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Mast Cells Acquire Monocyte-Specific Gene Expression and Monocyte-Like Morphology by Overproduction of PU.1

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PU.1 is a myeloid- and lymphoid-specific transcription factor that belongs to the Ets family. Recently, we found that overproduction of PU.1 in mouse bone marrow-derived hemopoietic progenitor cells induced monocyte-specific gene expression and caused their monocye-like morphological change. In the present study, PU.1 was overproduced by using retrovirus expression system in differentiated bone marrow-derived mast cells. By overexpression of PU.1, cell surface expression of MHC class II, CD11b, CD11c, and F4/80 was induced, accompanied by reduced expression of c-kit, a mast cell-specific marker. Morphology of PU.1-transfected cells was altered toward monocyte-like one. PU.1-overproducing cells acquired T cell stimulatory ability and showed an increase in response to LPS stimulation, while response through FcεRI was markedly reduced by overproduction of PU.1. These results suggest that the differentiated mast cells still have potential to display monocytic features. When PU.1 was overproduced in a different type of mast cell, peritoneal mast cells, similar monocyte-like morphological change, and the expression of CD11b and F4/80 were induced. However, surface level of CD11c and MHC class II was not affected. These results indicate that the potential capacity to exhibit monocytic features is different between both the mast cells. The Journal of Immunology, 2005, 174: 376–383.

PU.1 is an Ets family transcription factor and involved in lymphoid and myeloid cell development and specific gene regulation. PU.1 is expressed in lymphoid cells, macrophages, dendritic cells (DC),3 neutrophils, and mast cells in a cell type-specific manner (1). The necessity of PU.1 for generation of these lineages was shown by PU.1 knockout mouse that abolishes macrophage and B cell production and delays neutrophil and T cell production (2–4). The requirement of PU.1 for the development of DC and mast cells was also recently revealed by the analyses using PU.1 knockout mouse (5–7). It was also reported that expression level of PU.1 determines cell fate between B cells/macrophages (8) and neutrophils/macrophages (9). In addition, recent analyses demonstrated that overexpression of PU.1 in CD34+ human myeloid progenitors triggers development of Langerhans cells (LC) (10, 11).

Recently, we found that overproduction of PU.1 in mouse bone marrow-derived hemopoietic progenitor cells developing toward mast cells induced the expression of several monocyte-specific genes and caused morphological change (12). In this study, we conducted overexpression of PU.1 by retrovirus transfection system in differentiated mast cells, mouse bone marrow-derived mast cells (BMMC), and peritoneal mast cells (PMC), and examined its effect on the expression of monocyte- and mast cell-specific markers and the cell morphology. BMMC and PMC showed several monocytic characteristics by overproduction of PU.1, suggesting that PU.1 functions to promote monocyte-specific gene expression even in developed mast cells, and that developed mast cells still possess the potential capacity to exhibit monocytic features.

Materials and Methods

Cells

A retrovirus packaging cell, Plat-E (13), was maintained in DMEM (Sigma-Aldrich) supplemented with 10% heat-inactivated FBS (Sigma-Aldrich), 100 U/ml penicillin, 100 μg/ml streptomycin, 1 μg/ml puromycin (Sigma-Aldrich), and 10 μg/ml blasticidin (Funakoshi). To generate BMMC, bone marrow cells prepared from BALB/c (Japan SLC, Hamamatsu, Japan) were grown in RPMI 1640 (Sigma-Aldrich) supplemented with 10% heat-inactivated FBS, 100 μM 2-MA, 10 μM MEM nonessential amino acids solution (Sigma-Aldrich), 100 U/ml penicillin, 100 μg/ml streptomycin, and 10 μg/ml mitogen-stimulated splenocyte-condition medium (14). After 4-wk culture, >95% of cells were identifiable as mast cells by toluidine blue staining and as c-kit+/FceRI+ by flow cytometric analysis. Mouse PMC was prepared from whole peritoneal cells by density-gradient centrifugation techniques using metrizamide (Sigma-Aldrich) with >98% purity (15), and was maintained in RPMI 1640 supplemented with 10% FBS, 10% pokeweed mitogen-stimulated spleen-condition medium, 20 ng/ml mouse recombinant stem cell factor (Peprotech), and antibiotics (penicillin and streptomycin). Bone marrow-derived DC (BMDC) was prepared according to previously reported method (16, 17), with some modifications. In brief, bone marrow cells prepared from BALB/c were grown in RPMI 1640 supplemented with 10% FBS, 100 μM 2-MA, 10 μM MEM nonessential amino acids solution, antibiotics, 10 ng/ml mouse GM-CSF (PeproTech), and 10 ng/ml mouse IL-4 (PeproTech). Peritoneal macrophages were obtained from BALB/c, as described previously (18). Briefly, peritoneal exudate cells were harvested from mice, which received i.p. 2 ml of 4% thioglycollate (Sigma-Aldrich) 4 days before, by peritoneal lavage with ice-cold PBS. After 1-h culture on a plastic dish, nonadherent cells were collected and used as peritoneal macrophages.

