CD117 expression in gammopathies is associated with an altered maturation of the myeloid and lymphoid hematopoietic cell compartments and favorable disease features

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Supplementary data

Design and Methods

Multiparameter flow cytometry studies

Positivity for a particular antigen was recorded when 20% or more of the respective population of plasma cells showed a fluorescence intensity superior to the internal negative control (unstained bone marrow plasma cells); the percentage of CD117$^+$ plasma cells was systematically referred to (mono)clonal plasma cells. (Mono)clonal and normal plasma cells, lymphocytes, neutrophils, mast cells, CD117$^+$ precursors and different maturation-associated B-cell subsets were identified in bone marrow samples from all patients using either 4-color (41 symptomatic and 35 smoldering multiple myeloma patients) or 8-color (9 symptomatic multiple myeloma, 3 smoldering multiple myeloma and all monoclonal gammopathy of undetermined significance patients) immunostaining panels of fluorochrome-conjugated monoclonal antibodies based on the following combinations of reagents: fluorescein isothiocyanate (FITC)/phycoerythrin (PE)/peridinin chlorophyll protein-cyanin 5.5 (PerCP-Cy5.5)/allophycocyanin (APC) and FITC/PE/PerCP-Cy5.5/PE-Cyanin7 (PE-Cy7)/APC/APC H7/pacific blue (PB)/pacific orange (PO) –: CD38/CD56/CD19/CD45, CD38/CD27/CD45/CD28, β-2 microglobulin/CD81/CD38/CD117 and CD38/CD56/β-2 microglobulin/CD19/cytoplasmic immunoglobulin kappa light chain (cyIgκ)/cyIg lambda light chain (cyIgλ)/CD45/CD138, CD38/CD28/CD27/CD19/CD117/CD81/CD45/CD138, respectively.

(Mono)clonal and normal plasma cells were specifically identified on the basis of their unique CD19, CD38, CD45, CD56 and CD117 immunophenotypic profiles as previously described.[1] Mast cells were identified by their bright CD117 expression, CD117$^+$ erythroid and neutrophil precursors were identified on the basis of their CD117 expression and different light scatter and/or CD45 expression properties (CD117$^+$CD45$^{-/lo}$SSC^{lo} *vs.* CD117$^+$CD45^{lo}SSC^{int}), whereas pro-B and pre-B cells (both CD19$^+$CD38^{hi}) were each identified according to their characteristic CD45 expression and differentiated from mature B cells according to the CD19$^+$CD38$^-$ phenotype of the latter cells.[2,3]

In a subgroup of 26 bone marrow samples from symptomatic and smoldering multiple myeloma patients the distribution of different subsets of CD34$^+$ hematopoietic progenitor cells was also analyzed with an 8-color combination of monoclonal antibodies (CD20/CD45/CD34/CXCR4/CD19/CD10/CD27/CD38) aimed at the specific identification and enumeration of CD34$^+$ immature (CD34$^+$CD38$^{-/dim}$), myeloid (CD34$^+$CD19$^-$CD38$^+$) and lymphoid (CD34$^+$CD19$^+$CD38$^+$) cell subsets, within bone marrow CD34$^+$ hematopoietic progenitor cells.

Immunophenotyping of peripheral blood samples was carried out in parallel in a subgroup of 60 untreated symptomatic and smoldering multiple myeloma patients with the following 8-color combinations of monoclonal antibodies: PB/PO/FITC/PE/PerCP-Cy5.5/PE-Cy7/APC/alexafluor 700 (AF700); CD20/CD45/surface(s)IgM or sIgλ/sIgG, sIgA or sIgκ/CD19/CD10/CD27/CD38. Different B-cell subsets including immature (CD10$^+$CD27$^-$CD38$^+$), naïve (CD10$^-$CD27$^-$CD38$^-$) and memory (CD10$^-$CD27$^+$CD38$^-$) B cells, in addition to (mono)clonal and normal plasma cells (CD10$^-$CD38$^{+/++}$) were defined as recently reported.[4]

