Notch1-mediated Signaling Induces MHC Class II Expression through Activation of Class II Transactivator Promoter III in Mast Cells*

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Mast cells constitutively express Notch1 and Notch2 on the cell surface. Notch ligand Dll1 (Delta-like 1) stimulation induces MHC class II expression in mast cells and renders them as antigen-presenting cells. However, nothing is known about the mechanism by which Notch signaling induces MHC class II expression in mast cells. MHC class II genes are regulated by the class II transactivator (CIITA). In mice, transcription of the CIITA gene is controlled by three cell type-specific promoters (pI, pIII, and pIV). Here, we show that CIITA expression induced by Dll1 stimulation in mouse bone marrow-derived mast cells (BMMCs) depends critically on the signal mediated by Notch1 and that the most dominant promoter in Notch signaling-mediated CIITA expression in BMMCs is pIII, which is a lymphoid lineage-specific promoter. ChIP assays indicated that Notch signaling increased the binding of the transcription factor PU.1 to CIITA pIII in BMMCs. The knockdown of PU.1 expression using a specific siRNA suppressed Notch signaling-mediated CIITA expression, suggesting that PU.1 contributes to the expression of MHC class II induced by Notch signaling in mast cells. Furthermore, we show that a portion of freshly isolated splenic mast cells express MHC class II and that the most dominant promoter of CIITA in mast cells is pIII. These findings indicate that activation of CIITA pIII plays an important role in MHC class II expression in mast cells.

MHC class II molecules play a central role in the presentation of peptides to CD4+ T cells during initiation of the adaptive immune response. Constitutive expression of MHC class II molecules is limited to professional antigen-presenting cells (APCs), such as dendritic cells, macrophages, and B cells, whereas inducible expression of MHC class II molecules by activation with inflammatory cytokines is observed in various types of cells, such as T cells, keratinocytes, fibroblasts, and microglia cells (1–3). Recent findings indicate that mast cells and basophils also express MHC class II molecules and function as APCs. Mast cells and basophils can induce differentiation of Th2 (T helper type 2) cells (4–8). In contrast, dendritic cells alone are not sufficient to induce Th2 differentiation because the cells cannot produce IL-4 (9). Therefore, antigen presentation by mast cells and basophils may play an important role in induction and augmentation of Th2 cell response under some conditions. However, little is known about the regulatory mechanism of MHC class II expression in mast cells and basophils.

Transcription of MHC class II genes is controlled by the class II transactivator (CIITA), which is a transcriptional coactivator that functions as the master regulatory factor for all MHC class II genes (10). In mice, CIITA is transcriptionally regulated by three promoters: pI, pIII, and pIV. Each promoter possesses a distinct first exon that is alternatively spliced to the shared downstream exons, resulting in the formation of three types of CIITA (types I, III, and IV) with different 5′-untranslated regions and/or N-terminal sequences (11). The various CIITA isoforms contribute to the regulation of MHC class II expression in a given cell type and in a developmentally specific manner. Type I CIITA is predominantly expressed in conventional dendritic cells and macrophages, in contrast to the predominant expression of type III CIITA in B cells and plasmacytoid dendritic cells; the expression of type IV CIITA is induced by IFN-γ (12, 13).

MHC class II expression is not detected in steady-state mast cells. However, we found that MHC class II expression in mouse mast cells is induced by overproduction of the transcription factor PU.1 (14, 15). PU.1 plays an important role in mast cell-specific gene expression in a cooperative manner with GATA-1 and GATA-2, which are members of the GATA family of transcription factors and functionally antagonistic to PU.1 in other lineages (16–18). GATA-1 is expressed in erythroid cells, megakaryocytes, eosinophils, and mast cells and is involved in the development of these lineages (19, 20). GATA-2 is expressed at particularly high levels in progenitors of several hematopoietic lineages and is required for mast cell development (21, 22). Our previous studies show that increased expression of PU.1 and reduced expression of GATA-1/2 are required for expression of monocyte-related genes, such as MHC class II, in mast cells (14, 15). Recently, Schroeder et al. (23) reported that Notch signaling up-regulates PU.1 expression in immature hematopoietic progenitor cells. Notch proteins are epidermal growth factor-like transmembrane receptors, and four Notch
genes, Notch1–4, have been identified in mammals. We have confirmed that mouse mast cells constitutively express Notch1 and Notch2 proteins on the cell surface (4). Notch receptors bind to four ligands, Dll1 (Delta-like 1), Dll4 (Delta-like 4), Jag1 (jagged1), and Jag2 (jagged2), resulting in the generation of a proteolytic fragment of the intracellular region of Notch (NotchIC), which is transported to the nucleus as a signal transducer (24). Notch signaling regulates the progression of cell differentiation or specification of cell fates in various developmental systems (25). We have shown that MHC class II expression depends on the signal mediated by Notch1, regulated by Dll1/Notch signaling. To address this issue, we investigated the mechanism of CIITA expression regulated by Dll1/Notch signaling.

