70185, Sweden.
Email: josefin.sundh@oru.se

ORCID

Josefin Sundh https://orcid.org/0000-0003-1926-8464

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CD203c distinguishes the erythroid and mast cell-basophil differentiation trajectories among human FcεRI$^+$ bone marrow progenitors

To the Editor,

IgE molecules that bind their specific antigen crosslink FcεRI receptors present on mast cells and basophils. Downstream signaling results in cell activation and subsequent release of diverse compounds that exhibit potential to trigger allergic symptoms. Although mature FcεRI$^+$ cells have been extensively studied, less is known about the FcεRI$^+$ progenitors and their differentiation capacity.[1] Here, we analyzed the FcεRI$^+$ progenitor population from human bone marrow with multicolor flow cytometry and fate assays. The results revealed distinct subpopulations of FcεRI$^+$ progenitors, all showing capacity to form mast cells and basophil-like cells but not granulocytes or monocytes. The CD203c$^-$ subsets displayed erythroid potential, whereas the CD203c$^+$ subset did not, altogether providing early evidence for a common mast cell-basophil-erythroid differentiation trajectory in human, distinct from the granulocyte-monocyte axis.

The CD34$^+$ CD117$^+$ FcεRI$^+$ phenotype identifies the human mast cell progenitor population in blood.[2] Other characteristics include expression of the IL-3 receptor and the absence of CD45RA, positioning the cells among common myeloid progenitors (CMPs) when analyzing the progenitors with flow cytometry.[2,3] In contrast to blood, we recently demonstrated that CMPs$^{FcεRI+}$ in bone marrow do not exclusively form CD117^{hi} mast cells.[4] This observation warranted further characterization of the bone marrow CMPs$^{FcεRI+}$ phenotype and cell-forming potential.

Morphologic assessment following cell sorting and May-Grünwald Giemsa stain revealed that the CMP$^{FcεRI+}$ population was heterogenous (Figure 1A,C; see Methods S1 for materials and methods). Some cells exhibited a blast-like phenotype with little cytoplasm, whereas other displayed numerous metachromatic granules (Figure 1C). The cell heterogeneity prompted us to design a multicolor flow cytometry panel that further characterizes the progenitors. Analyzing the CD203c and integrin β7 expression patterns revealed subpopulations of CMP$^{FcεRI+}$ cells (Figure S1). Three CMP$^{FcεRI+}$ subpopulations—CD203c$^+$, integrin β7$^+$ CD203c$^-$, and integrin β7$^-$ CD203c$^-$ cells—exhibited distinct protein expression profiles and were studied further (Figure 1B,D). These three populations, along with CMPs$^{FcεRI-}$ and granulocyte/monocyte progenitors (GMPs), were sorted and cultured to investigate their cell-forming potential (Figure 2A). The five bone marrow progenitor populations were first cultured with IL-3 and IL-6. These conditions support mast cell progenitors from peripheral blood to form CD117^{hi} FcεRI$^+$ mast cells.[3] We analyzed the cultured bone marrow cells with a flow cytometry panel that distinguished three subsets of FcεRI$^+$ cells separated based on the CD117 expression, CD235a$^+$ erythroid cells, and

