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*J Immunol* 2013; 190:4500-4507; Prepublished online 27 March 2013;
doi: 10.4049/jimmunol.1203158
http://www.jimmunol.org/content/190/9/4500

Supplementary Material
http://www.jimmunol.org/content/suppl/2013/03/27/jimmunol.1203158.DC1

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Mast cells are well positioned in host tissue for detecting environmental signals, including allergens, leading to activation of the high-affinity IgE receptor FceRI, and initiating a signaling cascade that perpetuates the production of biologically potent mediators, including chemokines. We have identified a novel target of mast cell FceRI activity in the transcription factor early growth response 2 (Egr2) and sought to characterize its function therein. Egr2 was transiently activated following FceRI-mediated signaling, targeted the promoter of the chemokine CCL1, and was critical for allergen-induced mast cell CCL1 production. Egr2-deficient mast cells were incapable of directing CD4+ T cell migration via the CCL1–CCR8 axis. In a model of allergic asthma, reconstitution of mast cell–deficient mice with Egr2-deficient mast cells demonstrated that mast cell Egr2 was essential for migration of CD4+ T cells to the inflamed lung. These findings position Egr2 as a critical regulator of mast cell–directed CD4+ T cell migration.

The Journal of Immunology, 2013, 190: 4500–4507.

Received for publication November 15, 2012. Accepted for publication March 1, 2013.

This work was supported by the Canadian Institutes of Health Research and the Izaak Walton Killam Health Centre.

Address correspondence and reprint requests to Dr. Adam J. MacNeil and Dr. Tong-Jun Lin, Izaak Walton Killam Health Centre, Department of Pediatrics, 5850 University Avenue, Halifax, NS B3K 6R8, Canada. E-mail addresses: adam.macneil@dal.ca (A.J.M.) and tong-jun.lin@dal.ca (T.-J.L.)

The online version of this article contains supplemental material.

Abbreviations used in this article: BALF, bronchoalveolar lavage fluid; Egr, early growth response; LMC, liver-derived mast cell; SSH, suppression subtractive hybridization; TNP, trimethylpsyllate.

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.1203158

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Mast Cell FceRI-Induced Early Growth Response 2 Regulates CC Chemokine Ligand 1–Dependent CD4+ T Cell Migration

Zhengli Wu,*† Adam J. MacNeil,*†‡ Robert Junkins,*†‡ Bo Li,§ Jason N. Berman,*†‡ and Tong-Jun Lin*†‡
Materials and Methods

**Animals**

Egr2-deficient mice were generated as previously described (21). C57BL/6 mice were purchased from Charles River Laboratories (Wilmington, MA). Mast cell–deficient W-sh mice were obtained from The Jackson Laboratory (B6Cg-kit W-sh/HNHiJucBsmI NistIf4). Protocols were approved by the University Committee on Laboratory Animals, Dalhousie University, in accordance with the guidelines of the Canadian Council on Animal Care.

**Abs and reagents**

Abs to Egr1, Egr2, NF-κB p65, NF-κB p50, NFATc1, and NFATc2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). PerCP-Cy5.5–conjugated rat anti-mouse c-kit/CD117 mAb (IgG2b, κ), PerCP-Cy5.5–rat IgG2b, κ, FITC-conjugated rat anti-mouse IgE (IgG1), FITC-rat IgG1, allophycocyanin–CD4, FITC–CD25, allophycocyanin-rat IgG2a, κ, and FITC-rat IgM, κ were purchased from BD Biosciences (San Jose, CA). Abs to PE-Foxp3 and PE-rat IgG2b, κ were purchased from ebioscience (San Diego, CA). Alexa Fluor 594 goat and rabbit secondary Abs were from Invitrogen (Eugene, OR). Abs to CCL1, CCL3, CCL9, and TNF, as well as mouse cR1 and standards (DuoSet) for ELISA were from R&D Systems (Minneapolis, MN). Recombinant murine stem cell factor was purchased from PeproTech (Rocky Hill, NJ).

