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Impaired Mast Cell-Driven Immune Responses in Mice Lacking the Transcription Factor NFATc2

Marc Becker,* Valeska Heib,† Matthias Klein,* Fatma Doener,* Tobias Bopp,* Christian Taube,‡ Markus Radsak,‡ Hansjörg Schild,* Edgar Schmitt,* and Michael Stassen²*

The three calcium-dependent factors NFATc1, c2, and c3 are expressed in cells of the immune system and play pivotal roles in modulating cellular activation. With regard to NFATc2, it was reported that NFATc2-deficient mice display increased immune responses in several models for infection and allergy in vivo. This led to the assumption that NFATc2 is involved in the maintenance of immune homeostasis. Using the synthetic TLR7 agonist imiquimod as an adjuvant in epicutaneous peptide immunization, we observed that both the inflammatory reaction and the peptide-specific CTL response are severely impaired in NFATc2-deficient mice. Detailed analyses revealed that early production of proinflammatory cytokines, lymph node hypertrophy, and migration of Langerhans cells are strongly reduced in NFATc2-deficient animals. With the aid of mast cell-deficient mice and reconstitution experiments using mast cells derived from either NFATc2-deficient mice or wild-type controls, we were able to show that NFATc2 expressed in mast cells is critical for the initiation of inflammation, migration of Langerhans cells, and the development of full-blown CTL responses following epicutaneous immunization. Thus, NFATc2 is an important factor controlling mast cell accessory function at the interface of innate and adaptive immunity. The Journal of Immunology, 2009, 182: 6136–6142.

The NFAT family of transcription factors is widely expressed in numerous cell types and comprises the four genuine members NFATc1–c4 and the distantly related TonEBP. The latter controls the osmotic stress-induced expression of some cytokines, whereas the NFATc members of this family are activated by a calcium signaling pathway in which calcium-calmodulin complexes activate the cyclosporin-sensitive phosphatase calcineurin which then binds to and dephosphorylates NFAT in the cytosol. Dephosphorylated NFAT molecules translocate into the nucleus and cooperatively bind with their nuclear partners to DNA (1–3). NFATc1–c3 are expressed in the immune system and especially their role in T cells, which coexpress these factors, has been intensively studied.

Detailed analyses using mice lacking distinct NFAT factors revealed the development of quite disparate phenotypes. NFATc1-deficient mice die in utero due to defects in the generation of cardiac valves and septa (4). Blastocyst complementation assays showed that NFATc1−/−/Rag-1−/− chimeric mice have reduced numbers of thymocytes, impaired proliferation of peripheral lymphocytes, and reduced synthesis of Th2 cytokines (5). Mice lacking NFATc3 develop normally and are phenotypically inconspicuous, yet they have defects in positive selection of T cells due to increased apoptosis of thymocytes. Although diminished in number, an increased percentage of peripheral NFATc3−/− T cells display an activated phenotype, however, proliferation and cytokine production appeared normal (6). Mice deficient for NFATc2 were generated by three groups independently (7–9). No defects in the development of thymocytes were observed for NFATc2-deficient mice but there was a slight increase of T and B cells in the periphery accompanied by a modest splenomegaly. Interestingly, such mice were shown to develop enhanced immune responses in several models of infection and allergy (8, 10–13).

Double deficiency for NFATc2 and NFATc3 causes massive splenomegaly, lymphadenopathy, and a strong increase in serum IgG1 and IgE levels. Such mice show a dramatic increase in activated peripheral T cells able to overproduce cytokines independently of costimulatory signals. Ultimately, these animals develop an autoaggressive and fatal phenotype (14, 15). These studies suggested that NFATc2 and NFATc3 have important and partly overlapping functions in maintaining T cell homeostasis and tolerance (16). In this context, we were able to show that CD4⁺ T cells lacking both factors are able to escape suppression by CD4⁺ CD25⁺ T regulatory cells which might, at least partly, explain the development of lymphoproliferative disorder (17).