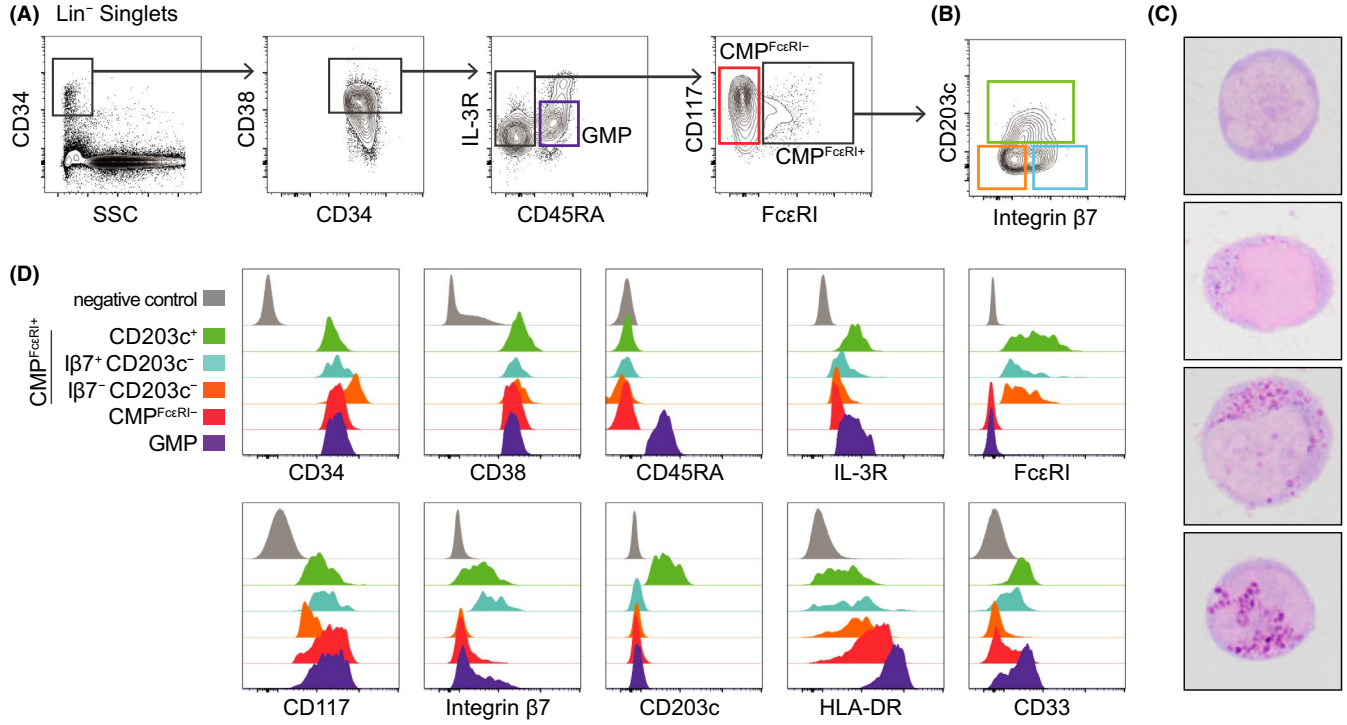

FIGURE 1 Bone marrow CMPs$^{FcεRI+}$ comprises three distinct progenitor populations. A, Flow cytometry analysis of human bone marrow cells. B, CD203c and integrin β7 distinguishes three distinct CMP$^{FcεRI+}$ subpopulations. C, May-Grünwald Giemsa staining of CMPs$^{FcεRI+}$. D, Surface expression analysis of CD203c$^+$ (green), Iβ7$^+$ CD203c$^-$ (blue), and Iβ7$^-$ CD203c$^-$ (orange) CMP$^{FcεRI+}$ compared with CMPs$^{FcεRI-}$ (red), and GMPs (purple). Negative controls (gray) represent internal control populations from the sample that are negative for the marker of interest. One representative bone marrow sample is shown

CD14$^+$ or CD15$^+$ granulocyte-monocyte output (Figure 2B visualizes the gating strategy). The CMP$^{FcεRI+}$ largely maintained their FcεRI$^+$ phenotype during culture (Figure 2C). None of the three CMP$^{FcεRI+}$ subpopulations produced pure populations of CD117^{hi} mast cells or CD117$^-$ basophil-like cells, but instead constituted a mix of cells with variable CD117 expression (Figure 2F-H).

The three CMP$^{FcεRI+}$ populations did not display granulocyte-monocyte potential (Figure 2D). By contrast, culture of CMPs$^{FcεRI-}$ and GMPs resulted in substantial granulocyte-monocyte output. The CMP$^{FcεRI-}$ population included progenitors with potential to upregulate FcεRI expression, suggesting that this population contains precursors of CMPs$^{FcεRI+}$. GMPs did not produce FcεRI$^+$ cells, indicating that this population lacks mast cell and basophil-forming capacity (Figure 2C). Bühring et al previously reported that the CD34$^+$ CD203c$^+$ progenitors exhibit mast cell-forming and high basophil-forming potential, agreeing with our results that the CD203c$^+$ subset of the CMPs$^{FcεRI+}$ form these cell types.[5] However, the CD34$^+$ CD203c$^+$ progenitors cultured in the study by Bühring et al exhibit residual granulocyte-monocyte-forming potential and were proposed to contain multipotent progenitors.[5] We sorted and cultured CD203c$^+$ cells from the CMP$^{FcεRI+}$ fraction, constituting cells that likely are more differentiated than CD34$^+$ CD203c$^+$ progenitors in general, which could explain the observation that CD203c$^+$ CMP$^{FcεRI+}$ cells lack granulocyte-monocyte potential.