**Histology**

Mouse liver-derived mast cells (LMCs) were obtained by culturing liver cells from newborn mice from Egr2<sup>−/−</sup> breeders. Egr2 deficiency results in neonatal or perinatal death (22). Briefly, liver tissue was removed to a sterile environment and was ground to produce a single-cell suspension in RPMI 1640 medium. Cells were collected, centrifuged at 500 × g for 5 min at 4˚C, and resuspended at a density of 0.5 × 10<sup>6</sup> cells/ml in complete medium (RPMI 1640 medium containing 10% FBS, 10% WEHI-3B–conditioned medium, 30 mg/ml stem cell factor, 50 U/ml each of penicillin and streptomycin, 50 μg/ml 2-ME, and 200 μM PGE<sub>2</sub>). An aliquot of cells from each mouse was used for genotyping. Nonadherent cells were resuspended in complete medium twice per week and transferred to a fresh flask once per week. After 5–6 wk, mast cell purity of >98% was achieved, as assessed by toluidine blue staining of fixed cytocentrifuged preparations. Sensitization, degranulation, and calcium mobilization were as previously described (20).

**Immunofluorescence**

Immunofluorescence was as described previously (18), except samples were incubated overnight with anti-Egr2 Ab (16 μg/ml) or rabbit serum (Cedarlane) at 4˚C.

**Electrophoretic mobility shift assay**

Nuclear protein extracts were isolated using a nuclear extract kit (Active Motif, Carlsbad, CA), according to the manufacturer’s protocol. EMSA was performed as previously described (18). The following synthesized double-stranded oligonucleotides (Sigma-Aldrich) were used as NF-κB–binding consensus sequences on mouse IL-6 promoter, 5′-TTATCCAGTGTTGGATTTTTCCCAATG-3′ and the mutant sequence, 5′-TTATCCAGTGTTGGATTTTTCCCAATG-3′; NFAT-binding consensus sequence on mouse IL-13 promoter, 5′-AAAGTGTGTCCTCAAGCCCTTTCCC-3′ and the mutant sequence, 5′-AAAGTGTGTCCTCAAGCCCTTTCCC-3′; and NFAT-binding consensus sequence on CCL1 promoter, 5′-TCAGCCAGGAGCTCCACGTTATTTTG-3′ and the mutant sequence, 5′-TCAGCCAGGAGCTCCACGTTATTTTG-3′ (italics indicate mutated bases). For competition assays, 1 μl nonradioabeled wild-type or mutant oligonucleotides (50-fold excess compared with radiolabeled probe) were added and incubated for 30 min before the addition of the radiolabeled probe. For blocking assays, 4 μg anti-NFATc1, anti-NFATc2, anti-p50, anti-p65, anti-Egr1, or serially diluted anti-Egr2 Abs were added and incubated at 4˚C for 30 min before the addition of the 32P radiolabeled probe. For quantifications, densitometry was conducted using Image J software.

**Chromatin immunoprecipitation**

Assays were performed as previously described (20). PCR consisted of 42 cycles of 30 s at 94˚C, 30 s at 59˚C, and 30 s at 72˚C. Primers for amplification of the CCL1 promoter region were 5′-GCCATTTGATGTTACTTCTC-3′ (forward) and 5′-ACCCACACGCGCAGCAGCA-3′ (reverse).

**IgE-mediated passive cutaneous anaphylaxis**

Mast cell–deficient W-sh mice were reconstituted with Egr2<sup>−/−</sup> (right ear) and Egr2<sup>−/−</sup> (left ear) LMCs. Eight months later, passive cutaneous anaphylaxis was conducted as previously described (20).

**Transwell migration assays**

Mast cell–conditioned cell-free supernatants were prepared using sensitized Egr2<sup>−/−</sup> and Egr2<sup>−/−</sup> LMCs (1 × 10<sup>5</sup>/ml) stimulated with trimethylphenyl (TNP)-BSA for 24 h in RPMI 1640 (Invitrogen) supplemented with 10% FBS and antibiotics. Splenic T cells were isolated from 6-wk-old C57BL/6 mice using a CD4<sup>+</sup>CD25<sup>+</sup> isolation kit (Miltenyi Biotec) allowing for purification of CD4<sup>+</sup>CD25<sup>+</sup> and CD4<sup>+</sup>CD25<sup>−</sup> cells. Isolated T cells were tested by flow cytometry with anti-CD4 and anti-CD25 or isotype control Abs. Assays were conducted using 5 μm polycarbonate filters in Costar 24-well Transwell chambers (Corning, Corning, NY) with 600 μl conditioned media in the lower chamber and 2 × 10<sup>5</sup> T cells in 100 μl in the upper chamber. Assays were conducted for 70 min at 37˚C, and cells that had migrated to the lower chamber were then counted. Net migration equals migrated cell number minus control migration cell number. Blocking Abs were used at a final concentration of 1 μg/ml and murine CCL1 protein was used at 100 ng/ml.