We previously reported that epicutaneous application of a cytotoxic T lymphocyte (CTL) epitope in combination with the synthetic TLR7 ligand imiquimod leads to the transport of applied Ag to draining lymph nodes (LNs) and mounts a full-blown CTL response (18). Imiquimod acts as an adjuvant, inducing both an inflammatory response and the emigration of Ag-presenting Langerhans cells (LCs) out of the epidermis. In the present study we show that inflammation, migration of LCs, development of LN hypertrophy, and the generation of a CTL response are severely impaired in NFATc2-deficient mice. Detailed analyses revealed that mast cells lacking NFATc2 are the cause of the observed phenomena, identifying NFATc2 as a critical factor for the accessory function of mast cells in vivo.

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3 Abbreviations used in this paper: CTL, cytotoxic T lymphocyte; BMMC, Bone marrow-derived mast cell; DC, dendritic cell; HGPRT, hypoxanthine-guanine phosphoribosyl transferase; LC, Langerhans cell; LN, lymph node; PCA, passive cutaneous anaphylaxis; TCI, transcutaneous immunization; KC, keratinocyte chemotractant.
Materials and Methods

Mice

Genetically mast cell-deficient Kit<sup>W<sub>62</sub>/Kit<sup>W<sub>68</sub>/mice, as well as the congenic Kit<sup>W<sub>62</sub>/Kit<sup>W<sub>68</sub> wild-type littersmates, were obtained by intercrossing heterogeneous Kit<sup>W<sub>62</sub>/Kit<sup>W<sub>68</sub> mice provided by Marcus Maurer (Charité-Universitätsmedizin Berlin, Berlin, Germany). NFATC2-deficient mice were provided by Edgar Serfling (University of Würzburg, Würzburg, Germany). These mice were generated in the laboratory of Laurie Glomcher (Harvard Medical School, Boston, MA). The TCR transgenic OT-I mice were obtained from The Jackson Laboratory and bred in our own animal facility. All mice were on a C57BL/6 genetic background and used at the age of 6–12 wk. Animal procedures were conducted in accordance with the institutional guidelines.

Generation of bone marrow-derived mast cells (BMMCs) and local reconstitution of mast cell-deficient mice

BMMCs were generated according to standard procedures (19). For local reconstitution, 5 × 10<sup>7</sup> BMMC were injected into the ear pinna of 6-wk-old Kit<sup>W<sub>62</sub>/Kit<sup>W<sub>68</sub> mice 6–8 wk before the experiments were conducted. The assessment of reconstitution efficiencies using the heparin-binding protein avidin was done as described by Heib et al. (20).

Generation of single-cell suspensions from murine ear tissue

Mice were killed and the ears were divided into dorsal and ventral halves. The two halves of one ear were incubated in a solution with 0.5 mg/ml Liberase (BD Pharmingen) and DNase (Sigma-Aldrich) in MEM for 90 min at 37°C. The two halves were shedded in 1 ml of MEM by a Medimachine (BD Pharmingen). The cell suspensions of the ears were collected in tubes, washed twice, and immunostained with FITC-conjugated anti-Gr-1 and PE-conjugated anti-MHC-II (BD Pharmingen).

Passive cutaneous anaphylaxis (PCA)

For the induction of PCA, both NFATC2-deficient and wild-type mice (C57BL/6) received intradermal injections of 1 μg of murine monoclonal anti-DNP IgE (clone SPE-7) in 50 μl of PBS in the right ear and 50 μl of PBS in the left ear as control. Mice were challenged 24 h later by i.v. injection of 50 μg of DNP-human serum albumin in 100 μl of PBS, including 0.5% Evans blue, into the tail vein (all reagents from Sigma-Aldrich). Mice were killed 30 min after i.v. injection of Ag and the ears including 0.5% Evans blue were harvested, weighted, and incubated in 200 μl of PBS. The two halves were shredded in 1 ml of MEM by a Medimachine and DNase (Sigma-Aldrich) in MEM for 90 min at 37°C. The two halves were shredded in 1 ml of MEM by a Medimachine and DNase (Sigma-Aldrich). The two halves were shredded in 1 ml of MEM by a Medimachine and DNase (Sigma-Aldrich). The two halves were shredded in 1 ml of MEM by a Medimachine and DNase (Sigma-Aldrich). The two halves were shredded in 1 ml of MEM by a Medimachine and DNase (Sigma-Aldrich).

