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c-Fos as a Regulator of Degranulation and Cytokine Production in FcεRI-Activated Mast Cells

Yu-Nee Lee,† Jan Tuckerman,‡ Hovav Nechushtan,‡ Gunter Schutz,§ Ehud Razin,²* and Peter Angel§

The AP-1 complex is composed of c-Jun and c-Fos and is a key component in the regulation of proinflammatory genes. Mast cells play a significant role in the initiation of many inflammatory responses, such as allergy and allergy-associated diseases. In the present work, we characterized the role of c-Fos in mast cell function by investigating IL-3-dependent cell proliferation, degranulation capability, and cytokine expression in c-Fos-deficient mice. In c-Fos-deficient mast cells, we found that FcεRI-mediated degranulation was significantly inhibited, which correlates with the reduced expression of SWAP-70, VAMP-7, and Synaptotagmin I genes, which are involved directly in the degranulation process. These findings show that c-Fos plays an important role in FcεRI-mediated regulation of mast cell function. The Journal of Immunology, 2004, 173: 2571–2577.

Mast cells are tissue-localized effector cells of the immune system. They are often the first cells to interact with airborne ingested Ags and noxious agents; therefore they are essential for the induction of local inflammatory responses in many tissues. Mast cells initiate the inflammatory response when the IgE-bound FcεRI receptors on the cell surface are cross-linked with Ag. These cells then immediately release a variety of lipid-derived mediators, amine derivatives, and proteoglycans (1). Furthermore, the relatively late release of cytokines by these activated cells seems to play an essential role in the inflammatory process. In addition to their role in innate immunity, mast cells can initiate the adaptive immune response by recruiting T cells to draining lymph nodes of an infected site (2).

Multiple transcription factors are induced in the cytoplasm in activated cells and then shuttled to the nucleus where they exert positive or negative control over cellular genes. The AP-1 complex, a transcriptionally active heterodimer of Fos and Jun proteins, is one of the most extensively analyzed transcription factors mediating gene regulation in response to extracellular signals. This transcription factor has been found to mediate gene regulation by various stimuli such as growth factors, cytokines, and tumor promoters. Fos proteins (c-Fos, FosB, Fra-1, Fra-2) heterodimerize with Jun proteins (c-Jun, JunB, JunD) to form AP-1, whereas Jun proteins can also homodimerize. AP-1 differs slightly from the Jun-Jun homodimer in DNA binding specificity and trans-activation functions (3, 4). Specific posttranslational modification of the protein subunits and protein/protein interactions in a given cell result in expression of specific, corresponding genes (5–8).

The AP-1 transcription complex has been previously shown to modulate the effect of other transcription factors. It is known that, in activated T cells, c-Fos regulates the transcription of various cytokines. The NF-AT is a protein complex that has been shown to cooperate with AP-1 in many cases (9). It was shown that the expression of IL-2, GM-CSF, IL-3, IL-4, and MIP1α mRNAs is absolutely dependent on cooperation between NF-AT and AP-1 (10). In addition, aggregation of their surface FcεRI in mast cells (11) potently induces c-Fos expression, suggesting that the activity of c-Fos might be implicated in mast cell activation. In rat basophilic leukemia cells and rat bone marrow-derived mast cells, it was shown that aggregation of FcεRI induced the binding of NF-AT and of AP-1 to the NF-AT- and AP-1-responsive elements on the DNA, and this binding was prevented with the addition of anti-Jun and anti-c-Fos Abs (12). These results demonstrate that NF-AT is induced by IgE-Ag in mast cells, and that c-Fos and c-Jun are part of the NF-AT DNA binding complex (12). In fact, mast cells contain two different kinds of NF-AT that are of distinct molecular mass compared with NF-AT derived from T cells (13, 14). These two subtypes of NF-AT cooperate either with AP-1 or members of the GATA family, as demonstrated on the TNF-α and IL-5 promoters, respectively (15).

Mice lacking c-Fos are viable and fertile. They lack osteoclasts, resulting in an osteopetrotic phenotype, but the differentiation and activity of the peripheral T cells are normal (16, 17). However, the involvement of c-Fos in the regulation of mast cell function in these mice has not yet been investigated. Thus, in the present work, we characterized a variety of parameters involved in mast cell function in c-Fos-deficient cells, including proliferation, degranulation capability, and expression of cytokines.

