

Original Article

Skewed Lymphocyte Subpopulations and Associated Phenotypes in Patients with Mastocytosis

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What is already known about this topic? Mast cell mediators affect lymphocyte differentiation and function, but lymphocyte subpopulations have not been thoroughly examined in patients with mastocytosis who exhibit expansion of mast cells and increase in mediator release.

What does this article add to our knowledge? Adult patients with indolent systemic mastocytosis exhibit immunologic phenotypic alterations compared with the normal adult population including lower absolute counts of peripheral CD8⁺ T cells and circulating natural killer cells.

How does this study impact current management guidelines? Skewed lymphocyte subsets are a feature of mastocytosis, suggesting the need for determining the lymphocyte immunophenotypic profile in patients with indolent systemic mastocytosis and associated infectious complications and/or autoimmune findings.

BACKGROUND: Mastocytosis is a clonal mast cell disorder associated with elevated mast cell mediators, which themselves have been reported to affect lymphocyte function. However, the impact of an expanded mast cell compartment on lymphocyte subpopulations, and their correlation with clinical phenotypes in patients with indolent systemic mastocytosis (ISM), has not been explored.

OBJECTIVE: To examine the immunophenotype of circulating lymphocytes in patients with ISM compared with healthy adult controls and examine relationships with aspects of clinical disease.

METHODS: We examined lymphocyte subsets in 20 adult patients with ISM and 40 healthy adult volunteers by multiparameter flow cytometry. Results were correlated with clinical characteristics.

RESULTS: Patients with ISM exhibited a significantly lower median frequency and absolute cell count of both circulating CD8⁺ T cells and natural killer cells accompanying a significantly increased ratio of CD4⁺/CD8⁺ T cells when compared with healthy volunteers. Stratification of our ISM patient cohort according to clinical manifestations revealed that CD19⁺CD21^{low}CD38^{low} B cells were significantly higher in patients with a history of autoimmune disease and counts of terminally differentiated CD4⁺ T cells were significantly higher in patients with osteoporosis or osteopenia.

CONCLUSIONS: Several circulating lymphocyte subpopulations in patients with ISM were significantly different when compared with healthy controls; in specific lymphocyte subsets, this lymphocyte skewing correlated with clinical observations including osteoporosis and autoimmune disease. These data suggest the need for further studies on abnormalities

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Abbreviations used

BST- Basal serum tryptase
 HC- Healthy control
 ISM- Indolent systemic mastocytosis
 NIH- National Institutes of Health
 NK- Natural killer
 TEMRA- T cells Effector Memory reexpressing RA

in lymphocyte subsets and the attendant clinical consequences in both mast cell proliferative and activation disorders. Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2019;■:■-■)

Key words: Mastocytosis; Mast cells; Mast cell activation; Tryptase; Lymphocyte; NK cell; T cell; Flow cytometry

INTRODUCTION

Indolent systemic mastocytosis (ISM), the most common mastocytosis variant in adults, is a clonal mast cell disorder associated with an increased number of mast cells in specific tissues including the bone marrow, liver, spleen, lymph nodes, and skin.¹ Patients with ISM generally suffer from episodes of mediator release and are managed symptomatically. Systemic effects include flushing, pruritus, hypotension, syncope, palpitations, and tachycardia. Mastocytosis is also associated with enlargement of the liver and spleen, and osteoporosis.² Mast cell mediators such as histamine, tryptase, and IL-6 are elevated in ISM and have been demonstrated in experimental models to alter lymphocyte function.³⁻⁵ This led us to examine the lymphocyte compartment in the peripheral blood of patients with ISM.

As will be shown, we found a previously uncharacterized immunophenotype of altered peripheral lymphocyte composition in patients with ISM. In addition, we identified clinical findings that correlated with specific peripheral blood lymphocyte subpopulations.

METHODS**Subjects**

Twenty patients aged 42 to 73 years with a diagnosis of ISM based on World Health Organization criteria⁶ were evaluated prospectively as part of routine care for patients with mastocytosis in our clinic at the National Institutes of Health (NIH) from January 2017 to January 2018 without preselection of patients and following informed consent on the NIH protocol 02-I-0277. All patients in this study were diagnosed with ISM and did not meet criteria for smoldering systemic mastocytosis, for example, basal serum tryptase (BST) level greater than 200 ng/mL and mast cell bone marrow burden greater than 30% + organomegaly. Furthermore, none of the patients included in this study exhibited any indication of organ dysfunction nor were any of the patients being treated with a cytoreductive agent. Eleven of the patients (55%) were female, and 9 (45%) were male. Forty healthy volunteers aged 18 to 69 years who have been standardized to determine the adult control population range of lymphocyte phenotype subsets for all NIH clinical studies were included as a control population for this study.^{7,8}

Flow cytometry

EDTA anticoagulated human peripheral blood samples were processed for flow cytometry in a Clinical Laboratory Improvement Amendments—certified laboratory at NIH using a whole blood lysis method, staining with fluorescent antibodies, collecting with a FACS Canto II (Becton Dickinson, San Jose, Calif), and analyzing using FCS Express software (De Novo, Glendale, Calif). Lymphocytes were identified on the basis of a gate established by forward and side scatter and confirmed using anti-CD45 and anti-CD14 (see Figure E1, A, in this article's Online Repository at www.jaci-inpractice.org). T-cell, B-cell, and natural killer (NK)-cell subsets were identified by directly conjugated mAbs. T cells: anti-CD3, anti-CD4, anti-CD8, anti-CD45RA, and anti-CD62L (Figure E1, B); B cells: anti-CD20, anti-CD19, anti-CD5, anti-CD10, anti-IgM, anti-CD38, and anti-CD27 (see Figure E2 in this article's Online Repository at www.jaci-inpractice.org); NK cells were identified by a combination of anti-CD16 and anti-CD56, evaluated on CD3-negative lymphocytes (Figure E1, C). Irrelevant, directly conjugated, murine IgG₁ was used to ascertain background staining. Most mAbs were obtained from Becton Dickinson, except for anti-CD45RA (Beckman-Coulter, Brea, Calif), anti-IgM (BioLegend, San Diego, Calif), and anti-CD4, anti-CD45, anti-CD14, anti-CD19, anti-CD10, and anti-CD27 (Life Technologies, Carlsbad, Calif).

KIT p.D816V allele-specific quantitative PCR

Genomic DNA was prepared from the peripheral blood of patients, and a mutation-specific real-time quantitative PCR assay was performed in our NIH Clinical Laboratory Improvement Amendments—certified laboratory to determine the percentage of cells carrying the *KIT* p.D816V mutation where an allelic frequency above 0.03% was considered positive, as described.⁹

Total BST quantification

Total BST levels were measured using a commercially available fluorescence enzyme immunoassay in a Clinical Laboratory Improvement Amendments—certified laboratory (Mayo Clinic, Rochester, NY).

Tryptase copy number genotyping

Detection of specific alpha (α) and beta (β) isoforms of secreted tryptase encoded by the genes *TPSB2* and *TPSAB1* was genotyped as described¹⁰ using droplet digital PCR analysis. Briefly, restriction-endonuclease-treated genomic DNA was prepared from patient specimens, and custom primers and probes for α - and β -tryptase were used in the detection of α - or β -tryptase encoding *TPSAB1* copies on a single allele using the PrimePCR droplet digital PCR copy number reference *AP3B1*, according to the manufacturer's specifications (Bio-Rad, Hercules, Calif).

Statistics

Two-tailed Mann-Whitney nonparametric analysis was used to compare flow cytometry parameters between patient groupings where *P* less than .05 was considered significant. Correlations were assessed using a nonparametric Spearman correlation, and significance was computed with a 2-tailed *P* value with a 95% CI. All statistical computations were performed using Prism software (GraphPad, La Jolla, Calif).

