Altered B-cell, plasma cell, and antibody immune profiles in blood of patients with systemic mastocytosis

Alba Pérez-Pons, MSc,[a,b,c,d] Ana Henriques, PhD,[d,e] Teresa Contreras Sanfeliciano, MD,[f] María Jara-Acevedo, PhD,[a,b,c,d,g] Paula Navarro-Navarro, MSc,[a,b,c,d,g] Andrés C. García-Montero, PhD,[a,b,c,d] Iván Álvarez-Twose, MD, PhD,[c,d,e] Quentin Lecrevisse, Eng,[a,b,c] Rafael Fluxa, Eng,[h] Laura Sánchez-Muñoz, MD, PhD,[d,e] Carolina Caldas, MSc,[a,b,d] Julio Pozo, MSc,[a,b] Óscar González-López, MSc,[a,b,d] Martín Pérez-Andrés, PhD,[a,b,c] Andrea Mayado, PhD,[a,b,c,d]* and Alberto Orfao, MD, PhD[a,b,c,d]*

Salamanca, Madrid, and Toledo, Spain

Background: Systemic mastocytosis (SM) is a heterogeneous disease characterized by an expansion of *KIT*-mutated constitutively activated mast cells (MCs) that release MC mediators, which might act on the tumor microenvironment including other immune cells.

Objective: To investigate the blood distribution of B-cell, plasma cell (PC), and antibody isotype compartments in patients with SM.

Methods: We used spectral flow cytometry and the EuroFlow Immunomonitoring panel and Lymphocyte Screening Tube to quantify B cells, PCs, and their subsets in blood of 108 patients with SM (35 bone marrow mastocytosis [BMM] cases, 64 indolent SM [ISM] cases, 9 aggressive SM [ASM] cases) versus 117 age-matched healthy donors and paired bone marrow samples of 31 patients with SM versus 17 controls, respectively. In parallel, IgM, IgD, IgG, IgA, and IgE plasma levels were measured.

Results: Compared with healthy donors, patients with SM showed increased immature B-cell production in bone marrow ($P = .003$) associated with greater release of pre–germinal center immature ($P < .001$) and naive $CD5^+$ B lymphocytes ($P < .001$) to blood, but a pronounced decrease in PC counts of all different IgH isotypes and subclasses ($P \leq .001$) together with overall increased IgM ($P = .001$) and IgD ($P < .001$) plasma levels. Different immune profiles were found per diagnostic subtype of disease with progressively greater counts in blood of immature B lymphocytes together with decreased $IgMD^+$, $IgG2^+$, $IgA1^+$, and $IgA2^+$ memory B cells ($P \leq .032$) and elevated IgM ($P = .017$) plasma levels in cases of ASM, increased IgM ($P = .001$) and IgD ($P = .001$) plasma levels in ISM cases, and exacerbated IgE ($P < .001$) with decreased IgG ($P = .008$) plasma levels in BMM cases.

Conclusions: Our results reveal a significant dysregulation of the B-cell and PC compartments in blood of patients with SM, consistent with distinctly altered antibody isotype profiles in plasma of patients with BMM versus ISM versus ASM. (J Allergy Clin Immunol 2025;155:628-39.)

Key words: *B lymphocyte, immunoglobulin, mast cell, plasma cell, systemic mastocytosis*

Systemic mastocytosis (SM) comprises a heterogeneous group of disorders that result from the clonal expansion of morphologically and immunophenotypically aberrant mast cells (MCs) that accumulate in one or more organ systems, such as the skin, bone marrow (BM), and gastrointestinal (GI) tract.[1-3] Based on the World Health Organization 2022 classification, SM is subdivided into bone marrow mastocytosis (BMM); indolent SM (ISM); smoldering SM; and 3 subtypes of advanced SM comprising aggressive SM (ASM), SM with an associated hematologic neoplasm, and mast cell leukemia (MCL).[4-6] Despite their great clinicobiologic heterogeneity, the majority (>90%) of patients with SM display the gain-of-function KIT^{D816V} mutation, in addition to other *KIT* mutations identified in approximately 1% to 4% of patients.[7] Those *KIT* mutations lead to constitutive activation of the *KIT* receptor and, ultimately, of the mutated MCs, with an increased release of MC mediators and inflammatory cytokines to the extracellular MC microenvironment, with pleiotropic effects on, for example, epithelial cells and other innate and adaptative immune cells.[8-14]

Because blood circulating MCs go undetected in physiologic conditions[15] and due to their broad tissue distribution and strategic perivascular localization, MCs possess unique immune functions in almost all human organs and tissues.[16] These functions are exerted via both the release of MC mediators[17,18] and direct cell-cell interactions, particularly with epithelial and endothelial cells, smooth muscle cells, and surrounding immune cells.[19] Thus, activation of MCs induces vasodilation with increased vascular and mucosal permeability[20,21] and local recruitment and activation of inflammatory cells and lymphocytes,[22] associated with the release of a wide repertoire of cytokines, chemokines, and growth factors such as IL-1, IL-3, IL-4, IL-5, IL-8, IL-10, GM-CSF, TGF-β, TNF-α, CCL2, and CCL5.[23] Of note, MCs might express a broad range of surface and cytoplasmic receptors (eg, CD43, CD80, CD86, and

From [a]Cancer Research Center, Department of Medicine and Cytometry Service, University of Salamanca, Salamanca; [b]Biomedical Research Institute of Salamanca, Salamanca; [c]Biomedical Research Networking Center Consortium, Madrid; [d]Spanish Network on Mastocytosis, Toledo and Salamanca; [e]Instituto de Estudios de Mastocitosis de Castilla La Mancha, Virgen del Valle Hospital, Toledo and Madrid; [f]the Department of Biochemistry, University Hospital of Salamanca, Salamanca; [g]Sequencing Service, University of Salamanca, Salamanca; and [h]Cytognos SL, Salamanca.

*These authors contributed equally to this work and are joint senior authors.

Received for publication April 30, 2024; revised September 18, 2024; accepted for publication October 7, 2024.

Available online October 16, 2024.

Corresponding author: Alberto Orfao, MD, PhD, Centro de Investigación del Cáncer, Campus Miguel de Unamuno, Paseo de la Universidad de Coimbra, 37007 Salamanca, Spain. E-mail: orfao@usal.es.

0091-6749

https://doi.org/10.1016/j.jaci.2024.10.005

Abbreviations used
ASM: Aggressive systemic mastocytosis
BM: Bone marrow
BMM: Bone marrow mastocytosis
GC: Germinal center
GI: Gastrointestinal
HD: Healthy donor
HVA: Hymenoptera venom allergy
$IgMD^+$: B cells expressing IgM and IgD immunoglobulin
ISM: Indolent systemic mastocytosis
MALT: Mucosa-associated lymphoid tissue
MBC: Memory B cell
MC: Mast cell
MCL: Mast cell leukemia
NK: Natural killer
PC: Plasma cell
SM: Systemic mastocytosis
smIg: Surface membrane Ig
WDSM: Well-differentiated systemic mastocytosis

CD40L) and cell-to-cell adhesion molecules (eg, intercellular adhesion molecule 1 and intercellular adhesion molecule 3), which also enable them to directly interact with both T and B lymphocytes.[18,24] Thus, MCs can release IL-13 and IL-4 and induce B cells to undergo class switching toward IgE-producing memory B cells (MBCs) and plasma cells (PCs)[16,18,22]; at the same time MCs can promote B-cell proliferation and differentiation to IgA-secreting PCs through direct CD40L-CD40 binding and the release of IL-5, IL-6, and TGF-β1.[16] Altogether, due to these interactions, MCs have an important role in IgE-mediated allergic responses, through IgE binding to the high-affinity IgE receptor (FCεRI) and subsequent triggering of MC degranulation, as well as in the homeostasis of commensal bacteria in, for example, the GI tract, where MC mediators also induce an increased permeability.[21,25]

In this regard, previous studies have reported altered innate immune responses in SM, associated with increased numbers of blood circulating activated, but functionally exhausted, monocytes and dendritic cells[26] and type 2 innate lymphoid cells,[27] together with decreased natural killer (NK) cell and CD8 cytotoxic T-lymphocyte counts.[28,29] More recently, uniquely altered $CD4^+$ T-cell immune profiles have also been described in SM that consist of decreased numbers of T_H1 cells and specific subsets of T_H2, T_H22, and T_H1-like regulatory T cells together with abnormally high T follicular helper cell counts.[29] Interestingly, the altered CD4 T-cell immune profiles were closely associated with both the diagnostic subtype of SM and the specific clinical manifestations of the disease, ie, anaphylaxis, Hymenoptera venom allergy (HVA), bone disease, pruritus, flushing, and GI symptoms.[29] Despite these findings, few studies have been reported so far that focused on the analysis of the B-cell compartment in blood of patients with SM.[29] This might be due to the fact that both normal serum immunoglobulin/antibody levels and normal peripheral blood and BM B-cell counts have been previously found in most patients with SM.[28-30]

In the present study, we investigated in detail for the first time to our knowledge the distribution of different subsets of blood circulating B lymphocytes, PCs, and antibody isotype PCs of a large cohort of patients with distinct diagnostic subtypes of SM. Our aims were to identify uniquely altered B-cell, PC, and antibody isotype profiles associated with SM and gain further insight into the potential role of the humoral response in the pathophysiology and clinical behavior of SM.