Application of imiquimod and measurement of ear thickness

The application of imiquimod was progressed by daily treatment of one ear with ∼40 mg Aldara 5% creme (3M Pharmaceuticals) for up to 6 days. The contralateral ear was treated with vehicle. The resultant ear swelling after local application was measured with a micrometer (Mitutoyo). Aldara contains 5% imiquimod ([1-(2-methylpropyl)-1H-imidazol-4,5-c]quinolin-4-amine) in vehicle (isooctadecanoic acid (20–30%), ethoxylated sorbitan monostearate (1–5%), octodecane-1-ol (1–5%), petroleum (1–5%), hexadecane-1-ol (1–5%), glycerol (1–5%), benzyl alcohol (1–5%), and water (40–60%).

Preparation of epidermal sheets

The dorsal and ventral halves of mouse ears were split and the dorsal halves were incubated in 0.5% dispase solution for 20 min (Dispase II; Roche). The epidermis was then removed, fixed in acetone, and stained with anti-MHC-II (clone M5.114.15.2) and goat anti-rat Ab labeled with Alexa594 (Molecular Probes). All washing steps were done in PBS/0.5% BSA. LC numbers were determined by fluorescence microscopy using Cell Imaging Software (Olympus) by counting at least three independent areas of 0.3 × 0.4 mm each. The values shown in Figs. 2A and 4D for LC migration refer to the reduction in LC numbers compared with the untreated contralateral ears.

PCR

Mouse ears were frozen in liquid nitrogen, total RNA was purified (RNaseasy kit; Qiagen), reverse transcribed into cDNA, and analyzed by PCR. PCR of the cDNA was performed using Advantage 2 Polymerase Mix (Clontech) with gene-specific primers: phosphoinositide 3-kinase p85α/HOG1 (HOGPRT), 5′-GGT GGA TAC AGG CCA GAC TTT GGT G-3′; HOGPRT.reverse − 5′-GAG GGT AGG CTG GCC TAT AGG CT-3′; mTNF-α.forward − 5′-TCT ACT GAA CTT CGG GGT GAT CGG TCC-3′; mTNF-α.reverse − 5′-AGA TAG CAA ATC GGC TGA CGG TGT GGG-3′; mIL-1β.forward − 5′-CACA ACA AGT AGT ATT CTC CAT G-3′; mIL-1β.reverse − 5′-GAT CCA CAC TCT CCA GCT GCA-3′. The PCR products were separated by electrophoresis on 2% agarose gels and visualized by ethidium bromide staining with UV light illumination. There were 30 HGPRT, 35 TNF-α, and IL-1β PCR cycles. To quantify MCP-1 and KC-mRNA, real-time PCR was performed using ABSOLUTE QPCR SYBR Green Mix (ABgene) and the specific primers (5 pmol each); mMCP-1.forward − 5′-CTC AGC CAG ATG CAG TTA ACG-3′; mMCP-1.reverse − 5′-TGT GGA TCA TCT TGG TG-3′; mKC.forward − 5′-CACCCA AAC GAA GTC ATA GC-3′; mKC.reverse − 5′-AGC CAG CTT GCA CAC AGG-3′.