TABLE I. Patients' demographic characteristics and clinical data

Patient no.	Age (y)	Sex	Clinical history										KIT D816V allelic frequency (%)	BST (ng/mL)	α/β tryptase copy no.
			Urticaria pigmentosa	Osteopenia	Osteoporosis	Splenomegaly	Hepatomegaly	Allergy	Anaphylaxis	Autoimmune	Chronic/recurrent infection	Malignancy			
ISM-1	68	F						✓	✓		✓	✓	24.17	87.5	1/3
ISM-2	64	F		✓	✓					✓	✓	✓	1.47	60.3	2/2
ISM-3	55	F	✓	✓				✓				✓	6.82	38	1/3
ISM-4*	62	M		✓					✓		✓		0.12	108	4/2
ISM-5	56	F	✓	✓	✓			✓					1.97	31.9	1/3
ISM-6	64	M	✓	✓									0.06	39.9	0/4
ISM-7	45	F	✓	✓				✓					0.06	28.2	1/3
ISM-8	55	M	✓	✓					✓	✓			0.05	51.1	3/2
ISM-9	67	F	✓	✓	✓			✓	✓	✓	✓		0.06	19.4	1/3
ISM-10	42	M	✓					✓	✓		✓		11.30	286	2/2
ISM-11	49	F	✓									✓	0.97	61.6	2/2
ISM-12	61	M	✓								✓	✓	3.68	63.9	1/3
ISM-13	71	M	✓	✓				✓	✓		✓		9.75	188	2/2
ISM-14	62	F	✓	✓	✓			✓		✓		✓	0.54	37.9	1/3
ISM-15	52	M	✓	✓		✓	✓	✓	✓	✓		✓	0.68	105	2/2
ISM-16	56	F	✓	✓				✓	✓	✓			<0.03	25.2	2/3
ISM-17	73	F	✓	✓	✓					✓	✓		<0.03	21.9	2/3
ISM-18	42	M	✓										0.13	20.6	1/3
ISM-19	67	F	✓	✓	✓	✓		✓		✓	✓		41.59	223	1/3
ISM-20	67	M	✓	✓		✓	✓			✓	✓	✓	0.29	236	3/2

Abnormal tryptase copy numbers due to a duplication in *TPSAB1* are shown in bold.

*Index patient ISM-4 was found to have severe peripheral B-cell lymphopenia.

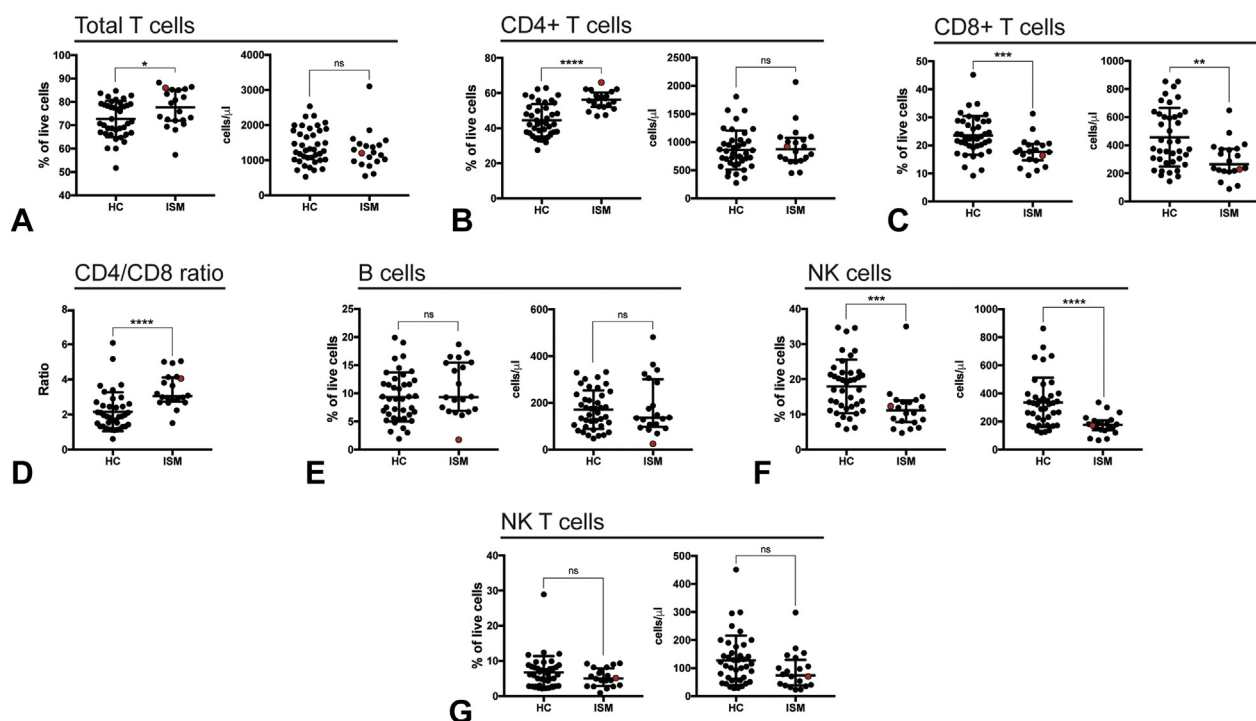


FIGURE 1. Peripheral lymphocyte populations in patients with ISM compared with HCs. (A-C, E, and F) The median frequency (%) and absolute count (cell/ μ L) $\pm$ interquartile range of peripheral blood lymphocytes in patients with ISM and healthy volunteer controls (HCs) as determined by surface marker expression for (Figure 1, A) CD45⁺CD3⁺ total T cells, (Figure 1, B) CD45⁺CD3⁺CD4⁺ T cells, (Figure 1, C) CD45⁺CD3⁺CD8⁺ T cells, (Figure 1, E) CD3⁺CD20⁺ B cells, (Figure 1, F) CD16⁺CD56⁺ NK cells, and (G) CD3⁺CD56⁺ NK T cells. Data from each patient are represented by an individual data point. (D) The median ratio of % CD3⁺CD4⁺ cells divided by the % CD3⁺CD8⁺ cells derived from data shown in Figure 1, B and C. Data points colored red identify index patient ISM-4 (see Table I). ns, Not significant. * $P < .05$. ** $P < .01$. *** $P < .001$. **** $P < .0001$.

RESULTS

Clinical manifestations and laboratory results

Clinical manifestations consistent with the diagnosis of ISM in our patient cohort included 17 patients (85%) with cutaneous lesions or maculopapular cutaneous mastocytosis, also known as urticaria pigmentosa, 15 patients (75%) with osteopenia, 6 patients (30%) with osteoporosis, and 3 patients (15%) with organomegaly (2 patients with splenomegaly and hepatomegaly, 1 patient with splenomegaly only) (Table I). The vast majority of our ISM patient cohort of 20 were treated with antihistamines (19), followed by leukotriene antagonists (5), and nonsteroidal anti-inflammatory drugs (8), all of which would not have an effect on patient lymphocyte subsets. Any patients treated with systemic steroids, anticancer medications, and monoclonal therapies that may alter the results were excluded from this study. All patients with osteoporosis were taking vitamin D supplements and patient ISM-19 was treated with a bisphosphonate several years before this study, both of which have been reported not to have any effect on the T-cell subset profiles.^{11,12}

On the basis of our evaluation and the medical record, patients were screened for a past or current history of autoimmune diseases, malignancy, chronic/recurrent infections (at least 2 episodes), and allergic diseases. Nine patients (45%) had a history of autoimmune disease (rheumatoid arthritis, 3; Raynaud's, 1; autoimmune thyroiditis, 4; other, 3); 8 patients (40%) had a history of malignancy (melanoma, 3; breast cancer, 2; basal

cell carcinoma, 1; possible polycythemia vera, 1; papillary thyroid tumor, 1); 10 patients (50%) had a history of chronic/recurrent infections (fungal, 1; bacterial pneumonia, 2; methicillin-resistant *Staphylococcus aureus* folliculitis, 1; meningitis, 1; herpes, 3; Lyme, 1; *Helicobacter pylori*, 1; *Clostridium difficile*, 1; tonsillitis, 1; diverticulitis, 2; urinary tract infection, 2); and 11 patients (55%) had a history of allergy (asthma, 4; atopic dermatitis, 2; drug allergy, 6; food allergy, 3; venom allergy, 4). Anaphylaxis was diagnosed in 8 patients (40%) by the referral physician using criteria from the summary report on anaphylaxis¹³ (see Table I).

