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Immunological Trigger of Mast Cells by Monomeric IgE: Effect on Microphthalmia Transcription Factor, STAT3 Network of Interactions[1]

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Microphthalmia transcription factor (MITF) and STAT3 are two transcription factors that play a major role in the regulation of growth and function of mast cells and melanocytes. We have previously provided experimental evidence regarding the functional cross-talk between MITF, protein inhibitor of activated STAT3, and STAT3 in response to cytokine activation of mast cells. Recent studies have demonstrated that binding of different IgE molecules to their FcεRI induces a spectrum of intracellular events in the absence of specific Ag. In this work, we show for the first time that, in mouse bone marrow-derived mast cells and in rat basophilic leukemia cells, monomeric IgE alone can induce the MITF-protein inhibitor of activated STAT3-STAT3 network of interactions and leads to phosphorylation of MITF at S73 and of STAT3 at both tyrosine 705 and S727. This phosphorylation increases the transcriptional activity of MITF and STAT3 as indicated by mRNA accumulation of their target genes such as *Bcl-2*, *granzyme B*, and c-*Myc*. Interestingly, MITF and STAT3 were not found to be obligatory factors in the anti-apoptotic response induced by IgE. Thus, the phenomenon that IgE alone was able to induce transcription factors that are essential for mast cell function could contribute to our understanding of the pathogenesis of allergy and its associated diseases. *The Journal of Immunology,* **2005, 175: 1450–1455.**

It has been known for decades that cross-linking of IgE bound to mast cell high affinity FcεRI with multivalent Ag initiates their activation by promoting FcεRI aggregation.

Studies on the survival effect of monomeric IgE showed that IgE binding alone to FcεRI induces the secretion of a variety of cytokines that enhance cell survival by an autocrine mechanism (1). This model was supported by the observation that the Akt and MAPK pathways could be activated by using monomeric IgE alone. Support for these in vitro phenomena come from in vivo studies that demonstrated that IgE Abs can promote immune sensitization to hapten in the skin, independently of Ag (2). Thus, the literature supplies us with good indications that stimulation of mast cells via FcεRI could be caused by binding of monomeric IgE.

The interplay between microphthalmia transcription factor (MITF)[3] and STAT3 in response to activation of mast cells by monomeric IgE is the focus of the present study.

MITF is a basic helix-loop-helix leucine zipper DNA-binding protein (3). Its gene resides at the *mi* locus in mice (4), and mutation of this gene results in deafness, bone loss, small eyes, and poorly pigmented eyes and skin (5). The primary cell types affected in MITF-deficient mice are mast cells, osteoclasts, and melanocytes (5). In humans, mutation in this gene causes Waardenburg syndrome type II (6). MITF regulates the expression of mouse mast cell protease (mMCP)-6 (7), mMCP-5 (8), c-*kit* (9), p75 nerve growth factor (8), granzyme B (10), tryptophan hydroxylase (11), and others. We have previously identified two MITF-interacting proteins, Hint (formerly known as protein kinase CI) (12) and protein inhibitor of activated STAT3 (PIAS3) (13), using the yeast two-hybrid system with the basic helix-loop-helix leucine zipper domain as a bait. These two MITF-associated proteins were shown to be repressors of MITF transcriptional activity (12, 13).

STAT3 is a transcription factor involved in signal transduction pathways that are activated by the IL-6 family of cytokines. It is tyrosine phosphorylated by JAK, translocates as a dimer into the nucleus, and activates specific genes (14). STAT3 signaling has been shown to prevent programmed cell death and enhance cell proliferation through regulating genes such as *Bcl-x$_L$*, *Mcl-l*, c-*Myc*, and *cyclin D1* (15–18).

PIAS3 was identified as inhibitor of both activated STAT3 (19) and MITF (13). We have previously provided experimental evidence regarding the functional cross-talk among MITF, PIAS3, and STAT3 in response to cytokine activation of mast cells. Furthermore, our results suggest that STAT3 acts in an indirect manner as a regulatory factor of MITF transcriptional activity. We showed that on IL-6/IL-6R or stem cell factor (SCF) activation, PIAS3 is mobilized from MITF to the activated STAT3, due to the phosphorylation of MITF at S409 (20).

In this work, we show that monomeric IgE alone can mediate the MITF-PIAS3-STAT3 network of interactions and lead to phosphorylation and transcriptional activation of MITF and STAT3 transcription factors.