Materials and Methods

Animals and cell culture

The c-Fos-deficient mice were generated by mixed background cross of Sv 129 with C57BL/6. c-Fos-deficient mice and their wild-type littermates were genotyped as described (16) and were housed in specific pathogen-free and light-, temperature (21°C), and humidity (50–60% relative humidity)-controlled conditions. Food and water were available ad libitum. The procedures for performing animal experiments were in accordance...
with the principles and guidelines of the ATBW (officials for animal welfare in Germany) and were approved by the Regierungspärisidendium (Karlsruhe, Germany).

Mouse spleen-derived mast cells (SMC) were obtained as previously described (18) from c-Fos-deficient mice and control littermates. We used spleen from one mouse per flask. Cells from control and c-Fos-deficient mice were grown in individual flasks according to the date the mice were killed. The cells were cultured at a starting density of 0.1 × 10^6 cells/ml at 37°C in a humidified atmosphere containing 5% carbon dioxide (CO₂). The culture medium was RPMI 1640 (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 2 mM L-glutamine, 2 mM nonessential amino acids, 100 µM penicillin, 100 µg/ml streptomycin, 50 µg/ml 2-ME, 10% FCS (Invitrogen Life Technologies), and 30 U/ml rIL-3 (R&D Systems, Minneapolis, MN). The nonadherent cells from the SMC cultures were transferred every 5 days into fresh IL-3-containing medium after verification that there were similar cell densities for the control and c-Fos-deficient cells. The cultures were maintained for 3–5 wk. Mast cells were identified by toluidine blue staining.

**FACS analysis**

Flow cytometric analysis of mast cells was performed by preincubating with anti-CD16/32 Abs (Fc block) and followed staining with rabbit polyclonal Ab for the α-chain of the human high affinity receptor for Ig E (FcεRⅠα; Upstate Biotechnology, Lake Placid, NY). After washing, cells were stained with FITC-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA) and with PE-conjugated anti-CD117 Ab (BD Pharmingen, San Diego, CA).

For DNA content analysis, 10^6 cells were fixed in 70% ethanol and washed twice with PBS and stained with propidium iodide (50 µg/ml in PBS; Sigma-Aldrich, St. Louis, MO). Data were collected and analyzed with a BD Biosciences (San Jose, CA) FACSCalibur system using CellQuest software (BD Biosciences).

**Proliferation assay**

The proliferation assay used was described previously (19). Cells were cultured in 200 µl of medium supplemented with doses of mouse IL-3 defined according to the requirements of each experiment. Cells from each treatment were divided into four replicates and were incubated for 24 h at 37°C in 5% CO₂ in round-bottom 96-well microtiter plates (Nunc, Roskilde, Denmark) at a density of 2 × 10^5 cells per well. The cells were labeled with [³H]thymidine (Amersham Biosciences, Piscataway, NJ) for the last 4 h at 37°C and transferred onto glass-fiber filter paper (Titerdek, Huntsville, AL). Thymidine incorporation was measured as described previously (18).

**Mast cell staining**

Mice were sacrificed by CO₂, and the abdomen was injected with 10 ml of normal saline i.p. After gentle abdominal massage, the peritoneal fluid was aspirated, 250 µl of the fluid was centrifuged, and the pellet was resuspended in 1 ml of normal saline (20). Then, 200 µl of this suspension was spun for 5 min in a cytospin centrifuge. The cells were stained by using a modification of the original Enerback protocol (20), which uses 0.5% Alcian blue and 0.3% acetic acid. They were then rinsed with water and counterstained with 0.1% safranin and 0.1% acid acetic.

**Ear sections**

Mast cell number per light field of standard microscope of ×100 magnification was determined as described previously (21). Ears from each of six mice per group (c-Fos–/– and wild type) were removed after death by cervical dislocation, fixed in 4% neutral buffered formalin for 24 h, immersed in 70% ethanol, and embedded in paraffin. Tissue sections of 6 µm from each ear were fixed on glass slides and stained with toluidine blue. Each section was examined at ×1000 magnification and was randomly counted in 10 different fields to determine the number of dermal mast cells. The average number of mast cells in 10 fields was determined, and the mean number of mast cells per field of ×100 magnification in each ear was calculated.