Plasmid construction

Plasmids, pMX-puro-PU.1 (12), and pMX-puro (19) were used to generate retrovirus vector to overproduce PU.1 tagged with 2× Flag at N terminus as per our previous reports (12, 20). The 2× Flag-tagged PU.1 cDNA sequence was isolated from pCR-2F-PU.1 (21) and subcloned into

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pMX-IG (22) using restriction endonucleases and a DNA ligation kit, version 1 (Takara Bio), to generate pMX-IG-PU.1.

Transfection
Infection of BMMC and PMC was performed, according to a previously reported method (12, 20). In brief, pMX-puro (mock vector) and pMX-puro-PU.1 (for the expression of wild-type PU.1) were transiently introduced into Plat-E with Fugene6 (Roche Diagnostics). BMMC after 4-wk culture and freshly prepared PMC were incubated with harvested culture medium of transfected Plat-E containing infectious viruses for 2 days in the presence of 10 μg/ml polybrene (Sigma-Aldrich). Infected cells were selected by culture in the presence of 1.2 μg/ml puromycin for 10–20 days. When pMX-IG and pMX-IG-PU.1 were used for transfection, transfecants that were obtained by the same method as that of pMX-puro-series described above were selected as GFP-positive cells.

Western blotting analysis
To detect endogenous PU.1, BMMC was stimulated by 1 μg/ml LPS or 100 mg/ml PMA (Sigma-Aldrich) for 0–48 h. Whole cells were subjected to Western blotting analysis. Rabbit polyclonal Ab against PU.1 (Santa Cruz Biotechnology) or mouse mAb against Flag-tag (Sigma-Aldrich) was used as the primary Ab. Alexa Fluor 680 goat anti-rabbit IgG or Alexa Fluor 680 goat anti-mouse IgG (Molecular Probes) was used as the secondary Ab. Infrared fluorescence on membranes was detected by Odyssey infrared imaging system (model ODY-9201-SC; LI-COR).

Flow cytometric analysis
The FITC- or PE-conjugated anti-mouse Abs against I-A^d, CD11b, CD11c, F4/80, and c-kit, all of which were purchased from BD Pharmingen, were used to stain each cell surface molecule after blocking Fc receptors with 2.4G2 (BD Pharmingen). Mouse IgE Ab (BD Pharmingen) conjugated with FITC was used to stain mouse FcεRI. To stain TLR2 and 4, anti-mouse TLR2 and 4 rat IgGmAbs purchased from HyCult Biotechnology were used as the first Ab, respectively, and FITC-conjugated rabbit Fab(ab')2 anti-rat IgG Ab (Valence Pharmaceuticals) was used as the second Ab. In the case of pMX-IG-series transfecants expressing GFP, cells were stained with PE-conjugated anti-mouse c-kit and allophycocyanin-conjugated anti-mouse CD11b, CD11c, or combination of biotin-labeled anti-mouse I-A^d and streptavidin-allophycocyanin (BD Pharmingen). For sorting of c-kit-positive or c-kit/FcεRI-double-positive cells, BD FACSAria Cell Sorter (BD Biosciences, San Jose, CA) was used. Cells stained with each Ab, as described previously (23, 24), were analyzed by FACSCalibur flow cytometer (BD Biosciences).

Morphological analysis
Electron microscopy and May-Grünwald-Giemsa’s staining were performed, as described previously (12).

LPS, peptidoglycan (PGN), and Ag/IgE stimulation
Culture medium of cells stimulated with LPS (from Escherichia coli; Sigma-Aldrich), PGN (from Staphylococcus aureus; Sigma-Aldrich), or Ag/IgE was harvested after 6-h incubation. Stimulation with Ag/IgE was performed according to a previously published method (25). Concentrations of IL-6 in the culture supernatant were determined by an ELISA kit, according to the manufacturer’s instruction (Genzyme Techne, Minneapolis, MN). β-Hexosaminidase-releasing assay was performed with previously reported method (25) to investigate degranulation activity.