Generation of lymph node cell suspensions and proliferation assay of OT-I T cells

One day before immunization, spleens of killed OT-I TCR transgenic mice were isolated and processed to single-cell suspensions. The spleen cells were labeled with 2.5 μM of CFSE (Molecular Probes) and injected i.v. Immunization of mice was performed on 2 consecutive days by mixing of 100 μg SIINFEKL with 40 μg preformed imiquimod cream per mouse per application. The mixture was applied to one ear of the anesthetized mouse. Two days after the first immunization, mice were killed and the auricular LN isolated. LN cell suspensions were generated by collagenase digestion as described previously (21) and immunostained with allophycocyanin-conjugated anti-CD8 (eBioscience).

Mouse dendritic cell (DC) generation and activation

Mouse immature DCs were generated from bone marrow according to standard protocols (22). On day 6, DCs were stimulated with 50 μg/ml TL1R3 ligand poly(I:C) (Amersham Biosciences) for 18 h. The activated DCs were loaded with SIINFEKL peptide (100 nM) for 1 h at 37°C. The peptide-loaded and activated DCs cells (10<sup>6</sup>) were injected i.p. into NFATC2<sup>−/−</sup> or NFATC2<sup>+/−</sup> control mice.

Transcutaneous immunization and in vivo cytotoxicity assay

Transcutaneous immunization was done according our standard protocol (23). In brief, mice were immunized on their shaved backs on 2 consecutive days with 60 mg with preformed imiquimod cream mixed with 100 μg SIINFEKL peptide per application. For detection of peptide-specific CD8<sup>+</sup> T cells, blood samples were collected 6 days after immunization. After a hypotonic lysis step, the cells were stained with PE-labeled SIINFEKL-H<sub>2</sub>K<sub>b</sub> tetramer and allophycocyanin-Cy7-conjugated anti-CD8. Propidium iodide was used to exclude nonviable cells from analysis.

Functional analysis of CTL responses induced by immunizations were performed by an in vivo cytotoxicity assay with differentially CFSE-labeled syngeneic target cells (0.4 μM and 4 μM CFSE; Molecular Probes). Six days after immunization (immunized with imiquimod or DC immunization), the CFSE<sup>low</sup> population was pulsed with 1 μM SIINFEKL and the CFSE<sup>high</sup> population was left without peptide. Some of these target cells (2 × 10<sup>7</sup>) were adoptively transferred into the immunized mice at a ratio of 1:1 (CFSE<sup>low</sup>:CFSE<sup>high</sup>). Eight or 20 hours (mice immunized with imiquimod or DC-immunized mice, respectively) after i.v. injection of the target cells, mice were killed and the spleens processed to single-cell suspensions. Specific in vivo lysis of target cells was evaluated as follows: specific lysis [%] = (No. of nonpeptide-pulsed cells − No. of peptide-pulsed cells)/No. of nonpeptide-pulsed cells.

Statistical analysis

Data are expressed as mean ± SD. Student’s t test was used to compare mean values between two experimental groups where appropriate. Values of p < 0.05 were considered significant.

Results

Delayed inflammation in mice lacking NFATC2

Topical treatment with imiquimod initiates an inflammatory skin response which can be measured as an increase in ear thickness against time. In wild-type mice, ear swelling can be detected 24 h after the initial application of imiquimod (Fig. 1A). In contrast, the inflammatory skin reaction is severely delayed in NFATC2-deficient mice, reaching on day 3 an extent comparable with that observed in wild-type animals on day 1.

It has been reported that pronounced inflammatory changes in the skin following topical application of imiquimod are based on a
massive increase in leukocytes in the dermis, mostly MHC-II$^+$, CD4$^+$, CD11c$^+$, and Gr-1$^+$ cells (24). By using FACS analyses, we were able to confirm these data (not shown), but in addition we also found a striking difference between wild-type and NFATc2-deficient mice with respect to the appearance of neutrophils in the inflamed tissue (Fig. 1B). In the latter, influx of neutrophils is markedly delayed, which is in accordance with data shown in Fig. 1A. Obviously, the early innate inflammatory response is delayed in mice lacking NFATc2. To investigate this phenomenon in more detail, we performed RT-PCR analyses using ear skin 2 h following the initial application of imiquimod. As depicted in Fig. 1, C and D, the expression of important proinflammatory mediators, TNF-$\alpha$, IL-1$\beta$, KC, and IFN-$\beta$, is severely impaired in NFATc2-deficient animals.