**OVA-induced asthma model**

Eight-week-old W-sh mice were reconstituted with either Egr2<sup>−/−</sup> or Egr2<sup>−/−</sup> LMCs (5 × 10<sup>5</sup>/mouse in 200 μl RPMI 1640) by i.v. injection. Five to 6 wk later, reconstituted mice were sensitized by i.p. injection with 10 μg OVA with 1 mg alum (Sigma-Aldrich) in 100 μl saline on days 1, 5, and 10, followed by intranasal challenge with 20 μg OVA in 20 μl saline on days 21, 25, 27, and 29. On day 30, lung tissue was collected for histological staining. Draining lymph nodes, lungs, and spleen were isolated, and cells were blocked with 1 μg/ml rat IgG and 1 μg/ml mouse IgG for 30 min prior to staining for surface CD4 and CD25 and subsequent permeabilization with Cytofix/Cytoperm (BD Biosciences) and intracellular staining for Foxp3 for 30 min each at room temperature. FACS analysis was conducted using WinMDI 2.9 or WinList 5.0 software.

**Bronchoalveolar lavage and lung processing**

Bronchoalveolar lavage fluid (BALF) was obtained by lavaging the lung with 1 ml phosphate buffer solution containing soybean trypsin inhibitor (100 μg/ml). Cells in the BALF were counted, and slides were prepared by cytospin (Cytospin 4; ThermoShandon) and stained with Diff-Quik (Dade Behring Diagnostics, Newark, DE). The right lung was homogenized at maximum speed 20 (PowerGen 125; Fisher Scientific) in 50 mmol/L HEPES buffer (4 μl/mg lung) containing soybean trypsin inhibitor (100 μg/ml) and cleared by centrifugation at 14,000 rpm.

**Eosinophil peroxidase and myeloperoxidase assays**

For eosinophil peroxidase assays, 75 μl substrate solution (3 mmol/L o-phenylenediamine dihydrochloride, 50 mmol/L chlorate-free HEPES [pH 8.0], 6 mmol/L KBr, and 8.8 mmol/L H<sub>2</sub>O<sub>2</sub>) was added to 75 μl of each sample. After 30 s, the reaction was stopped with 150 μl 2 mol/l H<sub>2</sub>O<sub>2</sub>, and the absorbance was read at 490 nm. For myeloperoxidase, 75 μl substrate solution (3 mmol/L fresh 3,3′,5,5′-tetramethylbenzidine dihydrochloride hydrate [Sigma-Aldrich], 0.12 mmol/L resorcinol [Sigma-Aldrich], and 2.2 mmol/L H<sub>2</sub>O<sub>2</sub>) was added to 75 μl of each sample. After 2 min, the reaction was stopped with 150 μl 2 mol/l H<sub>2</sub>O<sub>2</sub>, and the absorbance was read at 450 nm within 10 min.

**Statistical analysis**

The paired Student <i>t</i> test was used for statistical evaluation of data. Results were considered significant when <i>p</i> < 0.05. Data are expressed as means ± SEM.
Results

Mast cell activation via FcεRI induces transient Egr2 expression and activity

To identify genes with novel expression in mast cells activated through FcεRI, we employed an unbiased method termed suppression subtractive hybridization (SSH). For this assay, wild-type mast cells cultured from murine bone marrow were activated by sensitizing the cells with IgE specific for TNP-BSA and subsequently challenging these cells with this Ag to achieve mast cell activation through FcεRI. The Egr2 gene was among those clones identified by SSH, and a virtual Northern blot was performed on duplicate membranes containing cDNA from untreated and TNP-BSA–treated samples to confirm the enhanced expression of Egr2 in stimulated mast cells (Fig. 1A).

To characterize the kinetics of Egr2 mRNA expression in activated mast cells, we conducted real-time quantitative PCR studies. Following TNP-BSA treatment, Egr2 expression was induced by 15 min and reached its highest level by 30 min. By 60 min, Egr2 expression dropped considerably and returned to near basal levels by 180 min post–TNP-BSA stimulation (Fig. 1B). Egr2 transcription was similar to that of Egr1, but slightly delayed by comparison. PCR amplicons were also analyzed on agarose gel by ethidium bromide staining, and a representative gel is shown (Fig. 1C).