Ag presentation assay
Cells irradiated at a dose of 30 Gy were plated into 96-well round-bottom plates at 5-fold serial dilutions, each well of which contained splenic CD4^+ T cells from C57BL/6 mice at 3 × 10^5, and incubated for 2 days. [3H]Thymidine was then added (0.37 Mgbq/well), and incubation was continued for an additional 15 h.

Results
Overproduction of PU.1 in BMMC induced the expression of MHC class II, CD11b, CD11c, and F4/80, and suppressed the expression of c-kit
To examine the effect of overproduced PU.1 in mast cells, BMMC were transfected with retrovirus vector that directed the production of wild-type PU.1 or empty vector (Fig. 1A). In Western blotting analysis using anti-PU.1 Ab (Fig. 1B), apparent production of wild-type PU.1 was observed, while endogenous PU.1 was not detected under the conditions used (see Fig. 1B, control (without transfection) and mock), indicating that the cells transfected with pMX-puro-PU.1 expressed much larger amount of PU.1 than that of endogenous PU.1.

BMMC (top panel of Fig. 1C) and mock transfecants (second panel of Fig. 1C) expressed mast cell-specific markers (FceRI and c-kit), but not monocyte-specific molecules (MHC class II, CD11b, CD11c, and F4/80; bottom panel of Fig. 1C) on cell surface. In contrast, overproduction of PU.1 in BMMC induced the expression of MHC class II, CD11b, CD11c, and F4/80, while the expression of c-kit was slightly suppressed (third panel of Fig. 1C). Significant difference was not observed in the expression level of FceRI. These results indicated that overproduction of PU.1 in BMMC induced the expression of monocyte-specific molecules and suppressed c-kit expression.

Morphology of transfecants
To investigate the effect of PU.1 overproduction on the morphology of the cells, May-Grünwald-Giemsa staining of cytoplasm (Fig. 2A) and electron microscopy (Fig. 2B) were performed. The mock transfecants contained a large amount of granules as well as control BMMC. In contrast, PU.1-overproducing cells contained lesser amount of granules, but showed macrophage- and DC-like morphology characterized by vacuoles in the cytoplasm, larger veils, and lamellipodia extending from cell bodies. These observations showed that overproduction of PU.1 caused monocyte-like morphological change on BMMC.

Response to LPS and PGN stimulation
TLR4 signaling activated by LPS induces DC and macrophages to produce proinflammatory cytokines, including IL-6 (26). Although mast cells also produce IL-6 in response to LPS, the production level is quite lower than that of monocytes (27). We therefore analyzed the IL-6 production level of PU.1-overproducing cells by LPS stimulation (Fig. 3A). The amount of IL-6 produced by LPS stimulation was markedly increased in cells overproducing wild-type PU.1 as well as BMDC. In contrast, mock transfecants produced IL-6 at the level similar to that of BMMC.

To confirm the specificity of TLR signaling, we next analyzed the response to PGN, which activates cells via TLR2, by measuring IL-6 released from each transfecant (Fig. 3B). Overproduction of PU.1 resulted in markedly increased production of IL-6 in response to PGN stimulation.

Recently, it has been reported that the essential elements in the promoters of TLR2 and 4 genes were recognized by PU.1 (28, 29), suggesting the possibility that overproduction of PU.1 might enhance IL-6 production through increased expression of TLR2 and 4. We therefore analyzed cell surface expression of TLR2 and 4 in PU.1-overproducing cells (Fig. 3C). Although bone marrow-derived DC expressed an apparent amount of TLR2 and 4, PU.1-overproducing cells expressed TLR2 and 4 only slightly, which was similar to the case in BMMC.

These results suggest that PU.1 enhances IL-6 production by activating downstream process of TLR-LPS/PGN interaction in TLR signaling, but not by increasing the expression of TLRs.

Response to Ag/IgE stimulation
Mast cells also produce IL-6 in response to Ag/IgE stimulation through FceRI. Although a certain kind of monocyte expresses FceRI, the response of monocytes through FceRI is markedly reduced in comparison with that of mast cells (30). To examine the effect of PU.1 on response to Ag/IgE stimulation, we analyzed the level of IL-6 production of the cells stimulated by Ag/IgE (Fig. 4A). The amount of IL-6 produced from PU.1-overproducing cells was markedly reduced when compared with the case of mock
Transfectants and control BMMC. This result indicated that overproduction of PU.1 reduced IL-6 production by interfering the signal from FcγRI.