METHODS

Patients, controls, and samples

A total of 109 consecutive adults with SM (64 women and 44 men; median age of 55 years; age range, 27-77 years) diagnosed and retrospectively classified according to the World Health Organization 2022 updated criteria[4-6,31] at the reference centers of the Spanish Network on Mastocytosis (Mast Cell Unit, Hospital Virgen del Valle, Toledo, and Cancer Research Center, Salamanca, Spain) were studied. Patients included 35 with BMM, 64 with ISM, and 9 with ASMs. A 13-year-old patient diagnosed with a well-differentiated SM (WDSM) variant of MCL was also studied. In parallel, 117 age-matched (median age 43 years; age range, 19-78 years) healthy donors (HDs) with no past or present history of allergy or other immune disorders were studied as controls. Clinical and laboratory data (see Table E1 in this article's Online Repository at www.jacionline.org) of individual patients were that retrospectively collected from the patients' medical records included the presence versus absence of disease features such as anaphylaxis, HVA, bone disease (ie, osteopenia, osteoporosis, or other bone lesions such as osteolytic lesions and patchy or diffuse bone sclerosis), pruritus, flushing, GI symptoms, and history of *Helicobacter pylori* infection and other bacterial/viral infections (ie, recurrent urinary tract infections, recurrent respiratory infections, bilateral pneumonia, and recurrent COVID-19 infection). Before entering the study, each patient and HD gave their written informed consent to participate according to the Declaration of Helsinki. The study was approved by the local ethics committees.

Flow cytometry identification and quantification of B cells, PCs, and PC subsets in blood and BM

Blood and BM samples were separately collected in tubes containing K3 EDTA as anticoagulant. Blood samples were stained fresh (<24 hours after collection) with the 14-color Euroflow immune monitoring IgH isotype B-cell tube, and fresh BM samples were stained with the EuroFlow Lymphocyte Screening Tube (Table E2 in the Online Repository at www.jacionline.org), following the EuroFlow bulk lysis standard operating procedure (www.euroflow.org). In every blood sample, the IgH isotypes and subclasses were assessed using a cell surface membrane plus cytoplasmic staining, as described elsewhere.[32] Stained blood samples were measured in a 3-laser Aurora flow cytometer (Cytek Biosciences, Fremont, Calif) equipped with SpectroFlo software (Cytek Biosciences). BM samples were measured in a FACSCanto II flow cytometer (BD Biosciences, San Jose, Calif) equipped with FACSDiva software (BD Biosciences). For flow cytometry data analysis of both types of data files, Infinicyt software (Cytognos SL, Salamanca, Spain) was employed; the gating strategies for the individual cell population are detailed in the Methods section and Figs E1 and E2 in the Online Repository at www.jacionline.org.

Quantification of immunoglobulin levels in plasma

Soluble IgA, IgG, and IgM were measured using conventional nephelometry (Dimension Vista; Siemens Healthineers, Erlanger, Germany). For IgD and for IgE, the IgD Optilite kit (The Binding Site Group Ltd, Birmingham, UK) and the ImmunoCAP Total IgE (Thermo Fisher Scientific, Waltham, Mass) assays were respectively employed.

Statistical methods

The statistical significance of differences observed among groups was assessed by the Mann-Whitney *U* test for continuous variables or χ^2 and Fisher exact tests for categorical variables. The threshold for statistical significance was set at $P < .05$, with a false discovery rate correction for multiple comparisons of <10%. For all statistical analyses, IBM SPSS v28.0.0.0 software (IBM Corp, Armonk, NY) and MIDAS v2.0.5.d software (Cytognos SL) were used; figures were plotted using MIDAS v2.0.5.d and GraphPad Prism v8.0.2 (GraphPad Software, Boston, Mass). Normalization of leukocyte values by age was performed with MIDAS software, as previously described.[33]

RESULTS

Distribution of B cells and their subsets in blood and BM of patients with SM

Overall, patients with SM showed normal total B-cell counts in blood (vs HDs) and BM (vs normal BM samples). Despite this, detailed dissection of the B-cell compartment in blood of patients with SM revealed significantly increased numbers (normalized per age) of pre–germinal center (GC) B lymphocytes ($P = .009$) at the expense of both immature B cells ($P < .001$) and naive $CD5^+$ B cells ($P < .001$), but not the more mature and more represented naive $CD5^-$ B lymphocytes (Fig 1, *A*). Of note, progressively greater immature B-lymphocyte counts (increased over normal values) were observed in blood of patients with ISM ($P < .001$ vs HDs; $P = .032$ vs BMM patients) and patients with ASM ($P < .001$ vs HDs; $P < .001$ vs BMM patients; $P = .004$ vs ISM patients) (Fig 2, *A*; Table E3 in the Online Repository at www.jacionline.org), whereas the increase of naive $CD5^+$ B-cell counts in blood over normal values was restricted to ISM ($P = .002$) (Fig 2, *A*). Among patients with ISM, the numbers of immature ($P = .03$) and naive $CD5^+$ ($P = .046$) B lymphocytes were particularly elevated among patients with serum baseline tryptase ≥20 ng/mL (vs HDs and BMM patients who presented with <20 ng/mL) and among patients with a KIT^{D816V} variant allele frequency >1% (vs HDs and BMM patients with <1% variant allele frequency) (Fig E4 in the Online Repository at www.jacionline.org). Of note, normal naive B-cell counts were detected in the only case of WDSM variant of MCL analyzed (Fig E5 in the Online Repository at www.jacionline.org).

In line with the increased blood counts of pre-GC B lymphocytes observed in SM, significantly higher percentages of both immature I ($P = .031$) and immature II ($P = .003$) B lymphocytes (vs normal BM samples) were found in BM of patients with SM (Fig 3, *A*). This increased percentage of immature B lymphocytes in BM translated into a significantly higher immature B lymphocyte/myeloid cell ratio in SM cases ($P = .017$) versus normal BM samples, particularly among ISM cases ($P = .017$) (Fig 3, *B*), in the absence of significant differences for the other B-cell precursor and mature B-lymphocyte and PC subsets (Fig 3, *A* and *B*).

In contrast to immature and $CD5^+$ naive B cells, a pronounced decrease in total PC counts was observed in blood of patients with SM versus HDs (Fig 1, *A*). This decrease comprised all different IgH isotype and subclass subsets of PCs (ie, IgM^+, IgD^+, $IgG1^+$, $IgG2^+$, $IgG3^+$, $IgG4^+$, $IgA1^+$, and $IgA2^+$ PCs) ($P < .001$) (Fig 1, *C*; Table E3) and their maturation-associated populations of $CD20^+$ $CD138^-$, $CD20^-$ $CD138^-$, and $CD20^-$ $CD138^+$ PCs ($P < .001$) (Fig 1, *D*). Of note, very low numbers of IgM^-, IgD^-, IgA^-, and IgG^- PCs were found in blood (0.15%), at similar levels in BMM (0.14%), ISM (0.15%), and ASM cases (0.14%). These PC-associated findings contrasted with MBCs and their subsets, which showed normal levels in SM except for decreased counts (vs HDs) of unswitched surface membrane (sm) $IgMD^+$ MBCs ($P = .037$) and increased $smIgD^+$-only MBC numbers in blood ($P \leq .001$) (Fig 1, *B*).

Likewise, all diagnostic subgroups of the SM patient cohort showed significantly decreased numbers in blood of most PC subsets (ie, IgM^+, $IgG1^+$, $IgG2^+$, $IgG4^+$, $IgA1^+$, and $IgA2^+$ PCs) ($P \leq .008$) in blood, with the decrease being significantly more pronounced among ASM cases (Fig 2, *A*; Table E3) except for $IgG3^+$ PCs and IgD^+-only PCs, which were specifically decreased (vs HDs) in BMM ($P = .001$) and ISM ($P = .005$) cases and in ISM ($P = .001$) cases ($P < .001$), respectively (Fig 2, *A*). In addition, significantly lower $IgG2^+$ PC counts were found in blood of ASM cases versus BMM ($P = .025$) and ISM ($P = .017$) cases, whereas the IgD^+-only PC numbers in blood were significantly lower in ISM cases versus BMM ($P = .049$) cases (Fig 2, *A*). In this regard, a similar pattern was observed for the patient with a WDSM variant of MCL we analyzed, who also presented with decreased counts of circulating blood PCs, at the expense of decreased IgM^+, $IgG1^+$, $IgG2^+$, $IgG4^+$, $IgA1^+$, and $IgA2^+$ PCs (Fig E5). Although the distribution of MBCs in blood was frequently within the normal range, $smIgMD^+$ and $smIgG_2^+$ MBCs shared a similarly altered pattern with significantly decreased blood counts in ASM cases compared with HDs ($P = .038$ and $P = .017$, respectively) and BMM cases ($P = .038$ and $P = .021$, respectively), as well as $smIgG2^+$ MBCs in ISM cases ($P = .021$) (Fig 2, *A*).

Likewise, although overall normal $smIgA1^+$ and $smIgA2^+$ MBC counts were observed in blood of SM cases, ASM cases displayed significantly lower numbers of $smIgA1^+$ and $smIgA2^+$ MBCs compared with HDs ($P = .032$ and $P = .015$, respectively) and BMM cases ($P = .032$ and $P = .015$, respectively), as well as $smIgA2^+$ MBCs in ISM cases ($P = .019$) (Fig 2, *A*). Conversely, $smIgD^+$-only MBCs showed increased counts in blood over normal values in HDs in BMM ($P = .002$) and ISM ($P = .006$) (but not ASM) cases (Fig 2, *A*; Table E3).