Materials and Methods

Mice

Mouse colonies were established from transgenic mice (MITF$^{sp/sp}$ and MITF$^{sp/di}$) kindly provided by Dr. Lynne Lamoreux (College of Veterinary Medicine, Texas A&M University, College Station, TX). Mice carrying the

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[3] Abbreviations used in this paper: MITF, microphthalmia transcription factor; mMCP, mouse mast cell protease; BMMC, bone marrow-derived mast cells; PIAS3, protein inhibitor of activated STAT3; RBL, rat basophilic leukemia; wt, wild type; SCF, stem cell factor.

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MITF encoded by the mutant mi^{sp} are unable to incorporate exon 6a into the MITF message due to a splice site mutation (21). Failure to express exon 6a has little phenotypic effect in heterozygous or homozygous mutant mice. An effect is seen only when the mi^{sp} mutation is in combination with another mutation at the locus. The $MITF^{di}$ mutation arose in a cross between a PT female and an ethylnitrosourea-treated C3H/HeH male (22). MITF encoded by the mutant mi^{di} allele is lacking the Zip domain (23) due to a C to T transition in exon 8 at position 916, which introduces a premature stop codon between the basic helix-loop-helix and the leucine zipper domain. Heterozygous appear normal; however, when they are combined with the mi^{sp} mutant, the heterozygous have white coats with pigmented patches, which enables us to select and breed them. All experiments involving mice were reviewed and approved by our institute's review committee.

Cell culture and treatments

Femoral bone marrow cells derived from mice were cultured in IL-3-containing medium for 3 wk to generate bone marrow-derived mast cells (BMMC) as previously described (24). Rat basophilic leukemia (RBL) cells were cultured and maintained in a growth medium containing RPMI 1640, 10% FCS, 2 mM L-glutamine, 2 mM nonessential amino acids, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 50 μM 2-ME. All cells were grown in a humidified incubator at 37°C with 5% CO_2. RBL and BMMC cells were either triggered with anti-DNP IgE mAb alone (SPE-7; Sigma-Aldrich; Ag, or IgE derived from ascites of CAF1/J mice harboring the IgE hybridoma; Ref. 25) or sensitized first with IgE (2 h) and then challenged with DNP. IgE Ab was ultracentrifuged before use to remove aggregates.

Plasmids

The luciferase reporter plasmid, pSP72, containing the MITF-binding region of the promoter and the first exon of the mMCP-6 gene (−191 to +26), was generously provided by Professor Kitamura (Osaka, Japan). pcDNA-STAT3 flag tagged and the M67 pTATA *Tk-Luc* reporter gene were kindly provided by Dr. J. E. Darnell (Rockefeller University, New York, NY). The GFP-PIAS3 construct was kindly provided by Dr. L. Vassen (Universitatsklinikum Essen). The fidelity of all constructs was verified by direct sequencing.

Transient cotransfection and luciferase assay

RBL cells (5×10^6) were used in luciferase assay experiments. Cells were transfected using nucleofector technology (Amaxa Biosystems; Amaxa) solution R, program T-20. Cells were transfected with 5 μg of luciferase reporter gene (of MITF or STAT3). pcDNA alone (5 μg) was used as a nonspecific control. The cells were incubated in plates for 24 h, treated or not with IgE or IgE plus Ag, lysed, and assayed for luciferase activity. The luciferase activity was normalized to the total protein concentration. The normalized value was then divided by the luciferase activity obtained by cotransfection of the reporter with pcDNA alone. The ratio was expressed as the relative luciferase activity.

Coimmunoprecipitation

RBL cells were used in coimmunoprecipitation experiments. Cells were activated with IgE or with IgE plus Ag for 30 min. Cells ($5–10 \times 10^6$) were lysed by the addition of 300 μl of cold lysis buffer (0.01 M Tris-HCl (pH 7.4), 1% deoxycholate, 1% Triton X-100, 0.1% SDS, 0.15 M NaCl, and 0.25 μM PMSF) and 15 μl of protease inhibitors (Sigma-Aldrich). Cells were then homogenized, and their supernatants were collected after 15 min of centrifugation in a microcentrifuge at 4°C. Recovered lysates were incubated with anti-PIAS3 Ab (Santa Cruz Biotechnology) prebound to 15 mg of protein A/G agarose (Pierce Biotechnology) and incubated with agitation overnight at 4°C. Recovered immunocomplexes were washed three times with lysis buffer.