NFATc2-deficiency leads to impaired migration of LCs and reduced hypertrophy of draining LNs

In combination with the initiation of an inflammatory response, it was also reported recently that imiquimod induces the migration of LCs, important APCs in the epidermis (25). LCs form a dense cellular network that can be visualized by the staining of epidermal sheets with Abs to langerin (CD207), a C-type lectin selectively expressed in LCs (26). NFATc2-deficiency per se has no influence on the numbers of LCs, which is $\sim$900 LCs/mm$^2$ in both wild-type and knockout mice, but it severely impairs emigration of LCs out of the epidermis following topical treatment with imiquimod (Fig. 2A). Importantly, emigration of LCs is not only delayed in NFATc2-deficient mice but also remains reduced at later time points. Additionally, hypertrophy of draining LNs is severely reduced in the absence of NFATc2 as well (Fig. 2B).
Primed CTLs by transcutaneous peptide immunization (TCI) with imiquimod as adjuvant is impaired in NFATC2-deficient mice

Our observation that both emigration of LCs and hypertrophy of draining LNs are impaired in mice lacking NFATC2 prompted us to investigate whether the induction of an Ag-specific CTL response is also affected in these animals. As a model we chose the epitope SIINFEKL from chicken OVA (OVA257–264) administered in combination with imiquimod on the shaved backs of mice once daily on 2 consecutive days. Peripheral blood was collected on day 6 and stained for the presence of SIINFEKL–specific CTLs as depicted in Fig. 3A. In wild-type mice, expansion of Ag-specific CTLs can easily be followed over 1% of CTLs can be stained with H-2KbSIINFEKL tetramer. In contrast, expansion of CTLs is severely reduced in the absence of NFATC2 (0.36%). It should be mentioned that we did not find significant differences between NFATC2-deficient mice and their congenic littermates on a C57BL/6 background with respect to the total numbers of peripheral CTLs, monitored between 7- and 23-wk of age (data not shown).

Besides phenotypic staining of Ag-specific CTLs, their cytolytic activity can also be monitored in vivo. To this end, SIINFEKL-laden CFSE<sup>low</sup>-labeled syngeneic splenocytes were mixed in a ratio 1:1 with CFSE<sup>high</sup>-labeled splenocytes without Ag and injected i.v. Thereafter, lysis of SIINFEKL-laden target cells can be followed by a decrease in CFSE<sup>low</sup>-labeled cell numbers, whereas the CFSE<sup>high</sup>-labeled fraction serves as internal reference (Fig. 3B). According to the expectations, the appearance of high numbers of Ag-specific CTLs in wild-type mice is accompanied by a strong cytolytic activity in vivo, while the ability to initiate a cytolytic T cell response by transcutaneous immunization is severely impaired in mice lacking NFATC2. However, the ability to present Ag and to generate an efficient CTL response per se is unaffected in such animals. This can be concluded from data shown in Fig. 3C, where NFATC2<sup>−/−</sup> Ag-laden bone marrow-derived dendritic cells elicit full-blown cytolytic responses in both wild-type and NFATC2-deficient mice.

NFATC2 expressed in mast cells is critical to initiate inflammatory and adaptive immune responses

NFATC2 deficiency obviously does not impair the development of mast cells in vivo, which can be concluded from comparable mast cell numbers found in skin from wild-type and NFATC2-deficient mice (Table I). Furthermore, anaphylactic degranulation of mast cells following cross-linking of IgE is unaffected in mice lacking NFATC2. However, the ability to present Ag and to generate an efficient CTL response per se is unaffected in such animals. This can be concluded from data shown in Fig. 3C, where NFATC2<sup>−/−</sup> Ag-laden bone marrow-derived dendritic cells elicit full-blown cytolytic responses in both wild-type and NFATC2-deficient mice.