We further examined FcεRI-induced Egr2 expression at the protein level by immunofluorescence in IgE-activated wild-type LMCs. Following TNP-BSA stimulation, strong and primarily nuclear, expression of Egr2 protein is detected by 15 min and peaked at 30 min. By 60 min, Egr2 expression dropped off considerably and returned to near basal levels by 180 min through 360 min (Fig. 1D). As a control, immunofluorescence was also conducted using nonspecific rabbit serum as a primary Ab as well as in Egr2-deficient LMCs (described below) to evaluate the specificity of the anti-Egr2 Ab (Fig. 1D). Finally, we characterized the specific DNA-binding activity of Egr2 in our model (further information in Supplemental Fig. 1A, 1B). Collectively, these results indicate that active Egr2 is transiently expressed de novo in mast cells from 15–60 min following FcεRI-mediated activation.

Egr2 is not required for mast cell development or degranulation

To determine the role of Egr2 in mast cell function, wild-type and Egr2-deficient primary LMC cultures were established from fetal liver cells cultured in media conditioned with stem cell factor, IL-3, PGE2 to promote differentiation and proliferation of mast cells from liver-derived progenitor cells. PCR-based genotyping was carried out to confirm each culture (Supplemental Fig. 1C). Asessment of mast cell development was carried out using a toluidine blue stain that showed similar metachromatic staining and morphology between wild-type and Egr2-deficient LMCs (Supplemental Fig. 1D). To further examine the development of LMCs in vitro in the absence of Egr2, cultures were analyzed by flow cytometry for the presence of c-kit and IgE receptor expression. No deficit in expression of these mast cell markers was detected in Egr2-deficient LMCs (Supplemental Fig. 1E). These results indicate that mast cells develop normally in the absence of Egr2.

To investigate the role of Egr2 in early mast cell FcεRI activation-related functions, we first assessed mast cell degranulation in vitro by measuring the release of β-hexosaminidase. Egr2 deficiency did not alter LMC degranulation from wild-type levels, nor did it alter calcium influx (Fig. 2A, 2B). Finally, an in vivo model of passive cutaneous anaphylaxis was conducted in mast cell–deficient (W-sh) mice that had been reconstituted with wild-type and Egr2-deficient LMCs. No significant difference in dye leakage into tissue was observed when mice with wild-type LMCs were compared with Egr2-deficient LMCs (Fig. 2C). These results indicate Egr2 does not play a role in the early activation signals leading to mast cell degranulation in vitro or in vivo.

CCL1 production by FcεRI-activated mast cells is regulated by Egr2

Following evidence from protein array data suggesting a deficiency in CCL1 production in Egr2-deficient LMCs following IgE-mediated TNP-BSA stimulation, we conducted an ELISA analysis on cell-free supernatants. To examine the kinetics of cytokine and chemokine production, wild-type and Egr2-deficient LMCs were stimulated with TNP-BSA over a time course. Egr2-deficient LMCs displayed a significant defect in CCL1 production following FcεRI-mediated activation, whereas CCL3 and CCL9 remained unaltered by Egr2 deficiency (Fig. 3).

FIGURE 1. Mast cell activation via FcεRI induces transient Egr2 expression and activity. (A) RNAs from untreated and FcεRI-stimulated bone marrow–derived mast cells were reverse transcribed, creating cDNAs that were blotted and hybridized with either a GAPDH probe or an Egr2 probe. A representative Northern blot is shown. (B and C) RNA from murine mast cells activated via FcεRI (NT or 0, 15, 30, 60, 180 min) was used in real-time quantitative PCR. Egr2 and Egr1 mRNA expression was normalized to GAPDH and shown with SE (n = 3). PCR products were also separated on agarose gel and stained with ethidium bromide, and a representative gel is shown. (D) LMCs were stimulated through FcεRI by sensitizing with anti-TNP IgE and subsequently stimulating with TNP-BSA for 15, 30, 60, 90, 120, 180, and 360 min. Egr2 protein expression was detected by immunofluorescence using an anti-Egr2 Ab and DAPI as a nuclear stain. Immunofluorescence was also carried out using rabbit serum as the primary Ab control as well as in Egr2-deficient LMCs to demonstrate the specificity of the Egr2 Ab. Cells were examined using a fluorescence microscope (Nikon E600; Nikon, Tokyo, Japan) equipped with a DMX 1200 camera; original magnification ×100.
deficient LMCs. Data are expressed as mean ± SEM for the assessment of intracellular calcium flux following activation. A representative of n = 3 independent experiments is shown. (C) Mast cell–deficient (W-sh) mice were reconstituted locally with wild-type (right) or Egr2-deficient (left) LMCs in the ear tissue by intradermal injection. Reconstituted mice were then sensitized locally with anti-dinitrophenyl IgE, and the following day were challenged by i.v. injection of dinitrophenyl-BSA in a 1% solution of Evan’s blue dye for 30 min. Passive cutaneous anaphylaxis was determined by measuring dye leakage into the collected ear tissue using a spectrophotometer at 620 nm. Unreconstituted control (Wsh) data are also shown to demonstrate the inducible reaction. Data are expressed as ±SEM for n = 6 mice.

