 17, 18]. Lastly, it is interesting to note that polymorphisms in the TGFβ1 promoter segregate with asthmatic families [37]. These data support our interest in TGFβ1 as a mediator of mast cell homeostasis.

The timing of TGFβ1-mediated inhibitory effects is reminiscent of our work with IL-4 and IL-10, which diminished mast cell function and survival after 3 to 6 days of culture [21, 22, 30, 31, 32, 33]. We have postulated that this delay in inhibitory signaling may frame an “inflammatory window” during which mast cell responses elicit inflammation to control infection but after which mast cells are repressed to prevent tissue damage. The effects of TGFβ1 fit well with this theory. For example, TGFβ1 has been reported to elicit mast cell migration [38, 39]. There is also evidence that mast cell proteases activate latent TGFβ1 [40, 41]. Thus it is plausible that TGF acts to draw mast cells to an area of inflammation, where they serve a protective role. Subsequent to prolonged (3-day) stimulation with TGF, in part mediated by latent TGF activation by mast cell proteases, mast cell function and survival would be repressed. This feedback system would restore homeostasis and prevent chronic inflammatory disease.

Our results support the concept that TGF and other inhibitory cytokines normally function in a homeostatic fashion, controlling mast cell activity during an inflammatory response. It is plausible that loss of these control mechanisms through mutation of the cytokines, their receptors, or p53 contributes to inflammatory and autoimmune diseases. Our data emphasize the importance of understanding the molecular mechanisms controlling mast cell homeostasis.

Acknowledgments

This work was supported in part by grants to the Ryan laboratory from the National Institutes of Health (1RO1AI43433, 1R01CA91839).

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