In this study, we show that Dll1-induced CIITA and MHC class II expression depends on the signal mediated by Notch1, but not Notch2. We also show that Dll1/Notch signaling promotes binding of the transcription factor PU.1 to CIITA pIII, resulting in induction of the transcription of type III CIITA in mast cells. To the best of our knowledge, this is the first report resulting in induction of the transcription of type III CIITA in mast cells. To the best of our knowledge, this is the first report

**Experimental Procedures**

**Cells**—Bone marrow-derived mast cells (BMMCs) were generated from femoral bone marrow cells of BALB/c mice as described previously (26). Cells were incubated for 2–4 weeks in RPMI 1640 medium (Sigma) supplemented with 10% heat-inactivated FCS (Invitrogen), 100 units/ml penicillin, 100 µg/ml streptomycin, 10 mM sodium pyruvate, 10 µM minimum essential medium (MEM) nonessential amino acid solution (Invitrogen), 100 units/ml recombinant murine interleukin-3 (Wako Pure Chemical Industries, Osaka, Japan), and 0.5 units/ml recombinant murine stem cell factor (Wako Pure Chemical Industries) at 37 °C in a humidified atmosphere in the presence of 5% CO2. Mast cells were identified by flow cytometric analysis of cell-surface expression of c-Kit and the Fcε receptor I α-chain (FceRα). The murine A20 B cell line (27) was maintained in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 100 units/ml penicillin, 100 µg/ml streptomycin, and 100 µM 2-mercaptoethanol at 37 °C in a humidified atmosphere in the presence of 5% CO2.

**Sorting of Splenic Mast Cells**—Splenes were harvested from naive BALB/c mice, and single-cell suspensions were prepared. Red cells were removed from the suspensions using ammonium chloride lysis buffer. Spleen cells were stained with phycoerythrin-conjugated anti-mouse c-Kit mAb (2B8, BioLegend, San Diego, CA) and FITC-conjugated anti-mouse FceRα mAb (MAR-1, eBioscience) after blocking Fc receptors with anti-mouse CD16/CD32 mAb (2.4G2, Pharmingen). c-Kit^+ FcεRα^+ splenic mast cells were sorted on a FACSAria (BD Biosciences).

**Co-culture of BMMCs or A20 Cells with a CHO Cell Line**

**Expressing Notch Ligand**—BMMCs were cultured for 2 weeks, or A20 cells were co-cultured with a mouse Dll1-expressing CHO cell line (28, 29) or control CHO cells (a kind gift from Dr. S. Chiba, University of Tsukuba, Tsukuba, Japan) as described previously (4). In brief, the CHO cells were seeded at a density of 3.6 × 10^4 cells/cm^2 in plates and treated with 3 µg/ml mitomycin C (Sigma) for 3 h. Bone marrow cells were placed at a density of 1.2 × 10^6 cells/cm^2 into the plates and co-cultured with the CHO cells for 3–4 days in α-MEM (Invitrogen) supplemented with 10% heat-inactivated FCS, 100 units/ml penicillin, 100 µg/ml streptomycin, 100 µM 2-mercaptoethanol, 10 mM sodium pyruvate, 10 µM MEM nonessential amino acid solution, 100 units/ml recombinant murine IL-3, and 0.5 units/ml recombinant murine stem cell factor at 37 °C in a humidified atmosphere in the presence of 5% CO2. A20 cells were placed at a density of 1.2 × 10^4 cells/cm^2 into the plates and co-cultured with the CHO cells for 4 days in α-MEM supplemented with 10% heat-inactivated FCS, 100 units/ml penicillin, 100 µg/ml streptomycin, 100 µM 2-mercaptoethanol, 10 mM sodium pyruvate, and 10 µM MEM nonessential amino acid solution at 37 °C in a humidified atmosphere in the presence of 5% CO2.