Gel electrophoresis and Western blots

Proteins were resolved by 10% SDS-PAGE under reducing conditions and transferred to 0.45-μm pore size nitrocellulose membranes. Blots were probed with anti-PIAS3 (Santa Cruz Biotechnology), anti-MITF (C5-monoclonal; kindly provided by Dr. D. Fisher, Dana-Farber Cancer Institute and Children's Hospital, Boston, MA), anti-STAT3 (Santa Cruz Biotechnology), and anti-phosphoSTAT3 (Santa Cruz Biotechnology and Oncogene Research Products) Abs. Visualization of reactive proteins was by ECL (13).

Indirect fluorescent immunocytochemistry

RBL cells were cotransfected with GFP-PIAS3, STAT3 flag tagged, and MITF. Cells were grown on glass coverslips in six-well plates. After extensive washing with PBS, the cells were fixed with 1.5 ml of 3.7% formaldehyde in PBS for 10 min. The fixed cells were then washed with PBS and permeabilized with 1.5 ml of Triton X-100 diluted 1/2 with PBS containing 7.5 mg of BSA. After 45 min of blocking with normal donkey serum, the cells were stained with mouse anti-MITF and rabbit anti-flag followed by the addition of rhodamine-conjugated goat anti-mouse IgG and Cy5-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories). Fluorescence analysis was performed using the Zeiss LSM 410 confocal laser scanning system connected to a Zeiss Axiovert 135M microscope (Zeiss). Green fluorescence of GFP-labeled PIAS3 was excited with an argon laser (488 nm excitation line with 515 nm long pass barrier filter). Rhodamine-conjugated goat anti-mouse Ab was excited with a helium-neon laser (543 nm excitation line with 570 nm long pass barrier filter). Cy5-conjugated goat anti-rabbit was simultaneously excited with a helium-neon laser (633 nm excitation line).

Real time quantitative PCR

MITF- and STAT3-responsive genes were measured using real time quantitative PCR. Total RNA was extracted from BMMC cells, and mRNA levels of various genes were quantified by SYBR green incorporation (SYBR Green PCR Master Mix; Applied Biosystems). SYBR-green incorporation to double-stranded DNA permits the direct detection of PCR product after each amplification cycle (ABI Prism 7000 sequence detection system; Applied Biosystems). The genes with mRNA levels that were quantified by real time PCR are as follows: β-actin, STAT3 target genes: *Bcl-x_L*, *VEGF*, and c-*Myc*; MITF target genes: *Bcl-2*, *granzyme B*, c-*kit*, and tryptophan hydroxylase.

Flow cytometry apoptosis assay

To monitor apoptosis, BMMC cells were stained with FITC-labeled annexin V and propidium iodide using an annexin V-FLUOS staining kit (Roche). Flow cytometric analysis of the stained cells was performed with FACScan.

Results

Interplay between PIAS3, STAT3, and MITF on IgE stimulation

We have previously shown in mast cells that on IL-6/IL-6R or SCF activation, PIAS3 is mobilized from MITF to the activated STAT3. Thus, to investigate whether this interplay occurred due to stimulation of mast cells with IgE or IgE plus Ag, RBL cells were activated and lysed, and PIAS3 was immunoprecipitated with anti-PIAS3 and immunoblotted with anti-MITF or anti-STAT3. To prevent aggregates, IgE Ab was ultracentrifuged before use.

As shown in Fig. 1*A*, an increase in the association of PIAS3 with STAT3 was observed after exposure of mast cells to soluble IgE. However, in IgE- plus Ag-activated cells, the increase in PIAS3-STAT3 interactions was significantly lower than in cells treated with IgE alone. As expected, a decrease in the association between PIAS3 and MITF was observed, regardless of whether the cells were activated by IgE or IgE plus Ag. Stripping of the membranes and reprobing with anti PIAS3 revealed a similar amount of PIAS3 bound to the beads. The same amount of MITF or STAT3 was detected in each of the lysates as revealed by probing the lysates with anti-MITF and anti-STAT3 as control (not shown).