NFATC2<sup>−/−</sup> mice displayed an impaired CTL response after transcutaneous peptide immunization with imiquimod as adjuvant. A. Mice were treated with peptide plus imiquimod or peptide plus vehicle once daily on two consecutive days on their shaved backs. On day 6, peripheral blood was stained for peptide-specific CD8<sup>+</sup> T cells using a fluorescently labeled SIINFEKL–specific tetramer. The depicted numbers represent the percentages of Ag-specific cells within the CD8<sup>+</sup> population. B. Animals from the experiment described in A were injected i.v. on day 6 with CFSE-labeled syngeneic splenocytes. CFSE<sup>low</sup>-labeled splenocytes were previously pulsed with the antigenic SIINFEKL peptide and mixed at a ratio 1:1 with CFSE<sup>high</sup>-labeled splenocytes. Lysis of peptide-laden target cells in spleen cell suspensions was determined by FACS analysis 8 h after adoptive transfer. Specific lysis [%] = (number of cells nonpeptide pulsed – number of peptide-pulsed cells)/number of cells nonpeptide pulsed. C. Wild-type or NFATC2-deficient mice were immunized i.p. with peptide-laden DC generated from NFATC2-deficient mice in vivo. Six days after immunization, cytolytic activity against SIINFEKL-pulsed syngeneic splenocytes was assessed as described in B. Shown are representative FACS stainings and means (±SD) from eight to ten animals per group.

Table 1. Engraftment of mast cell-deficient mice with BMMCs derived from different donor strain<sup>a</sup>

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mast Cells/mm Ear Cartilage</th>
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<tr>
<td>Kit&lt;sup&gt;W-sh/W-sh&lt;/sup&gt;</td>
<td>36.1 (±2.51)</td>
</tr>
<tr>
<td>Kit&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td>33.9 (±4.91)</td>
</tr>
<tr>
<td>NFATC2&lt;sup&gt;−/−&lt;/sup&gt;→Kit&lt;sup&gt;W-sh/W-sh&lt;/sup&gt;</td>
<td>32.2 (±6.98)</td>
</tr>
<tr>
<td>NFATC2&lt;sup&gt;−/−&lt;/sup&gt;→Kit&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td>30.9 (±4.24)</td>
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<sup>a</sup>Mice were reconstituted intra pinna with BMMCs from different donors, as described in the methods section. After 6 weeks, ear sections were stained with avi-din-fluorophor conjugate and mast cell numbers determined. Shown are the means (±SD) from four individuals per group. ND, Not detectable.
To investigate the role of mast cells in vivo we used mast cell-deficient KitW-sh/W-sh (“sash”) mice, which can be reconstituted with BMMCs derived from either wild-type or NFATc2-deficient mice (29). Six weeks following local reconstitution of the ear skin, comparable mast cell numbers can be found in sash mice engrafted with either wild-type or NFATc2-deficient mast cells. In addition, there is no difference in mast cell numbers between wild-type and NFATc2-deficient mice (Table I).

As depicted in Fig. 4C, reconstitution of sash mice with BMMCs from wild-type mice restores the inflammatory response to imiquimod, whereas reconstitution with NFATc2−/− mast cells closely resembles the delayed inflammation seen in sash mice. Furthermore, emigration of LCs cannot be restored following transfer of NFATc2-deficient mast cells (Fig. 4D). Using this model we recently reported (20) that mast cell-derived cytokines and TLR7 expressed in mast cells are critical for the early onset of inflammation (TNF-α) and emigration of LCs (IL-1β). Production of these cytokines is severely impaired in sash mice and can be restored following transfer of BMMCs derived from wild-type but not from NFATc2-deficient mice (Fig. 4E).

Reconstitution of sash...
mice with wild-type BMMCs is critical to restore the production of early cytokines (IL-1β, TNF-α) following topical application of imiquimod. However, BMMCs only marginally produce IL-1β mRNA and no TNF-α mRNA upon treatment with imiquimod in vitro. To explain this discrepancy, it is reasonable to assume that mast cells are able to change their characteristics following transfer in vivo, determined by the local microenvironment (30).

