

Original article

IgE-receptor activation induces survival and Bfl-1 expression in human mast cells but not basophils

Background: The contribution of mast cells to the pathology of allergic diseases are facilitated by their long life span in tissue and ability to regranulate. Bcl-2 genes are one of the main regulators of cell death and survival. The aim of this study was to elucidate the mechanisms responsible for mast cell survival in allergy.

Methods: Bcl-2 family gene expression in human mast cells and basophils was analyzed by ribonuclease protection assay and by reverse-transcriptase polymerase chain reaction. Cell survival was measured by mixing cells with the vital dye, trypan blue, and the number of living cells was enumerated. Apoptotic cells were measured by a Cell Death Detection ELISA.

Results: We found that cross-linking of FcεRI on human cord blood cultured mast cells (CBCMCs) promoted cell survival and induced expression of the pro-survival gene Bfl-1. CBCMCs were found to express both Bfl-1 and Bfl-1S, two splicing variants of Bfl-1. Bfl-1 induction was mediated through Syk, PI3-kinase and intracellular calcium mobilization, since piceatannol, wortmannin and EDTA, respectively, significantly reduced Bfl-1 expression levels. In contrast to CBCMCs, no evidence was found for Bfl-1 expression and survival promotion in human basophils.

Conclusions: Immunoglobulin E (IgE)-dependent activation-induced mast cell survival was correlated with Bfl-1 gene upregulation, providing a possible explanation for mast cell longevity in allergic reactions.

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Mast cells and basophils share certain similarities in structure, function and activation (1, 2). They store histamine in their granules and are positive for metachromatic staining. Both cell types express on their surfaces the high-affinity immunoglobulin E (IgE) receptor, FcεRI, which can be cross-linked by multivalent allergen binding to IgE. Upon FcεRI cross-linking, both populations of cells are activated and immediately degranulate and secrete their granular constituents that are stored in the cytoplasmic granules of the cell, as well as synthesize and release lipid mediators, cytokines and chemokines on a slower basis. All these features define the two cell types as effector cells in IgE-associated immune responses, such as those that contribute to asthma and allergic disorders.

One of the biological characteristics of mast cells in allergic pathology is that these cells have the capacity to recover and regranulate after IgE-receptor mediated activation (3, 4). We have previously demonstrated that

the antiapoptotic protein A1, a member of the Bcl-2 family, is required for mouse mast cells to survive IgE-mediated activation (5), which provides a possible explanation, among other suggestions (6), for the observed survival promotion.

The A1 gene was identified to possess structural homology to Bcl-2 (7) and to be an early-response gene expressed in hematopoietic tissues and cell lineages under various pro-inflammatory stimulations (7, 8). In contrast to other hematopoietic cells, A1 is constitutively expressed in neutrophils (9). The human homologue of the mouse A1 is Bfl-1 (10, 11). Bfl-1 and A1 share 72% amino acid identity and function through similar mechanisms. Expression of the Bfl-1 gene is induced in cultured endothelial cells by phorbol ester, endothelial growth factor and inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-4 (IL-4) suggesting a possible role for Bfl-1 during inflammation (11–13). In contrast, A1 is induced in mast cells by FcεRI-mediated activation but not by treatment with TNF-α, stem cell factor (SCF), nerve growth factor (NGF), IL-4 or granulocyte-macrophage colony-stimulating factor (GM-CSF) (5). A similar finding is reported

Abbreviations: CBCMC, human cord blood cultured mast cells; RPA, ribonuclease protection assay; RT-PCR, reverse-transcriptase polymerase chain reaction.

for naive, activated and posteffector T cells that express A1 upon TCR engagement, but not by cytokine treatment (14). A1 can interact with and thereby probably neutralize several proapoptotic proteins, including Bax and Bim (15, 16), which are proteins reported to promote apoptosis in mast cells both *in vitro* and *in vivo* (17, 18). Bfl-1 functions at the mitochondria to delay endothelial apoptosis in response to tumor necrosis factor (19) and is indicated to sequester truncated Bid (tBid) thereby blocking collaboration between tBid and Bax or Bak (20).

Several publications have linked a physiological role of A1 or Bfl-1 to protecting certain types of cells against death stimuli. For example, A1 protects myeloid cells and lymphocytes from apoptosis (21, 22). Enhanced expression of A1 in myeloid cells is critical for the BCR/ABL-mediated cell protection from apoptosis induced by growth factor withdrawal (23). Furthermore, Bfl-1 suppresses p53-induced apoptosis and permits cell proliferation *in vitro* (24, 25).