Soluble immunoglobulin levels in plasma of patients with SM

Overall, normal total immunoglobulin levels were found in plasma of patients with SM (Fig 4, *A*). However, more detailed analysis per immunoglobulin isotype revealed increased (vs HDs) IgM ($P = .001$) and IgD ($P < .001$) (but not IgE, IgA, or IgG) antibody levels in plasma of patients with SM (Fig 4, *A*). Of note, the higher IgM plasma levels observed in patients with SM were due to their increase in plasma (vs HDs) in ISM ($P = .001$) and ASM ($P = .017$) cases, but not BMM cases, whereas greater IgD serum levels (vs HDs) were restricted to BMM ($P = .027$) and ISM ($P = .001$) cases (Fig 4, *A*). In addition,

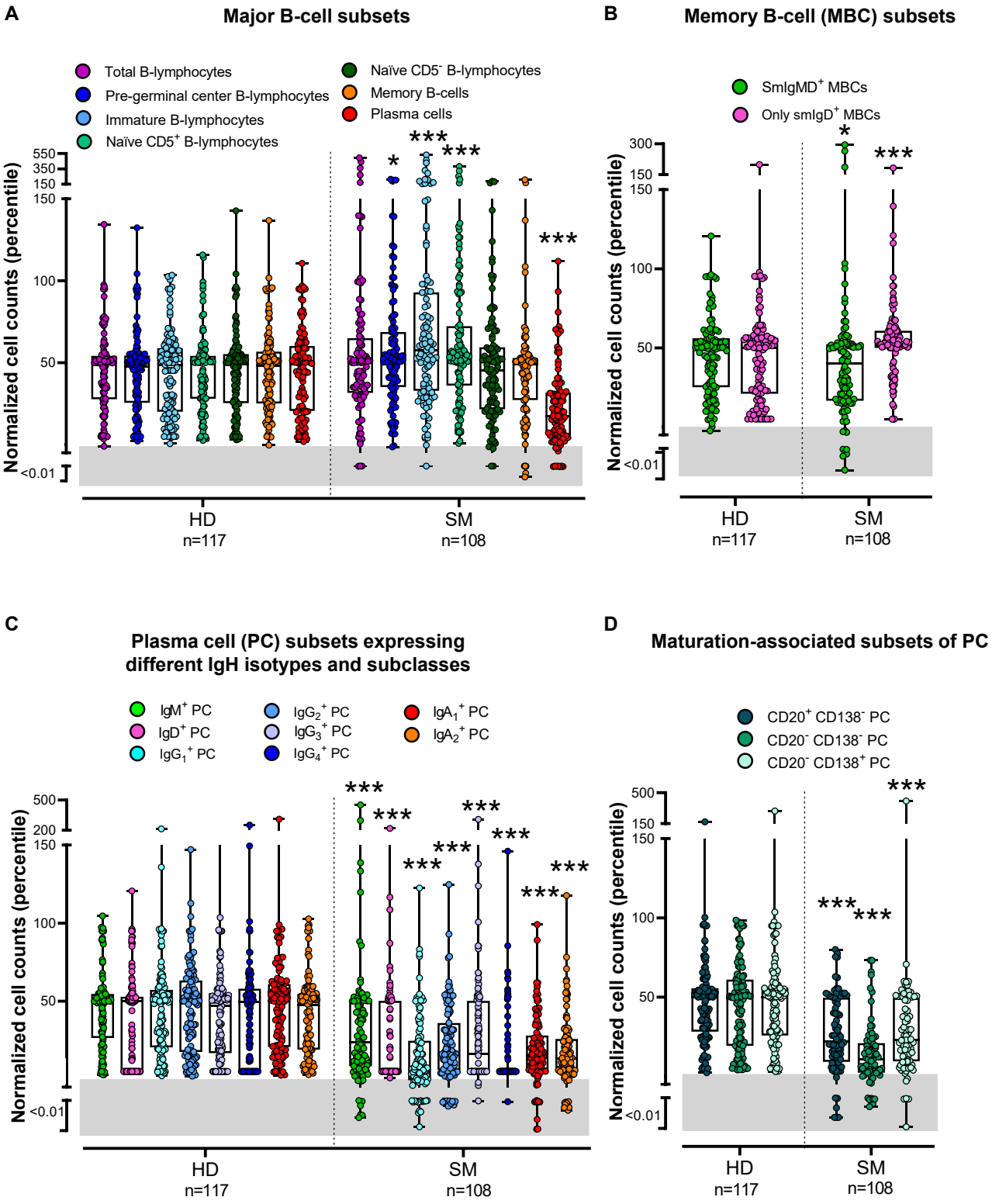

FIG 1. Distribution of different maturation-associated and IgH isotype and subclass-related subsets of B lymphocytes and PCs in blood of patients with (n=108) vs HDs (n=117). **(A)** Major subsets of B lymphocytes including total B lymphocytes, pre-GC B lymphocytes (ie, immature, naive $CD5^+$, and naive $CD5^-$ B lymphocytes), MBCs, and PCs. **(B)** $smIgMD^+$ and $smIgD^+$ MBC. **(C)** PC subsets expressing distinct IgH isotypes and subclasses: IgM^+-only, IgD^+-only, $IgG1^+$, $IgG2^+$, $IgG3^+$, $IgG4^+$, $IgA1^+$, and IgA_2^+. **(D)** Maturation-associated subsets of PCs defined based on their CD138 and CD20 expression profile. *Notched boxes* correspond to normalized box plots based on median (5th-95th) percentile values observed among age-matched HDs that extend from the 25th to 75th percentile values; the *lines in the middle* and *vertical lines* correspond to median values and the 10th and 90th percentiles, respectively, and *dots* represent each individual case analyzed. The *gray* area represents data below the HD 99.9th percentile values. $*P < .05$, $**P < .010$, $***P < .001$.

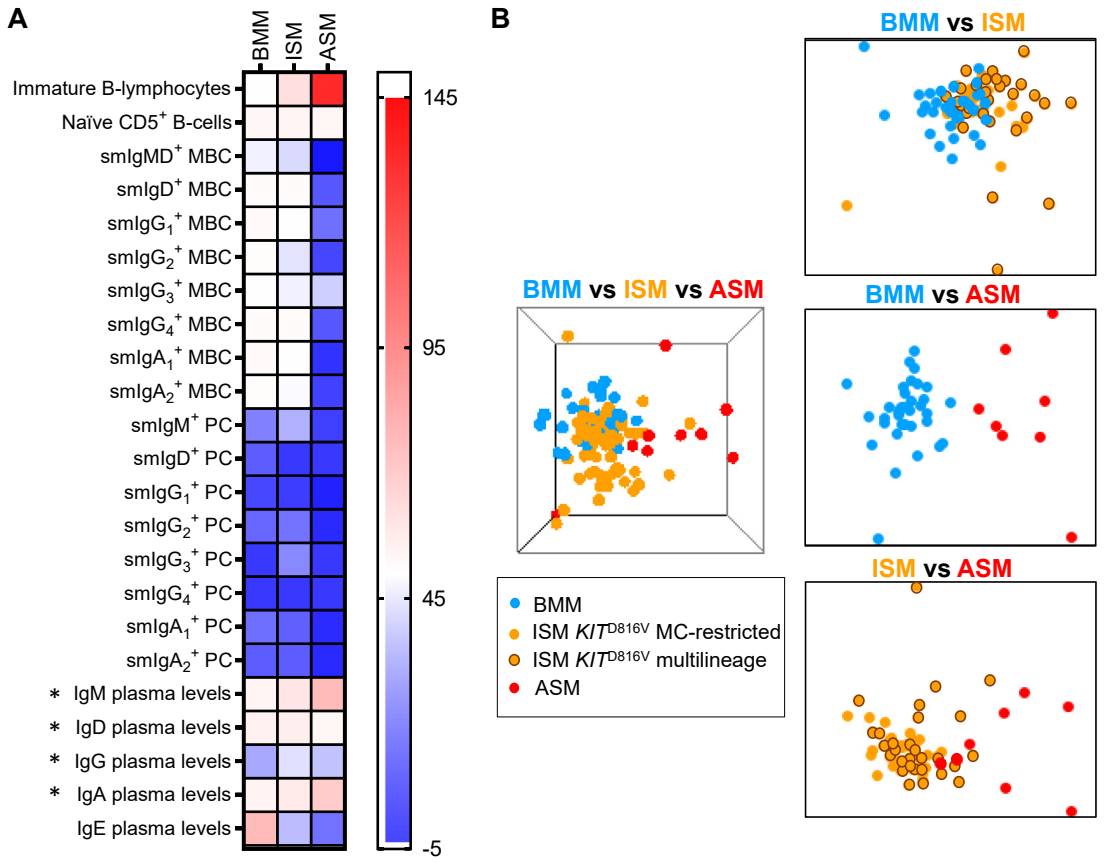

FIG 2. Overall plasma immunoglobulin and blood B-cell immune profile of patients with SM (n = 108) distributed according to the distinct diagnostic subtypes of the disease based on multivariate canonical analyses. **(A)** Heatmap showing color-coded squares based on percentile values normalized against age-matched HDs. **(B)** Three-dimensional and two-dimensional plot representations of multivariate canonical analyses of BMM vs ISM vs ASM cases; each individual *circle* represents a single case expressed as age-normalized percentile values found among healthy donors. Data on canonical analysis plots are based on multivariate canonical analyses of the 15 different cellular parameters that showed statistically significant differences between the groups in the univariate analysis plus antibody plasma levels; variables not included are indicated with an *asterisk*. ASM is depicted in *red*, BMM is depicted in *blue*, and ISM is depicted in *orange* with or without a *dark surrounding circle* in cases showing multilineage or MC-restricted KIT^{D816V}, respectively.

patients with BMM (vs HDs) displayed abnormally low IgG ($P = .008$) together with significantly increased IgE plasma levels compared with HDs ($P < .001$), ISM cases ($P < .001$), and ASM cases ($P < .001$) (Fig 4, *A*). To confirm whether the increased IgE levels were a unique feature of BMM or were due to the high prevalence of anaphylaxis in those patients, patients with BMM and ISM were further stratified according to the presence versus absence of anaphylaxis. Our results showed that patients with BMM who had anaphylaxis had significantly higher IgE plasma levels than patients with ISM ($P < .0001$) who also presented with anaphylaxis, supporting a close association between increased IgE serum levels and BMM (Fig E3). Of note, no significantly altered IgA antibody levels in plasma were found in patients with SM (Fig 4, *A*). Altogether, the above-mentioned alterations translated into significantly increased IgE/IgG and IgE/IgA ratios in plasma of BMM ($P = .001$ and $P = .012$, respectively), ISM ($P < .001$ for both ratios) and ASM ($P = .005$ and $P = .006$, respectively) cases versus HDs (Fig 4, *B*). Meanwhile, a decreased IgG^+ IgA/IgM ratio was found in patients with SM ($P = .001$), specifically among ISM cases ($P = .001$) (Fig 4, *B*).

