Transcription factor E3, a major regulator of mast cell–mediated allergic response

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Background: Microphthalmia transcription factor, an MiT transcription family member closely related to transcription factor E3 (TFE3), is essential for mast cell development and survival. TFE3 was previously reported to play a role in the functions of B and T cells; however, its role in mast cells has not yet been explored.

Objective: We sought to explore the role played by TFE3 in mast cell function.

Methods: Mast cell numbers were evaluated by using toluidine blue staining. FACS analysis was used to determine percentages of Kit and FcεRI double-positive cells in the peritoneum of wild-type (WT) and TFE3 knockout ($TFE3^{-/-}$) mice. Cytokine and inflammatory mediator secretion were measured in immunologically activated cultured mast cells derived from either knockout or WT mice. *In vivo* plasma histamine levels were measured after immunologic triggering of these mice.

Results: No significant differences in mast cell numbers between WT and $TFE3^{-/-}$ mice were observed in the peritoneum, lung, and skin. However, $TFE3^{-/-}$ mice showed a marked decrease in the number of Kit^+ and $Fc\varepsilon RI^+$ peritoneal and cultured mast cells. Surface expression levels of FcεRI in $TFE3^{-/-}$ peritoneal mast cells was significantly lower than in control cells. Cultured mast cells derived from $TFE3^{-/-}$ mice showed a marked decrease in degranulation and mediator secretion. *In vivo* experiments showed that the level of plasma histamine in $TFE3^{-/-}$ mice after an allergic trigger was substantially less than that seen in WT mice.

Conclusion: TFE3 is a novel regulator of mast cell functions and as such could emerge as a new target for the manipulation of allergic diseases. (J Allergy Clin Immunol 2012;129:1357-66.)

Key words: *Allergy, mast cells, degranulation, histamine, cytokine secretion, transcription factor E3 knockout mice*

Mast cells, the primary effectors of immediate-type allergic reactions, reside in peripheral tissues that directly associate with the external environment. In most allergic reactions the allergen is recognized by IgE, which is bound to the surface high-affinity receptor for IgE (FcεRI) on mast cells. Aggregation of this receptor initiates the intracellular signals that lead to the release of multiple granular mediators, as well as cytokines that are responsible for the immediate hypersensitivity reaction.

Kit/CD117 (Kit) is another cell-surface receptor on mast cells that is crucial for mast cell differentiation, proliferation, and survival. The expression of Kit is mainly regulated by microphthalmia transcription factor (MITF), a member of the MiT family of transcription factors. The MiT family of transcription factors is composed of 4 closely related members: MITF, transcription factor E3 (TFE3), TFEB and TFEC.[1]

It is well established that MITF, the most investigated protein of this family, plays a critical role in the regulation of mast cell differentiation.[2-4] Genetic and biochemical studies have revealed a functional overlap of MiT activity in certain developmental lineages.[5-8] Specifically, Jenkins' group[7] demonstrated, by using knockout mice, that although mutation of either MITF or TFE3 in mice does not disrupt osteoclast development, mutation of both genes or the presence of a dominant negative MITF resulted in severe osteopetrosis. Phylogenetic analysis shows that of the 4 MiT family members (see Fig E1 in this article's Online Repository at www.jacionline.org), TFE3 is the closest family member to MITF. In light of the previous observations regarding MITF and TFE3 redundancy[7] and the similarity between MITF and TFE3, the role played by TFE3 in mast cell function was explored in this work.

TFE3 has been shown to play a major role in the immune system because of its regulation of CD40 ligand expression in

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Abbreviations used
BMMC: Bone marrow–derived mast cell
ChIP: Chromatin immunoprecipitation
Gzmb: Granzyme B
Mcpt4: Mast cell protease 4
MITF: Microphthalmia transcription factor
PE: Phycoerythrin
siRNA: Small interfering RNA
TFE3: Transcription factor E3
Tpsb2: Mast cell protease 6
WT: Wild-type

T cells.[9] In addition, a considerable decrease in the expression of the low-affinity receptor for IgE (CD23) on B cells derived from stem cells deficient in TFE3 was observed.[10] This receptor has an important role in the control of allergic diseases.[11] It was previously reported that TFE3 compensated for MITF deletion in the development of osteoclasts in MITF-deficient mice[7] and that these 2 transcription factors have overlapping roles in their functions as transcriptional mediators in the macrophage colony-stimulating factor pathway.[7,12]

Using TFE3-deficient mice and knocking down TFE3 in mast cells derived from MITF-deficient mice, we explored the specific role played by TFE3 in mast cell function. The results of these 2 combined approaches suggest that TFE3 compensates for the lack of MITF by means of regulation of MITF target genes. Furthermore, TFE3 was found to be a novel regulator of the α subunit of the high-affinity IgE receptor (FcεRIα). Together, these findings indicate that TFE3 has a critical role in mast cell function.

