Demonstration of human mast cell progenitors in the bone marrow

To the editor

Mast cells are tissue-resident cells widely recognized for their role in asthma and allergy. In inflammatory diseases, mast cells accumulate and become activated in particular sites of affected tissues such as, for example in the bronchial smooth muscles of allergic asthmatics.1 Using in vivo models, we and others have shown that these inflammation-induced mast cells originate from mast cell progenitors (MCp).2 For decades, human mast cells have been differentiated in vitro from uncommitted progenitor cells isolated from, for example peripheral blood and bone marrow (BM). In the current study, a population of MCp in the BM of healthy adults was identified, which was more frequent, and had lower surface expression of integrin β7 than the recently described MCp population in the blood.3 Integrin β7 is required for transmigration of mouse MCp into the lung.4 Thus, we speculate that integrin β7 is upregulated on the human MCp upon release from the BM in preparation for their transmigration from the blood to the peripheral tissues.

Using flow cytometry, BM samples from healthy donors were investigated for the possible presence of a MCp population. In comparison with peripheral blood samples from the same individuals (n = 11), the BM was enriched in lineage (Lin−) CD34hi CD117+ FcεRI+ cells and Lin− CD34hi cells and Lin− CD34hi CD117+ cells (Figure 1A). A population of Lin− CD34hi CD117+ FcεRI+ cells was found in healthy BM (Figure 1D), which was similar but 14-fold more frequent than the known MCp population in peripheral blood (Figure 1B and I). In one set of experiments, Lin− CD34hi CD117+ FcεRI+ cells from the BM and blood from seven donors were isolated by fluorescence-activated cell sorting (FACS) and either cultured in a myeloid-erythroid cytokine cocktail for 7 days or analysed directly. The primary BM Lin− CD34hi CD117+ FcεRI+ cells demonstrated immunofluorescence for mast cell tryptase (Figure 1E). Re-analysis of the respective progeny cells by flow cytometry revealed that all cells had an intermediate CD117 expression after culture and that a proportion of them had lost FcεRI surface expression (Figure 1F and J). The loss of FcεRI expression upon in vitro culture of the human peripheral blood MCp during the same culture conditions was reported previously,3 and FcεRI+ mast cells have been demonstrated in vitro and shown residing in the alveolar parenchyma in vivo.5 Regardless, there was no difference in the expression of FcεRI between the BM and blood progenies (39.8% ± 10.2% and 52.7% ± 12.0%, respectively). The BM progeny cells had a similar mast cell-like morphology, determined by May-Grünwald-Giemsa staining (Figure 1G), as the blood progeny cells (Figure 1K). Further, the progeny cells from both sources expressed various levels of tryptase (Figure 1H and L). Altogether,
FIGURE 1  MCp in the BM are similar but more frequent than their counterparts in the blood. A and B. The frequency of BM and blood populations. C. Analysis of Lin− CD34hi CD117+ FcεRI+ cells by qPCR using beta-actin (ACTB) as endogenous control. Each donor is labelled by a unique symbol. KIT, CPA3 and HPGDS were undetectable in one sample. D and I. Gating strategy for the Lin− CD14− CD34hi CD117+ BM (D) and blood cells (I). The primary cells (E) and progenies were visualized with an anti-tryptase mAb (H, L). The progenies were analysed by flow cytometry (F, J) or May-Grünwald-Giemsa (G, K). Means ± SD (**P < .001, ***P < .0001)
Figure 2  Blood MCp have higher surface expression of integrin β7 than those in the BM. A and B. The frequency of MCp correlates with the frequency of CD34+ cells in the BM and blood. C. The number of MCp progenies from five blood and BM donors after 7 d in culture was quantified by flow cytometry. Each donor is depicted with a unique symbol. D-G. The expression of Ki-67 (D), ST2 (E), integrin β7 (Int β7) (F), and IL-3R (G) relative to the appropriate isotype control. The histograms are representative of three-four experiments. Means ± SD (*P < .05)
the progenies of the Lin⁻ CD34⁺CD117⁺ FcεRI⁺ cells from the BM and blood were similar in terms of morphology and surface expression of CD117 and FcεRI, indicating that both populations consisted of MCp. To verify this, mRNA expression of mast cell-related genes was assessed in MCp from three donors. Both MCp populations expressed similar levels of mRNA of CD117 (KIT), the FcεRI alpha chain (FCER1A), carboxypeptidase A3 (CPA3), hematopoietic prostaglandin D synthase (HPGDS), serglycin (SRGN) and tryptase alpha/beta I/II (TPSAB1/TPSB2) (Figure 1C).

As both the CD34⁺ cells and the MCp were more frequent in the BM than in the blood, we investigated whether the MCp frequency was related to the frequency of CD34⁺ cells. Indeed, the levels of MCp correlated with the frequency of CD34⁺ cells in BM and blood from 11 donors (Figure 2A and B). To assess the proliferation capacity, 50 BM and blood MCp from 7 donors were cultured in a myeloid-erythroid cytokine cocktail for 7 days, before quantification by flow cytometry. The MCp proliferation capacity was variable between donors and the number of progenies after culture did not differ between the MCp from blood (866 ± 375) and BM (1028 ± 433) (Figure 2C). On average, the MCp divided approximately four times. The proliferation capacity of the MCp populations in BM (n = 4) and blood (n = 3) was also compared by determining the protein expression of the intracellular proliferation marker Ki-67. Most MCp from both sources were positive for Ki-67 (Figure 2D). As the MCp were in a highly proliferative state but divided poorly in vitro, our culture system was insufficient in supporting mast cell proliferation. Next, the surface expression of the IL-33 binding receptor ST2, integrin β7 and the IL-3 receptor (IL-3R) on primary blood and BM MCp from the same three donors was determined by flow cytometry. The membrane-bound ST2 receptor was expressed on mouse MCpβ and human in vitro-derived mast cells. However, the human MCp from BM and blood lacked or had minimal ST2 surface expression (Figure 2E). Mouse MCp express integrin β7 and the α4β7 integrin is functionally important for the transmigration of MCp into the lung in a mouse model of allergic airway inflammation. In humans, α4β7 is required for transmigration of lymphocytes into the gut and a therapeutic target in inflammatory bowel diseases. Interestingly, the surface expression of integrin β7 was three times higher in the blood MCp than in the BM MCp (Figure 2F). We speculate that the MCp upregulates integrin β7 expression as they leave the BM and that integrin α4β7 is involved in the transmigration of human circulating MCp into tissues. As surface expression of the IL-3R was reported on SSC⁺ CD14⁺ CD34⁺ CD117⁺hi/hi FcεRI⁺ blood MCp, the surface expression of IL-3R was compared between BM and blood MCp. In all donors, the relative IL-3R expression was higher in the BM MCp compared to the blood counterparts (Figure 2G).