unaffected (Fig. 3). IL-6 and TNF production were reduced at later time points, but the impairment did not reach significance (Fig. 3). It is also notable that mast cell CCL1 production displays delayed kinetics in comparison with the other cytokines examined, consistent with a requirement for de novo gene expression preceding CCL1 production. These results indicate that mast cell CCL1 production is preferentially regulated by Egr2.

Mast cell activation through FcεRI results in the activation of a host of transcription factors and signaling pathways, including NF-κB, NFAT, and MAPK, which cooperate to deliver the full mast cell response to IgE Ags (10, 23, 24); however, these pathways were not significantly affected by Egr2 deficiency in LMCs (Supplemental Fig. 2).

Egr2 binds to the promoter of the CCL1 gene following FcεRI-mediated mast cell activation

To examine the direct interaction of Egr2 on CCL1 gene transcription, a DNA probe was created using an Egr consensus-binding sequence from the promoter of CCL1 (Fig. 4A). Specific Egr2 binding to this sequence was demonstrated by EMSA using nuclear proteins from wild-type LMCs treated with TNP-BSA (Fig. 4B). Additional dsDNA EMSA probes specific to putative binding sites in the CCLI promoter were also created and tested, but no Egr2 binding was detected at these positions (Supplemental Fig. 3A, 3C). An anti-Egr2 Ab significantly blocked the interaction of the CCLI promoter probe with Egr2 nuclear protein in activated mast cells, and unlabeled and mutant competitor probes demonstrated the specificity of Egr binding to the probe (Fig. 4B). Finally, the Egr2-binding EMSA probe was used to characterize the kinetics of Egr2 activity on the CCLI promoter sequence (Supplemental Fig. 3D), demonstrating a peak binding at 1 h, followed by a persistent interaction lasting at least to 6 h.

To demonstrate Egr2 binding to the endogenous CCLI promoter in FcεRI-activated mast cells, chromatin immunoprecipitation was performed in untreated and TNP-BSA–stimulated LMCs using an Egr2 Ab and primers specific to the CCLI promoter. Following TNP-BSA treatment, a strong induction of Egr2 binding to the

FIGURE 4. Egr2 directly interacts with the CCLI promoter following FcεRI-mediated mast cell activation. (A) CCLI gene promoter with location and sequence of Egr2-binding EMSA probe. (B) Nuclear proteins extracted from wild-type LMCs that had been sensitized and stimulated with TNP-BSA for 1 h or left untreated (NT) were used in EMSA with a 32P-labeled dsDNA probe (above). Mast cell activation (TNP-BSA 1 h) induced a strong Egr2 interaction with the CCLI promoter probe. Competition with unlabeled (E) and mutant (Em) probes was used to demonstrate specificity, and an anti-Egr2 Ab partially blocked Egr2 binding. A representative from n = 3 experiments is shown. (C) Chromatin immunoprecipitation (ChIP) was conducted using either an anti-Egr2 or IgG control Ab in LMCs left untreated or stimulated for 1 h. Immunoprecipitated (IP) chromatin was analyzed by PCR using primers specific to the CCLI promoter. Samples from chromatin pre-IP were used as input controls. Shown is a representative gel from n = 3 experiments.
CCL1 promoter is detected (Fig. 4C). Additional primers with specificity in the CCL1 promoter were also tested, but no specific Egr2 binding at these sites was detectable (Supplemental Fig. 3A, 3B). These results indicate that Egr2 interacts with the CCL1 promoter following mast cell activation, suggesting that Egr2 mediates CCL1 production through a direct interaction.