METHODS

Mice

VGA-9$^{tg/+}$ and TFE3$^{(fcr)}$ mice were kindly provided by H. Arnheiter (National Institutes of Health, Bethesda, Md). Mice carrying the *tg*/*tg* mutation have an insertion of approximately 50 copies of a transgene integrated inside the MITF-M promoter and are unable to express MITF.[13] Normal littermates of *tg*/*tg and* TFE3$^{(fcr)}$ mice were determined by means of genomic DNA extraction from tails and subsequent PCR analysis. Primers for detection of the transgenes are specified in the Methods section in this article's Online Repository at www.jacionline.org.

Cells

For bone marrow–derived mast cells (BMMCs), femoral bone marrow cells derived from wild-type (WT) C3H, TFE3$^{(fcr)}$, *tg*/*tg*, and *tg*/+ mice were cultured for 4 to 8 weeks to generate BMMCs, as previously described (see additional information in the Methods section in this article's Online Repository).[12] The level of differentiation to BMMCs was verified by using toluidine blue staining.

For details about resident peritoneal mast cells, see additional information in the Methods section in this article's Online Repository.

Gene silencing

TFE3 siGENOME SMARTpool small interfering RNA (siRNA) oligomers were purchased from Dharmacon (Thermo Fischer, Waltham, Mass). SiRNA was delivered by means of electroporation with Amaxa nucleofection solution V, program T-17 (Amaxa Biosystems, Cologne, Germany). BMMCs (1.5×10^6) were electroporated with either 0.2 nmol/L siRNA (approximately 3 μg) or scrambled nonrelevant siRNA as control. Protein levels were monitored 24, 48, and 72 hours after transfection to evaluate silencing of TFE3 protein. The levels of TFE3 silencing were determined for each biological repeat independently by using real-time PCR and varied from 50% to 70%.

Antibodies

Anti-TFE3 H-300 rabbit polyclonal antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, Calif); clone MAR-1 of phycoerythrin (PE)–conjugated anti-FcεRI and clone 2B8 of PE-CY5–conjugated anti-Kit antibodies were purchased from eBioscience (San Diego, Calif).

Immunologic activation

BMMCs were treated with 1 μg/mL IgE anti-DNP (Sigma-Aldrich, St Louis, Mo) for 2 hours and then washed 3 times in serum free medium and incubated with DNP-albumin (Sigma) for 2 to 8 hours. Cells were then collected and analyzed for the various procedures.

PCR and real-time PCR

BMMCs were immunologically activated, and then RNA was extracted with the RNeasy Kit (Qiagen GmbH, Hilden, Germany), followed by DNAse treatment (Qiagen). RNA was quantified with a Nanodrop spectrophotometer and reverse transcribed with random primers (Promega, Madison, Wis) and MMLV reverse transcriptase (Invitrogen, Carlsbad, Calif).

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) from *tg*/*tg* BMMCs was performed as previously described.[14] For further details, see the Methods section in this article's Online Repository.

Measurements of secreted mediators

BMMCs (3×10^6) were treated with 1 μg/mL IgE anti-DNP (Sigma-Aldrich) for 4 hours and then washed 3 times in PBS and incubated with 50 ng/mL DNP-albumin (Sigma) for 20 hours in complete growth medium. Supernatants were collected, and IL-4, IL-6, granzyme B (Gzmb), and TNF-α content was measured by using ELISA kits (eBioscience). Cells were counted by using flow cytometry, and readings were normalized to total protein levels of the cell lysates. The BD cytometric bead array (BD Biosciences, San Jose, Calif) was also used as a second method to measure secreted mediator levels by means of flow cytometry.

In vivo histamine release assay

TFE3$^{-/-}$ mice and WT control animals were sensitized with 3 μg of the murine IgE anti-DNP mAb SPE-7 by means of intravenous injection in the tail vein. Twenty-four hours later, the mice were challenged with intravenous injection of DNP-BSA (250 μg per mouse). Blood histamine levels were determined by using competitive ELISA (IBL International Gmbh, Hamburg, Germany) on approximately 100 μL of blood 1.5 minutes after antigen challenge.

Further method details

Other methods used were flow cytometry and immunofluorescence; histological analysis of mast cells; and the β-hexosaminidase release assay. The details of these methods can be found in the Methods section in this article's Online Repository.

Statistical analysis

Statistical analysis was conducted with Microsoft Excel 2007 software (Microsoft, Redmond, Wash). Mutant mice and their normal littermates were compared by using 2-tailed Student *t* tests. Data are reported as means ± SEs.