Using confocal laser scanning, we determined the in vivo interactions between PIAS3, MITF, and STAT3 in RBL cells stimulated with IgE alone or with IgE plus Ag. The cells were cotransfected with GFP-PIAS3, MITF, and flag tagged STAT3. Resting and activated cells were fixed and stained with mouse anti-MITF and rabbit anti-flag, which were detected by staining with rhodamine-conjugated goat anti-mouse and Cy5 conjugated goat anti-rabbit Abs, respectively. As shown in Fig. 1*B*, MITF and PIAS3 in resting cells were colocalized in the nucleus (cyan dots), whereas most of STAT3-flag tagged was detected in the cytoplasm. IgE treatment induced STAT3 to partially colocalize with PIAS3 and MITF in the nucleus. IgE plus Ag also caused the translocation of

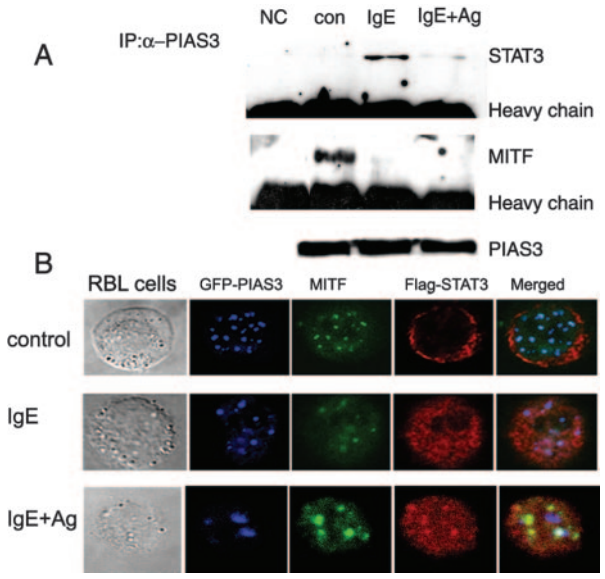

FIGURE 1. Interplay between PIAS3, STAT3, and MITF on IgE stimulation. *A*, RBL cells were stimulated with IgE or IgE plus Ag for 1 h at 37°C. Cells were lysed, and PIAS3 was immunoprecipitated (IP) with anti (α)-PIAS3 and immunoblotted with anti-STAT3 (top blot), anti-MITF (lower blot) and reprobed with anti-PIAS3 (indicated beneath MITF blots). NC, Isotype-matched control (con) Ab. One representative of three experiments is shown. *B*, RBL cells were cotransfected with GFP-PIAS3, MITF, and STAT3-flag tagged. Cells were incubated with IgE or IgE plus Ag for 1 h. The immunostaining was performed with anti-MITF and anti-flag Abs using rhodamine (green)- and Cy5 (red)-labeled secondary Abs, respectively. The cells were analyzed by laser confocal microscopy. The nuclear colocalization is shown in the right panels (white dots for triple colocalization and cyan for MITF and PIAS3 colocalization).

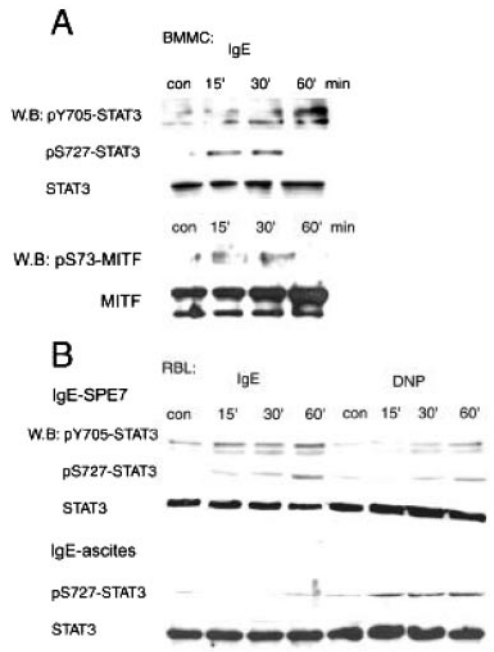

FIGURE 2. IgE stimulates the phosphorylation of MITF and STAT3 transcription factors. *A*, BMMC were deprived of IL-3 for 2 h and then treated with IgE alone for the indicated times, and the total cell lysates were subjected to Western blot (W.B.) analysis with phospho-specific Abs to STAT3 and MITF. The blots were then reprobed with Abs against STAT3 and MITF to confirm equal loading. *B*, RBL cells were treated with IgE alone (SPE7 clone or ascites derived) or IgE plus Ag for the indicated times, and the total cell lysates were subjected to Western analysis with phospho-specific Abs to STAT3. The blots were then reprobed with Abs against STAT3 to confirm equal loading. con, Control.