Immune B-cell, PC, and soluble immunoglobulin profiles in blood of patients with SM

Multivariate canonical analysis confirmed the existence of unique humoral immune B-cell and PC profiles in blood of BMM versus ISM versus ASM cases (Fig 2, *B*). These included progressively increased BM production associated with a greater release of immature (pre-GC) B cells to blood, but a marked decrease in blood of both $smIgMD^+$ MBC counts and circulating PC numbers (of virtually all isotypes, subclasses, and maturation stages) numbers, from BMM to ISM and, particularly, ASM cases, in parallel to uniquely altered plasma immunoglobulin isotype patterns in the different diagnostic subtypes of SM (Fig 2, *A*).

Association between immune B-cell, PC, and immunoglobulin profiles in blood and clinical findings in patients with SM

Patients with SM with a prior history of *H pylori* infection ($P = .024$), lymphadenopathy ($P = .011$), osteoporosis ($P = .004$),

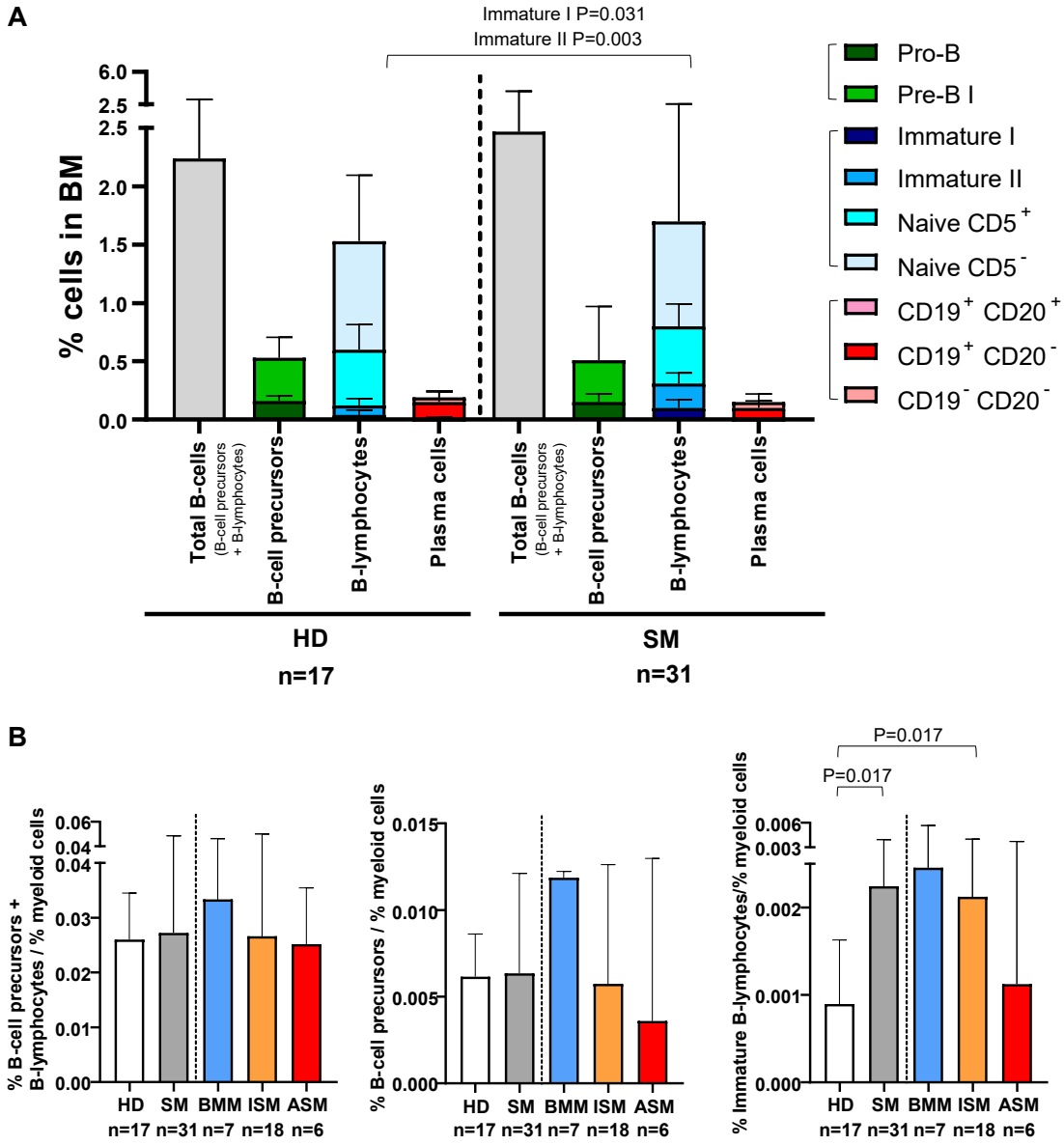

FIG 3. Distribution of the major populations of B-cell precursors, maturing and mature B lymphocytes, and PCs in BM of patients with SM (n = 31) compared with HDs (n = 17). **(A)** Relative distribution of total B cells and their subsets, including B-cell precursors (pro-B and pre-B), smIg$^+$ (immature I, immature II, naive CD5$^+$, and naive CD5$^-$) B lymphocytes, and PCs (classified into different maturation stages according to the pattern of expression of CD19 and CD20). **(B)** Ratio between the percentage of total B cells (B-cell precursors plus B lymphocytes), B-cell precursors, or immature B lymphocytes divided by the percentage of myeloid cells in BM. Percentage data are median values and interquartile range.

and hepatomegaly ($P = .005$) showed greater counts of immature B lymphocytes in blood compared with patients with SM who did not present with these clinical features (Fig 5, *A*). In turn, a more pronounced decrease in (total and/or subset) PC counts was observed among patients with SM who presented with several other specific features of the disease: (1) patients who had pruritus showed lower IgM$^+$ ($P = .022$), IgG2$^+$ ($P = .024$), IgG3$^+$ ($P = .016$), and IgA1$^+$ ($P = .039$) PC counts in blood; (2) patients who had flushing had lower IgM$^+$ ($P = .032$) and IgG1$^+$ ($P = .019$) PC counts; (3) patients presenting with (vs without) splenomegaly had lower IgG2$^+$ ($P = .033$) and IgA1$^+$ ($P = .017$) PC counts; (4) patients presenting with (vs without) hepatomegaly had decreased IgD$^+$-only ($P = .044$) and lower IgA2$^+$ ($P = .036$) PC counts; and (5) patients with a history of *H pylori* infection had lower IgG1$^+$ ($P = .013$) PC counts (Fig 5, *B*). Similarly, patients with a history of prior relevant (recurrent and/or severe) viral/bacterial infection other than *H pylori* also showed lower IgG1$^+$ ($P = .012$), IgG2$^+$ ($P = .021$), and IgM$^+$ ($P = .019$) PC counts (Fig 5, *B*). As expected, IgE plasma levels were significantly more elevated among patients who had anaphylaxis ($P = .002$), HVA ($P = .005$), and bone sclerosis ($P = .002$) (Fig 5, *C*). Of note, an increasing frequency of multilineage involvement of T lymphocytes (by *KIT*^{D816V}) was

