Analysis of the immune system of multiple myeloma patients achieving long-term disease control by multidimensional flow cytometry

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ABSTRACT

Multiple myeloma remains largely incurable. However, a few patients experience more than 10 years of relapse-free survival and can be considered as operationally cured. Interestingly, long-term disease control in multiple myeloma is not restricted to patients with a complete response, since some patients revert to having a profile of monoclonal gammopathy of undetermined significance. We compared the distribution of multiple compartments of lymphocytes and dendritic cells in the bone marrow and peripheral blood of multiple myeloma patients with long-term disease control (n=28), patients with newly diagnosed monoclonal gammopathy of undetermined significance (n=23), patients with symptomatic multiple myeloma (n=23), and age-matched healthy adults (n=10). Similarly to the patients with monoclonal gammopathy of undetermined significance and symptomatic multiple myeloma, patients with long-term disease control showed an expansion of cytotoxic $CD8^+$ T cells and natural killer cells. However, the numbers of bone marrow T-regulatory cells were lower in patients with long-term disease control than in those with symptomatic multiple myeloma. It is noteworthy that B cells were depleted in patients with monoclonal gammopathy of undetermined significance and in those with symptomatic multiple myeloma, but recovered in both the bone marrow and peripheral blood of patients with long-term disease control, due to an increase in normal bone marrow B-cell precursors and plasma cells, as well as pre-germinal center peripheral blood B cells. The number of bone marrow dendritic cells and tissue macrophages differed significantly between patients with long-term disease control and those with symptomatic multiple myeloma, with a trend to cell count recovering in the former group of patients towards levels similar to those found in healthy adults. In summary, our results indicate that multiple myeloma patients with long-term disease control have a constellation of unique immune changes favoring both immune cytotoxicity and recovery of B-cell production and homing, suggesting improved immune surveillance.

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Introduction

With the introduction of high dose therapy/ autologous stem cell transplantation as well as novel agents (up-front), most patients with multiple myeloma (MM) achieve a transient remission; however, the vast majority relapse within a median of 3 years from diagnosis.[1] Thus, long-term follow-up studies in the setting of high dose therapy/ autologous stem cell transplantation show that only a small fraction of MM patients (6-18%) remain relapse free for 10 years or more, and these patients are now considered as being operationally cured.[2-4] Interestingly, this operational cure is not restricted to patients in complete response, since those who revert to having a monoclonal gammopathy of undetermined significance (MGUS)-like profile may also achieve long-term disease control (LTDC), despite persistence of a residual M-component.[4] Recent clinical and molecular data suggest that some features may help to identify this group of LTDC-MM patients such as an evolving smoldering pattern, a gene expression profile signature of MGUS and the CD2 molecular subtype.[5,6] Collectively, these findings suggest that in addition to anti-myeloma therapy, other factors may play a critical role in disease control.

For decades, increasing evidence has shown that the immune system is dysfunctional and impaired in active MM.[7-10] Accordingly, B-cell precursors and normal plasma cells are compromised, and immune paresis is also a consistent finding in newly-diagnosed MM patients.[11] In turn, effector cells such

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as natural killer (NK) cells and cytotoxic CD8+ T cells are expanded in both the bone marrow (BM) and peripheral blood (PB), but they are unable to control disease progression, suggesting a marked immunosuppressive microenvironment.[12,13] Moreover, dendritic cells (DC) have also been reported to be altered in MM, with reductions in circulating myeloid DC (m-DC) and plasmacytoid DC (p-DC), lower expression of co-stimulatory molecules and impaired induction of allogeneic T-cell responses.[14-16]

Since these immune defects are invariably present in active MM, we hypothesized that the immune system may play a critical role in LTDC-MM patients. To explore this possibility, in the present study we compared the distribution of many different subsets of T- B - and NK-lymphocytes and DC in both the BM and PB of MM patients who achieved long term disease control (LTDC-MM) *versus* the distributions in patients with newly diagnosed MGUS and symptomatic MM, as well as in healthy adults of similar age.

Design and Methods

Patients, controls and samples

A total of 74 patients with plasma cell disorders were prospectively studied. The group under investigation was composed of LTDC-MM patients (n=28), defined as either: (i) MM patients achieving complete response after up-front therapy and remaining relapse free for >5 years (n=11), or (ii) patients achieving a near complete response or partial response and remaining progression-free for ≥3 years without therapy (n=17) (MGUS-like profile). As controls, newly-diagnosed MGUS patients (n=23) and symptomatic MM patients (n=23), as well as age-matched healthy adult volunteers (n=10) undergoing orthopedic surgery, were studied. All samples from controls and patients were collected after informed consent had been given by each individual, according to the local ethical committee requirements and the Helsinki Declaration. Most LTDC-MM patients (90%) were enrolled in the GEM 2000 protocol and, therefore, received six induction cycles of VBMCP/VBAD followed by high-dose therapy/autologous stem cell transplantation and maintenance with interferon/prednisone. At the time of the present study, 85% of these patients were off any anti-myeloma therapy for >4 years. The median time from diagnosis was 9 years (range, 5 to 20 years). The study was conducted on paired BM and PB samples collected at the same time for each individual. Overall, a total of 168 samples were obtained with the following distribution per group: LTDC-MM (median age of 70 years; range, 52 to 85 years), 56 samples; MGUS (median age of 71 years; range, 43 to 93 years), 46 samples; symptomatic MM (median age of 74 years; range, 50 to 89 years), 46 samples and; healthy adults (median age of 64 years; range, 40 to 89 years), 20 samples.