**Degranulation assay**

IgE sensitization was conducted by incubating 2 × 10⁶ cells with 30 µg of monoclonal IgE against dinitrophenol (DNP; Sigma-Aldrich) for 2 h at 37°C in RPMI 1640 medium. The cells were washed once with 1 ml of Tyrode buffer and resuspended in the same buffer. Fifty-microliter aliquots of this cell suspension were put into microfuge tubes containing increasing amounts of human serum albumin (HSA) conjugated to DNP (HSA-DNP) or ionomycin (Sigma-Aldrich) for 45 min. The release of β-hexosaminidase was then determined in triplicate in a 96-well plate. The plate was read in ELISA reader at a wavelength of 495 nm. The results were expressed as the net percentage release of hexosaminidase, as previously described (22).

**Real-time quantitative PCR**

For experiments investigating cytokine mRNA production and release, the cells were preincubated for 2 h with IgE at the same concentration as above and then incubated with 100 ng/ml HSA-DNP in tissue culture medium for 6 h (23, 24). The cells were then centrifuged and the supernatants were collected and frozen at −70°C for later cytokine determination. The activated mast cell pellets were lysed directly with 1 ml of Tri Reagent, and total RNA was isolated and purified according to manufacturer’s instruction (Sigma-Aldrich).

The mRNA transcript levels were measured using real-time quantitative PCR. Total RNA was extracted from resting and activated mast cells derived from control and c-Fos-deficient mice. mRNA of various genes were quantified by SYBR Green incorporation (SYBR Green PCR Master Mix; Applied Biosystems, Foster City, CA). SYBR Green incorporation to dsDNA permits the direct detection of PCR product after each amplification cycle (Applied Biosystems Prism 7000 sequence detection system). The specificity of the amplification was controlled by electrophoresis.

**Results**

**General characterization of mast cells derived from c-Fos-deficient mice**

Because c-Fos-deficient mice develop osteopetrosis and lack bone marrow cavities (17), the spleen was isolated from wild-type and mutant mice, and the cells were cultured in presence of IL-3. A purity of 95% of SMC was observed after 3 wk in culture, as determined by toluidine blue staining and by FACS analysis using Abs for FcεRI and CD117 (Table I). The expression of surface FcεRI in c-Fos-deficient mast cells was similar to that expressed in the wild-type mast cells.

Cell cycle profile was determined by propidium iodide staining and FACS analysis. No significant differences (p > 0.05) were detected in the cell cycle profile of c-Fos-deficient SMC from wild-type SMC (Table I). However, a slight reduction (p < 0.1) in the percentage of G₂/S cells and an increased number of cells in the G₃ phase were detected in c-Fos-deficient SMC (Table I), indicating that mutant mast cells exhibit either an accelerated G₂/S-to-G₃ transition or a reduced efficiency of G₂/M-to-mitosis transition, or both. In addition, no significant difference was detected in the number of mast cells recorded over a period of 15 wk in mast cells derived from wild-type or c-Fos-deficient mice (Fig. 1A).

The rate of SMC proliferation derived from wild-type and c-Fos-deficient mice was determined by incubating the cells for 48 h with increasing concentration of IL-3. Proliferation was assessed by [³H]thymidine incorporation into DNA and no difference in this

<table>
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<th>Table I. Flow cytometry analysis of mast cells*</th>
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*SMC from wild-type and c-Fos-deficient mice were stained by propidium iodide staining to determine the cell cycle profile. Surface expression of FcεRI and c-kit (CD117) was analyzed using Abs against FcεRI and c-kit in order to determine the percentage of mast cells. Student’s t test was carried out to verify whether there is significant difference between wild-type and c-Fos-deficient SMC for the cell cycle results. The p value is 0.099; thus, there is no significant difference between the groups.
A parameter was detected between the wild-type and the mutant SMC (Fig. 1B).

Peritoneal and tissue mast cells

The phenotype of peritoneal mast cells was determined in the c-Fos-deficient mice. Peritoneal mast cells differ in many respects from cultured precursor-derived mast cells and represent a type of mature “connective tissue mast cell,” which can be detected by the presence of heparin in their granules (20). If heparin-containing mast cells are dependent on c-Fos for their maturation, one would not expect to see peritoneal mast cells from c-Fos-deficient mice stained with safranin. However, we found that the peritoneal mast cells from mouse peritonemum from both wild-type and c-Fos-deficient mice were stained with safranin (Fig. 2A). We determined whether there is any change in the number of heparin-containing mast cells in the ears of c-Fos-deficient mice. There was no difference in mast cell number between the c-Fos-deficient and the wild-type ear sections (Fig. 2B), which indicates that c-Fos does not play a critical role in the maturation and development of mast cells in vivo.