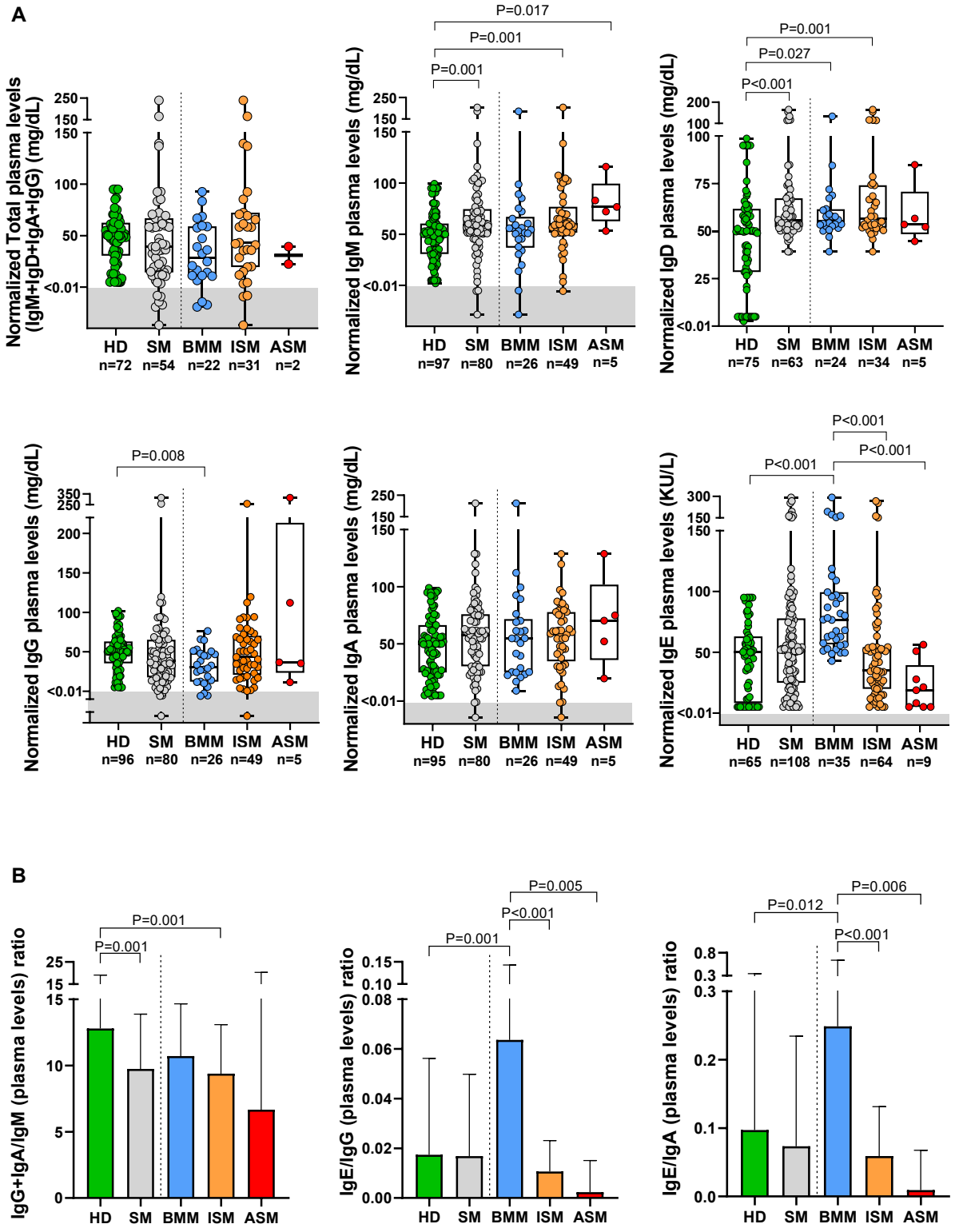

FIG 4. Soluble IgM, IgD, IgG, IgA (mg/dL), and IgE (KU/L) levels in plasma of HDs and patients with SM classified according to the distinct diagnostic subtypes of the disease. **(A)** Total immunoglobulins and plasma levels of IgM, IgD, IgA IgG, and IgE isotypes. **(B)** Ratio between the IgA^+ IgG/IgM, IgE/IgG, and IgE/IgA levels in plasma. *Notched boxes* correspond to normalized box plots based on median (5th-95th) percentile values observed among age-matched HDs that extend from the 25th to 75th percentile values; the *lines in the middle* and *vertical lines* correspond to median values and the 10th and 90th percentiles, respectively, and *dots* represent each individual case analyzed color-coded according to the different diagnostic subtypes of SM (SM *[gray]*, BMM *[blue]*, ISM *[orange]*, ASM *[red]*) vs HD *(green)*. The *gray* area represents data below HD 99.9th percentile values. Data on the IgA^+ IgG/IgM, IgE/IgG, and IgE/IgA plasma level ratios are median and interquartile range values.

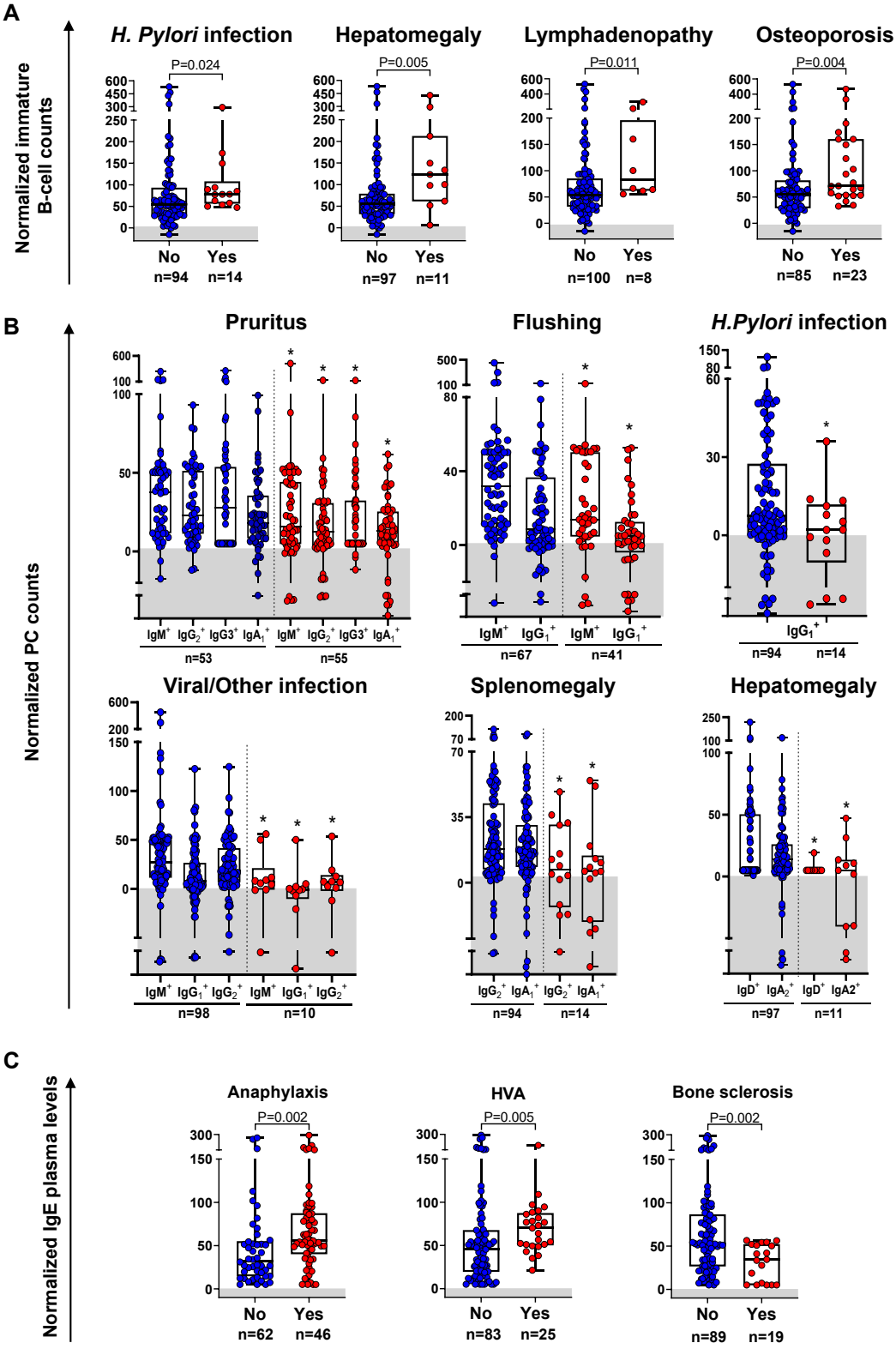

FIG 5. Distribution of immature B-lymphocyte counts **(A)** and PC subset counts **(B)** in blood and IgE plasma levels **(C)** in patients with SM (n = 108) classified according to the presence vs absence of different clinical features of the disease. *Notched boxes* extend from the 25th to 75th percentile values, the *lines in the middle* and *vertical lines* correspond to median values and the 10th and 90th percentiles, respectively, and *dots* represent each individual case analyzed color-coded according to the absence *(blue)* vs presence *(red)* of each individual clinical feature. The *gray* area represents data below HD values. Other bacterial/viral infections included recurrent urinary tract infections, recurrent respiratory infections, bilateral pneumonia, and recurrent COVID-19 infection).

observed from BMM (2/21, 9.5%) to ISM (15/47, 31.9%) and ASM (6/8, 75%) cases.

DISCUSSION

MCs from patients with SM harbor *KIT* mutations, mostly *KIT*^{D816V}.[5] Such mutations have been shown to induce constitutive activation of MCs[34,35] and the release of MC mediators to the extracellular environment, where they exert effects on other innate and adaptive immune cells in addition to epithelial cells and other tissue resident cells.[36-38] Thus, constitutive activation of MCs in patients with SM has long been associated with symptoms of variable MC mediator release (eg, pruritus, diarrhea, flushing, and anaphylaxis)[3,17] and parallel (local and systemic) direct and indirect (eg, via increased mucosal permeability) immune dysregulation.[27-30] In this regard, recent studies from our group and others have reported increased counts of constitutively activated but exhausted blood circulating monocytes[26] and type 2 innate lymphoid cells,[27] together with decreased NK cell and TCD8 cytotoxic (memory and effector) lymphocyte counts[28,29] and uniquely altered TCD4$^+$ lymphocyte subset immune profiles in patients with BMM, ISM, and ASM. These later alterations consisted of decreased counts in blood of T_H1 TCD4$^+$ lymphocytes and specific subsets of T_H2 (effector memory [EM]), T_H22 (terminal effector [TE]), and T_H1-like regulatory cells, together with abnormally high T follicular helper cell counts, and emerged as being closely associated with several specific clinical manifestations of the disease.[29] Despite these innate cell and T-cell alterations, overall normal serum immunoglobulin levels and normal total B-cell counts have been recurrently found in blood and BM of patients with SM,[28-30] with limited data available on the distribution of B-cell and PC subsets in blood of patients with SM. Altogether, this points out the need for a more in-depth analysis of the different maturation and functional/effector B-cell populations for better understanding of immune dysregulation in SM and its relationship with the clinical manifestations of the disease.