Median immunoglobulin levels were within the normal range in the ISM group as follows: IgM, 119.5 mg/dL (NIH Clinical Center DLM normal range, 40-230 mg/dL); IgE, 9.7 mg/dL (0-90 mg/dL); IgA, 205 mg/dL (70-400 mg/dL); and IgG, 993.5 mg/dL (700-1700 mg/dL). All liver enzymes were within normal limits.

The *KIT* p.D816V mutation was detected in the peripheral blood of 18 of the 20 patients in our ISM cohort (Table I). The median allelic frequency of *KIT* p.D816V in our ISM cohort was 0.612% (25th percentile, 0.06%; 75th percentile, 6.04). The median BST level for our ISM patient cohort was 55.7 ng/mL (25th percentile, 29.13 ng/mL; 75th percentile, 107.3 ng/mL), well above the normal reference range of 0.0 to 11.5 ng/mL, with 19 of 20 patients (95%) exhibiting a value greater than 20 ng/mL, which is a minor criterion for the diagnosis of ISM.

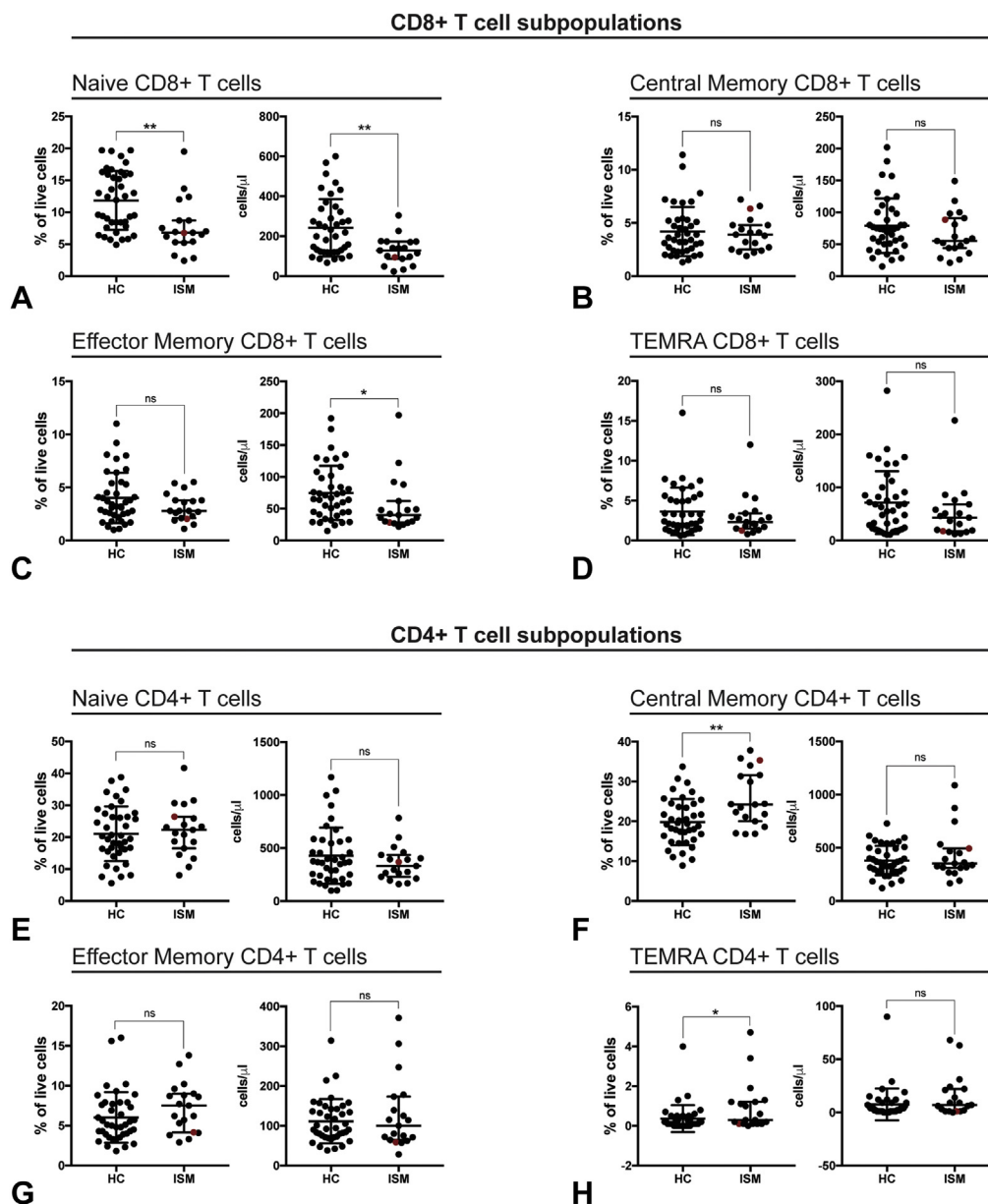


FIGURE 2. Peripheral T-cell populations in patients with ISM compared with HCs. The median frequency (%) and absolute count (cell/ μ L) $\pm$ interquartile range of peripheral blood (**A-D**) CD3⁺CD8⁺ and (**E-H**) CD3⁺CD4⁺ naive (CD62L⁺CD45RA⁺), central memory (CD62L⁺CD45RA⁻), effector memory (CD62L⁻CD45RA⁻), and effector (CD62L⁻CD45RA⁺) subsets, in patients with ISM and healthy volunteer controls (HCs). Data from each patient are represented by an individual data point. Data points colored red identify index patient ISM-4 (see [Table I](#)). ns, Not significant. * $P < .05$. ** $P < .01$.

Analysis of peripheral lymphocyte phenotype

As an initial screening approach, we determined the frequency and absolute lymphocyte count (T cells, B cells, NK cells, and NK T cells) in the peripheral blood of patients with ISM and compared these results with those in a group of healthy adult controls (HCs). Correlation analysis of patient age within the ISM cohort did not show any significant association with any lymphocyte subsets measured (data not shown). Although the median frequency of total (CD45⁺CD3⁺) peripheral T cells was significantly higher than that of HCs, there was no difference in the overall absolute total T-cell count

between the 2 groups ([Figure 1, A](#)). By further distinguishing between CD4⁺ and CD8⁺ peripheral T cells ([Figure 1, B-D](#)), our analysis revealed that patients with ISM exhibit a significantly increased CD4⁺/CD8⁺ T-cell ratio compared with HCs ([Figure 1, D](#)). We found that the median frequency of CD4⁺ T cells was increased ([Figure 1, B](#)), whereas both the frequency and absolute number of peripheral CD8⁺ T cells were significantly decreased in patients with ISM compared with HCs ([Figure 1, C](#)). Not all peripheral lymphocyte subsets were affected because total B cells (CD19⁺ or CD20⁺) were not significantly altered in patients with ISM ([Figure 1, E](#)). Finally,