Egr2-dependent mast cell CCL1 production drives CD4+ T cell migration in vitro

Mast cells can direct the migration of CD4+CD25+ T regulatory cells through the CCL1–CCR8 axis (7, 25). To investigate the role of Egr2-dependent mast cell CCL1 in T cell migration, an in vitro Transwell migration assay was conducted. Splenic CD4+ T cells were isolated (Fig. 5A, 5B), and CD4+ cells were then further purified into CD25+ and CD25- populations. Transwell migration assays were conducted with 24-h TNP-BSA–stimulated wild-type or Egr2-deficient LMC-conditioned media in the lower chamber. CD4+CD25+ T cell migration was significantly impaired in response to media conditioned with Egr2-deficient mast cells, with 8-fold more cells migrating across the membrane in response to wild-type mast cell–conditioned media (Fig. 5C). Migration in response to mast cell–conditioned media was significantly dependent on Egr2 until the wild-type media had been serially diluted to 12.5% (8-fold) (Fig. 5C).

To examine the contribution of mast cell–produced CCL1 in CD4+CD25+ T cell migration, we added either specific Abs to CCL1 or CCL9, or isotype control Abs to wild-type mast cell–conditioned media in our Transwell migration assay. Interestingly, CCL1 Ab significantly reduced T cell migration to levels consistent with that of Egr2-deficient mast cell media shown in Fig. 5C, whereas anti-CCL9 and isotype control Abs had no blocking effect on T cell migration (Fig. 5D). Finally, T cell migration assays were conducted using Egr2-deficient mast cell–conditioned media with purified rCCL1 protein added at a final concentration of 100 ng/ml. Reconstitution of Egr2-deficient mast cell media with CCL1 restored the migration of CD4+CD25+ and CD4+CD25- T cells to that detected in response to wild-type mast cells (Fig. 5E). These results indicate that in vitro, mast cell–dependent
were then evaluated for CD4+ T cell subsets, including the T LMCs, we stained sectioned lung tissues and evaluated skin and constituted with wild-type control LMCs (Fig. 6A). To evaluate the restricted goblet cell hyperplasia compared with mice reconstituted with either wild-type or Egr2-deficient LMCs. Reconstituted mast cell–deficient mice reconstituted with Egr2-deficient LMCs. 

To evaluate the significance of Egr2-dependent mast cell CCL1 production on T cell migration in vivo, an OVA-induced model of asthma was conducted in mast cell–deficient W-sh mice that had been reconstituted with wild-type and Egr2-deficient LMCs, reconstituted mice from the same asthma model was then further analyzed for Th2 cytokine levels, and significant defects in IL-4, IL-6, IL-10, IL-13, and CCL1 were detected in lung and BALF as well as a defect in IL-5 levels in the lung (Fig. 7A–F), consistent with reduced CD4+ T cells in the lung. Airway hyperresponsiveness was also assessed in these OVA-challenged mice, but no significant difference was detected with airway hyperresponsiveness reaching 5.01 ± 0.48- and 6.09 ± 0.54-fold change over baseline at 50 mg/ml methacholine challenge for mice reconstituted with wild-type and Egr2-deficient LMCs, respectively (n = 6). Finally, eosinophil and neutrophil infiltration was evaluated by measuring the activity of eosinophil peroxidase and myeloperoxidase, respectively, in the lung tissue of these mice, and no significant defect was detected (Fig. 7G, 7H). Collectively, these results suggest that Egr2 specifically directs mast cell production of CCL1 and that this mechanism is critical for CD4+ T cell homing in the cellular response to allergic inflammation (Fig. 8).

CD4+ T cell migration through the CCL1–CCR8 axis is specifically controlled by Egr2-dependent mast cell production of CCL1. 