Here we investigated in depth the distribution of different B-cell and PC subsets in blood in parallel to immunoglobulin isotype plasma levels in a large cohort of SM patients, aiming at better understanding of the role of B cells and the humoral response in the pathophysiology and clinical behavior of SM. Overall, our results confirm previous data[28-30] that showed normal (total) B-cell counts in blood and BM and overall normal immunoglobulin levels in plasma of patients with SM (vs HDs). However, more detailed analysis of the different B-lymphocyte and PC subsets revealed uniquely altered profiles associated with different diagnostic subtypes of the disease. Thus, a progressively higher increase of both immature and naive CD5$^+$ B-lymphocyte counts, in parallel to a more marked decrease in PC counts (of all IgH subclasses and maturation subsets), was found in patients with SM, increasing from BMM to ISM and ASM. These findings suggest the existence of increased BM production and release to blood of immature and early-naive (ie, CD5$^+$) B lymphocytes in SM, which is progressively more prominent among ISM and ASM (vs BMM) cases. Of note, such increased production of immature B lymphocytes was confirmed in BM of patients with SM supporting their release in greater numbers to blood, particularly among ASM cases. Although we did not investigate the presence of *KIT*^{D816V} on purified B cells, such progressively increased B-cell production does not seem to be related to a greater fraction of *KIT*-mutated B cells, as an increased frequency of involvement of T lymphocytes by the *KIT* mutation was associated with similar or lower naive T-cell counts (Fig 6 in the Online Repository at www.jacionline.org). This increased B-cell production also translated into an elevated ratio between the number of immature B lymphocytes and myeloid cells produced in the BM, which is in contrast to the decreased B-cell production previously reported for (other) myeloproliferative disorders.[39,40]

Despite the aforementioned increased production and release of immature and naive CD5$^+$ B lymphocytes to blood, this did not translate into a greater number in blood of the more mature (and major) population of naive CD5$^-$ B lymphocytes. These findings suggest that the increased production of B lymphocytes reported here might be associated with either a greater naive B-lymphocyte turnover or an increased migration of naive B cells into lymphoid tissues. In line with this latter hypothesis, patients with SM presenting with organomegalies (eg, lymphadenopathy and hepatomegaly) and peripheral tissue disorders (eg, *H pylori* and other severe/current infections) showed significantly greater levels in blood of pre-GC immature and CD5$^+$ naive, but not CD5$^-$ naive, B lymphocytes, supporting an increased migration of the more mature CD5$^-$ naive B cells to secondary lymphoid organs, including peripheral (lymphoid) tissues such as mucosa-associated lymphoid tissue (MALT).

In contrast to pre-GC B lymphocytes, decreased PC counts in blood was a hallmark of SM of any diagnostic subtype (including the only WDSM variant case investigated); this was not mirrored in BM where normal percentages of PCs were found. At the present time, it is well established that PCs recently produced in lymphoid tissues, such as lymph nodes, migrate (via blood) to the BM and other peripheral tissues where they secrete antibodies of different isotypes that can be detected in plasma and other body secretions/fluids.[41,42] However, whereas IgG PCs predominantly migrate to inflammatory sites and the BM, IgA PCs are primarily located in widespread mucosal regions (where they respond to a broad spectrum of antigens present in, eg, the GI and the respiratory tracts).[41,42] Altogether, our results suggest that the deeply decreased counts of blood circulating PCs observed in patients with SM might not be directly linked only to the migration of these cells as long-lived PCs to the BM, but also linked to an increased migration and/or generation of PCs in MALT (eg, the GI and respiratory mucosa and/or the skin). In this regard, significantly lower counts of blood circulating PCs of specific isotypes were found among patients presenting with specific MC mediator release–related symptoms (ie, pruritus and flushing were associated with lower IgM$^+$; IgG1$^+$, IgG2$^+$, and IgG3$^+$; and IgA1$^+$ PCs), organomegalies (patients with splenomegaly and hepatomegaly had lower IgG2$^+$, IgA1$^+$ and IgA2$^+$, and IgD$^+$ PCs), and recurrent bacterial/viral infections. *H pylori* and other severe/recurrent infections (eg, recurrent urinary tract infections, recurrent respiratory infections, bilateral pneumonia, and recurrent COVID-19 infection) were specifically associated with lower IgM$^+$, IgG1$^+$, and IgG2$^+$ PC counts compared with patients with SM who presented without those clinical features. These findings, together with the presence of overall normal total immunoglobulin plasma levels, suggest the existence of an increased migration of recently produced PCs of all isotypes to BM and peripheral tissues (eg, MALT), potentially associated with a locally increased PC production, in line also with the (potentially) increased tissue migration of pre-GC B lymphocytes, discussed above. Although further peripheral tissue studies are needed to

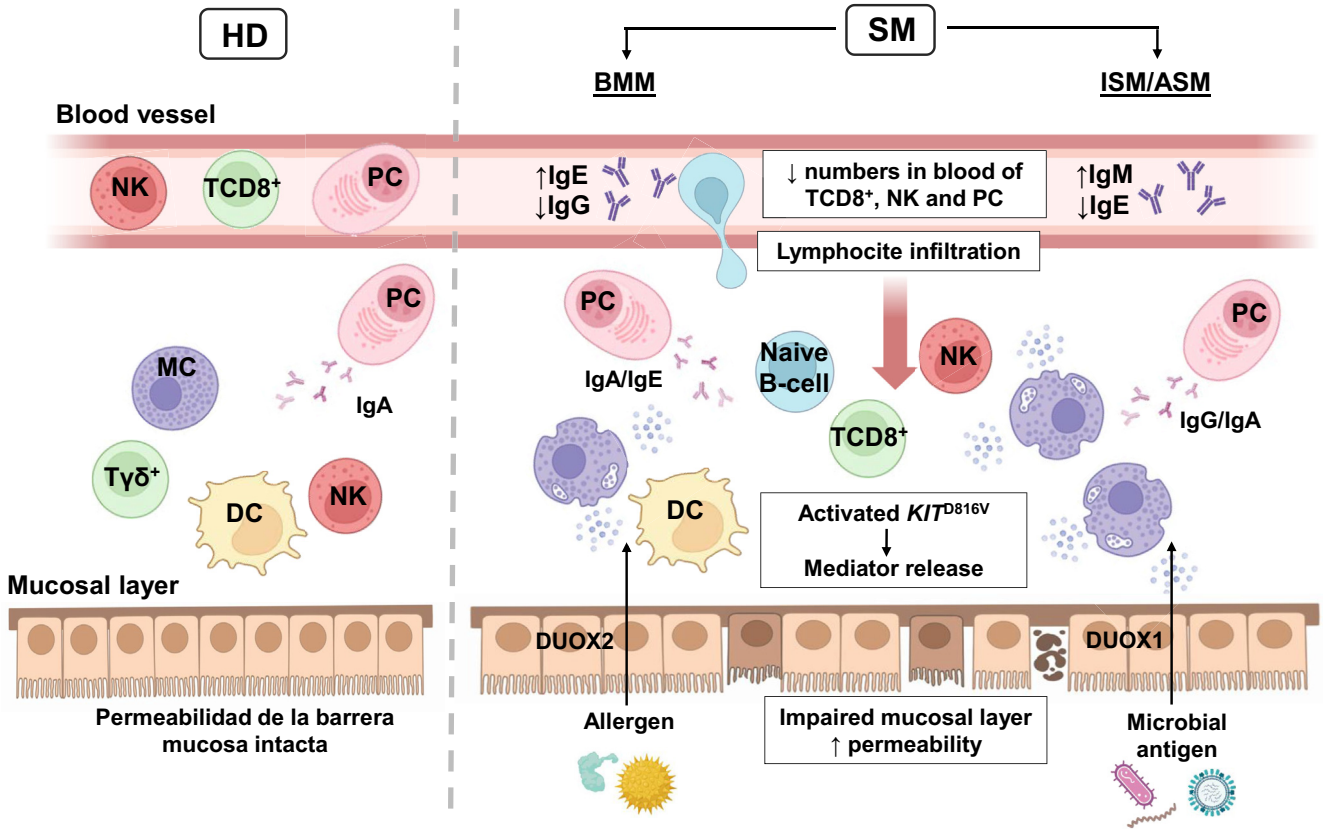

FIG 6. Schematic representation of the dysregulated B-cell, PC, and antibody isotype compartments observed in blood and plasma of patients with different diagnostic subtypes of SM. In patients with SM, chronic activation of (eg, mucosal) tissues infiltrated by *KIT*-mutated MCs associated with the local release of MC mediators might cause an increased mucosal permeability and, ultimately, distinctly dysregulated mucosal antigen trafficking. Depending on the predominance of allergen vs commensal microbial antigen stimuli and the induction of type I vs type II immune responses, different subsets of blood circulating immune cells are recruited locally, such as NK cells, $CD8^+$ T cells, naive B cells, and PCs, which might lead to the dysregulated humoral response and to the different B-cell, PC, and antibody isotype immune profiles observed in our cohort among the distinct diagnostic subtypes of SM.