STAT3 to the nucleus, but colocalization of PIAS3 with MITF and STAT3 was less prominent.

IgE stimulates the phosphorylation of MITF and STAT3 transcription factors

The signaling potential of IgE trigger in mast cells was investigated by the exposure of BMMC to IgE alone (SPE-7 clone) for various periods of time after the cells were deprived of IL-3 for 2 h. We first followed MITF and STAT3 phosphorylation sites at MITF-S73 (26), STAT3-Y705 (27), and STAT3-S727 (28). As can be seen in Fig. 2*A*, 5 μg/ml IgE induced MITF phosphorylation at S73 and STAT3 phosphorylation at both S727 and Y705. The kinetics of STAT3 phosphorylation at Y705 and MITF phosphorylation were different.

A similar pattern of STAT3 phosphorylation was observed at both sites in RBL cells exposed to IgE. However, a decrease in the intensity of Y705-STAT3 phosphorylation was observed when FcϵRI was aggregated via IgE-DNP trigger (Fig. 2*B*).

In addition, another set of experiments were conducted using ascites recovered IgE (25). This IgE was previously described as poorly cytokinergic IgE (29). The signaling potential of that IgE isoform was investigated by following STAT3 phosphorylation at STAT3-S727 site (28). We found that IgE did not induce STAT3 phosphorylation at S727 (Fig. 2*B*).

Real time PCR showing IgE enhances MITF transcriptional activity

Real time PCR was performed to investigate whether IgE alone could enhance MITF transcriptional activity in BMMC derived from wild-type (wt) mice. The cells were exposed to IgE and than to Ag for 6 h. Changes in the level of RNA of six potential target genes previously shown to be regulated by MITF in mast cells (9–11, 30) were determined. Of the six MITF target genes, the mRNA accumulation of two, *granzyme B* and *Bcl-2*, was significantly elevated on exposure to IgE or IgE and Ag (Fig. 3*A*). The mRNA accumulation of c-*Kit* showed significant elevation only when the cells were exposed to IgE and than triggered with Ag. The mRNA levels of mMCP-4 and mMCP-5 did not show significant accumulation after IgE or IgE plus Ag trigger. Unexpectedly, mRNA accumulation of tryptophan hydroxylase was reduced both in mast cells triggered by IgE alone and those triggered by IgE plus Ag.

Real time PCR experiments were conducted to determine whether the elevation in mRNA expression of *Bcl-2* and *granzyme B* is specifically attributed to MITF transcriptional activity. For that, we used BMMCs derived from MITF mutated mice ($MITF^{di/di}$) that lack the MITF-Zip domain. This motif has been shown to be essential for MITF transcriptional activity (23). In IgE-activated BMMC derived from these $MITF^{di/di}$ mice, no accumulation of mRNA was observed in the MITF target genes (*Bcl-2, granzyme B*) (Fig. 3*B*). This strongly indicates that IgE induced MITF-mediated gene expression in vivo.

IgE mediates STAT3 transcriptional activity in mast cells

Real time PCR was performed to determine whether IgE could enhance STAT3 transcriptional activity in BMMC derived from wt mice. These cells were exposed to IgE or to IgE and Ag. Three STAT3 target genes were analyzed (c-*Myc, VEGF, Bcl-*x_L) (9, 10, 15–17, 30–32). Of these three STAT3 target genes, the mRNA