Egr2-dependent mast cell CCL1 regulates CD4+ T cell migration in allergic asthma

To evaluate the significance of Egr2-dependent mast cell CCL1 production on T cell migration in vivo, an OVA-induced model of asthma was conducted in mast cell–deficient W-sh mice that had been reconstituted with either wild-type or Egr2-deficient LMCs. Mast cell–deficient mice reconstituted with Egr2-deficient LMCs displayed a deficit in immune cell infiltration into the lung and restricted goblet cell hyperplasia compared with mice reconstituted with wild-type control LMCs (Fig. 6A). To evaluate the degree of mast cell reconstitution by wild-type and Egr2-deficient LMCs, we stained sectioned lung tissues and evaluated skin and lung tissues by flow cytometry for mast cell content and found no discrepancy between groups (Supplemental Fig. 4). These mice were then evaluated for CD4+ T cell subsets, including the T regulatory–dependent transcription factor Foxp3, at the draining lymph node, lung, and spleen by flow cytometry. A significant deficit in all CD4+ T cell subsets analyzed (CD25+ Foxp3+) was detected at the draining lymph node and in the lung, whereas cell numbers at the spleen remained unaffected (Fig. 6B–D). The cellular content of the BALF was also assessed revealing an Egr2-dependent defect in mononuclear cells, whereas eosinophil numbers were not impaired (Fig. 6E). Collectively, these data suggest a defective CCL1–CCR8 migratory axis in vivo. Lung tissue was

![Figure 7](http://www.jimmunol.org/)

**FIGURE 7.** Mast cell Egr2 is critical in directing Th2 cytokine production in the lung during allergic asthma, but is not required for eosinophilia. (A–F) W-sh mice reconstituted with either wild-type or Egr2-deficient LMCs were used in a model of asthma. The production of Th2 cytokines IL-4, IL-5, IL-6, IL-10, IL-13, and CCL1 in the lung and BALF was assessed by ELISA. *p < 0.05, **p < 0.01. (G and H) Lung tissue from Egr2+/+ and Egr2−/− mast cell–reconstituted mice from the same asthma model was used to measure the activity of eosinophil peroxidase (EPO), a marker of eosinophil infiltration, and myeloperoxidase (MPO) activity, a marker of neutrophil infiltration. Data are expressed as ±SEM, n = 6.

![Figure 8](http://www.jimmunol.org/)

**FIGURE 8.** Mast cell FcεRI-induced Egr2 regulates CCL1 production in the asthmatic lung, driving CD4+ T cell migration, and enhancing Th2 cytokines and inflammatory pathology. Egr2 represents a novel FcεRI-induced transcription factor in mast cells and specifically regulates mast cell CCL1-dependent CD4+ T cell migration via the CCL1–CCR8 axis, resulting in enhanced Th2 cytokine production and allergic pathology in the inflamed lung tissue. NM, Nuclear membrane; PM, plasma membrane.

Discussion

Previous work has demonstrated the fundamental importance of de novo gene expression as a mechanism for mast cell responses to IgE-mediated FcεRI aggregation. When mast cells are treated with the general protein synthesis inhibitor, cyclohexamide, prior to activation, related mast cell secretory responses are significantly impaired, indicating that newly synthesized proteins are critical in the production of mast cell mediators (26). More recently, we have described the significance of de novo expressed Egr1 in the production of several key cytokines (18). In the study presented in this work, we demonstrate a novel role for the de novo expression of the transcription factor Egr2 in specifically directing the production of the critically important chemokine CCL1 following FcεRI-mediated mast cell activation. We further demonstrate that CD4+ T cell migration to sites of allergen-activated mast cells through the CCL1–CCR8 axis is significantly dependent on mast cell Egr2 expression (Fig. 8).

The Egr family of transcription factors functions in directing transcriptional events for a broad array of cell type– and signaling context–specific events, including differentiation, proliferation, and response to extracellular signals. Target gene promoters include the vascular endothelial and platelet-derived growth factors (27, 28), as well as regulators of hematopoietic differentiation (29, 30) and neuronal development (31). In this work, we demonstrate
a novel finding, that 

CCL1 is a target of Egr2 in FceRI-activated mast cells, and, upon induction, Egr2 can directly bind to the promoter of 

CCL1 and drive gene expression. Egr2 deficiency results in a specific and significant defect in mast cell CCL1 production with functional consequences for chemotactic responses to allergic inflammation. Notably, a previous microarray analysis of TCR-induced genes supporting T cell anergy listed both Egr2 and CCL1 as upregulated, although no direct association was identified (32).

Although further study is required, this finding may suggest that Egr2 can regulate CCL1 expression in other cell type–specific contexts, including promotion of T cell anergy, raising the possibility of Egr2 as a critical regulator of CCL1 expression in a broader scope of cellular functions.