Here we describe that Bfl-1, the human homologue of the mouse A1 gene, is upregulated after IgE-receptor activation in human mast cells but not basophils and provide evidence that implicates Bfl-1 as a mediator of activation-induced mast cell survival.

Materials and methods

Mast cell cultures

Cord blood cultured human mast cells (CBCMCs) were differentiated from cord blood cells as described by Saito *et al.* (26). The cells were cultured for 8–10 weeks in RPMI 1640 (Sigma, St Louis, MO, USA) supplemented with 10% fetal calf serum (FCS) (Gibco, Paisley, UK), 50 ng/ml SCF, 10 ng/ml IL-6 (PeproTech, London, UK), 2 mM L-glutamine, 100 IU/ml penicillin and 50 µg/ml streptomycin (Sigma). The purity of human mast cells was >90% by tryptase staining. The growth factor independent human mast cell line HMC-1.1 was maintained in IMDM (Sigma) supplemented with 10% FCS (Gibco), glutamine, antibiotics and 1.2 mM alpha-thioglycerol (Sigma).

Basophil isolation

Peripheral blood basophils were isolated using a basophil isolation kit from Miltenyi Biotec (Surrey, UK).

Activation of mast cells and basophils *in vitro*

Human myeloma IgE (ND) and mouse anti-human IgE (clone 346) (kindly provided by Dr Torbjörn Karlsson, Pharmacia Diagnostics, Uppsala, Sweden) were used to activate CBCMCs as well as primary basophils. Cells were incubated at 1×10^6 cells/ml with 1 µg/ml IgE overnight, washed twice with PBS, plated at 1×10^6 cells/ml, and challenged with 2 µg/ml anti-IgE for various time points at 37°C. The IgE overnight incubation was carried out in normal culture medium for the respective cells and the anti-IgE cross-linking challenge was carried out in RPMI medium supplemented with 0.2% BSA (Sigma) at 37°C. Alternatively, cells were

activated by ionomycin (Sigma) treatment. Cells were washed, resuspended at 10^6 cells/ml in the same activation medium as mentioned above, and stimulated for 2 h with 1 µM of ionomycin. After activation, cells were examined for survival rates or cell pellets were harvested for RNA preparation. For inhibition studies, cells were activated for 2 h in medium containing piceatannol (50 µg/ml), wortmannin (100 nM), or EDTA (1 mM) (Calbiochem, Nottingham, UK).

Cell survival assay

For cell viability assays, the cell suspension was mixed with the vital dye, trypan blue, and the number of living cells was enumerated. Cell apoptosis was measured by Cell Death Detection ELISA (Roche Diagnostics, Mannheim, Germany), quantitatively detecting the mono- and oligo-nucleosomes released into culture supernatant.

Bfl-1 mRNA expression analysis

Total cellular RNA was isolated using the TriPure isolation reagent (Roche Diagnostics). To quantitatively measure the expression of Bfl-1, ribonuclease protection assay (RPA) was performed using the hAPO-2c multi-probe set from the RiboQuant System (PharMingen, San Diego, CA, USA) following the supplier's recommended protocol. Quantification of mRNA expression was determined using a phosphorimaging device, and levels of each gene transcript were quantified by MacBas V2.2 (Fuji Photo Film Co. Ltd, Tokyo, Japan). In some experiments, reverse-transcriptase polymerase chain reaction (RT-PCR) was performed. RNA was reverse-transcribed by oligo-p(dT)15 priming using AMV reverse transcriptase (first strand cDNA synthesis kit for RT-PCR; Roche Diagnostics) according to the manufacturer's instruction. PCR was performed for 30 cycles, each consisting of 30 s of denaturation at 94°C, 45 s of annealing at 52°C and 1 min of polymerization at 72°C by a DNA thermal cycler (Perkin Elmer 2400, Norwalk, CT, USA). The PCR primers used for fragment amplification of both human Bfl-1 and Bfl-1S were 5'-TCCACCAGGCAGAAGATGAC-3' (upper) and 5'-TCTGGAAGGTCAAGTTACAT-3' (lower) spanning a region of 665 bp. For glyceraldehyde-3-phosphate dehydrogenase amplification (GAPDH), as a control for the integrity of cDNA, a 452-bp fragment was generated with primers as described (27). PCR products were electrophoresed at 100 V/15 cm for 1 h in a 2% agarose gel (Fig. 5), or at 30 V/15 cm overnight in a 3% agarose gel so as to identify the Bfl-1S band (Fig. 3) which is only 56 bp longer than Bfl-1. Ethidium bromide staining was used to visualize the DNA bands.