No or few erythroid cells developed from any of the starting populations in the IL-3 and IL-6 conditions (Figure 2E), which is in agreement with lack of sufficient stimulus for erythroid development. Through a combined single-cell RNA sequencing and cell culture-based approach, Tusi et al[6] recently identified progenitors with combined erythroid and mast cell-basophil output in mouse bone marrow. Hence, we investigate whether the CMP$^{FcεRI+}$ populations exhibited erythroid potential. We cultured the CMPs$^{FcεRI+}$ with the myeloerythroid-promoting cytokines IL-3, IL-6, SCF, EPO, and GM-CSF. Cell culture assays in these conditions revealed that all CMP$^{FcεRI+}$ subpopulations still maintained FcεRI$^+$ cells (Figure 2B-C). Notably, we observed clear erythroid output when culturing the two CD203c$^-$ subpopulations (Figure 2E). A population of FcεRI$^-$ CD117^{hi} cells was also present in cultures of the

FIGURE 2 Cell fate assays reveal the cell-forming potential of the CMP$^{FcεRI+}$ populations. A, Schematic diagram indicating the cell fate assay methodology. B, Gating strategy of the cultured cells. Cells cultured for 5-6 d with the myeloerythroid-promoting cytokines IL-3, IL-6, SCF, EPO, and GM-CSF are shown. Percentages of (C) FcεRI$^+$, (D) CD14$^+$ or CD15$^+$, and (E) CD235a$^+$ (Glycophorin A$^+$) cells after 5-6 d of culture. Percentages of (F) CD117^{hi} FcεRI$^+$ mast cells, (G) CD117$^+$ FcεRI$^+$ precursors, and (H) CD117$^-$ FcεRI$^+$ basophils after 5-6 d of culture. Panels C-H display cells cultured with IL-3 and IL-6 (left) or the myeloerythroid-promoting cytokines IL-3, IL-6, SCF, EPO, GM-CSF (right). The cell fate assay was performed from 3-4 donors per population and condition as indicated

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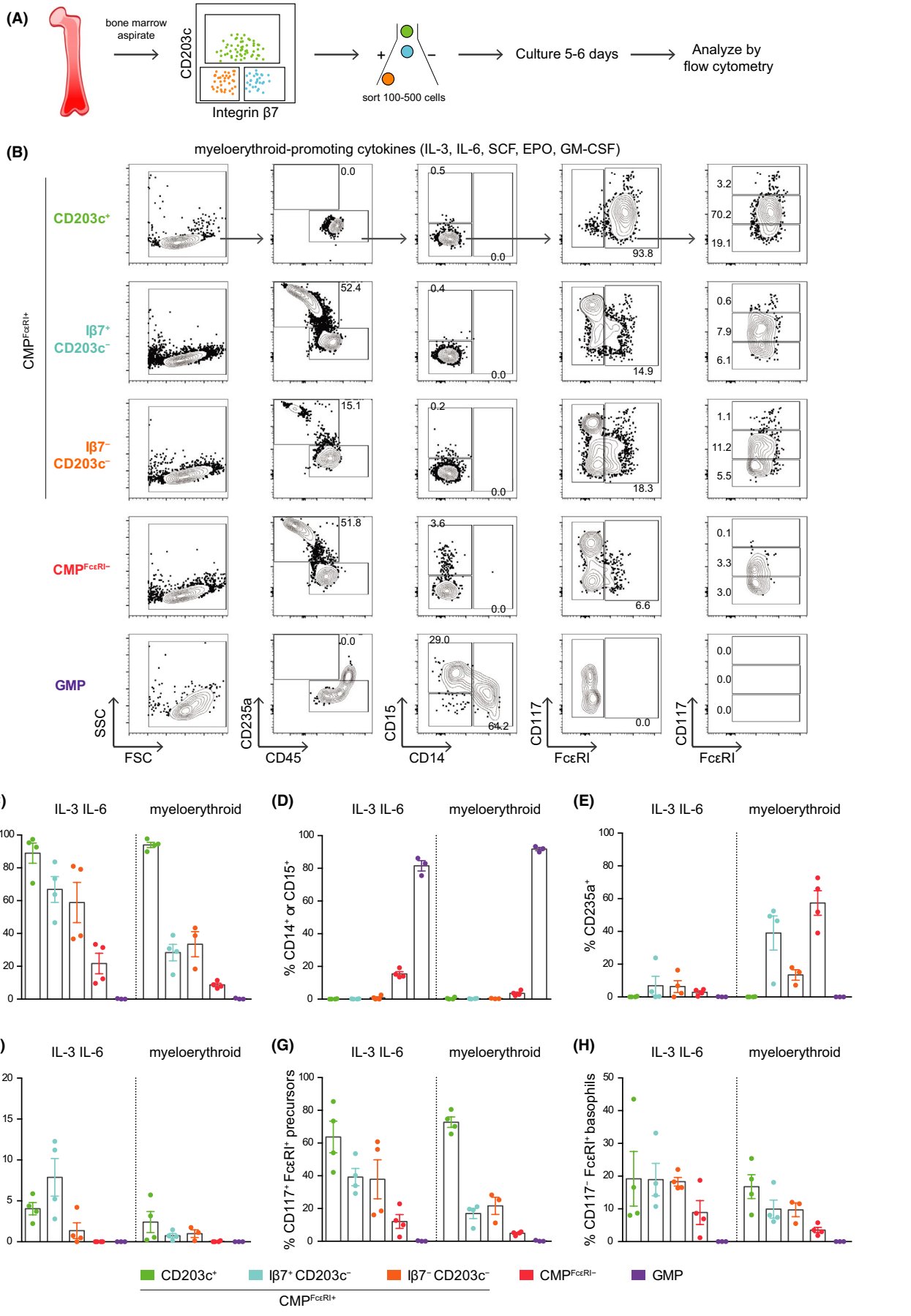