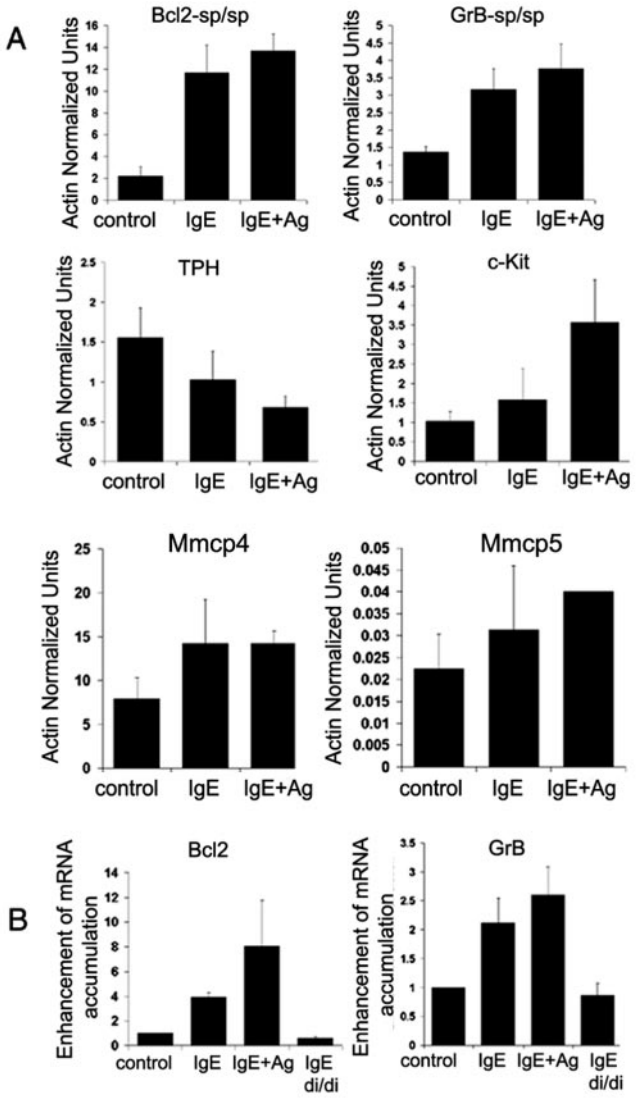

FIGURE 3. IgE enhances MITF transcriptional activity. *A*, Quantitative RT-PCR analysis of MITF target genes (c-*Kit*, *Bcl-2*, *granzyme B* (*GrB*), and *TPH*), from IgE- or IgE and Ag (DNP)-activated BMMC, derived from wt mice. mRNA levels were normalized to actin and measurements were performed in triplicates. *B*, Quantitative RT-PCR analysis of MITF target genes (*Bcl-2, granzyme B*) in IgE-activated BMMCs, derived from wt or MITF$^{di/di}$ mice. Results are represented as accumulation of mRNA of the target genes.

accumulation of only one (c-*Myc*) was significantly elevated, after cells were exposed to each one of the stimuli (Fig. 4*A*).

To determine whether the elevation in mRNA of c-*Myc* is specifically attributed to STAT3 transcription factors, real time PCR experiments were performed in BMMC, where the function of STAT3 was disrupted by STAT3 inhibitor peptide (33). No accumulation of c-*Myc* mRNA was observed in IgE-activated BMMC that were treated with STAT3 inhibitor (Fig. 4*B*). Thus, IgE induced the expression of STAT3-mediated genes in mast cells.

Luciferase assay showing that IgE enhances MITF and STAT3 transcriptional activity

To further assess the ability of IgE alone to enhance MITF and STAT3 transcriptional activity, RBL cells were transfected with MITF reporter gene (*191*) or STAT3 reporter gene (*M67*). The cells were deprived of FCS and treated with IgE for 6 h, and the luciferase activity was measured. The results were normalized against protein concentration. As shown in Fig. 5, IgE triggering induced transcriptional activity by 2- to 3-fold in both cells transfected with MITF reporter gene and those transfected with *STAT3* reporter gene.

MITF and STAT3 do not affect IgE-dependent antiapoptotic effect

It was previously shown that BMMC go into apoptosis when growth factors such as IL-3 are removed from the medium (1, 34). This apoptosis is significantly attenuated by IgE. The role played by MITF and STAT3 in IgE-dependent mast cell survival was determined by flow cytometry on cells stained with annexin V to detect early apoptotic cells and with propidium iodide to detect dead cells. As shown in Fig. 6, IgE was able to rescue the cells from apoptosis observed in cells depleted from IL-3 for 24 h in BMMC derived from MITF$^{di/di}$ mice and in those derived from wt mice. BMMC cells that were treated with specific STAT3 inhibitor peptide were also rescued from apoptosis on IgE treatment. Thus, MITF and STAT3 are not obligatory factors of the antiapoptotic effect induced by IgE.

Discussion

In the present study, we have demonstrated that monomeric IgE alone induces signaling, which results in the transcriptional regulation of MITF and STAT3 transcription factors by PIAS3.

These new data are an extension of our previous work, in which we showed that in mast cells and melanocytes, PIAS3 is mobilized from MITF to activated STAT3 on exposure to IL-6/IL-6R or SCF (20). Here we show that IgE alone, without Ag, mediates this network of interactions.