Our results demonstrate that the FceRI-induced transcription factor Egr2 can dictate mast cell–dependent CD4+ T cell migration. In a model of allergic asthma, we demonstrated that mast cell Egr2 is required for the recruitment of CD4+ T cells in vivo. Although splenic cell numbers were unaffected, CD4+ T cell numbers at the draining lymph node and local site of inflammation (lung) were dramatically impaired among all subsets examined, indicating that mast cell Egr2 is required to direct CD4+ T cell migration during allergic inflammatory events in the lung. Consistent with this, Th2 cytokine levels in the lungs of asthmatic mice were significantly impaired, demonstrating that this mast cell Egr2-dependent defect in CD4+ T cell recruitment affects the cytokine milieu in the allergically inflamed lung. Indeed, CD4+ T cells are significant contributors in inflammatory processes in the lung mucosa in asthma (33, 34), suggesting that mast cell Egr2 is a key regulator of the asthmatic phenotype. However, our results show a more marked impairment of CD4+ T cell recruitment than the detected impairment in Th2 cytokines in the lung, suggesting that cell types other than CD4+ T cells are contributing to the Th2 cytokine milieu in the asthmatic lung. Indeed, reports have shown that mast cells, eosinophils, and other types of T cells, including CD8+, NKt, and γδT cells, can contribute to the cytokine milieu in the lung during asthma (35). In addition, a series of work recently characterized a new population of innate helper cells that have been shown to significantly contribute to the cytokine milieu in Th2-driven responses (36, 37). Our results further support a role for these other cell types contributing to the total levels of Th2 cytokines in the lung, not to the level attainable when CD4+ T cells also contribute.

Mast cells are an abundant source of CCL1 following FceRI-mediated activation and are critically important in the chemotactic recruitment of CCR8-expressing CD4+ T cells (25, 38), a relationship central in the development of asthmatic mucosal inflammation in the lung (7). We have detected a deficiency of CCL1 in the lungs of asthmatic mice with Egr2-deficient mast cells (Fig. 7F). It is notable, however, that, like Th2 cytokines, the defect in total CCL1 in the lung is less severe when compared with the defect in CD4+ T cell recruitment (Fig. 6C, 6E), suggesting a possible threshold level of CCL1 being required to initiate significant CD4+ T cell recruitment, and that this threshold may be represented in the difference in CCL1 levels we see between the wild-type and Egr2-deficient mast cell–reconstituted mice in our model. Interestingly, the CCL1–CCR8 axis not only regulates allergic inflammation in the lung (7), but is also critical in mast cell–dependent recruitment of T cells to sites of atopic skin inflammation (8), suggesting a likely role for Egr2 in the pathology of an additional IgE-directed disease. Notably, a recent study examined the significance of Egr2 in CD4+ T cell immune responses and demonstrated that Egr2 was not required for normal CD4+ T cell function, distinctly highlighting the cell type specificity of Egr2 in the mast cell–CD4+ T cell relationship (39).

The direct regulatory role of Egr2 on the CCL1–CCR8 axis identified in this work indicates that Egr2 may indeed be an attractive target for the development of novel therapeutics for the treatment of allergic inflammatory conditions such as asthma and atopic dermatitis. CCL1 expression is known to be elevated in the lung of asthmatic patients (40). Expression of the CCL1 receptor, CCR8, is elevated in asthma (41), and small molecule inhibitors of CCR8 inhibit human T cell migration (25), signifying that the reciprocal blockade of CCL1 production should impair human CD4+ T cell migration. The CCL1–CCR8 axis is also relevant in other diseases, including multiple sclerosis and cancer, yet a link to Egr2 remains unclear (42, 43). Interestingly, however, Egr2 is required for the expression of a myriad of myelinating genes in neurons (31), and mutations in Egr2 are associated with Charcot-Marie-Tooth disease, a condition in which impaired Egr2 DNA binding results in nerve demyelination (44), bearing phenotypic similarities to multiple sclerosis, although seemingly mechanistically distinct.

In summary, we have identified Egr2 as a novel de novo expressed transcription factor actively binding to the promoter of CCL1 following FceRI-mediated mast cell activation. Egr2 is required for CCL1 production by mast cells and directly regulates the CCL1–CCR8 mast cell–CD4+ T cell migratory axis in allergic inflammation. Our results define a novel function for Egr2 in the regulation of CCL1 expression that may have context beyond allergy and asthma.

Acknowledgments

We thank Sandy Edgar for technical assistance with the SSH assay.

Disclosures

The authors have no financial conflicts of interest.

References