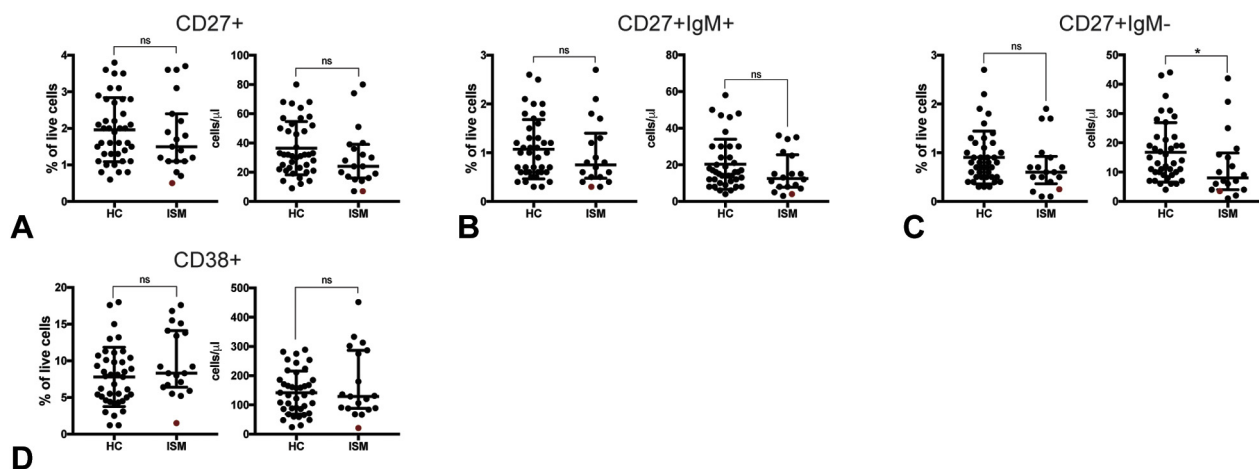
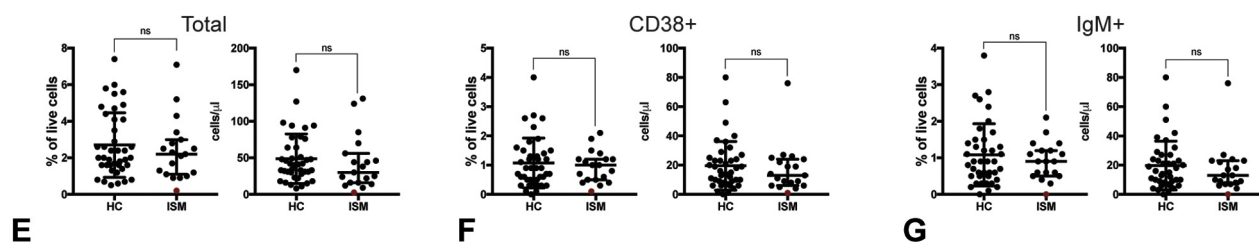
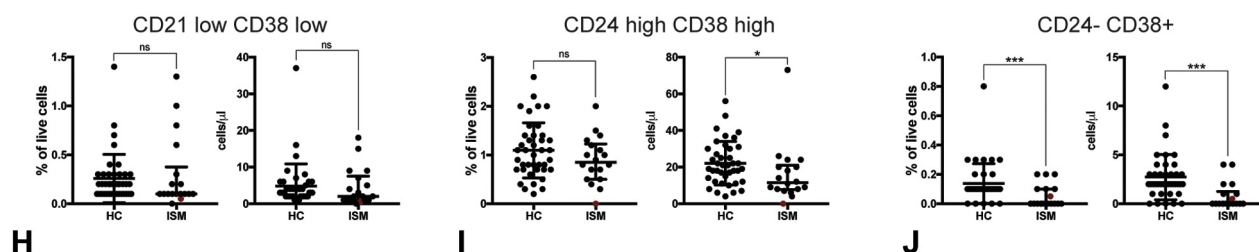
Gated on CD20⁺ B cellsGated on CD20⁺CD10⁺ transitional B cellsGated on CD19⁺ B cells

FIGURE 3. Peripheral B-cell populations in patients with ISM compared with HCs. The median frequency (%) and absolute count (cell/ μ L) $\pm$ interquartile range of peripheral blood (A) CD3⁺CD20⁺CD27⁺ cells, (B) CD3⁺CD20⁺CD27⁺IgM⁺ cells, (C) CD3⁺CD20⁺CD27⁺IgM⁺ cells, (D) CD3⁺CD20⁺CD38⁺ cells, (E) CD3⁺CD20⁺CD10⁺ cells, (F) CD3⁺CD20⁺CD10⁺CD38⁺ cells, (G) CD3⁺CD20⁺CD10⁺IgM⁺ cells, (H) CD3⁺CD19⁺CD21^{low}CD38^{low} cells, (I) CD3⁺CD19⁺CD24^{high}CD38^{high} cells, and (J) CD3⁺CD19⁺CD24⁺CD38⁺ cells in patients with ISM and healthy volunteer controls (HCs). Data from each patient are represented by an individual data point. Data points colored red identify index patient ISM-4 (see Table I). ns, Not significant. * $P < .05$. *** $P < .001$.

we found that both the median frequency and absolute count of CD3⁺CD56⁺ NK cells (but not CD3⁺CD56⁺ NK T cells) were significantly lower in the ISM patient cohort compared with HCs (Figure 1, F and G).

Further analysis of specific T-cell subsets (naive, memory, and effector cells) revealed that the elevated CD4⁺/CD8⁺ ratio that was observed in patients with ISM was largely due to a significantly reduced number of circulating naive (CD62L⁺CD45RA⁺) CD8⁺ T cells (Figure 2, A). Although the median absolute number of all circulating CD4⁺ T-cell subsets measured in patients with ISM was not significantly different

from that in HCs (Figure 2, E-H), we noted that the elevated frequency of total CD4⁺ T cells in our ISM patient cohort was reflected in both the central memory (CD62L⁺CD45RA⁺) (Figure 2, F) and CD4⁺ T cells Effector Memory reexpressing RA (TEMRA)-cell (CD62L⁺CD45RA⁺) (Figure 2, G) compartments.

In contrast to the differences in the T-cell and NK-cell compartments, differences in peripheral B-cell subsets (eg, naive and switched memory cells) between patients with ISM and HCs were less striking (Figure 3). The significant differences in B-cell subsets between patients with ISM