**Purification of BMMCs**—After co-culture with the CHO cells, BMMCs (>98% purity) were purified by magnetic cell sorting (MACS) (Miltenyi Biotec, Bergisch Gladbach, Germany) using magnetic microbead-conjugated anti-mouse CD117/c-Kit mAb or anti-mouse MHC class II (I-A) mAb (Miltenyi Biotec) according to the manufacturer's instructions.

**Retroviral Constructs and Transfection of BMMCs**—Murine Notch1^IC^ cDNA (30) or Notch2^IC^ cDNA (31) (a gift from Dr. S. Chiba) was subcloned into the retrovirus vector pMXs-puro (32), which was a gift from Dr. T. Kitamura (University of Tokyo, Tokyo, Japan). Transduction of the vectors into BMMCs was performed according to a previously reported method (33). In brief, a retrovirus-packing cell line, PLAT-E, was transfected with each retrovirus vector using FuGENE 6 (Roche Diagnostics). The conditioned medium was concentrated by centrifugation, and then BMMCs that had been cultured for 2 weeks were incubated with the concentrated medium for 2 days in the presence of 10 µg/ml Polybrene (Sigma). Infected cells were selected by additional culture in the presence of 1.2 µg/ml puromycin for 14 days.

**Flow Cytometric Analysis**—BMMCs purified by MACS or freshly isolated spleen cells were stained with phycoerythrin-conjugated anti-mouse I-A^d^ mAb (AMS-32.1, Pharmingen), FITC-conjugated anti-mouse FceRα mAb (MAR-1), and/or PerCP (Peridinin Chlorophyll Protein complex)/Cy5.5-conjugated anti-mouse c-Kit mAb (2B8) after blocking Fc receptors with anti-mouse CD16/CD32 mAb (2.4G2). The expression of cell-surface markers was analyzed on a FACSCalibur (BD Biosciences).

**Quantitative Real-time PCR**—BMMCs purified by MACS or freshly isolated spleen mast cells were lysed with QIAshredder (Qiagen, Hilden, Germany), and total cellular RNA was purified from lysates using an RNaseasy kit (Qiagen) according to the manufacturer's instructions. First-strand cDNA was synthesized from 1 µg total RNA with random hexamers as a primer.
using SuperScript III RNase H− reverse transcriptase (Invitrogen). Quantitative real-time PCR was performed with the StepOne real-time PCR system (Applied Biosystems, Foster City, CA) using TaqMan Universal PCR Master Mix and Assays-on-Demand gene expression products for the I-A α-chain (H2-Aa, Mm00439211_m1), I-E α-chain (H2-Ea, Mm00772352_m1), common CIITA (Ciita, Mm00482919_m1), Hes-1 (hairy and enhancer of split 3; Hes1, Mm01342805_m1), PU.1 (Sfp1, Mm00488342_m1), GATA-1 (Gata1, Mm00484678_m1), and GATA-2 (Gata2, Mm00492300_m1), which were purchased from Applied Biosystems. The mRNA expression levels were quantified with the comparative method using StepOne software and normalized against the housekeeping gene Gapdh.

The mRNA levels of type III CIITA (Mm01342720_m1) and type IV CIITA (Mm01349324_m1), which were purchased from Applied Biosystems, and type I CIITA, which was originally constructed using the customized service of Applied Biosystems, were quantified with the absolute standard curve method using StepOne software. To standardize mRNA concentrations, transcript levels of a housekeeping gene were quantified with the absolute standard curve from Applied Biosystems, and type I CIITA, which was originally constructed using the customized service of Applied Biosystems, were quantified with the absolute standard curve method using StepOne software. To standardize mRNA concentrations, transcript levels of Gapdh were quantified in parallel of each sample. The copy number of each mRNA was corrected by normalization based on the copy number of Gapdh mRNA as follows: normalized copy number = (copy number of mRNA of interest/copy number of Gapdh mRNA) × 10^6.