In conclusion, we establish the presence of a MCp population in the BM of healthy subjects, which is more frequent in numbers compared to peripheral blood MCp from the same donors. Blood and BM MCp have a similar expression of mast cell-related genes and demonstrate a mast cell-like phenotype after in vitro culture. However, the blood MCp have three times higher surface expression of integrin β7 than the BM MCp, indicating that the circulating MCp are preparing to transmigrate into tissues.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

MS and JH conceived the idea and designed the study; JU recruited the healthy donors and arranged the sampling; MS performed the experiments; PAA-V. performed one set of experiments; MS, PAA-V., and JH analysed the data; MS and J.H wrote the manuscript; JU and PAA-V critically reviewed the manuscript and approved of submission.

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To the Editor,

Exercise-induced bronchoconstriction (EIB) refers to acute airway narrowing that occurs in association with physical activity and is prevalent in both elite and recreational athletes.\(^1,2\) It is important to accurately detect EIB to reduce its potential impact on respiratory health and sporting performance.\(^3,4\) However, diagnosis is clinically challenging due to the limited value of self-reported respiratory symptoms\(^5\) and broad differential diagnosis associated with exertional breathing difficulty.\(^6\) It is, therefore, recommended that EIB should be objectively confirmed via indirect bronchoprovocation prior to initiating treatment.\(^7\) In this context, exercise challenge testing (EX) and eucapnic voluntary hyperpnoea (EVH) are the most commonly employed diagnostic tests, with a positive result most typically defined as a pre-post challenge reduction in lung function; ie ≥10% fall in FEV\(_1\).\(^7\) However, the most appropriate diagnostic threshold currently remains unclear on the basis that the ‘normative’ airway response to EX appears to be mild bronchodilation (primarily due to withdrawal of vagal cholinergic tone)—whereas the highly provocative stimulus of EVH typically elicits bronchoconstriction.\(^8\) Accordingly, to date, there remains a lack of consensus regarding the optimal or ‘gold-standard’ approach to assessment,\(^9\) which in turn, presents a potential for misdiagnosis; ie over- and under-detection.

The primary aim of this study was, therefore, to compare the airway response to EX (conducted in a controlled dry environment) against an EVH challenge. An evaluation of current\(^7\) and revised diagnostic thresholds\(^8\) was undertaken to determine the impact of any proposed modification to EIB screening outcome. We hypothesized that the achieved ventilation and severity of bronchoconstriction would be greater following EVH in comparison to EX.

The study was conducted as a multi-site randomised trial. Following approval from local research ethics committees, sixty-three recreationally active individuals (≥5 hours endurance training per week) (male: n = 47) provided written informed consent. At the beginning of each visit, exertional respiratory symptoms and eosinophilic airway inflammation were assessed via interview and fractional exhaled nitric oxide (FeNO), respectively, followed by either an EX or EVH challenge. Spirometry was performed in triplicate at baseline and in duplicate at 3, 5, 7, 10 and 15-minutes post challenge. A positive diagnosis for EIB was defined by ≥10% fall in FEV\(_1\), at two consecutive time points for both EX and EVH\(^7\) and ≥15% fall in FEV\(_1\) at one time point for EVH\(^7\) (for detailed overview of study methodology refer to online supplement).

Fourteen participants (22%) had a prior diagnosis of asthma ± EIB. Despite this, all participants had normal resting lung function with no evidence of airflow limitation (FEV\(_1\) predicted >80% and FEV\(_1\)/FVC > 70% predicted). Over half of the cohort (63%) reported exertional respiratory symptoms, and twenty-six (41%) had elevated FeNO (>25 ppb). Clinical characteristics and baseline lung function are presented in Table 1.

Fifty-eight (92%) and forty-four (70%) participants achieved a \(V_E \geq 60\%\) predicted MVV (ie the accepted minimal ventilatory load for a valid test) for EVH and EX, respectively. Although power output during EX (260 ± 57 W) was lower than the calculated target (323 ± 92 W; \(P < .01\)), all participants achieved a mean heart rate >80% predicted maximum (162 ± 11 beats min\(^{-1}\)). Despite this, \(V_E\) for EX (93 ± 19 L min\(^{-1}\)) was lower than EVH 106 ± 22 L min\(^{-1}\) (\(P < .01\); Table 1).

The mean fall in FEV\(_1\) was greater following EVH (−7.9% ± 6.9%) in comparison to EX (−1.9 ± 7.1; \(P < .01\)), with a reduction in FEV\(_1\) observed following EVH in almost all participants (94%). In contrast, EX elicited bronchodilation in over half of the cohort (53%).

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**SUPPORTING INFORMATION**

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

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