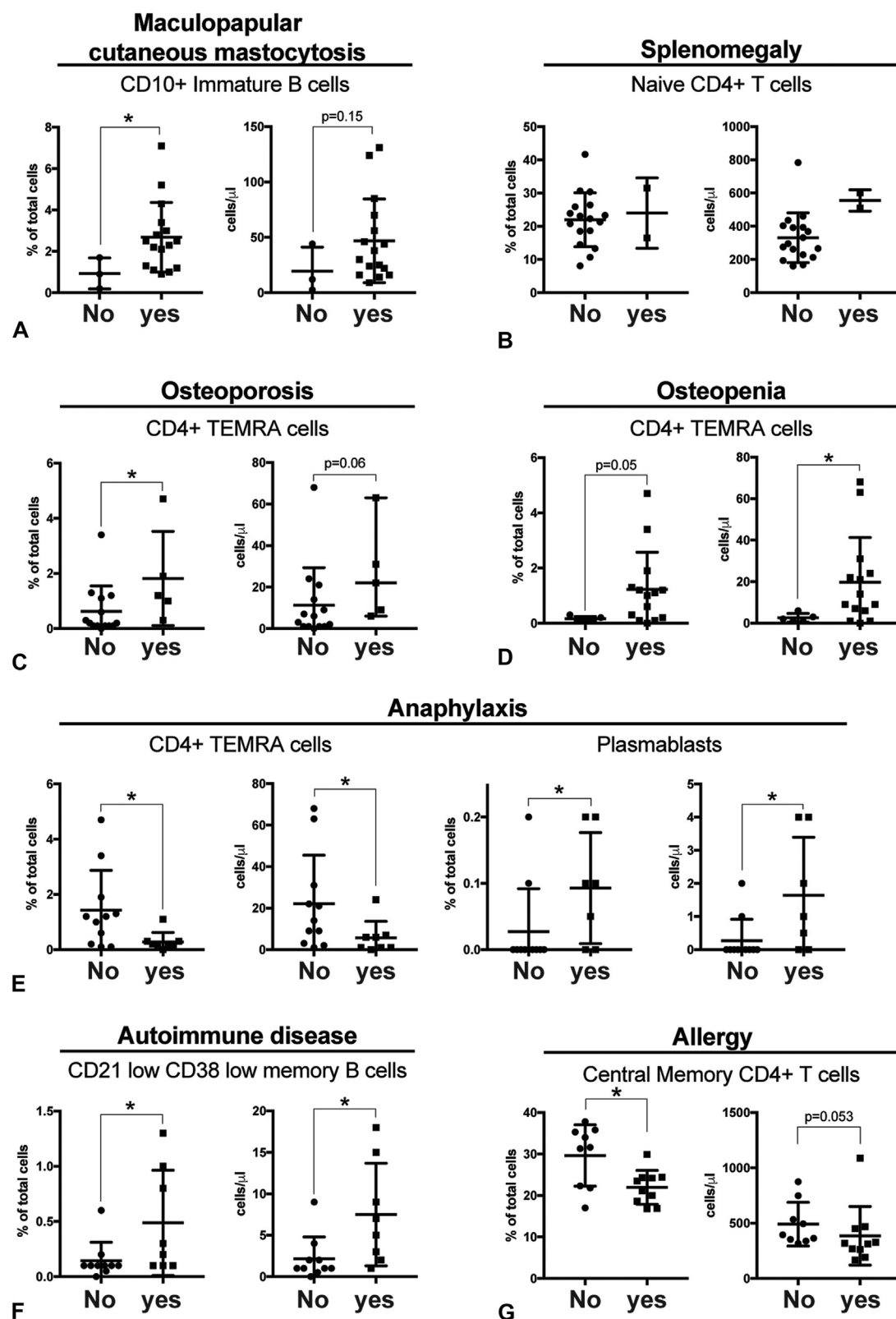


FIGURE 4. Peripheral lymphocyte populations in patients with ISM grouped according to clinical manifestations. The cohort of 20 patients with ISM was stratified on the basis of presence (yes) or absence (no) of various clinical manifestations (see Table I), and all peripheral lymphocyte subsets shown in Figures 1 and 2 were compared between clinical groupings within the ISM cohort using 2-tailed Mann-Whitney nonparametric analysis. (A-G) The median frequency (%) and absolute count (cell/μL) ± interquartile range of peripheral blood lymphocytes positive for the indicated phenotypic markers that were found to be significantly different among the indicated clinical groupings. * $P < .05$.

and HCs that were observed include lower absolute counts of $CD20^+CD27^+IgM^-$ (Figure 3, C) and $CD19^+CD24^{high}CD38^{high}$ (Figure 3, J) cells as well as both lower frequency and absolute count of $CD19^+CD24^-CD38^+$ cells (Figure 3, J). Although most patients with ISM in our cohort exhibited relatively normal peripheral B-cell counts, our analysis did identify 1 patient (ISM-4, Table I) with an almost complete absence of most B-cell populations in circulation (Figure 1, E, and Figure 3, red data point). Despite these findings, this patient maintains an active and healthy lifestyle with an uneventful history of minor inflammatory and infectious diseases, which include recurrent sinusitis, diverticulitis, and prostatitis; other lymphocyte populations as well as quantitative serum immunoglobulin levels were normal. This patient is currently being assessed to further characterize the etiology of this B-cell lymphopenia.

Correlation of peripheral lymphocyte counts with tryptase and *KIT* p.D816V allelic frequency

We next stratified the ISM cohort using 2 surrogate markers associated with mastocytosis disease severity (*KIT* p.D816V allelic frequency and BST) to determine whether lymphocyte phenotypic subsets segregate with these markers.^{14,15} Quantitative assessment of *KIT* p.D816V mutational burden (for individual patient values, see Table I) is of prognostic significance in predicting patient survival,^{6,16} and BST levels (see also Table I for values) generally correlate with the clinical severity of disease.¹⁷ Although we did indeed observe a significant positive correlation between *KIT* p.D816V allelic frequency and BST levels ($R^2 = 0.27$; $P = .019$), as has been reported for patients with indolent disease,¹⁸ we did not find any significant correlations between either BST levels or *KIT* p.D816V allelic frequency and the frequency or absolute count of any peripheral lymphocyte population measured (data not shown).

In addition to BST values, we determined the α - and β -tryptase genotypes of the patients in this study because a gene-dosage effect between the number of *TPSAB1* copies, BST levels, and severity of related clinical symptoms in some individuals had been reported.¹⁹ We found that 5 patients in our ISM cohort had hereditary alpha tryptasemia, defined by increased *TPSAB1* copy number (Table I, last column, bolded). On average, BST levels do appear higher among individuals with increased *TPSAB1* copy number than among those with normal copy number (J. J. Lyons, MD, unpublished data, 2019), although there was no significant difference in average BST levels between these groups within the ISM patient cohort evaluated for this study (data not shown). One of 5 patients with ISM with hereditary alpha tryptasemia who were evaluated had hepatosplenomegaly, and 2 of 5 had BST levels greater than 100 ng/mL; however, any potential conclusion drawn from these data would be limited by sample size. Furthermore, there was no significant association between the composition of peripheral lymphocyte subsets, and increased *TPSAB1* copy number was detected although the sample size was small (data not shown).

Associations between peripheral lymphocyte composition and clinical findings

We then stratified the results according to a spectrum of clinical manifestations associated with mastocytosis (see Table I, clinical history). Our analysis revealed several associations between the abundance of specific peripheral lymphocyte subsets

and several clinical parameters. We found that patients with urticaria pigmentosa (also known as maculopapular cutaneous mastocytosis) ($n = 17$, Table I) exhibited a significantly higher frequency of circulating $CD20^+CD10^+$ immature B cells ($P = .036$; Figure 4, A), whereas the few patients in the ISM cohort exhibiting splenomegaly ($n = 2$, Table I) were found to have higher absolute numbers of naive $CD4^+$ T cells ($CD62L^+CD45RA^+$) ($P = .047$, Figure 4, B). The frequency and total counts of TEMRA $CD4^+$ T cells were found to be significantly higher in patients with osteoporosis ($n = 5$, Table I) ($P = .038$, Figure 4, C) or osteopenia ($n = 15$, Table I) ($P = .045$, Figure 4, D). In contrast, patients with a documented history of systemic anaphylaxis ($n = 8$, Table I) exhibited lower numbers of circulating TEMRA $CD4^+$ T cells (absolute count, $P = .034$) alongside elevated numbers of circulating plasmablasts (absolute count, $P = .028$) (Figure 4, E).

Analysis of additional clinical groupings in our ISM cohort (as described earlier) revealed that patients with a history of autoimmune disease ($n = 8$, Table I) had significantly higher $CD19^+CD21^{low}CD38^{low}$ memory B cells ($P = .015$, Figure 3, E) and patients with moderate to severe allergy ($n = 11$, Table I) exhibited a lower frequency of central memory $CD4^+$ T cells ($CD62L^+CD45RA^-$) ($P = .028$, Figure 4, G). Finally, patients with a history of chronic or recurrent infections ($n = 10$, Table I) exhibited significantly decreased median counts of several circulating lymphocyte subsets including $CD4^+CD62L^-CD45RA^-$ effector memory T cells ($P = .0411$, Figure 5, A), transitional B cells ($CD19^+CD24^{high}CD38^{high}$) ($P = .009$, Figure 5, B), class-switched memory B cells ($CD20^+CD27^+IgM^-$) ($P = .009$, Figure 5, C), and several populations of immature $CD20^+CD10^+$ B cells (Figure 5, D-F).