Degranulation

Degranulation is considered to be a major function of mast cells, and can be triggered by FcεRI cross-linking. Wild-type or c-Fos-deficient SMC were preincubated with IgE against DNP and subsequently challenged with increasing doses of DNP. The efficiency of degranulation was measured by the release of the indicative enzyme β-hexosaminidase, present in the secretory granules of these cells. Degranulation was significantly lower in c-Fos-deficient SMC compared with wild-type cells (Fig. 3). Interestingly, upon incubation of cells for 20 min at 37°C with ionomycin (Ca^{2+} ionophore), both wild-type and c-Fos-deficient SMC revealed the same percentage of degranulation (data not shown). Because ionomycin was able to rescue the degranulation defect in the c-Fos-deficient mice, it appears that c-Fos is essential for the expression/function of key molecules in FcεRI signaling.

The accumulation of SWAP-70, VAMP-7, and Synaptotagmin I (SYT I) mRNA in c-Fos-deficient SMC

Because the FcεRI-mediated exocytosis process in the c-Fos-deficient SMC was impaired, we determined the level of transcripts of genes that are known to be involved in the degranulation process. The mRNA levels of SWAP-70 (25, 26), VAMP-7 (27), SYT I (28, 29), and SYT II (30, 31) were determined by real-time PCR. The mRNA levels of SWAP-70, VAMP-7, and SYT I from resting SMC were analyzed, and their levels are significantly lower in c-Fos-deficient SMC compared with the wild-type SMC (Fig. 4A). The SYT-II mRNA level was very low in resting c-Fos-deficient SMC and was not expressed in resting wild-type SMC (Fig. 4B).

FIGURE 1. SMC proliferation in response to IL-3. A, SMC were cultured at starting density of 0.1 × 10^6 cells/ml in the presence of IL-3 as described in Materials and Methods. Total number of cells was counted every 5–6 days. The graph represents average of total cell number from three different mice from each group. B, SMC were incubated for 24 h with medium containing increasing doses of IL-3. [3H]Thymidine incorporation was determined in the c-Fos-deficient and wild-type SMC (mean ± SE; n = 3).

FIGURE 2. Peritoneal and tissue mast cells from c-Fos-deficient and wild-type mice. A, Light micrographs of peritoneal mast cells from wild-type mice and from c-Fos-deficient mice. Peritoneal cells were stained with Alcian blue and counterstained with safranin. B, Tissue mast cells of the ear from wild-type and c-Fos-deficient mice. Microsections of the ear were stained with toluidine blue. Stained mast cells were counted in 10 different light fields from six different mice in each group.

FIGURE 3. Degranulation of c-Fos-deficient and wild-type SMC. DNP Ag dose-dependent net percentage release of β-hexosaminidase from IgE-sensitized c-Fos-deficient and wild-type SMC. One representative experiment of three is shown.
Cytokines and other gene expression in IgE-Ag-activated c-Fos-deficient SMC

In vitro activation of mast cells by cross-linking FcεRI with IgE and Ag induces the synthesis and release of various cytokines (33). When we measured the level of cytokine transcripts in resting and IgE-Ag-activated c-Fos-deficient SMC by real-time PCR, an increase of up to 15-fold in IL-5, IL-6, IL-10, IL-13, MIP-1α, TNF-α, and GM-CSF mRNA was observed in wild-type, but not in the c-Fos-deficient SMC (Fig. 5). In addition, the IL-3 and IL-4 mRNA levels were significantly induced in the c-Fos-deficient SMC compared with the wild type upon activation via FcεRI. These data implicate the function of c-Fos in the regulatory machinery that controls the de novo synthesis of cytokines upon immunological trigger in mast cells.

The expression of c-Kit, tyrosine kinase receptor for stem cell factor, is one of the key characteristic of mast cells (34). The binding of stem cell factor to c-Kit mediates proliferation, differentiation, migration, activation, and survival of mast cells (35, 36). As can be seen in Fig. 4C, the c-Kit mRNA was hardly elevated upon activation in wild-type SMC, whereas in c-Fos-deficient mast cells, a >2-fold increase was observed. It is important to note that the differences in c-Kit transcripts are not at the level of basal expression, but rather due to an accumulation of c-Kit mRNA upon immunological stimulation.