Sequencing

PCR fragments derived from Bfl-1 and Bfl-1S amplification were extracted from agarose gels (Qiagen, Hilden, Germany) and inserted into pGEM-T Easy Vector (Promega, Madison, WI, USA), which were subsequently transformed into competent RapidTransTM bacteria (Active motif, Carlsbad, CA, USA). Sequencing was performed using M13 forward and reverse primers.

Statistics

Statistics were calculated using an analysis of variance (ANOVA), followed by multiple comparison using Fisher's method: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Values presented are the means $\pm$ SD or otherwise as indicated.

Results

Survival promotion of CBCMC by FcεRI cross-linking

Human mast cells, e.g. CBCMCs, are growth factor dependent and in the absence of exogenous growth factors in the culture medium they will die. However, upon IgE receptor cross-linking, they survive better than unstimulated control cells in the absence of SCF, IL-6 and serum as demonstrated in Fig. 1A. Survival of activated CBCMCs increased by 80% compared with resting control cells measured 24 h after the initiation of the activation when growth factors were withdrawn. At 48 h, the increase in survival of activated CBCMCs over resting control cells reached as high as about 250%. Consistent with the trypan blue exclusion measurement, nucleosome release assay also indicated that the activated cells were substantially less apoptotic than control cells as shown in Fig. 1B. Nucleosome release from activated cells could be inhibited by 50% compared with resting cells.

The pro-survival gene Bfl-1 is upregulated upon FcεRI activation

Since we have previously shown that the antiapoptotic Bcl-2 family protein A1 is required for mouse mast cells

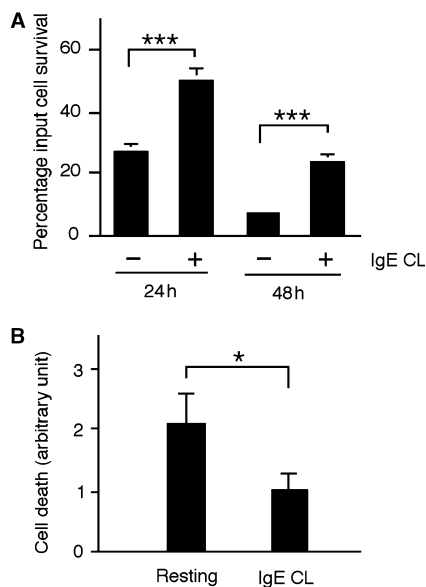


Figure 1. Survival promotion in human mast cells by cross-linking of FcεRI (IgE CL). Human CBCMCs were either stimulated through FcεRI cross-linking or left untreated (resting) in starvation medium deprived of serum and growth factors. (A) Viability was determined by trypan blue exclusion and presented as the percentage of input cells still alive when examined at the indicated time points. One experiment done in triplicate is presented which is representative of at least five similar experiments. (B) Apoptosis was measured by detecting the nucleosomes released into the culture supernatant 10 h after activation. One experiment done in triplicate is shown which is representative of three similar experiments.

to survive IgE-mediated allergic activation (5), we hypothesized that the human homologue of mouse A1, Bfl-1, might be regulated in human mast cells after FcεRI cross-linking, which might provide a possible explanation for the survival promotion seen in Fig. 1. Therefore, we analyzed by RPA the transcript expression levels of some of the Bcl-2 family members including Bfl-1. Upon IgE receptor cross-linking, a clear upregulation of Bfl-1 and Mcl-1 could be observed (Fig. 2A). Quantification of the induction revealed a prominent expression of Bfl-1 and to a lesser extent Mcl-1 after 2 h of FcεRI cross-linking (Fig. 2B). After 2 h of FcεRI cross-linking, the Bfl-1 expression was at its maximal at about 100-fold, compared with resting cells, then the expression was reduced after 6 h, and returned to background level after 12 h (Fig. 2C). For Mcl-1, the peak induction was also seen after 2 h, which represented about four to fivefold upregulation (Fig. 2B).

Recently, Ko et al. reported on an alternative splicing variant of Bfl-1, i.e. Bfl-1S (28). Bfl-1S was found to be predominately expressed in lymphoid tissues. However, the expression of Bfl-1S in mast cells was not investigated. We were, therefore, interested in investigating whether human mast cells also express the Bfl-1 splicing variant. As shown in Fig. 3, resting CBCMCs expressed only the long-form of Bfl-1 whereas activated CBCMCs expressed both Bfl-1 and Bfl-1S. Sequencing of the PCR bands confirmed the identity of both Bfl-1 and Bfl-1S. Similarly to resting CBCMCs, the human mast cell line HMC-1.1 expressed only Bfl-1 (Fig. 3).