ChIP Assay—ChIP assays were performed as described previously (34) using antibodies against mouse PU.1 (T-21), IRF-4 (M-17), and IRF-8 (C-19) (Santa Cruz Biotechnology, Santa Cruz, CA). Results were quantified by real-time PCR primers and a TaqMan probe for the promoter region of the mouse type III CIITA gene (sense, 5'-CAGCTTCTGTTGGT-CACCCAAT-3'; antisense, 5'-TCCCTTAGCCACACAGCTT-3'; and probe, 5'-FAM-CAAGAGGAACTGAATTT-Minor Groove Binder-3'). To average samples between experiments and to apply statistics, ChIP-immunoprecipitated DNA was normalized to the amount of a specific fragment in the DNA before immunoprecipitation in each assay. The data were then plotted as -fold over an irrelevant antibody control.

Western Blot Analysis—BMMCs purified by MACS were collected and lysed by the direct addition of sample buffer (62.5 mM Tris-HCl (pH 6.8), 10% glycerol, 2% SDS, 0.1 mg/ml bromphenol blue dye, and 10% 2-mercaptoethanol). The cell lysates were electrophoretically resolved on a 10% SDS-polyacrylamide gel and transferred onto a polyvinylidene fluoride membrane (Millipore, Bedford, MA). Antibodies against PU.1 (T-21), GATA-1 (N6), GATA-2 (H-116), and actin (C-2), which were purchased from Santa Cruz Biotechnology, were used as primary antibodies. Alexa Fluor 680- or IRDye 800-conjugated anti-mouse or anti-rabbit IgG antibody (Protein Probes, Eugene, OR) was used as a secondary antibody. Infrared fluorescence on the membrane was detected by the infrared imaging system Odyssey (LI-COR Biosciences, Lincoln, NE).

RNA Interference Experiments—BMMCs were treated with siRNA directed against Pu.1 (Oligo ID MSS247676, Invitrogen) or non-targeting control siRNA (Invitrogen), both at 500 nm. The siRNAs were transfected into 2 × 10^6 BMMCs with Nucleofector II (Amaxa, Koln, Germany) set at Program Y-001 using a mouse macrophage Nucleofector kit (Amaxa) according to the manufacturer’s instructions. After 24 h of transfection, the BMMCs were co-cultured with Dll1-expressing or control CHO cells for 3 days.

RESULTS

Dll1/Notch Signaling Induces Transcription of MHC Class II-related Genes in BMMCs—MHC class II expression on BMMCs is not observed at steady state but is induced by signaling from Notch receptors mediated by Dll1 (4). We first analyzed the expression profiles of MHC class II-related genes in Dll1-stimulated BMMCs. As shown in Fig. 1A, the transcription levels of both I-A and I-E MHC class II molecules were confirmed to be higher in Dll1 BMMCs, which were co-cultured with Dll1-expressing CHO cells for 4 days, compared with control BMMCs, which were co-cultured with control CHO cells. The mRNA level of common CIITA was also increased in the Dll1 BMMCs, indicating that Dll1/Notch signaling induces the transcription of CIITA, the master regulator of MHC class II genes, in mast cells.

Expression of MHC Class II-related Genes Depends on Notch1-mediated Signaling in BMMCs—Mouse mast cells, such as BMMCs and peritoneal mast cells, express Notch1 and Notch2 proteins at high levels on the cell surface (4). To determine which of these Notch receptors is involved in MHC class II expression in mast cells, we analyzed the mRNA levels of MHC class II-related genes in BMMCs transfected with Notch1IC or Notch2IC.

Hes-1 is a basic helix-loop-helix transcriptional repressor and a well known Notch1IC target gene (35, 36). As shown in Fig. 1B, modest but significant up-regulation of mRNA expression
of Hes-1 was observed in both Notch1IC- and Notch2IC-transfected BMMCs, indicating that gene transcription was induced by Notch signaling in both the transfected BMMCs. In contrast to Hes-1, mRNA expression of both I-A and I-E MHC class II molecules and common CIITA were observed in BMMCs transfected with Notch1IC, but not Notch2IC (Fig. 1B). These results indicate that MHC class II-related gene expression depends on Notch1 signaling in mast cells.