The most outstanding consequence of NFATc2-deficiency solely in mast cells is the inability of such animals to develop adequate cytolytic responses as depicted in Fig. 4, F and G. In these experiments, the ears of sash mice were locally reconstituted with BMMCs derived from the indicated strains and imiquimod plus peptide was applied to the ears of the animals. TCI via the ears was chosen because systematic reconstitution of sash mice by i.v. injection of BMMCs does not lead to the development of dermal mast cells (29).

Immunization was followed by measuring the expansion of adoptively transferred CFSE-labeled T cells from OT-I mice expressing a SIINFEKL-specific TCR. As detailed in Fig. 4, F and G, proliferation of labeled T cells after TCI is severely impaired in mast cell-deficient sash mice and can only be restored following transfer of wild-type, but not of NFATc2−/−, mast cells.

Thus, NFATc2 expressed in mast cells plays important roles in various aspects of mast cell-mediated immunity in vivo.

Discussion

It was reported that NFATc2-deficient mice are prone to mount-enhanced immune responses in several models, including allergic pleurisy (8, 12), infection with Nippostrongylus brasiliensis (11), and allergic asthma (13). Supplemented by studies performed in vitro, it can be concluded that such aggravated responses are most likely due to the enhanced reactivity of NFATc2−/− peripheral T cells with respect to proliferation and production of cytokines.

Despite the expression of NFATc1−c3 in mast cells, information on their role in these cells is scarce. We previously reported that BMMCs that lack NFATc3 are unimpaired with regard to de novo synthesis of cytokines, proliferation, and release of preformed mediators. Yet, BMMCs from NFATc2-deficient mice display a strong reduction in the production of TNF-α and IL-13, whereas their degranulation is unimpaired (27). Obviously, individual NFAT family members can perform cell-type-specific functions, which is corroborated in a recent study (31) demonstrating reduced production of several cytokines by NFATc2-deficient mucosal T cells. This leads to protective effects of NFATc2-deficiency in experimental colitis (31). Such cell-type-specific functions of distinct NFATs are supposedly based on their cooperation with various nuclear partners, acting as coactivators or repressors of NFAT function (1, 32).

Mast cells play an important role at the interface of innate and adaptive immunity, i.e., these cells are able to initiate and accelerate inflammation and to promote the development of adaptive responses (33). IgE-independent activation of mast cells, e.g., via TLRs, is an impressive example for their versatility. This was exemplified in a recent study in which we demonstrate that TLR7-mediated activation of dermal mast cells by imiquimod initiates fast inflammation of the skin, migration of LCs, and finally contributes to the development of an adequate CTL response against applied Ag (20).

Mast cell-derived cytokines such as TNF-α were shown to mediate a variety of important mast cell functions, like their ability to promote migration of APCs, hyper trophy of LN s, attraction of neutrophils, and modulation of T cell activity (33). Most likely by driving the production of important cytokines, NFATc2 enables mast cells to participate in these diverse immunological reactions.

This can also be concluded from experiments in which transfer of wild-type BMMCs, but not of NFATc2-deficient BMMCs, in sash mice restores production of TNF-α and IL-1β mRNAs following topical application of imiquimod. With regard to the ability of mast cells to promote CTL responses in our model, it is reasonable to assume that mast cells act at the level of the APC. We previously reported that mast cell-derived IL-1β is crucial for the migration of LCs to the draining LNs (20). In line with this, selective ablation of LCs in the presence of mast cells before TCI severely impairs the development of a CTL response in vivo, demonstrating that LCs act as important APCs (P. Lopez and M. P. Radask, in preparation).

Obviously, NFATc2 is an important factor controlling the activation of mast cells and appears to be an attractive target for therapeutic intervention in mast cell-mediated diseases.

Disclosures

The authors have no financial conflict of interest.

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