confirm our hypothesis, emerging evidence indicates that although MC infiltration of peripheral tissues, such as the skin and the GI tract, is a frequent finding in patients with SM, there is not a direct correlation between MC levels (as reflected by the presence of the KIT^{D816V} mutation, serum tryptase levels, or the histologic findings in GI biopsies) and the frequency and severity of, for example, GI symptoms.[43] Moreover, MC infiltrates observed in GI and liver biopsy specimens from patients with SM frequently coexist with mixed inflammatory infiltrates consisting of lymphocytes, macrophages, and, to a lesser extent, eosinophils.[43,44] Such lymphoid infiltrates have been observed in >80% of patients with both nonadvanced and advanced cases of SM who underwent a liver biopsy.[45] Altogether, these data further support the notion that decreased PC counts in blood of patients with SM and involvement of other organs (eg, skin and GI tract) might be due to an increased migration and/or local production of PCs in peripheral tissues, potentially triggered by an increased mucosal permeability and the greater microbial/allergen exposure induced by the locally increased release of mediators by *KIT*-mutated infiltrating MCs. This could lead to locally increased antibody production with overall normal plasma immunoglobulin levels. In line with these findings, an expansion of IgH class-switched MBCs has been reported in gut tissues of individuals with a hereditary alpha-tryptasemia–positive genotype, which is known to be more prevalent among patients with SM.[46]

In-depth analysis of antibody levels in plasma of patients with SM also revealed an altered profile with elevated amounts of IgM and IgD, particularly among patients with BMM and ISMs. The existence of increased IgM plasma levels further supports the hypothesis of an increased encountering of new antigens that trigger primary B-cell responses in SM; meanwhile the potential mechanisms underlying the greater IgD plasma levels warrant further investigations. Interestingly, however, increased IgM and IgD plasma levels were associated with uniquely different profiles for the other immunoglobulin isotypes in BMM cases versus ISM and ASM cases. Patients with BMM displayed significantly increased IgE levels together with decreased amounts of IgG and normal IgA, which translated into elevated IgE/IgG and IgE/IgA ratios compared with HDs, patients with ISM and patients with ASM. In contrast, patients with ISM showed normal IgE and IgG plasma levels, in parallel to elevated IgM and IgD values, leading

to a decreased IgG^+ IgA/IgM ratio, whereas patients with ASM displayed lower IgE plasma levels together with increased IgM values. Altogether, these data point out an increase in IgE type II immune responses, with decreased IgG levels, in patients with BMM, consistent with the greater frequency of allergic responses (approximately 90% of patients had anaphylaxis associated in half of the cases with IgE-mediated HVA as diagnostic trigger) observed in this diagnostic subtype of SM.[44,47,48] Interestingly, however, the increased IgE plasma levels found in patients with BMM do seem to be a specific feature of this disease subtype, as patients with ISM who also presented with anaphylaxis showed significantly lower IgE plasma levels (vs BMM cases), similar to those found in age-matched HDs. Meanwhile, patients with ISM, particularly cases of ASM, displayed a shift toward IgM/IgG, but not IgE, type I antibody immune responses against newly encountered commensal bacteria and other microbes (Fig 6). Independently of the cause of these different immune response profiles, our data support the notion that the heterogeneity of MC mediator release–associated symptoms cannot be solely explained by the presence of variable numbers of KIT^{D816V}-mutated MCs and the tumor MC burden,[43,49] supporting a key role for other bystander immune cells and the local or systemic immune response profiles in determining and modulating such symptoms, as previously indicated by others.[29] Of note, preliminary data obtained in patients with advanced SM suggest that *in vivo* inhibition of KIT^{D816V} by targeted therapies may not reverse these immune alterations, at least in the short term (data not shown).

Recent studies have demonstrated an increase in multiple markers that reflect an abnormal (eg, GI) mucosal permeability in ISM, in close association with specific clinical findings.[50] Of note, disruption of the normal (eg, GI) mucosal barrier induced by specific MC mediators and locally produced cytokines (eg, TNF-α, interferon, or IL-1β) that are increased in SM[26,51] have also been reported to enhance mucosal permeability (eg, in the GI tract),[26,51] which, in turn, may substantially affect antigen trafficking and modulate the close interaction between the gut microbiome (shaped mostly by IgA^+ PCs) and the local versus systemic immune responses.[52,53] Our data suggest that the different immune profiles observed among the distinct diagnostic subtypes of SM might result from different antigen trafficking at the MALT and mucosal tissues, potentially triggered by chronic activation of tissue-infiltrating *KIT*-mutated MCs, associated with the local release of MC (epithelial and other immune cell) mediators. This might ultimately lead to a greater local increased recruitment of naive B cells and the production of antibody-secreting PCs of different isotypes, depending on the predominance of allergen versus commensal microbial antigen stimuli, the oxidative (eg, DUOX1 vs DUOX2 oxidase and signaling) potential of epithelial cells, and the induction of type I versus type II immune responses.[54] Further investigations focused on the study of local mucosal immune responses in SM are critical to better understand the role of the mucosal barrier in SM and to confirm our hypothesis (Fig 6).

In summary, we report here for the first time a significant dysregulation of the B-cell and PC compartments in blood of patients with SM in association with distinctly altered antibody isotype profiles in plasma of patients with distinct diagnostic subtypes of SM. Further investigations are needed to better understand the precise underlying pathophysiologic mechanisms involved in the alteration of the B-cell compartment in SM and its contribution to the variable clinical behavior of the disease.

DISCLOSURE STATEMENT

This study was supported by grants from Instituto de Salud Carlos III (ISCIII; Madrid, Spain) and co-funded by the European Union (grant numbers PI19/01166, PI22/01657, and CB16/12/00400 for the Biomedical Research Networking Center Consortium [CIBERONC] program) and the Subprograma Estatal de Infraestructuras de Investigación y Equipamiento Científico Técnico de 2019 del Ministerio de Ciencia, Innovación y Universidades (Madrid, Spain) co-funded by the European Union (grant number EQC2019-005419-P). A.P.-P. was supported by a grant from the Government of Castilla y León (Valladolid, Spain; Orden EDU/556/2019) co-funded by the European Regional Development Fund (FEDER BDNS, Identif.: 422058). P.N.-N. was supported by a grant from the Government of Castilla y León (Valladolid, Spain; Orden EDU/875/2021) co-funded by the European Social Fund (FEDER BDNS, Identif.: 540787). O.G.-L. was supported by a grant from ISCIII (PFIS: FI20/00116-AES 2017-2020, BOE 28/03/2019-Orden CNU/354/2019) co-funded by the European Union (European Social Fund Plus). A.M. was supported by the CIBERONC program (contract number PRF-2869).

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

We thank the Spanish National DNA Bank Carlos III (Salamanca, Spain; biobank ID B.0000716; supported by ISCIII and co-founded by the European Union [grant number PT23/00086]; www.bancoadn.org) and the Biobank at the Hospital Universitario de Toledo (Toledo, Spain; BioB-HUT; biobank ID B.0000520) for providing plasma samples from patients with SM and HDs.

Key messages

- **Patients with SM show a significant dysregulation of blood circulating B-cell and PC compartments together with distinctly altered immunoglobulin isotype profiles in plasma.**
- **Different immune B-cell, PC, and immunoglobulin isotype profiles were found among patients with BMM, ISM, and ASM.**

REFERENCES

1. Horny HP, Sotlar K, Valent P, Hartmann K. Mastocytosis: A disease of the hematopoietic stem cell. Dtsch Arztebl Int 2008;105:686-92.
2. Valent P, Akin C, Sperr WR, Horny HP, Arock M, Metcalfe DD, et al. New insights into the pathogenesis of mastocytosis: Emerging concepts in diagnosis and therapy. Annu Rev Pathol 2023;18:361-86.
3. Zanotti R, Tanasi I, Crosera L, Bonifacio M, Schena D, Orsolini G, et al. Systemic mastocytosis: Multidisciplinary approach. Mediterr J Hematol Infect Dis 2021;13:e2021068.
4. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia 2022;36:1703-19.