Regulation of Bfl-1 expression and cell survival is dependent on PI3-kinase activity

To investigate down-stream signaling pathways involved in the regulation of Bfl-1 expression upon FcεRI activation, we used piceatannol to block Syk and wortmannin to block PI-3 kinase. The upregulation of Bfl-1 triggered by FcεRI cross-linking for 2 h was inhibited by both piceatannol and wortmannin (Fig. 4A,B), suggesting that the regulation of Bfl-1 is via Syk- and PI-3 kinase-dependent pathways. To address whether Bfl-1 upregulation is dependent on calcium influx, we cross-linked the IgE receptors in the presence of EDTA, which is a chelating agent that blocks calcium influx. Our result showed that EDTA could significantly block the induction of Bfl-1 (Fig. 4A,B).

We also investigated whether Bfl-1 could be upregulated by signals other than IgE receptor cross-linking. As shown in Fig. 4A, incubation with ionomycin, a calcium ionophore, could also upregulate Bfl-1, thus further suggesting that Bfl-1 induction is calcium dependent.

We also investigated the effect of wortmannin, piceatannol and EDTA on the activation-induced mast cell survival. Similarly to its inhibitory effect on Bfl-1 expression, wortmannin totally inhibited the FcεRI-induced survival promotion (Fig. 4C). EDTA and piceatannol

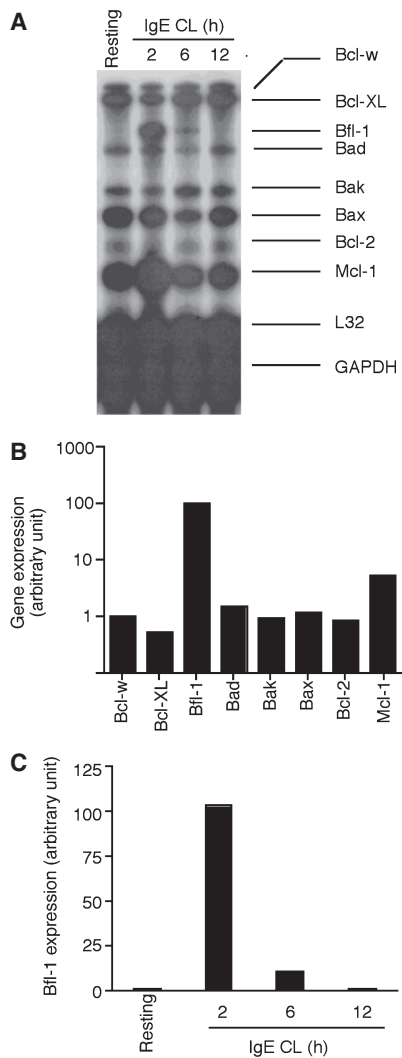


Figure 2. Bfl-1 gene expression by human mast cells. (A) RNA was isolated from CBCMCs either untreated (resting; time point 0) or activated through FcεRI cross-linking (IgE CL) for the time points as indicated and RPA was performed to demonstrate the kinetics of Bcl-2 family gene transcript levels. (B) Phosphoimaging signals presented in A are shown as the ratio of Bcl-2 family members to L32, a house keeping gene, after 2 h of IgE CL (C) and Bfl-1 to L32 after indicated time points. The figures are based on one representative experiment out of at least three.

were toxic at longer exposure and, therefore, it was not possible to evaluate their effects on activation-induced survival (data not shown).

Absence of Bfl-1 expression in human basophils

Besides mast cells, basophils also express the FcεRI receptor and can thus be activated through IgE-receptor cross-linking similarly as mast cells. It is generally accepted that basophils, compared with mast cells, are short-lived. In contrast to CBCMCs, peripheral blood basophils lack activation-induced survival enhancement

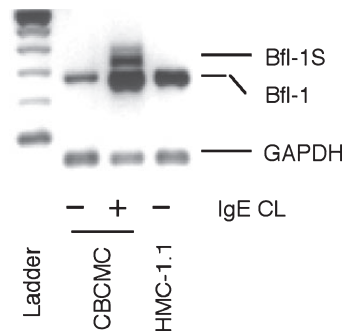


Figure 3. Bfl-1 and Bfl-1S expression in mast cells. The splice variants were examined by RT-PCR. CBCMC were activated by FcεRI cross-linking and HMC-1.1 is a human mast cell line.

in starvation medium (Fig. 5A). Nucleosome release analysis indicated that resting and activated basophils underwent apoptosis at the same rate (data not shown). We, therefore, examined whether basophils upregulated the Bfl-1 gene upon activation of FcεRI. In contrast to CBCMCs purified basophils expressed no Bfl-1 after IgE receptor cross-linking (Fig. 5B). These peripheral blood basophils released histamine upon FcεRI activation (data not shown), thus confirming that they were properly activated.