Degranulation by cross-linking of FcγRI is one of the major characteristics of mast cells. Therefore, degranulation of the cells overproducing PU.1 was analyzed (Fig. 4B). Control BMMC and mock transfectants were degranulated by stimulation with Ag/IgE to the same degree. In contrast, degranulation of the cells overproducing PU.1 was markedly decreased as well as BMDC. From these results, it was concluded that overproduction of PU.1 reduced response to the stimulation through FcγRI in mast cells.

**T cell stimulation activity**

Overproduction of PU.1 induced marked expression of MHC class II, which is a hallmark of APCs, on mast cells, as shown in Fig. 1C. To examine whether induced MHC class II possesses function, allostimulatory activity of transfectants cocultured with allogenic C57BL/6 CD4+ T cells was measured. Thymidine incorporation of T cells was significantly increased when PU.1-overproducing cells were cocultured (Fig. 5).

**Effect of overproduction of PU.1 on PMC**

PMC is connective tissue-type mast cell, which is more maturated than mucosal-type mast cell, BMMC. To examine the effect of PU.1 on connective tissue-type mast cells, PU.1 was overexpressed in freshly isolated PMC by the retrovirus vectors. The expression level of PU.1 in transfectants was increased, as was the case in BMMC (data not shown). May-Grünwald-Giemsa staining indicated that overproduction of PU.1 caused morphological change of the cells: decrease in granules accompanied by formation of vacuoles, larger veils, and lamellipodia (Fig. 6A). Expression profile of the cell surface markers on PU.1-overproducing PMC (Fig. 6B) was somewhat different from the case of BMMC. Similar to the case in BMMC, CD11b and F4/80 were expressed and c-kit expression was suppressed on PU.1-overproducing PMC. However, expression of CD11c and MHC class II was not induced in the transfectant. Thus, increased expression of PU.1 induced several monocyte-like changes even in PMC, but the effect of PU.1 was negligible on some target molecules such as CD11b and MHC class II in PMC.

**Transfection of sorted mast cells using internal ribosome entry site-GFP system to eliminate the contamination of monocyte lineages**

Ten- to 20-day culture was required to select transfectants by puromycin, when plasmids pMX-puro-series were used for retrovirus transfection, as described above. Although the purity of mast cells is high (>95% for BMMC and >98% for PMC, as described in Materials and Methods), the possibility that monocytes and/or

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**FIGURE 1.** Overexpression of PU.1 in BMMC induces monocyte-specific gene expression. A, Schematic structure of PU.1. PU.1 is composed of the acidic region (33–73), the Gln-rich region (74–99), the Pro, Glu, Ser, and Thr-rich (PEST) region (117–166), and the Ets domain (167–271). The amino acid residue numbers are that of rat PU.1 numbering (21).PU.1 that is overproduced by retrovirus vector is tagged with 2×Flag at its N terminus. B, Western blotting analysis of BMMC and transfectants. A total of 5×10⁵ cells was applied onto each lane. Control, normal BMMC after 6-wk culture; mock, BMMC transfected with mock vector (pMX-puro); PU.1, BMMC transfected with retrovirus vector encoding PU.1 cDNA (pMX-puro-PU.1). Transfectants were selected as puromycin-resistant cells by 10- to 20-day culture in the presence of puromycin (1.2 μg/ml). C, Cell surface expression of mast cell- and monocyte-specific molecules. Thin-line histogram represents cells with each Ab. Thin-line histogram indicates control with 2.4G2 alone. Control (top panel), normal BMMC without infection; mock (second panel), BMMC transfected with pMX-puro; PU.1 (third panel), BMMC transfected with pMX-puro-PU.1; BMDC (bottom panel), mouse BMDC. A representative result of five independent experiments is shown.
monocyte progenitors contaminated mast cell suspensions could not be excluded completely. This issue raised a possibility that contaminating monocytes and/or monocyte progenitors that were transfected with retrovirus directing to produce PU.1 proliferated more vigorously than transfected mast cells in the culture period. Therefore, to eliminate this possibility, we used purified BMMC that was collected as c-kit+/H11001/Fc/H9280RI/H11001 cells by sorting for transfection. After 14-day culture for selection of transfectants, 98% of mock transfectants were c-kit+/H11001/Fc/H9280RI/H11001, while c-kit+/H11001/Fc/H9280RI/H11001 population of ~94% was obtained from PU.1 transfectant (Fig. 7A). PU.1-overproducing cells expressed CD11c, CD11b, and MHC class II, which were not expressed on mock transfectants. These