Fluorescence in situ *hybridization and DNA ploidy analyses*

Briefly, *IGH* gene rearrangements were identified with a panel of LSI *IGH* dual-color break-apart rearrangement probes (Vysis, Downers Grove, IL, USA). Presence of del(13q) and del(17p) was analyzed with the LSI 13 (RB1) and LSI P53 (17p13.1) probes (Vysis), respectively. Flow cytometry DNA ploidy analyses were based on a double staining for nuclear DNA (propidium iodide) and surface plasma cell antigens (anti-CD38 and anti-CD138 monoclonal antibodies). The DNA index was defined to be the ratio between the modal channel of the G0-G1 peak of plasma cells and the G0-G1 peak of the remaining residual normal cell populations. Hypodiploidy was defined when the DNA index was 0.9 or lower, hyperdiploidy when it was 1.08 or higher; all other cases with a DNA index greater than 0.9 and less than 1.08 were classified as diploid.

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Online Supplementary Table S1. Distribution of peripheral blood cell subsets and hemoglobin concentration in CD117$^+$ *versus* CD117$^-$ MM patients.

Variable	CD117$^+$ (n=32)	CD117$^-$ (n=56)	*P* value
N. of leukocytes (×10^9/L)	4.9 (2.6-8.1, 23)	5.6 (1.9-9.0, 45)	NS
N. of lymphocytes (×10^9/L)	1.9 (1.1-3.9, 21)	1.5 (0.6-3.9, 36)	NS
N. of neutrophils (×10^9/L)	2.5 (0.8-4.2, 21)	2.9 (1.4-6.2, 35)	0.04
Hemoglobin (g/L)	116 (82-147, 22)	116 (80-148, 44)	NS
M-PC (×10^6/L)	0.78 (0-19.32, 16)	0.73 (0-108.75, 44)	NS
N-PC (×10^6/L)	0.49 (0-4.02, 15)	0.47 (0-5.21, 42)	NS
B cells (×10^6/L)	56 (6-164, 16)	94 (3-485, 43)	NS
Immature (×10^6/L)	1.6 (0.1-4.5, 16)	1.1 (0-26.2, 43)	NS
Naïve (×10^6/L)	34 (2-118, 16)	57 (1-340, 43)	NS
Memory (×10^6/L)	28 (4-61, 16)	27 (2-143, 43)	NS
IgM$^+$ (×10^6/L)	14 (2-41, 13)	13 (0-100, 39)	NS
IgM$^-$ (×10^6/L)	11 (3-24, 13)	13 (2-75, 39)	NS

Results expressed as median (range, number of evaluable patients). M-PC [(mono) clonal plasma cells], N. (number), N-PC (normal plasma cells), NS (not significant).

Online Supplementary Table S2. Ratio between the number of peripheral blood neutrophils and hemoglobin levels and the percentage of bone marrow CD117$^+$ neutrophil and erythroid precursors in CD117$^+$ *versus* CD117$^-$ MM and MGUS patients.

Variable	MM CD117+ (n=32)	MM CD117- (n=56)	*P* value	MGUS CD117+ (n=13)	MGUS CD117- (n=5)	*P* value
N. of PB neutrophils (×10^9/L) / % of BM CD117$^+$ precursor ratio	1.2 (0.4-4.9, 21)	2.1 (0.7-14.4, 35)	<0.001*	4.2 (1.5-11.6, 8)	3.7 (0.8-4.9, 5)	NS
N. of PB neutrophils (×10^9/L) / % of BM CD117$^+$ neutrophil precursor ratio	3.9 (1.2-22.1, 21)	6.6 (1.5-82.5, 35)	0.003*	15.0 (2.6-28.3, 8)	11.5 (2.3-13.7, 5)	NS
Hemoglobin (g/L) / % of BM CD117$^+$ erythroid precursor ratio	191 (80-664, 22)	236 (87-1258, 44)	NS	350 (141-688, 8)	575 (143-1355, 5)	NS

*Results expressed as median (range, number of evaluable patients), whereas percentages of CD117$^+$ precursors refer to all BM cells. *These differences remained significant if considering only SMM patients (data not shown). BM (bone marrow), MGUS (monoclonal gammopathy of undetermined significance), MM (multiple myeloma), N. (number), NS (not significant), PB (peripheral blood).*