Because both the mi transcription factor (MITF)- and c-Fos-deficient mice show osteopetrotic symptoms (17, 37), we determined the expression of MITF, an essential regulator of the development and differentiation of mast cells, osteoclasts, and melanocytes, which are affected in MITF-deficient mice (38). MITF mRNA levels showed a 4-fold increase in activated wild-type, but not in c-Fos-deficient SMC (Fig. 4C); thus, c-Fos acts as a negative regulator of MITF expression in activated mast cells.

Discussion

In general, c-Fos and c-Jun are known as positive regulators of cell proliferation; however, the results of studies in certain specific cells, such as in the nervous system (39) and in mast cells (40), suggest that these two protooncogenes might play a negative role in cell growth. In mast cells, inhibition of cell growth by c-Jun was suggested that these two protooncogenes might play a negative role in cell growth. In mast cells, inhibition of cell growth by c-Jun was suggested that these two protooncogenes might play a negative role in cell growth. In mast cells, inhibition of cell growth by c-Jun was inhibited for a short period of time (11). Our results show that, although there is no difference in IL-3-dependent proliferation rate of the c-Fos-deficient SMC (Fig. 1), there seems to be an indication (0.05 < p < 0.1) for growth arrest in c-Fos-deficient SMC (Table I). It was logical to expect that, if c-Fos acts as a negative regulator of proliferation, then we should observe an increase in mast cell growth in the c-Fos-deficient SMC. However, we observed that, in the c-Fos-deficient cells, there was a slight decrease in mast cell at the G2 phase. Countless interactions of AP-1 with other cellular components such as NF-AT, the existence of a plethora of physiological and pathological stimuli, and the microenvironment are all responsible for activating AP-1 proteins. Therefore, additional experiments should be performed to elucidate the exact regulatory mechanism in which the components of AP-1 control the role of mast cell proliferation.

The significant reduction in degranulation that is observed in our study is not due to the cellular decrease in β-hexosaminidase content but rather due to the defect in the release of the secretory granules. The mRNA accumulation of another granular mast cell enzyme, CPA, remains unchanged in the c-Fos-deficient mast cells, which implies that the granular contents are not changed due to c-Fos deficiency (Fig. 4C). These data strengthen the assumption that the impairment due to c-Fos depletion lies in the granular release process rather than in the assembly of the secretory granules or in the synthesis of the granular content.

There are several proteins that are involved in the degranulation process that might be targeted by c-Fos in immunologically triggered SMC. One of these proteins is SWAP-70, and similar results

These data strongly suggest that c-Fos serves as a positive regulator of SWAP-70 and the granule membrane-bound proteins VAMP-7 and SYT-1 in resting mast cells.

In contrast to the altered expression of these membrane-bound proteins, the mRNA level of carboxypeptidase A (CPA), one of the representative digestive enzyme found in the secretory granules of mast cells (32), did not differ in wild-type and c-Fos-deficient SMC (Fig. 4C).

**Discussion**

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There are several proteins that are involved in the degranulation process that might be targeted by c-Fos in immunologically triggered SMC. One of these proteins is SWAP-70, and similar results.
of dysfunction in mast cell degranulation was observed in the SWAP-70-deficient mice (25) as shown in c-Fos-deficient mice (Fig. 3). Moreover, it was reported that primary cultures of fibroblasts derived from SWAP-70-deficient mice show reduced membrane ruffling induced by epidermal growth factor (26), and thus the possible involvement of SWAP-70 in IgE-Ag-mediated degranulation is suggested by our current study. Both SWAP-70 and c-Fos seem to be specifically required for the degranulation machinery via FcεRI signal transduction. Furthermore, c-Fos seems to be a regulator of SWAP-70, at least on the transcriptional level, because the transcript of SWAP-70 is reduced in resting c-Fos-deficient mast cells compared with the wild-type mast cells (Fig. 4A).

VAMP-7, which belongs to the SNARE protein family, is another protein that is involved in the exocytosis of the secretory granules of mast cells (27, 41). It is localized in the granule membranes of mast cells and relocates to the plasma membrane upon IgE-Ag stimulation. It is interesting to observe that the transcript level of this protein is significantly decreased in resting c-Fos-deficient SMC compared with the wild-type cells (Fig. 4A), which clearly indicated that c-Fos serves as a positive regulator of VAMP-7 expression and thus affects the degranulation process.