DISCUSSION

Mast cells continue to emerge as key players in various physiologic and pathologic processes reflective of their functional plasticity and ability to release a broad array of bioactive mediators.^{5,20,21} Although it has been shown that mast cells and their secreted products are capable of modulating lymphocyte number,^{3,22} to date there has been no study focused on an analysis of lymphocyte populations in patients with mastocytosis who have both an increase in mast cell burden and an excess production of mediators. As we show in our prospective study, patients with systemic mastocytosis do exhibit several significant alterations in peripheral lymphocyte composition. We observed a significantly lower median frequency and absolute cell count of both circulating $CD8^+$ T cells and NK cells in our ISM cohort compared with normal healthy adults, a previously uncharacterized immunophenotype for patients with mastocytosis. Further analysis in this ISM cohort also uncovered significant associations between the frequency and/or absolute counts of several peripheral lymphocyte subsets with specific clinical parameters.

Despite our findings that patients with mastocytosis have lower counts of circulating $CD8^+$ T cells and NK cells compared with HCs, patients with systemic mastocytosis generally do not suffer from severe viral or bacterial infections that are characteristic of more severe deficiencies in these cell types.²³ Indeed, only 3 patients (15%) in our ISM cohort had a history of severe viral infections. However, patients from our ISM cohort with any history of either chronic or recurrent viral or bacterial infections (50%) did exhibit significantly lower absolute counts of several

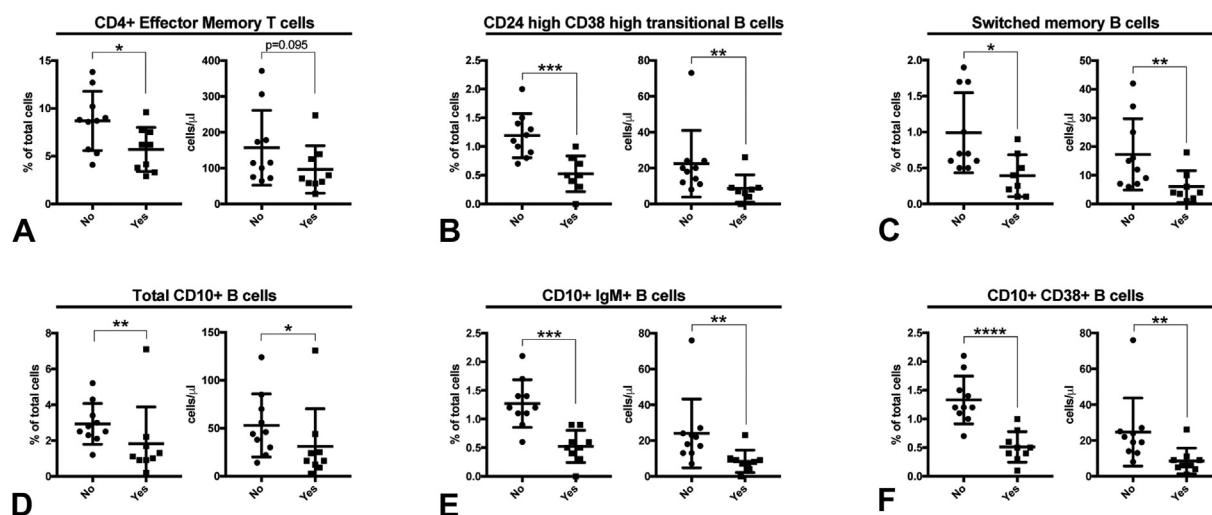


FIGURE 5. Peripheral lymphocyte populations in patients with ISM grouped according to a clinical history of recurrent/chronic infections. The cohort of 20 patients with ISM was stratified on the basis of presence (yes) or absence (no) of chronic or recurrent infectious disease (see Table I), and all peripheral lymphocyte subsets shown in Figures 1 and 2 were compared between clinical groupings within the ISM cohort using 2-tailed Mann-Whitney nonparametric analysis. (A-F) The median frequency (%) and absolute count (cell/μL) $\pm$ interquartile range of peripheral blood lymphocytes positive for the indicated phenotypic markers that were found to be significantly different among patients grouped as either (–) or (+). * $P < .05$. ** $P < .01$. *** $P < .001$. **** $P < .0001$.

circulating B-cell populations (Figure 5). Such observations suggest that further study is warranted to determine whether the association of these circulating lymphocyte populations with susceptibility to infection has prognostic value for patients with systemic mastocytosis.

By stratifying our ISM patient cohort on the basis of a series of additional clinical parameters, we found that terminally differentiated TEMRA cells were significantly higher in patients with osteoporosis or osteopenia (Figure 4, C and D). Although no significant differences were observed when comparing counts of circulating TEMRA cells between patients with ISM and HCs (Figure 2, D and H), we did find that this subpopulation was significantly lower in those patients with ISM with a history of severe anaphylaxis (Figure 4, E). TEMRA cells are a T-cell subset that exhibit a limited proliferative capacity, are generally negative for the cell surface markers CD27 and CD28, and express markers of cellular senescence.^{24,25} The observation that patients in our ISM cohort exhibiting osteopenia/osteoporosis have increased circulating TEMRA cells compared with patients with normal bone density is not without precedent.²⁶ In a recent study examining the pathogenesis of osteoporosis in 107 patients with rheumatoid arthritis and 75 patients without rheumatoid arthritis with primary osteopenia/osteoporosis, elevated frequencies of CD4⁺CD28[–] senescent T cells compared with patients with normal bone mass were observed in both groups.²⁶ The authors went on to show that stimulation of T cells with a mast cell–associated cytokine IL-15 led to increased expression of RANKL, a mediator implicated in bone loss.^{27,28} Although the pathogenesis of osteoporosis in patients with mastocytosis has been attributed to an increase in circulating mast cell–associated products, the specific mediators and pathways involved remain largely uncharacterized.²⁹ Given our findings it is tempting to speculate that elevated mast cell–associated products may contribute to bone remodeling by modulating specific T-cell subsets in patients with mastocytosis.³⁰

Patients in our ISM cohort with a history of autoimmune disease also had significantly higher CD19⁺CD21^{low}CD38^{low} memory B cells (Figure 4, F). CD21^{–/low} B cells in humans have been described in several disorders associated with chronic inflammation.^{31–34} It has been posited that an increased frequency of CD21^{–/low} B cells observed in patients with HIV, hepatitis C, and malaria infections, as well as common variable immunodeficiency and in various autoimmune diseases, could be due to an expansion of this B-cell subset in response to chronic activation.³⁵ Although there was no significant difference in circulating CD19⁺CD21^{low}CD38^{low} cells when comparing patients with ISM to HCs (Figure 3, H), it is possible that mast cells or mast cell products in these patients could further modulate preexisting autoimmune conditions³⁶ associated with chronic activation of lymphocytes.

We thus found a number of associations between the quantity of specific circulating lymphocyte subpopulations and specific clinical conditions in patients with ISM. Our cohort included a significant population of patients with autoimmune and inflammatory phenotypes, which, given the rarity of mastocytosis, may be somewhat skewed by referral bias. These observations suggest the need for larger studies with possible disease-matched controls and investigation into how an expanded mast cell population and elevated levels of mast cell–derived mediators result in lymphocyte skewing and how such alterations might affect specific aspects of clinical disease and prognosis. These findings are relevant not only to mast cell proliferative disorders but also to diseases of mast cell activation and associated pathology.

REFERENCES

- Carter MC, Metcalfe DD, Komarow HD. Mastocytosis. *Immunol Allergy Clin North Am* 2014;34:181–96.
- Metcalfe DD, Mekori YA. Pathogenesis and pathology of mastocytosis. *Annu Rev Pathol* 2017;12:487–514.