Discussion

We and others have previously reported the ability of allergic stimulus, FcεRI aggregation, to enhance mouse mast cell survival (5, 6, 29). We have also identified a crucial role for the Bcl-2 family gene A1 in this survival enhancement (5). Considering the fact that there exist certain significant discrepancies between human and mouse mast cells, it is of interest to compare the survival and A1/Bfl-1 expression patterns in the two species. This study shows that cross-linking of FcεRI on human mast cells leads to an upregulation of the human homologue of the mouse A1 gene, Bfl-1, which is concomitant of activation-induced survival (Figs 1 and 2). However, for the other Bcl-2 family members, the patterns of regulation following IgE-mediated activation are different in the two species. Most noticeably, besides A1 upregulation, transcript levels of Bcl-XL is also increased in activated mouse mast cells (5), whereas in human mast cells the Bcl-XL level is not affected by IgE-receptor cross-linking (Fig. 2A). Instead, Mcl-1 is upregulated in activated human mast cells (Fig. 2A). Both Bcl-XL and Mcl-1 are pro-survival members of the Bcl-2 family, but the role of these proteins in mast cell biology remains to be investigated.

In contrast to the mouse where there exist three functional genes encoding A1-a, A1-b and A1-c, only one gene exists for human Bfl-1 (30). Instead the human Bfl-1 exists in two splicing variants, i.e. Bfl-1 and its