**Notch Signaling Activates Transcription of Type III CIITA in Mast Cells**—Mouse CIITA expression is controlled by three independent promoters: pl, pIII, and pIV. To elucidate which promoters of CIITA are activated by Notch signaling, we quantitated the mRNA levels of three transcripts (type I, III, and IV CIITA) in Dll1 BMMCs by real-time PCR using different type-specific primer pairs. Cell-surface expression of the MHC class II molecule I-A^d_1 protein was observed in ~21.6% of Dll1 BMMCs co-cultured with Dll1-expressing CHO cells for 4 days (Fig. 2A). In the Dll1 BMMCs, the transcription of type III and type IV CIITA was detected, and type III CIITA was the most dominant transcript (Fig. 2B). These results indicate that MHC class II-related gene expression of CIITA is controlled by three independent promoters: pl, pIII, and pIV.

**Notch Signaling Promotes PU.1 Binding to CIITA pIII in Mast Cells**—In B cells, the type III CIITA promoter is transactivated by the Ets family transcription factor PU.1 and its activating partner IRF-4, which bind to an Ets-binding site in pIII. In activated T cells, an unidentified Ets family member has been shown to interact with the site (2, 37). In mast cells, PU.1 is an important regulator of cellular functions and development (20, 38, 39). Thus, we analyzed the amount of PU.1 and its activating partners, IRF-4 and IRF-8, that bind to pIII in I-A^d_1 Dll1 BMMCs by ChIP assay. The amount of PU.1 bound to the Ets site in Dll1 BMMCs was significantly higher than that in control BMMCs (Fig. 3A). In contrast, we could not detect significant differences between Dll1 and control BMMCs in the amount of IRF-4 and IRF-8 binding to the site (Fig. 3A).

To determine whether the increase in PU.1 binding to pIII by Notch signaling is specific for mast cells, we next analyzed the amount of PU.1 bound to the site in A20 B cells co-cultured with Dll1-expressing or control CHO cells. Steady-state A20 cells expressed I-A^d_1, Notch1, and Notch2 at high levels and expressed Notch3 and Notch4 at low levels (data not shown). The amount of PU.1 bound to pIII in A20 cells was modestly but significantly increased by co-culture with Dll1-expressing CHO cells, whereas that of IRF-4 and IRF-8 was modestly decreased (Fig. 3B). However, no significant change in the expression level of I-A^d_1 on A20 cells by co-culture with Dll1-expressing CHO cells was observed (data not shown). These results indicate that Notch signaling significantly up-regulates the recruitment of PU.1 to pIII in B cells, but not as strikingly as that in stimulated mast cells, because of the high amount of PU.1 recruitment in steady-state B cells, resulting in an insignificant effect on MHC class II expression in B cells.

**PU.1 Contributes to Notch Signaling-mediated CIITA Expression**—Fig. 3 shows that Notch signaling increased the amount of PU.1 bound to CIITA pIII in BMMCs. To demonstrate the contribution of PU.1 to Notch signaling-mediated CIITA expression in BMMCs, we examined the effect of specific knockdown of PU.1. As shown in Fig. 4A, expression of the PU.1 protein was markedly reduced in BMMCs from 16 h to 5 days following specific siRNA introduction. The PU.1 protein was at non-detectable levels especially in BMMCs from 1 to 3 days after siRNA introduction. Therefore, we started a co-culture of BMMCs with Dll1-expressing CHO cells 24 h after siRNA introduction. The expression of mRNA for common CIITA was reduced by approximately 50% (Fig. 4B) in Dll1 BMMCs introduced PU.1-specific siRNA. The data indicate that PU.1 is involved in CIITA expression induced by Notch signaling in mast cells.
Notch1 Signal Activates CIITA pIII in Mast Cells

A

FIGURE 4. PU.1 contributes to CIITA expression induced by Dll1/Notch signaling in BMMCs. BMMCs cultured for 2 weeks were co-cultured with Dll1-expressing or control CHO cells. After 3 days of co-culture, the BMMCs were harvested and purified by MACS using magnetic microbead-conjugated anti-CD117/c-Kit mAb. The level of mRNA for common CIITA in siRNA-treated cells was assessed by quantitative real-time PCR. Data are represented as means of normalized copy numbers ± S.D. of triplicate measurements.