3. Mekori YA, Hershko AY, Frossi B, Mion F, Pucillo CE. Integrating innate and adaptive immune cells: mast cells as crossroads between regulatory and effector B and T cells. *Eur J Pharmacol* 2016;778:84-9.
4. Valitutti S, Espinosa E. Cognate interactions between mast cells and helper T lymphocytes. *Self Nonsell* 2010;1:114-22.
5. 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.
6. Valent P, Akin C, Metcalfe DD. Mastocytosis: 2016 updated WHO classification and novel emerging treatment concepts. *Blood* 2017;129:1420-7.
7. Valiathan R, Deeb K, Diamante M, Ashman M, Sachdeva N, Asthana D. Reference ranges of lymphocyte subsets in healthy adults and adolescents with special mention of T cell maturation subsets in adults of South Florida. *Immunobiology* 2014;219:487-96.
8. Kuehn HS, Boisson B, Cunningham-Rundles C, Reichenbach J, Stray-Pedersen A, Gelfand EW, et al. Loss of B cells in patients with heterozygous mutations in IKAROS. *N Engl J Med* 2016;374:1032-43.
9. Kristensen T, Vestergaard H, Moller MB. Improved detection of the KIT D816V mutation in patients with systemic mastocytosis using a quantitative and highly sensitive real-time qPCR assay. *J Mol Diagn* 2011;13:180-8.
10. Lyons JJ, Yu X, Hughes JD, Le QT, Jamil A, Bai Y, et al. Elevated basal serum tryptase identifies a multisystem disorder associated with increased TPSAB1 copy number. *Nat Genet* 2016;48:1564-9.
11. Mao X, Hu B, Zhou Z, Xing X, Wu Y, Gao J, et al. Vitamin D levels correlate with lymphocyte subsets in elderly patients with age-related diseases. *Sci Rep* 2018;8:7708.
12. Rossini M, Adams S, Viapiana O, Fracassi E, Ortolani R, Vella A, et al. Long-term effects of amino-bisphosphonates on circulating gammadelta T cells. *Calcif Tissue Int* 2012;91:395-9.
13. Sampson HA, Munoz-Furlong A, Campbell RL, Adkinson NF Jr, Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: summary report—second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. *Ann Emerg Med* 2006;47:373-80.
14. Furitsu T, Tsujimura T, Tono T, Ikeda H, Kitayama H, Koshimizu U, et al. Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product. *J Clin Invest* 1993;92:1736-44.
15. Nagata H, Worobec AS, Oh CK, Chowdhury BA, Tannenbaum S, Suzuki Y, et al. Identification of a point mutation in the catalytic domain of the proto-oncogene c-kit in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder. *Proc Natl Acad Sci U S A* 1995;92:10560-4.
16. Jara-Acevedo M, Teodosio C, Sanchez-Munoz L, Alvarez-Twose I, Mayado A, Caldas C, et al. Detection of the KIT D816V mutation in peripheral blood of systemic mastocytosis: diagnostic implications. *Mod Pathol* 2015;28:1138-49.
17. Akin C, Metcalfe DD. Surrogate markers of disease in mastocytosis. *Int Arch Allergy Immunol* 2002;127:133-6.
18. Matito A, Morgado JM, Alvarez-Twose I, Sanchez-Munoz L, Pedreira CE, Jara-Acevedo M, et al. Serum tryptase monitoring in indolent systemic mastocytosis: association with disease features and patient outcome. *PLoS One* 2013;8:e76116.
19. Lyons JJ. Hereditary alpha tryptasemia: genotyping and associated clinical features. *Immunol Allergy Clin North Am* 2018;38:483-95.
20. Gilfillan AM, Beaven MA. Regulation of mast cell responses in health and disease. *Crit Rev Immunol* 2011;31:475-529.
21. Krystel-Whittemore M, Dileepan KN, Wood JG. Mast cell: a multi-functional master cell. *Front Immunol* 2015;6:620.
22. Merluzzi S, Betto E, Ceccaroni AA, Magris R, Giunta M, Mion F. Mast cells, basophils and B cell connection network. *Mol Immunol* 2015;63:94-103.
23. Mace EM, Orange JS. Emerging insights into human health and NK cell biology from the study of NK cell deficiencies. *Immunol Rev* 2019;287:202-25.
24. Lugli E, Goldman CK, Perera LP, Smedley J, Pung R, Yovandich JL, et al. Transient and persistent effects of IL-15 on lymphocyte homeostasis in nonhuman primates. *Blood* 2010;116:3238-48.
25. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 1999;401:708-12.
26. Fessler J, Husic R, Schwetz V, Lerchbaum E, Aberer F, Fasching P, et al. Senescent T-cells promote bone loss in rheumatoid arthritis. *Front Immunol* 2018;9:95.
27. Okabe I, Kikuchi T, Mogi M, Takeda H, Aino M, Kamiya Y, et al. IL-15 and RANKL play a synergistically important role in osteoclastogenesis. *J Cell Biochem* 2017;118:739-47.
28. Jones DH, Kong YY, Penninger JM. Role of RANKL and RANK in bone loss and arthritis. *Ann Rheum Dis* 2002;61:ii32-i39.
29. Rossini M, Zanotti R, Viapiana O, Tripi G, Orsolini G, Idolazzi L, et al. Bone involvement and osteoporosis in mastocytosis. *Immunol Allergy Clin North Am* 2014;34:383-96.
30. Orinska Z, Maurer M, Mirghomizadeh F, Bulanova E, Metz M, Nashkevich N, et al. IL-15 constrains mast cell-dependent antibacterial defenses by suppressing chymase activities. *Nat Med* 2007;13:927-34.
31. Terrier B, Joly F, Vazquez T, Benech P, Rosenzweig M, Carpentier W, et al. Expansion of functionally anergic CD21-/low marginal zone-like B cell clones in hepatitis C virus infection-related autoimmunity. *J Immunol* 2011;187:6550-63.
32. Isnardi I, Ng YS, Menard L, Meyers G, Saadoun D, Srdanovic I, et al. Complement receptor 2/CD21- human naive B cells contain mostly autoreactive unresponsive clones. *Blood* 2010;115:5026-36.
33. Wehr C, Eibel H, Masilamani M, Illges H, Schlesier M, Peter HH, et al. A new CD21low B cell population in the peripheral blood of patients with SLE. *Clin Immunol* 2004;113:161-71.
34. Saadoun D, Terrier B, Bannock J, Vazquez T, Massad C, Kang I, et al. Expansion of autoreactive unresponsive CD21-/low B cells in Sjogren's syndrome-associated lymphoproliferation. *Arthritis Rheum* 2013;65:1085-96.
35. Thorarinsdottir K, Camponeschi A, Cavallini N, Grimsholm O, Jacobsson L, Gertsson I, et al. CD21(-/low) B cells in human blood are memory cells. *Clin Exp Immunol* 2016;185:252-62.
36. Latar I, Koufany M, Hablot J, Loeuille D, Netter P, Jouzeau JY, et al. Association between rheumatoid arthritis and systemic mastocytosis: a case report and literature review. *Clin Rheumatol* 2016;35:2619-23.