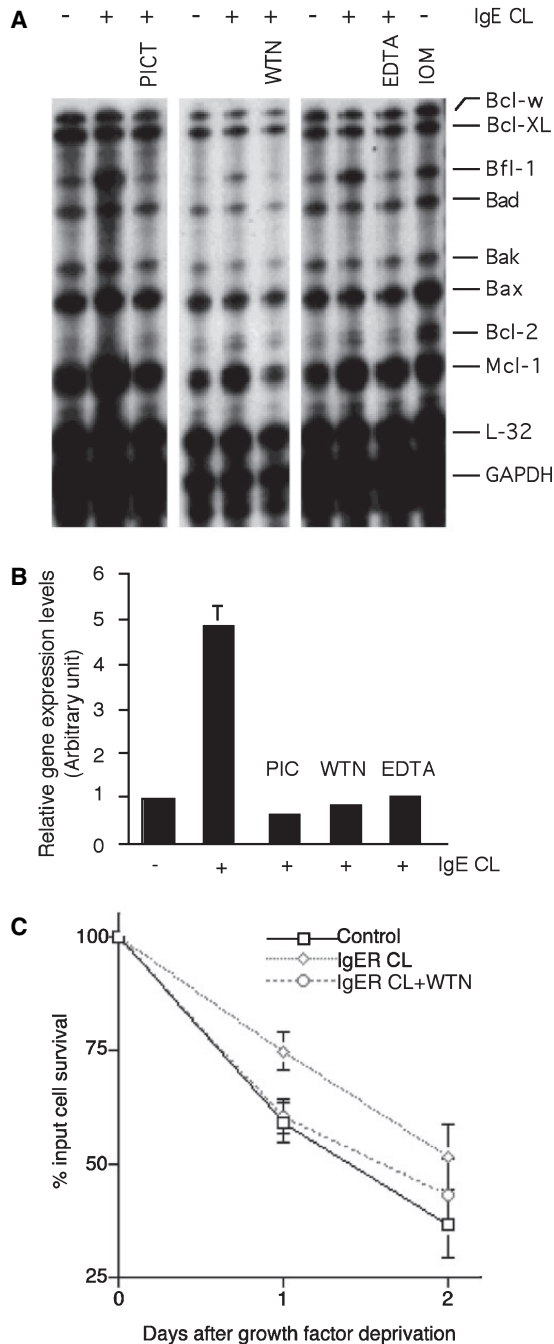


Figure 4. Effects of inhibitors on Bfl-1 induction and mast cell survival. (A) RPA was performed on RNA derived from unstimulated CBCMCs or CBCMCs activated by FcεRI cross-linking (IgE CL) for 2 h in the presence of various inhibitors as indicated. PICT, piceatannol; WTN, wortmannin and EDTA. CBCMCs were also treated with 1 μM of ionomycin (IOM) for 2 h. (B) Densitometric analyses in arbitrary units are shown as the Bfl-1/L32 ratio. Bfl-1 constitutive expression was taken as 1. Although the left, middle and right panels in (A) were based on experiments using cells from different donors, the fold induction of Bfl-1 after IgE-mediated activation was similar. The error bar on the second column in (B) indicates mean ± SEM of the three measurements.

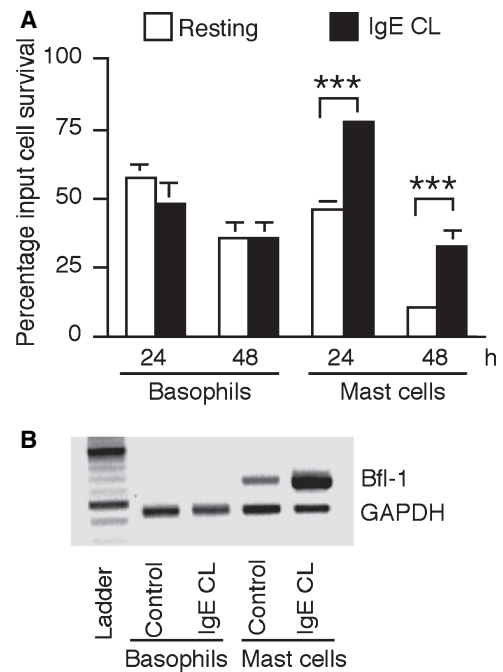


Figure 5. Bfl-1 expression by basophils and basophil survival after activation. (A) Survival rates of human peripheral blood basophils and CBCMCs were measured as explained in Fig. 1A. One experiment done in triplicate is presented which is representative of three similar experiments. (B) Peripheral blood basophils and CBCMCs were compared by RT-PCR for their expression of Bfl-1 after activation through FcεRI cross-linking (IgE CL).

shorter splicing form Bfl-1S (28). It has been documented that Bfl-1S protects T-leukemia cells treated by staurosporine (28). We hypothesize that Bfl-1S might also have a protective role in human mast cells, e.g. CBCMCs since Bfl-1S is strongly induced in FcεRI-activated CBCMCs. It is interesting that HMC-1.1, despite its autoactivated Kit receptor, does not express Bfl-1S, but only Bfl-1, indicating that Bfl-1 is not regulated by SCF-Kit interaction. Similarly, A1 is not induced by SCF-treatment (5).

In our inhibition studies, we observed that 2 h exposure of CBCMCs to piceatannol, wortmannin and EDTA inhibited the expression of Bfl-1. Wortmannin also inhibited activation-induced survival in CBCMCs (Fig. 4), whereas the effect of EDTA and piceatannol on activation-induced survival could not be evaluated due to their toxic effect on mast cells during longer exposure (data not shown). However, our data suggest a correlation between survival retardation and inhibition of Bfl-1 expression in human mast cells. Therefore, similar to the murine model (5), there may be a possible role for Bfl-1 in human mast cell survival in allergy. These data also suggest that signals via Syk and PI-3 kinase as well as calcium influx are critical for the induction of Bfl-1. We

have previously shown that PI-3 kinase pathway and calcium influx are also critical for A1 induction in mouse mast cells after activation by IgE (5).

Both mast cells and basophils are known to be capable of initiating and perpetuating immunological and inflammatory responses. Basophils are thought to be the circulating counterpart of mast cells, since they, like mast cells, express FcεRI and release histamine upon coaggregation of FcεRI (2). Mast cells not only survive the activation, but can be activated repeatedly (4). Mast cells are long lived, where the half-life of rat mucosal mast cells has been calculated to be about 40 days (31). Mouse connective tissue mast cells may have even longer life-span (32). In humans, mast cells survive in tissues for several months (33). This stands in contrast to basophils, which in similarity to other granulocytes have a relatively short life-span (1). Other cell types that play a role in inflammation including neutrophils, T cells and macrophages undergo apoptosis during the inflammation (34, 35). In this study, we compared the Bfl-1 gene expression in human mast cells and basophils. The result clearly demonstrated that human basophils, in contrast to mast cells, do not express Bfl-1 at the transcript level after IgE

receptor aggregation (Fig. 5). The lack of Bfl-1 expression in basophils after allergic stimulation might provide an explanation for the short life span of these cells compared to their long-lived tissue counterpart, mast cells.