FIGURE 3. IL-6 production in response to the stimulation through TLR2 and 4. IL-6 production level in response to LPS stimulation (A) and PGN stimulation (B) was enhanced by overproduction of PU.1, whereas cell surface expression level of TLR2 and 4 was not affected by overproduction of PU.1 (C). A and B, IL-6 concentration in culture medium after 6 h of stimulation was measured by ELISA. A representative result performed with triplicate was shown as mean ± SD. Similar results were observed in other three independent experiments. C, Histogram of thick line represents cells stained with anti-TLR2 or 4 as first Ab and FITC-conjugated anti-rat IgG Ab as second Ab. Thin-line histogram indicates control cells incubated with second Ab alone. Similar profile was observed in another independent experiment.
results were consistent with the results of BMMC without sorting (Fig. 1C). In addition, CD11c-, CD11b-, and MHC class II-positive cells also expressed mast cell markers, c-kit and FcεRI. This result suggested that the cells expressing CD11c, CD11b, and MHC class II originated from mast cells.

To eliminate the possibility that a few contaminating monocytes and/or its progenitors grew as a major population through 20 days of culture, we constructed plasmids, pMX-IG and pMX-IG-PU.1. By infection with retrovirus, transfectants were detected as GFP cells within 2 days after transfection (Fig. 7B). GFP population of BMMC infected with the retrovirus generated from pMX-IG-PU.1 showed reduced expression of c-kit, while GFP population and GFP mock expressed c-kit at the level same as that of GFP mock. PU.1-overproducing cells detected as GFP expressed CD11c and MHC class II, while most of the GFP population of mock transfectants did not express these molecules. These observations excluded the possibility that contaminating monocytes were the source of cells expressing monocyte-specific molecules. From these results, we concluded that overproduction of PU.1 in BMMC suppressed c-kit expression and induced the expression of CD11c and MHC class II.

Up-regulation of endogenous PU.1 in mast cells by LPS or PMA stimulation

Because PU.1 autoregulates its own promoter (31), the stimulation signal to activate PU.1 protein would further accelerate overexpression of PU.1. Actually, the signal to activate PU.1 protein by LPS (32) and PMA (33) induces the up-regulation of PU.1 in monocytes. Therefore, we hypothesized that mast cells and/or its progenitors might be converted to monocyte-like cells in vivo under a certain condition when either activation or up-regulation of PU.1 is induced. To evaluate the effect of LPS and PMA on PU.1 expression level in mast cells, endogenous PU.1 proteins were analyzed after stimulation of mast cells with LPS or PMA by Western blotting. Intensity of the band migrating at 39 kDa was markedly increased from 4 h after LPS or PMA stimulation and decreased to background level by 24-h incubation (Fig. 8). An additional band migrating slower than the major band of ~43 kDa also appeared from 4 to 8 h after LPS or PMA stimulation. Considering that PU.1 is shown to be present in several forms with
FIGURE 6. Overproduction of PU.1 in PMC. A. May-Grünwald-Giemsa staining of transfected PMC and peritoneal macrophages. ×1000. B. Cell surface expression of mast cell- or monocyte-specific molecules. Thick-line histogram represents cells with each Ab. Thin-line histogram indicates control with 2.4G2 alone.

Discussion
PU.1 is a transcription factor involved in the lymphoid- and myeloid-specific gene regulation and the development of these cell lineages. Expression level of PU.1 determines cell fate between B cells/macrophages (8) and neutrophils/macrophages (9). In addition, overexpression of PU.1 in CD34⁺ human myeloid progenitors promotes LC development (10, 11). In recent analysis, we found that monocyte-specific gene expression and monocyte-like morphological change were induced by overproduction of PU.1 in mouse bone marrow-derived mast cell progenitors (12, 20, 21). In this study, overproduction of PU.1 caused several monocyte-like characteristics on both BMMC and PMC, suggesting that developed mast cells still have the capacity to exhibit monocyte-like features.