B

FIGURE 5. Dll1/Notch signaling reduces GATA-1 and GATA-2 expression in BMMCs. BMMCs cultured for 2 weeks were co-cultured with Dll1-expressing or control CHO cells. The levels of mRNA for Pu.1, Gata1, and Gata2 in the purified cells were assessed by quantitative real-time PCR. The values obtained (mean ± S.D. of triplicate measurements) were normalized to mRNA for Gapdh and are expressed relative to control BMMCs transfected with control siRNA. **, p < 0.005 as determined by Student’s t test.

Notch Signaling Reduces GATA-1 and GATA-2 Expression Levels in Mast Cells—To elucidate the mechanism of the increased recruitment of PU.1 to pIII following Notch stimulation, we compared the expression levels of PU.1 between I-A^d^ Dll1 BMMCs and control BMMCs. The mRNA (Fig. 5A) and protein (Fig. 5B) expression of PU.1 in I-A^d^ Dll1 BMMCs was almost comparable with that in control BMMCs, whereas that of GATA-1 and GATA-2 was markedly reduced in I-A^d^ Dll1 BMMCs compared with control BMMCs. Considering that GATA proteins inhibit the function of PU.1 by forming an inactive PU.1-GATA-1 or PU.1-GATA-2 complex (16–18), Notch signaling may increase the rate of active PU.1 by suppressing the expression of GATA-1 and GATA-2.

Splenic Mast Cells Express MHC Class II and Type III and Type IV CIITA—We next attempted to assess whether CIITA pIII contributes to the expression of MHC class II in mast cells in vivo. Although freshly isolated peritoneal mast cells from the peritoneal cavity of naive mice had no detectable levels of MHC class II expression (data not shown), ~38.1% of splenic mast cells in freshly isolated spleen cells expressed MHC class II (Fig. 6A). Similar to that observed in Dll1 BMMCs, the transcription of type III and type IV CIITA was detected in splenic mast cells, and type III CIITA was the most dominant transcript (Fig. 6B). These results suggest that the activation of CIITA pIII contributes to the expression of MHC class II in mast cells.

DISCUSSION

We have previously shown that stimulation with the Notch ligand Dll1 significantly induces MHC class II expression in BMMCs (4). We have also shown that BMMCs express Notch1 and Notch2 constitutively. In this study, we demonstrated that CIITA and MHC class II molecule expression was dependent on signaling mediated by Notch1. Thus, in mast cells, Notch1 is the receptor that mediates stimulation with Dll1 and induces MHC class II expression. On the other hand, recent studies have reported that Notch2-mediated signaling is required for mast cell development and intraepithelial localization of mast cells (36, 40). Although Hes-1 is a common target gene of Notch1 and Notch2, the preferred targets of Notch1 and
Notch2 are usually different (41). In this study, CIITA and MHC class II expression was induced by Notch1 signaling, but not Notch2 signaling (Fig. 1). Therefore, it is likely that characteristics of mast cells are modulated by a balance between Notch1 and Notch2 signaling.

We detected type III and type IV CIITA transcription in Dll1 BMMCs. Type III CIITA was the dominant transcript (Fig. 2B), suggesting that Notch signaling activates mainly pIII in mast cells. pIII is a lymphoid promoter essential for the expression of CIITA in B cells and plasmacytid dendritic cells, whereas pI is a myeloid promoter regulating expression in macrophages and conventional dendritic cells. Our results indicate that the mechanism of Notch signaling-mediated MHC class II expression in mast cells is distinct from that in other myeloid lineage cells. The expression of type IV CIITA was also clearly observed in Dll1 BMMCs. Kambayashi et al. (5) have reported that MHC class II expression is induced in mouse mast cells upon stimulation with IFN-γ and lipopolysaccharide. Therefore, considering that pIV is an IFN-γ-dependent promoter, the activation of pIV may be further activated in IFN-γ-stimulated mast cells.