Our study suggests that activation-induced mast cell survival during allergic reaction is regulated by the pro-survival gene Bfl-1. Blockade of Bfl-1 function may be beneficial in the long-term management of mast cell-mediated inflammations such as asthma and allergy.

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References

1. Nilsson G, Costa JJ, Metcalfe DD. Mast cells and basophils. In: Gallin JI, Snyderman R, editors. *Inflammation: Basic Principles and Clinical Correlates*, 3rd edn. Philadelphia, PA: Lippincott Williams & Wilkins, 1999:97–117.
2. Galli SJ. Mast cells and basophils. *Curr Opin Hematol* 2000;**7**:32–39.
3. Kobayashi T, Asboe-Hansen G. Degranulation and regranulation of human mast cells. An electron microscopic study of the whealing reaction in urticaria pigmentosa. *Acta Derm Venereol* 1969;**49**:369–381.
4. Xiang Z, Block M, Lofman C, Nilsson G. IgE-mediated mast cell degranulation and recovery monitored by time-lapse photography. *J Allergy Clin Immunol* 2001;**108**:116–121.
5. Xiang Z, Ahmed AA, Moller C, Nakayama K, Hatakeyama S, Nilsson G. Essential role of the prosurvival bcl-2 homologue A1 in mast cell survival after allergic activation. *J Exp Med* 2001;**194**:1561–1569.
6. Yoshikawa H, Nakajima Y, Tasaka K. Glucocorticoid suppresses autocrine survival of mast cells by inhibiting IL-4 production and ICAM-1 expression. *J Immunol* 1999;**162**:6162–6170.
7. Lin EY, Orlofsky A, Berger MS, Prystowsky MB. Characterization of A1, a novel hemopoietic-specific early-response gene with sequence similarity to bcl-2. *J Immunol* 1993;**151**:1979–1988.
8. Orlofsky A, Somogyi RD, Weiss LM, Prystowsky MB. The murine antiapoptotic protein A1 is induced in inflammatory macrophages and constitutively expressed in neutrophils. *J Immunol* 1999;**163**:412–419.
9. Moulding DA, Akgul C, Derouet M, White MR, Edwards SW. BCL-2 family expression in human neutrophils during delayed and accelerated apoptosis. *J Leukoc Biol* 2001;**70**:783–792.
10. Choi SS, Park IC, Yun JW, Sung YC, Hong SI, Shin HS. A novel Bcl-2 related gene, Bfl-1, is overexpressed in stomach cancer and preferentially expressed in bone marrow. *Oncogene* 1995;**11**:1693–1698.
11. Karsan A, Yee E, Kaushansky K, Harlan JM. Cloning of human Bcl-2 homologue: inflammatory cytokines induce human A1 in cultured endothelial cells. *Blood* 1996;**87**:3089–3096.
12. Karsan A, Yee E, Harlan JM. Endothelial cell death induced by tumor necrosis factor-α is inhibited by the Bcl-2 family member, A1. *J Biol Chem* 1996;**271**:27201–27204.
13. Gerber HP, Dixit V, Ferrara N. Vascular endothelial growth factor induces expression of the antiapoptotic proteins Bcl-2 and A1 in vascular endothelial cells. *J Biol Chem* 1998;**273**:13313–13316.
14. Vershelde C, Walzer T, Galia P, Biemont MC, Quemeneur L, Revillard JP et al. A1/Bfl-1 expression is restricted to TCR engagement in T lymphocytes. *Cell Death Differ* 2003;**10**:1059–1067.
15. Sedlak TW, Oltvai ZN, Yang E, Wang K, Boise LH, Thompson CB et al. Multiple Bcl-2 family members demonstrate selective dimerizations with Bax. *Proc Natl Acad Sci U S A* 1995;**92**:7834–7838.
16. Chen L, Willis SN, Wei A, Smith BJ, Fletcher JI, Hinds MG et al. Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. *Mol Cell* 2005;**17**:393–403.
17. Maurer M, Tsai M, Metz M, Fish S, Korsmeyer SJ, Galli SJ. A role for Bax in the regulation of apoptosis in mouse mast cells. *J Invest Dermatol* 2000;**114**:1205–1206.