Another protein known to play an important role in the exocytosis of mast cells is SYT I (28, 29), which acts as one of the Ca^{2+} sensors in the synaptic vesicle and mediates exocytosis (42). The expression of SYT I increases in stimulated human mast cells as was demonstrated by immunohistochemistry (29). In this study, we observed that the expression of SYT I was elevated in resting SMC (Fig. 4A), thus leading to the assumption that c-Fos is also involved in the up-regulation of SYT I. In contrast, SYT II, another Ca^{2+} sensor that acts as a negative regulator of mast cell exocytosis (30) seems to be negatively regulated by c-Fos (Fig. 4B).

Because SYT-I and VAMP-7 are calcium sensors, it is predicted that c-Fos-deficient SMC will not be able to degranulate upon treatment with ionomycin. However, it is interesting to note that there is no significant difference in the degranulation in c-Fos-deficient SMC compared with the wild-type cells (data not shown). This result may be explained by functional redundancy of other members in these protein families, which could be activated by responding to a large increase in intracellular calcium through an ionomycin stimulus.

It is well known that a wide range of cytokine genes are induced upon immunological activation of mast cells (33). Interestingly,
the majority of the proinflammatory cytokines are positively regulated by c-Fos as shown by experiments on their mRNA accumulation (Fig. 5). Our results are in agreement with reports that the mRNA levels of proinflammatory mediators such as IL-6 (43, 44), TNF-α (45), and GM-CSF (46) are positively regulated by AP-1 in a variety of cells. These cytokines are known to participate in allergic diseases, and it is known that TNF-α released from mast cells especially play a role in the development of rheumatoid arthritis (47) and in protection from acute bacterial peritonitis (48).

However, it is important to note that the mRNA expression pattern was found to be different in IL-3 and IL-4 compared with IL-10 and other cytokines (Fig. 5), although these three cytokines, the Th2 cytokines, have been shown in many studies to behave in a similar manner (49, 50). This shows that IL-3 and IL-4, unlike other cytokines, are negatively regulated by c-Fos at the transcriptional level. It has been shown previously that IL-3 and IL-4 are especially critical in supporting helminth-induced reactive mast cell hyperplasia (51, 52). Our studies indicate that c-Fos might play a substantial role in preventing the induction of IL-3 and IL-4 production and thus helminth-induced reactive mast cell hyperplasia. However, c-Fos induces transcription of other cytokines leading to other allergic disorders. It seems that c-Fos controls the type of immunological response in mast cells via differential production of cytokines (Fig. 6).

The significantly reduced induction of MITF gene upon activation of mast cells in the absence of c-Fos (Fig. 4C) is noteworthy because both MITF and c-Fos-deficient mice have the same physiological defect leading to osteopetrosis (17, 37). It was claimed that the association of c-Fos with MITF interferes with the nuclear translocation of c-Fos (53). Our results indicate that c-Fos acts as a positive regulator of MITF expression upon mast cell activation. This suggests that, upon mast cell activation, the elevated c-Fos localizes to the nucleus, and binds to AP-1-responsive elements including the promoter of MITF. However, MITF associates with c-Fos and negatively regulates its function by preventing its localizaton to the nucleus.

The most important role of c-Fos in mast cells appears to be the regulation of the degranulation process of the secretory granules upon immunological trigger. Preformed mediators such as CPA are not altered; however, the newly synthesized mediators of various cytokines are affected by c-Fos. From our results, we can postulate the following: upon immunological stimulation, c-Fos, a member of the early gene family, is among the first genes to be affected, and its transcription and protein level are elevated. Then, c-Fos trans-activates various genes including proinflammatory cytokines (IL-5, IL-6, IL-10, IL-13, TNF-α, GM-CSF, and MIP-1α) and MITF, which is an important transcription factor in the development and differentiation of mast cells. Furthermore, c-Fos positively regulates the expression of SWAP-70, VAMP-7, and SYT I genes that are involved in the exocytosis of granules in mast cells and in other cells (Fig. 6). All of these proteins cooperatively regulate the degranulation process, a physiological function that is critical in mast cells and in the early immune response.

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