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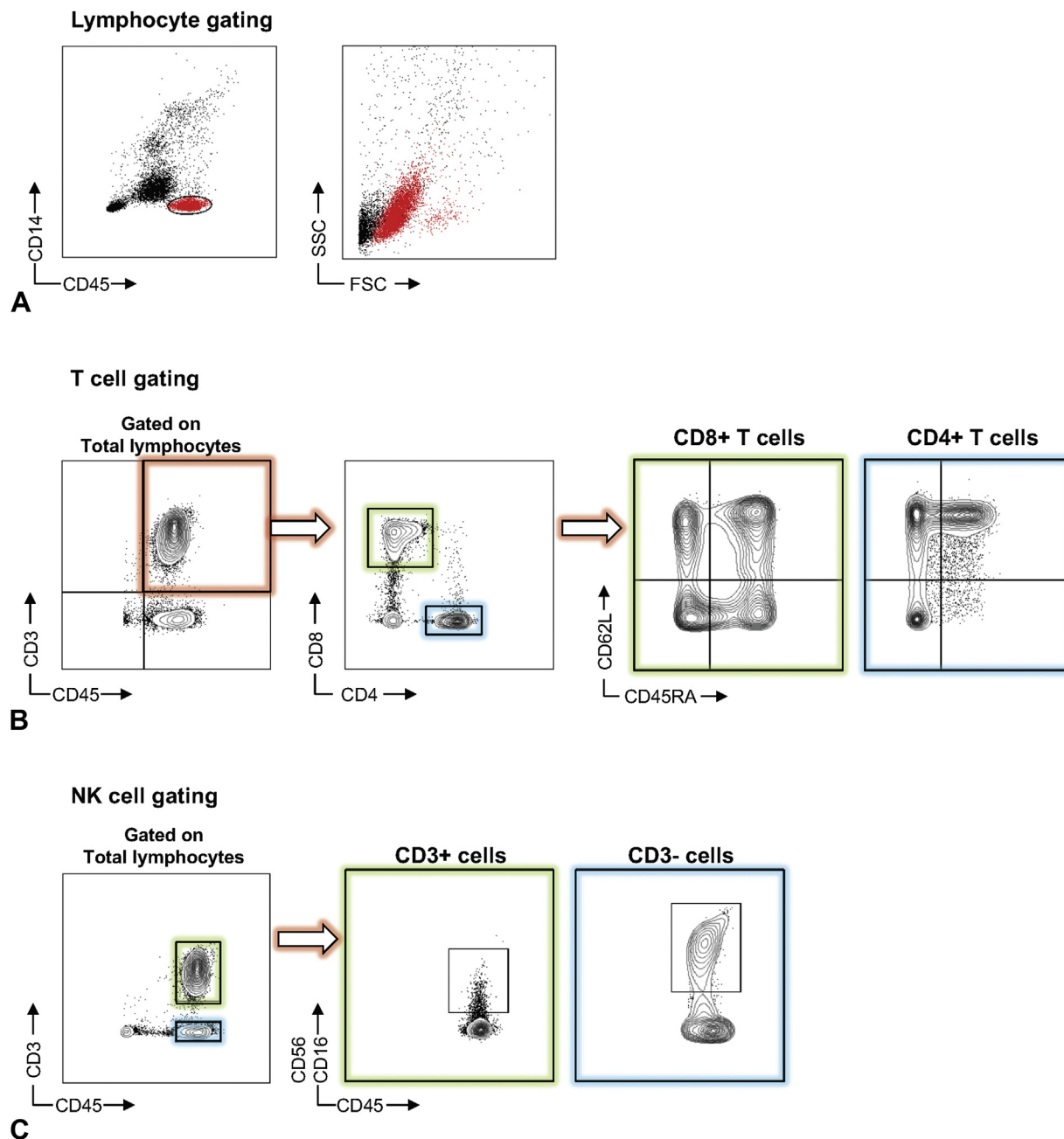


FIGURE E1. Basic gating strategy for identification of T cells and NK cells used by the NIH Clinical Center. (A) CD14/CD45 expression and forward- and side-scatter profile for CD45⁺CD14⁻ lymphocytes (shown in red). (B) Representative gating used to identify naive, memory, and effector subpopulations of CD3⁺CD45⁺ CD8⁺ and CD4⁺ T cells based on CD62L and CD45RA expression. (C) Representative gating used to identify CD3⁻CD45⁺CD56/CD16^{bright} NK cells and CD3⁺CD45⁺CD56/CD16^{int} NK T cells. All gates shown were established using background fluorescence obtained with isotype- and fluorophore-matched control antibodies. *FSC*, Forward scatter; *SSC*, side scatter.

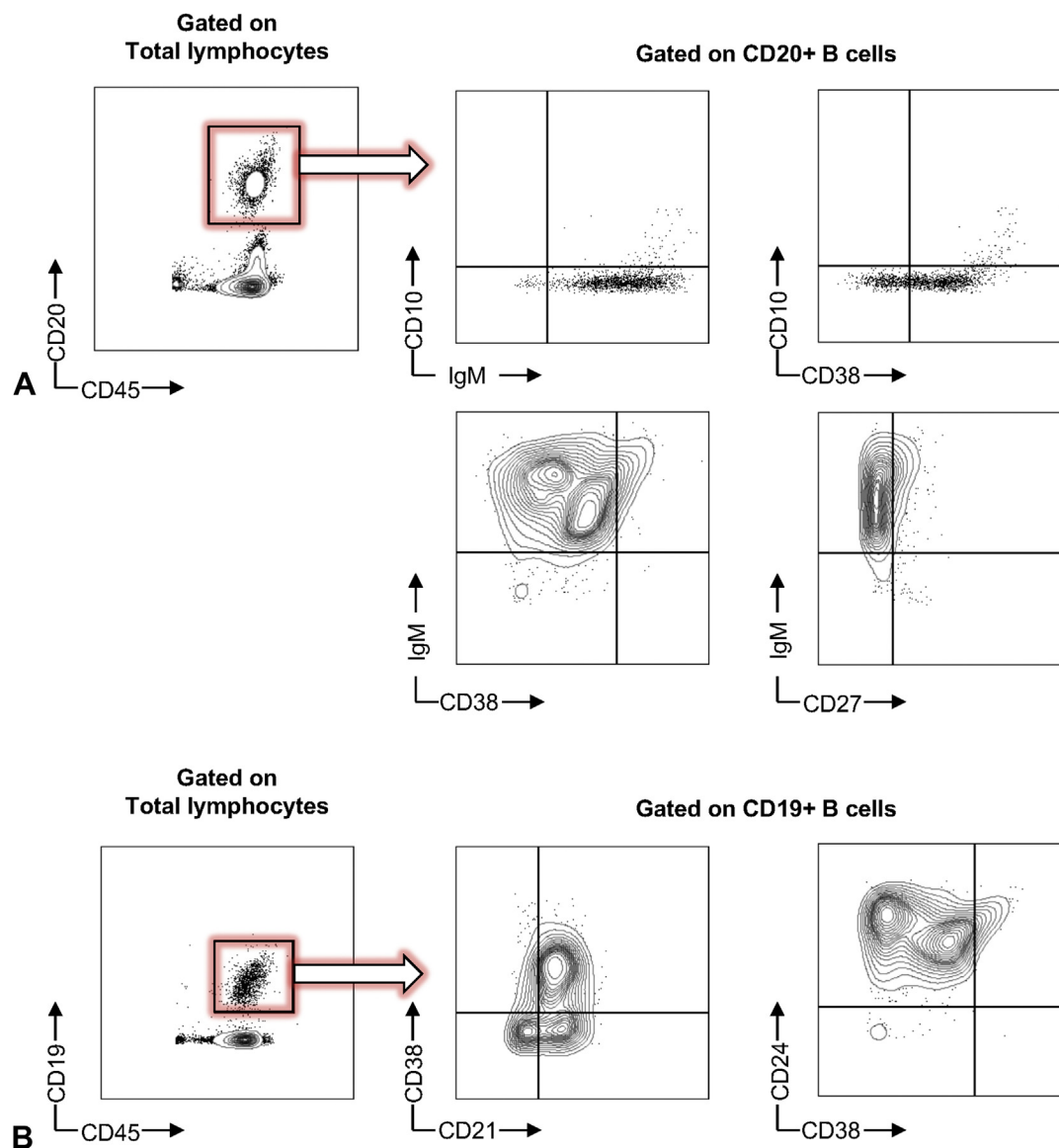


FIGURE E2. Basic gating strategy for identification of B-cell subsets used by the NIH Clinical Center. **(A)** Representative gating for CD10, CD38, and CD27 and surface IgM expression on CD45⁺CD20⁺ peripheral lymphocytes. **(B)** Representative gating for CD21, CD24, and CD38 expression on CD45⁺CD19⁺ peripheral lymphocytes. All gates shown were established using background fluorescence obtained with isotype- and fluorophore-matched control antibodies.