18. Alfredsson J, Puthalakath H, Martin H, Strasser A, Nilsson G. Proapoptotic Bcl-2 family member Bim is involved in the control of mast cell survival and is induced together with Bcl-XL upon IgE-receptor activation. *Cell Death Differ* 2005;**12**:136–144.
19. Duriez PJ, Wong F, Dorovini-Zis K, Shahidi R, Karsan A. A1 functions at the mitochondria to delay endothelial apoptosis in response to tumor necrosis factor. *J Biol Chem* 2000;**275**:18099–18107.
20. Werner AB, de Vries E, Tait SW, Bontjer I, Borst J. Bcl-2 family member Bfl-1/A1 sequesters truncated bid to inhibit its collaboration with pro-apoptotic Bak or Bax. *J Biol Chem* 2002;**277**:22781–22788.
21. Lin EY, Orlofsky A, Wang HG, Reed JC, Prystowsky MB. A1, a Bcl-2 family member, prolongs cell survival and permits myeloid differentiation. *Blood* 1996;**87**:983–992.
22. Chuang PI, Morefield S, Liu CY, Chen S, Harlan JM, Willerford DM. Perturbation of B-cell development in mice overexpressing the Bcl-2 homolog A1. *Blood* 2002;**99**:3350–3359.
23. Nieborowska-Skorska M, Hoser G, Kossev P, Wasik MA, Skorski T. Complementary functions of the antiapoptotic protein A1 and serine/threonine kinase pim-1 in the BCR/ABL-mediated leukemogenesis. *Blood* 2002;**99**:4531–4539.
24. D'Sa-Eipper C, Subramanian T, Chinnadurai G. bfl-1, a bcl-2 homologue, suppresses p53-induced apoptosis and exhibits potent cooperative transforming activity. *Cancer Res* 1996;**56**:3879–3882.
25. D'Sa-Eipper C, Chinnadurai G. Functional dissection of Bfl-1, a Bcl-2 homologue: anti-apoptosis, oncogene-cooperation and cell proliferation activities. *Oncogene* 1998;**16**:3105–3114.
26. Saito H, Ebisawa M, Tachimoto H, Shichijo M, Fukagawa K, Matsumoto K et al. Selective growth of human mast cells induced by Steel factor, IL-6, and prostaglandin E2 from cord blood mononuclear cells. *J Immunol* 1996;**157**:343–350.
27. Tsai M, Miyamoto M, Tam SY, Wang ZS, Galli SJ. Detection of mouse mast cell-associated protease mRNA. Heparinase treatment greatly improves RT-PCR of tissues containing mast cell heparin. *Am J Pathol* 1995;**146**:335–343.
28. Ko JK, Lee MJ, Cho SH, Cho JA, Lee BY, Koh JS et al. Bfl-1S, a novel alternative splice variant of Bfl-1, localizes in the nucleus via its C-terminus and prevents cell death. *Oncogene* 2003;**22**:2457–2465.
29. Kitaura J, Xiao W, Maeda-Yamamoto M, Kawakami Y, Lowell CA, Kawakami T. Early divergence of Fc epsilon receptor I signals for receptor up-regulation and internalization from degranulation, cytokine production, and survival. *J Immunol* 2004;**173**:4317–4323.
30. Hatakeyama S, Hamasaki A, Negishi I, Loh DY, Sendo F, Nakayama K. Multiple gene duplication and expression of mouse bcl-2-related genes, A1. *Int Immunol* 1998;**10**:631–637.
31. Enerback L, Lowhagen GB. Long term increase of mucosal mast cells in the rat induced by administration of compound 48/80. *Cell Tissue Res* 1979;**198**:209–215.
32. Fukuzumi T, Waki N, Kanakura Y, Nagoshi J, Hirota S, Yoshikawa K et al. Differences in irradiation susceptibility and turnover between mucosal and connective tissue-type mast cells of mice. *Exp Hematol* 1990;**18**:843–847.
33. Garriga MM, Friedman MM, Metcalfe DD. A survey of the number and distribution of mast cells in the skin of patients with mast cell disorders. *J Allergy Clin Immunol* 1988;**82**(3 Pt 1):425–432.
34. Akgul C, Moulding DA, Edwards SW. Molecular control of neutrophil apoptosis. *FEBS Lett* 2001;**487**:318–322.
35. Bjorkerud S, Bjorkerud B. Apoptosis is abundant in human atherosclerotic lesions, especially in inflammatory cells (macrophages and T cells), and may contribute to the accumulation of gruel and plaque instability. *Am J Pathol* 1996;**149**:367–380.