5. Valent P, Akin C, Hartmann K, Alvarez-Twose I, Brockow K, Hermine O, et al. Updated diagnostic criteria and classification of mast cell disorders: A consensus proposal. Hemasphere 2021;5:e646.
6. Valent P, Horny HP, Escribano L, Longley BJ, Li CY, Schwartz LB, et al. Diagnostic criteria and classification of mastocytosis: A consensus proposal. Leuk Res 2001;25:603-25.
7. Garcia-Montero AC, Jara-Acevedo M, Teodosio C, Sanchez ML, Nunez R, Prados A, et al. KIT mutation in mast cells and other bone marrow hematopoietic cell lineages in systemic mast cell disorders: A prospective study of the Spanish Network on Mastocytosis (REMA) in a series of 113 patients. Blood 2006;108:2366-72.
8. Castells M, Metcalfe DD, Escribano L. Diagnosis and treatment of cutaneous mastocytosis in children: Practical recommendations. Am J Clin Dermatol 2011;12:259-70.
9. Tefferi A, Pardanani A. Systemic mastocytosis: Current concepts and treatment advances. Curr Hematol Rep 2004;3:197-202.
10. Escribano L, Akin C, Castells M, Schwartz LB. Current options in the treatment of mast cell mediator-related symptoms in mastocytosis. Inflamm Allergy Drug Targets 2006;5:61-77.
11. Coppé JP, Desprez PY, Krtolica A, Campisi J. The senescence-associated secretory phenotype: The dark side of tumor suppression. Annu Rev Pathol 2010;5:99-118.
12. Coppé JP, Patil CK, Rodier F, Sun Y, Muñoz DP, Goldstein J, et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol 2008;6:2853-68.
13. Bischoff SC. Physiological and pathophysiological functions of intestinal mast cells. Semin Immunopathol 2009;31:185-205.
14. Zhao XO, Sommerhoff CP, Paivandy A, Pejler G. Mast cell chymase regulates extracellular matrix remodeling-related events in primary human small airway epithelial cells. J Allergy Clin Immunol 2022;150:1534-44.
15. Henriques A, Muñoz-González JI, Sánchez-Muñoz L, Matito A, Torres-Rivera L, Jara-Acevedo M, et al. Frequency and prognostic impact of blood-circulating tumor mast cells in mastocytosis. Blood 2022;139:572-83.
16. da Silva EZ, Jamur MC, Oliver C. Mast cell function: A new vision of an old cell. J Histochem Cytochem 2014;62:698-738.
17. Li JY, Ryder CB, Zhang H, Cockey SG, Hyjek E, Moscinski LC, et al. Review and updates on systemic mastocytosis and related entities. Cancers (Basel) 2023;15:5626.
18. Dileepan KN, Raveendran VV, Sharma R, Abraham H, Barua R, Singh V, et al. Mast cell-mediated immune regulation in health and disease. Front Med (Lausanne) 2023;10:1213320.
19. Elieh Ali Komi D, Grauwet K. Role of mast cells in regulation of T cell responses in experimental and clinical settings. Clin Rev Allergy Immunol 2018;54:432-45.
20. Ashina K, Tsubosaka Y, Nakamura T, Omori K, Kobayashi K, Hori M, et al. Histamine induces vascular hyperpermeability by increasing blood flow and endothelial barrier disruption in vivo. PLoS One 2015;10:e0132367.
21. Albert-Bayo M, Paracuellos I, González-Castro AM, Rodríguez-Urrutia A, Rodríguez-Lagunas MJ, Alonso-Cotoner C, et al. Intestinal mucosal mast cells: Key modulators of barrier function and homeostasis. Cells 2019;8:135.
22. Elieh Ali Komi D, Wöhrl S, Bielory L. Mast cell biology at molecular level: A comprehensive review. Clin Rev Allergy Immunol 2020;58:342-65.
23. Akdis M, Aab A, Altunbulakli C, Azkur K, Costa RA, Crameri R, et al. Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: Receptors, functions, and roles in diseases. J Allergy Clin Immunol 2016;138:984-1010.
24. Woźniak E, Owczarczyk-Saczonek A, Placek W. Psychological stress, mast cells, and psoriasis—is there any relationship? Int J Mol Sci 2021;22:13252.
25. Krystel-Whittemore M, Dileepan KN, Wood JG. Mast cell: A multi-functional master cell. Front Immunol 2016;6:620.
26. Pérez-Pons A, Jara-Acevedo M, Henriques A, Navarro-Navarro P, García-Montero AC, Álvarez-Twose I, et al. Altered innate immune profile in blood of systemic mastocytosis patients. Clin Transl Allergy 2022;12:e12167.
27. van der Ploeg EK, Hermans MAW, van der Velden VHJ, Dik WA, van Daele PLA, Stadhouders R. Increased group 2 innate lymphoid cells in peripheral blood of adults with mastocytosis. J Allergy Clin Immunol 2021;147:1490-6.e2.
28. Kulinski JM, Eisch R, Young ML, Rampertaap S, Stoddard J, Monsale J, et al. Skewed lymphocyte subpopulations and associated phenotypes in patients with mastocytosis. J Allergy Clin Immunol Pract 2020;8:292-301.e2.
29. Pérez-Pons A, Teodosio C, Jara-Acevedo M, Henriques A, Navarro-Navarro P, García-Montero AC, et al. T-cell immune profile in blood of systemic mastocytosis: Association with disease features. Allergy 2024;79:1921-37.
30. Makovoz A, Cousins KL, Dominguez Aldama LN, Bolan H, Metcalfe DD, Komarow HD, et al. Assessment of low immunoglobulin levels and clinical manifestations in patients with mastocytosis. J Allergy Clin Immunol Glob 2023;2:105-10.
31. Zanotti R, Bonifacio M, Lucchini G, Sperr WR, Scaffidi L, van Anrooij B, et al. Refined diagnostic criteria for bone marrow mastocytosis: A proposal of the European competence network on mastocytosis. Leukemia 2022;36:516-24.
32. Blanco E, Pérez-Andrés M, Arriba-Méndez S, Contreras-Sanfeliciano T, Criado I, Pelak O, et al. Age-associated distribution of normal B-cell and plasma cell subsets in peripheral blood. J Allergy Clin Immunol 2018;141:2208-19.e16.
33. Criado I, Nieto WG, Oliva-Ariza G, Fuentes-Herrero B, Teodosio C, Lecrevisse Q, et al. Age- and sex-matched normal leukocyte subset ranges in the general population defined with the EuroFlow Lymphocyte Screening Tube (LST) for monoclonal B-cell lymphocytosis (MBL) vs. non-MBL subjects. Cancers (Basel) 2022;15:58.
34. Rajan V, Prykhozhij SV, Pandey A, Cohen AM, Rainey JK, Berman JN. KIT D816V is dimerization-independent and activates downstream pathways frequently perturbed in mastocytosis. Br J Haematol 2023;202:960-70.
35. Raghav PK, Singh AK, Gangenahalli G. A change in structural integrity of c-Kit mutant D816V causes constitutive signaling. Mutat Res 2018;808:28-38.
36. Yu Y, Blokhuis BR, Garssen J, Redegeld FA. Non-IgE mediated mast cell activation. Eur J Pharmacol 2016;778:33-43.
37. Gri G, Frossi B, D'Inca F, Danelli L, Betto E, Mion F, et al. Mast cell: An emerging partner in immune interaction. Front Immunol 2012;3:120.
38. Cardamone C, Parente R, Feo GD, Triggiani M. Mast cells as effector cells of innate immunity and regulators of adaptive immunity. Immunol Lett 2016;178:10-4.
39. Benjamin D, Shohat B, Pinkhas J, Joshua H. B- and T-lymphocyte subpopulations in polycythemia vera. A study of 100 patients. Clin Immunol Immunopathol 1979;13:378-82.
40. Sørensen AL, Bjørn ME, Riley CH, Holmstrøm M, Andersen MH, Svane IM, et al. B-cell frequencies and immunoregulatory phenotypes in myeloproliferative neoplasms: Influence of ruxolitinib, interferon-α2, or combination treatment. Eur J Haematol 2019;103:351-61.
41. Kunkel EJ, Butcher EC. Plasma-cell homing. Nat Rev Immunol 2003;3:822-9.
42. Husband AJ, Gowans JL. The origin and antigen-dependent distribution of IgA-containing cells in the intestine. J Exp Med 1978;148:1146-60.
43. Hsieh FH. Gastrointestinal involvement in mast cell activation disorders. Immunol Allergy Clin North Am 2018;38:429-41.
44. Mican JM, Di Bisceglie AM, Fong TL, Travis WD, Kleiner DE, Baker B, et al. Hepatic involvement in mastocytosis: Clinicopathologic correlations in 41 cases. Hepatology 1995;22:1163-70.
45. Rossignol J, Canioni D, Aouba A, Bulai-Livideanu C, Barete S, Lancesseur C, et al. Histological characterization of liver involvement in systemic mastocytosis. Liver Int 2024;44:1680-8.
46. Konnikova L, Robinson TO, Owings AH, Shirley JF, Davis E, Tang Y, et al. Small intestinal immunopathology and GI-associated antibody formation in hereditary alpha-tryptasemia. J Allergy Clin Immunol 2021;148:813-21.e7.
47. Jackson CW, Pratt CM, Rupprecht CP, Pattanaik D, Krishnaswamy G. Mastocytosis and mast cell activation disorders: Clearing the air. Int J Mol Sci 2021;22:11270.
48. Zanotti R, Tanasi I, Bernardelli A, Orsolini G, Bonadonna P. Bone marrow mastocytosis: A diagnostic challenge. J Clin Med 2021;10:1420.
49. Sokol H, Georgin-Lavialle S, Canioni D, Barete S, Damaj G, Soucie E, et al. Gastrointestinal manifestations in mastocytosis: A study of 83 patients. J Allergy Clin Immunol 2013;132:866-73.e1-3.
50. Komarow HD, Brenchley JM, Eisch AR, Young ML, Scott LM, Kulinski JM, et al. A study of microbial translocation markers in mastocytosis. Clin Exp Allergy 2021;51:369-72.
51. Meyer F, Wendling D, Demougeot C, Prati C, Verhoeven F. Cytokines and intestinal epithelial permeability: A systematic review. Autoimmun Rev 2023;22:103331.
52. Fasano A. All disease begins in the (leaky) gut: Role of zonulin-mediated gut permeability in the pathogenesis of some chronic inflammatory diseases. F1000Res 2020;9:F1000 Faculty Rev-69.
53. Zogorean R, Wirtz S. The yin and yang of B cells in a constant state of battle: Intestinal inflammation and inflammatory bowel disease. Front Immunol 2023;14:1260266.
54. van der Vliet A, Danyal K, Heppner DE. Dual oxidase: A novel therapeutic target in allergic disease. Br J Pharmacol 2018;175:1401-18.