CD203c$^-$ subpopulations, cells with dim CD45 expression that likely constituted erythroid precursors (Figure S2A-B).

We cannot exclude that unipotent erythroid progenitors contaminated the CMP$^{FcεRI+}$ sort gate. However, performing single-cell culture experiments to resolve this question is complicated by the poor proliferation capacity of human mast cell progenitors,[2] making it difficult to identify and characterize mixed colonies. Though, it is worth to point out that erythrocytes were not present in cultures derived from the CD203c$^+$ subpopulation across cell fate assays of 4 donors, suggesting that CD203c upregulation is associated with loss of erythroid potential.

Progressive loss of proliferation capacity with mast cell differentiation may explain the relatively low frequency of mast cells derived from CD203c$^+$ compared with integrin β7$^+$ CD203c$^-$ CMP$^{FcεRI+}$ cells in IL-3 and IL-6 conditions.

Resolving the differentiation trajectories from hematopoietic stem cells to FcεRI$^+$ mast cells and basophils could significantly improve our understanding of, for example, IgE-mediated allergic diseases as well as the mast cell-driven disease systemic mastocytosis. The frequency of putative bone marrow mast cell progenitors was recently demonstrated to be elevated in systemic mastocytosis patients compared with healthy subjects.[7] Thus, the establishment of flow cytometry gating strategies for the identification of progenitors with basophil and mast cell-forming capacity can help us to understand why mature cells accumulate in disease. Taken together, the results presented here provide early evidence that the mast cell and basophil differentiation trajectories are closely linked to the erythropoiesis in human. Further studies on the topic are warranted, and fate assays of individual cells coupled with single-cell transcriptomics represent a promising way forward.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

Jennine Grootens[1]
Johanna S. Ungerstedt[2,3]
Chenyan Wu[1]
Kerstin Hamberg Levedahl[4]
Gunnar Nilsson[1,5]
Joakim S. Dahlin[1]

[1]Department of Medicine Solna, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden
[2]Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden
[3]Hematology Center, Karolinska University Hospital, Stockholm, Sweden
[4]Division of Hematology, Uppsala University Hospital, Uppsala, Sweden
[5]Department of Medical Sciences, Uppsala University, Uppsala, Sweden

Correspondence
Joakim Dahlin and Gunnar Nilsson, NKS BioClinicum, J7:30, Visionsgatan 4, Solna 171 64, Sweden.
Emails: joakim.dahlin@ki.se; gunnar.p.nilsson@ki.se

ORCID

Jennine Grootens https://orcid.org/0000-0003-4469-8589
Gunnar Nilsson https://orcid.org/0000-0001-6795-5512
Joakim S. Dahlin https://orcid.org/0000-0003-3007-9875

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.