The activation of CIITA pIII in B cells and activated T cells is promoted by binding of an Ets motif-binding factor, such as PU.1, to the promoter region (2, 37). We detected a significant increase in the amount of PU.1 at an Ets-binding site in pIII when BMMCs were stimulated with Dll1 (Fig. 3A). This is consistent with our previous reports showing that MHC class II expression is induced in BMMCs overexpressing PU.1 (14, 15, 33, 39). However, it is still unclear whether some binding partners of PU.1 are required for CIITA pII activation in mast cells. In B cells, PU.1 binds to the Ets-binding site in pIII as a heterodimer complex with IRF-4 (37). Considering that the IRF-4 protein was not detected in BMMCs in our preliminary Western blot analysis (data not shown), PU.1 may act alone or in concert with other transcription factors to activate CIITA pIII. In any case, Notch signaling-mediated CIITA expression in BMMCs was suppressed by knockdown of PU.1 expression with specific siRNAs (Fig. 4), supporting the importance of the recruitment of PU.1 to pIII. In contrast, in A20 B cells, Notch signaling led to only a slight increase in the recruitment of PU.1 to pIII (Fig. 3B). A20 cells constitutively express MHC class II. Because the amount of PU.1 bound to pIII in steady-state A20 cells was sufficiently high to express type III CIITA, Notch signaling might have insignificant effects on PU.1 recruitment. Therefore, PU.1 recruitment to pIII induced by Notch signaling is critical for MHC class II expression in mast cells.

Schroeder et al. (23) have shown that Notch signaling up-regulates PU.1 expression in immature hemopoietic progenitor cells. Although we detected no significant up-regulation of PU.1 expression and a marked reduction in expression of GATA-1 and GATA-2 in BMMCs stimulated with Dll1 (Fig. 5), PU.1 might be transiently up-regulated in BMMCs. Because PU.1 inhibits GATA-1/2 expression (16–18), a transient increase in PU.1 expression probably decreased the expression of GATA-1 and GATA-2 in BMMCs. Both GATA-1 and GATA-2 directly interact with the PU.1 Ets domain and repress PU.1-dependent transcription (16–18). Therefore, a relative increase in PU.1 and a decrease in GATA-1 and GATA-2 may lead to an increase in the active form of PU.1. It is assumed that an increase in the recruitment of PU.1 to pIII in BMMCs stimulated with Dll1 is caused by an increase in the active form of PU.1. Further analyses are required to understand the mechanism of regulating GATA-1 and GATA-2 expression by Notch signaling in mast cells. In addition, knockdown of GATA-1 and GATA-2 expression alone could not induce CIITA expression in BMMCs (data not shown). The induction of CIITA expression in mast cells may require not only an increase in active PU.1 but also some other transcriptional regulators induced by Notch signaling.

Furthermore, we have shown that MHC class II expression was induced in a portion of splenic mast cells (Fig. 6A). The expression of type III and type IV CIITA was detected in splenic mast cells, and type III CIITA was the dominant transcript. The expression pattern of CIITA isoforms in splenic mast cells is similar to that in Dll1 BMMCs, suggesting that the activation of CIITA pIII plays an important role in MHC class II expression in mast cells. We observed the expression of Notch1 and Notch2 on the cell surface of freshly isolated splenic mast cells (data not shown). Therefore, Notch signaling may contribute to the expression of MHC class II in splenic mast cells.

In this study, we demonstrated the cellular function of Notch1 signaling in BMMCs. A recent report has indicated that Notch2 signaling determines the fate of myeloid progenitors for mast cell-producing progenitors via coordinate up-regulating Hes-1 and GATA-3 (36). Mast cells circulate through the vascular system as immature progenitors and then as mature progenitors in peripheral tissues (42, 43). Thus, mast cell differentiation may be promoted by contact with Notch ligand–expressing cells in peripheral tissues. In addition, mast cells are suspected to acquire APC functions by Notch1 signaling induced by contact with Dll1. Recently, Tsuchiya et al. (44) reported that the mRNAs for MHC class II molecules are expressed in mast cells isolated from mouse stomach mucosa. Therefore, further analyses using murine models of food allergies or nematode infections should be used to elucidate the roles of mast cells as APCs in the adaptive immune response.

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