We observed that the addition of Ag to IgE-sensitized cells resulted in cellular responses with regard to this network different than those observed when the cells were treated with IgE alone. Using confocal laser scanning microscope, we saw that in resting cells MITF and PIAS3 were colocalized in the nucleus, whereas most of STAT3-flag tagged was detected in the cytoplasm. IgE treatment induced STAT3 translocation to the nucleus, and all three proteins were found to partially colocalize in the nucleus in dotted complexes. However, although IgE plus Ag also caused the translocation of STAT3 to the nucleus, the colocalization of STAT3 and MITF with PIAS3 was less prominent. Furthermore, coimmunoprecipitation experiments showed the same pattern. An increase in the association of PIAS3 with STAT3 was observed after exposure of mast cells to soluble IgE, whereas in IgE- plus Ag-activated cells, the increase in PIAS3-STAT3 interactions was significantly smaller.

We have demonstrated here that monomeric IgE alone can lead to phosphorylation of MITF and STAT3 transcription factors. STAT3 phosphorylation at Y705 and S727 have been shown to be critical for full transcriptional activity (28). Phosphorylation-dephosphorylation plays a major role in the assembly and disassembly of multiprotein complexes. For example, phosphorylation of MITF at S73 triggers the recruitment of the coactivator P30.0 (35). In activated melanocytes, an alanine substitution at either S73 or S409 produced transcriptionally inactive MITF, whereas MAPK and Rsk-1, which phosphorylate MITF at S73 and S409, respectively, promote its activation followed by its degradation (26, 36).

We used IgE from an SPE-7 clone. This Ab was used by Kalesnikoff et al. (1) in their study showing that IgE alone induces the secretion of a variety of cytokines and triggers multiple signaling pathways. Our results show that IgE from SPE-7 clone is able to induce transcription of genes such as *Bcl-2* and c-*Myc*, which are responsible for cell survival and proliferation. Such transcription

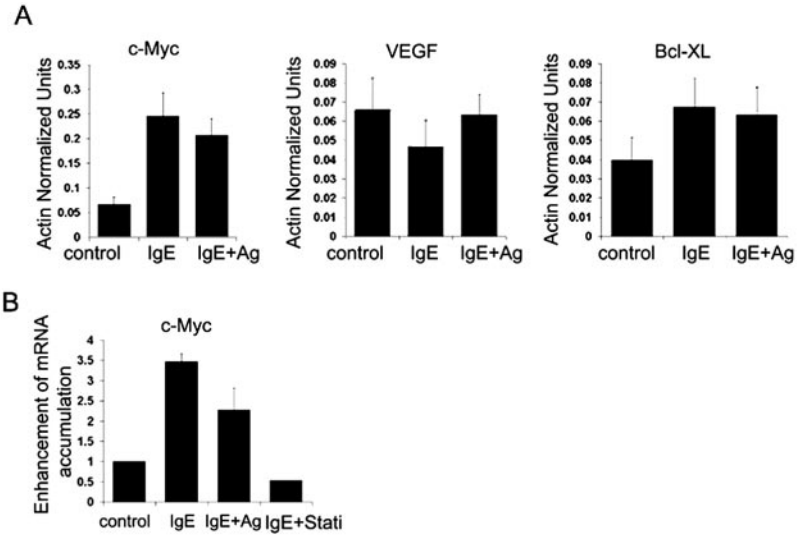

FIGURE 4. IgE enhances STAT3 transcriptional activity. *A*, Quantitative RT-PCR analysis of STAT3 target genes (c-*Myc*, *Bcl-x*$_L$, and *VEGF*) from IgE- or IgE plus Ag (DNP)-activated BMMC, derived from wt mice. RNA levels were normalized to actin and measurements were performed in triplicates. *B*, Quantitative RT-PCR analysis of STAT3 target gene (c-*Myc*) in IgE-activated BMMCs, treated with a specific STAT3 inhibitor peptide. Results are represented as accumulation of mRNA of the target genes.

could be due to the induction of the activity of MITF and STAT3 transcription factors. In contrast, Asai et al. (34) claimed that monomeric IgE does not promote DNA synthesis. This could be explained, as suggested by Kitaura et al. (29), if different IgE molecules induce different cellular responses. Although highly cytokinergic IgE (SPE-7) can efficiently induce signal transduction and production of cytokines, poorly cytokinergic IgEs have a weaker effect. A recent study showed that the SPE-7 Ab can adopt different Ag-binding conformations before Ag binding and that binding of different Ags can induce isomerization of the binding site, leading to high affinity complexes with a deep or narrow site (37). Therefore, it is possible that IgE can induce FcεRI aggregation in the absence of the Ag, because IgE is known to have specificity when the Fab of an FcεRI-bound IgE molecule interacts with a neighboring FcεRI-bound IgE, directly or indirectly. Moreover, such isomerization can explain the different biological events produced by IgE alone vs IgE plus Ag.