Although the overproduction of PU.1 decreased the function and morphology of mast cells, PU.1 overproduction did not decrease the expression of FcεRI, a typical marker for mast cells. In most cases, PU.1 inhibits GATA-1 to function and vice versa, possibly by forming an inactive PU.1/GATA-1 complex (34), and therefore, only either PU.1 or GATA-1 is expressed in a cell. For example, in monocyte/granulocyte lineage development from hematopoietic stem cells, stimulation signals such as GM-CSF up-regulates PU.1 expression, which subsequently inhibits the function of GATA-1 in these cells (34–36). However, mast cells produce both PU.1 and GATA-1, and both transcription factors trans activate the promoter of FceRI α-chain (37). GATA-1 also positively regulates the transcription of another FceRI-specific component, FceRI β-chain (38). In our present study, we observed that BMMC transfecants overproducing PU.1 still expressed GATA-1 at similar level as that of mock transfecants (data not shown). Therefore, we assume that GATA-1 present in the cell might assure FcεRI expression even in BMMC overproducing PU.1. The mechanism allowing the copresence of both GATA-1 and PU.1 in mast cells is unclear at present. Considering that cooperative function between GATA-1 and PU.1 is involved in mast cell-specific gene regulation (7, 37, 39), we speculate that this synergistic effect may be essential for mast cell development. In any case, further detailed analysis will be required to clarify these points.

Overproduction of PU.1 induced apparent expression of CD11b and F4/80, and suppressed c-kit expression in PMC. However, the PU.1 overproduction did not induce expression of CD11c and MHC class II in PMC, which was in contrast to the case of BMMC overproducing PU.1. These results indicated that PMC possessed lower capacity to express monocyte-specific gene than that of BMMC. We assume that this discrepancy may reflect the difference in the expression profile of other transcription factors between these cells. A transcription factor, C/EBPα, might be one of the candidates, because C/EBPα is reported to inhibit development of myeloid progenitor cells to DC, which is induced by PU.1, and direct them to macrophage and granulocyte lineages (11).

Overexpression of PU.1 in BMMC induced hyperproduction of IL-6 in response to stimulation signal through TLR2 and 4. It is suggested that PU.1 is involved in the transcriptional regulation of TLR2 and 4 (28, 29). However, expression level of TLR2 and 4 in BMMC was not affected by overproduction of PU.1 (Fig. 3). In addition, production of IL-6 in response to Ag/IgE stimulation was reduced (Fig. 4A). These results suggest that PU.1 regulates IL-6 expression in a complex manner. Considering that PU.1 is activated by stimulation with LPS (32), PU.1 might function in the downstream process in TLR signaling.

PU.1 autoregulates its own promoter (31). Therefore, the stimulation signal to activate PU.1 protein would further accelerate up-regulation of PU.1. Therefore, we hypothesize that mast cells and/or its progenitors might be converted to monocyte-like cells in vivo under a certain condition when either activation or overproduction of PU.1 is induced. We have shown that LPS and PMA stimulation induced PU.1 production in mast cells, which suggests that mast cells might be potential sources for macrophages and/or DC under certain conditions in vivo. This is the first observation in mast cells showing up-regulation of PU.1 by stimulation of LPS and PMA. However, the production of PU.1 induced by LPS or PMA stimulation was transient and markedly lower in level than that of PU.1 produced by retrovirus system. We assume that this is the reason for failure of the stimulations in converting mast cells to...
monocytes. To prove this possibility, further analysis will be required to optimize conditions of PU.1 induction in mast cells, which are suitable for obtaining monocyte-specific gene expression.

Recently, the presence in vivo of FcεRI-positive monocytes, DC, and LC, all of which possess the Ag presentation ability, was shown (40, 41). Considering that the marked up-regulation of FcεRI expression is observed specifically on LC and DC in lesional skin of atopic dermatitis (40, 41), we assume that above-mentioned signal to activate PU.1 might be a possible cause of the appearance of FcεRI-positive monocytes, which are developed from mast cells or its progenitors in vivo. Therefore, characterization of the mechanisms for the regulation of FcεRI expression on monocyte might give important information for prevention of atopic dermatitis.

GM-CSF (16) and M-CSF (42) induce development of hematopoietic progenitor cells toward DC and macrophage. In the process of monocyte development, the signal through these factors is transduced to Fes and subsequently to PU.1 (43) to induce the expression of the receptors for GM-CSF (44) and M-CSF (45). Actually, retrovirus-mediated expression of PU.1 in macrophage prepared from GM-CSF-null mice rescued the defect in differentiation (46). Thus, it is likely that PU.1-mediated gene expression is a key event in monocyte development. However, the mechanism for both activation of PU.1 and up-regulation of PU.1 expression is mostly unknown. Therefore, further detailed analyses on upstream and downstream signaling of PU.1 are required for revealing the mechanism for monocyte lineage development.

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