These in vitro phenomena are supported by in vivo studies that have demonstrated that IgE Abs can induce up-regulation of FcεRI surface expression in mast cells and basophils (38, 39) and promote immune sensitization to hapten in the skin, independently of Ag (2).

STAT3 signaling has been shown to prevent programmed cell death and enhance cell proliferation through regulating genes involved in cell growth and apoptosis, including *Bcl-x*$_L$, *Mcl*-l, c-*Myc*, and *cyclin D1* (15–18). The ability of activated STAT3 to enhance MITF activity by recruiting PIAS3 might play an important role in mast cells, because MITF has an essential role in mast cell function and development (40–42).

We have demonstrated that monomeric IgE can suppress apoptosis, independent of MITF or STAT3, although these transcription factors were shown to regulate transcription of genes that are important for mast cell survival (*Bcl-2*, c-*Myc*). Therefore, the exact mechanism by which exposure to monomeric IgE can enhance mast cell survival still remains unclear.

Disclosures

The authors have no financial conflict of interest.

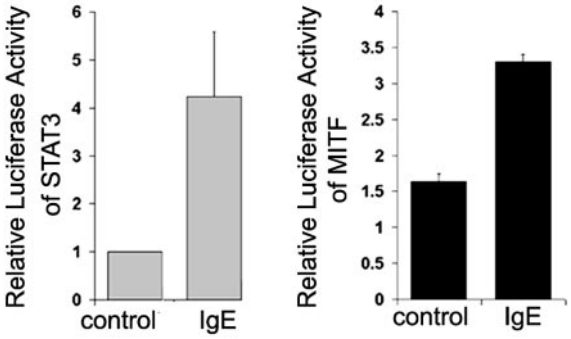

FIGURE 5. IgE induces MITF and STAT3 transcriptional activity. RBL cells were transfected with STAT3 reporter gene (*M67*) or MITF reporter gene. Cells were triggered with 5 μg/ml IgE for 6 h. Luciferase activity of lysed cells was measured and normalized against protein concentration. Values are means ± SE of three experiments.

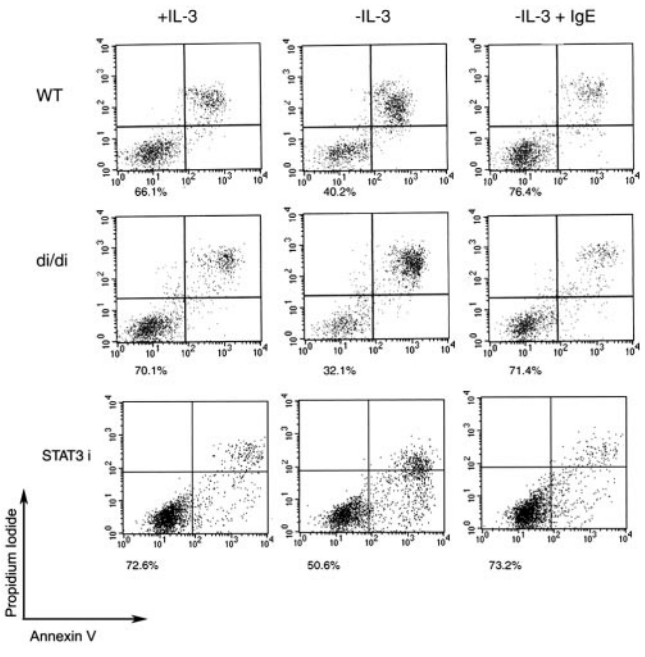

FIGURE 6. MITF and STAT3 do not affect IgE-dependent antiapoptotic effect. BMMC, in the absence of IL3, were incubated with or without 5 μg/ml monomeric IgE for 24 h. Control includes cells incubated with IL-3 but without IgE. Cells were stained with FITC-annexin V and propidium iodide followed by flow cytometric analysis. Percentages of annexin V-propidium iodide-stained cells are shown.

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