Multiparametric flow cytometry immunophenotypic studies

EDTA-anticoagulated fresh BM and PB samples (approximately 1 mL) from each subject were immunophenotyped during the first 24 h, using an eight-color direct immunofluorescence technique previously described in detail.[17,18] The monoclonal antibody combinations – Pacific blue (PacB), fluorescein isothiocyanate (FITC), Pacific orange (PacO), phycoerythrin (PE), peridinin chlorophyll protein-cyanin 5 (PerCPCy5), PE-cyanin 7(PECy7), allophycocyanin (APC), Alexafluor 700 (AF700) or APCH7 used with the aim of quantifying and characterizing B cells, T cells, NK cells and DC, as well as their subsets, were as follows: (i) CD4, CD45, CD45RA, CD127, CD8, CD25, CD56, CD3; (ii) HLADR, CD45,

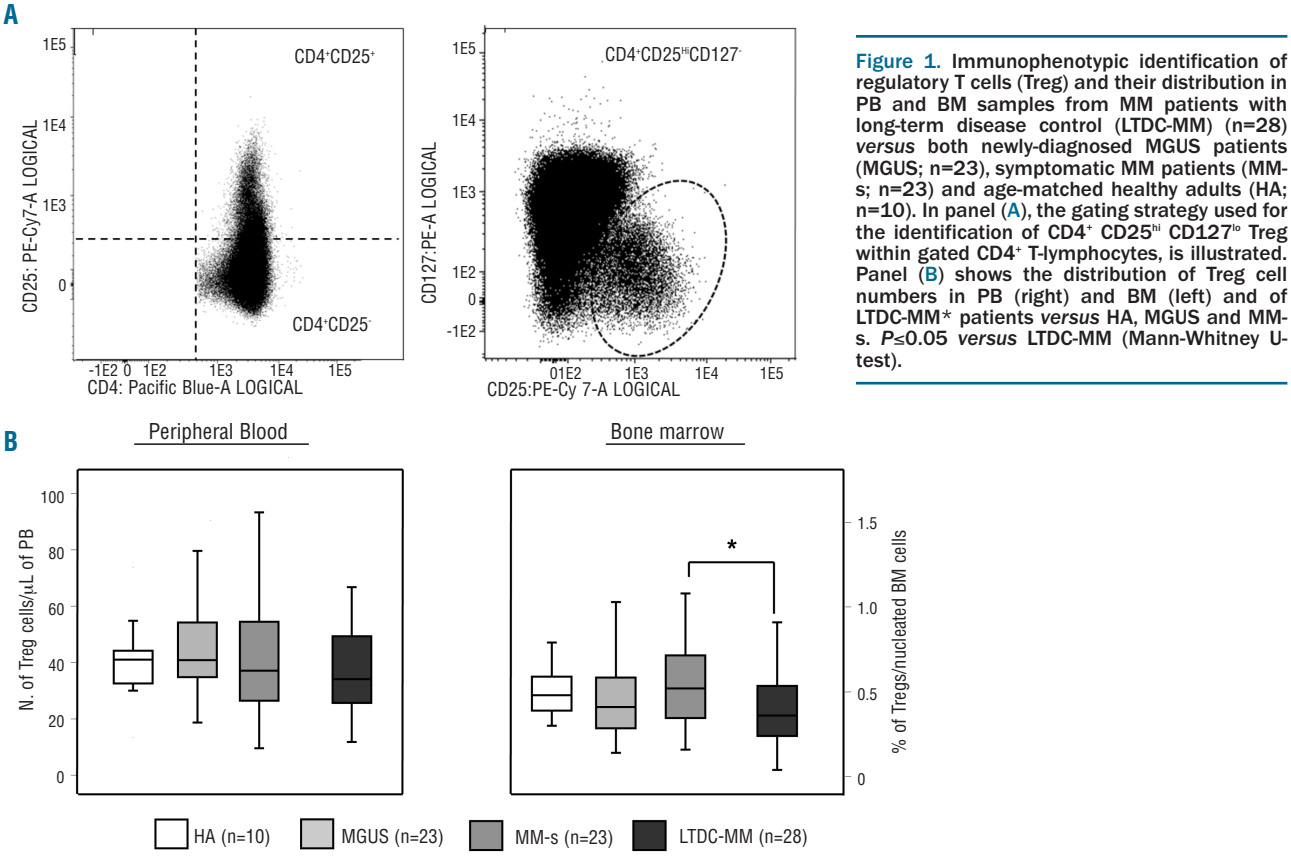

Figure 1. Immunophenotypic identification of regulatory T cells (Treg) and their distribution in PB and BM samples from MM patients with long-term disease control (LTDC-MM) (n=28) *versus* both newly-diagnosed MGUS patients (MGUS; n=23), symptomatic MM patients (MM-s; n=23) and age-matched healthy adults (HA; n=10). In panel (A), the gating strategy used for the identification of $CD4^+$ $CD25^{hi}$ $CD127^{lo}$ Treg within gated $CD4^+$ T-lymphocytes, is illustrated. Panel (B) shows the distribution of Treg cell numbers in PB (right) and BM (left) and of LTDC-MM* patients *versus* HA, MGUS and MM-s. $P \leq 0.05$ *versus* LTDC-MM (Mann-Whitney U-test).

CD16, CD33, CD34, CD117, CD123, CD14; (iii) CD20, CD45, CD62L, CXCR4, CD19, CD27, CD10+TCRγδ, CD38; (iv) CD20, CD45, surface membrane immunoglobulin (SmIg)G, SmIgA, CD19, CD27, SmIgM, CD38; CD45, CD38, CD56, CD138, CD19, CyK+TCRγδ and Cyλ. Based on these combinations the following populations of lymphocytes were identified in PB and BM samples: total $CD3^+$ T cells and their major $CD3^+/CD4^+$, $CD3^+/CD8^+$ and $TCR\gamma\delta^+$ cell subsets plus $CD4^+/CD25^+/CD127^-$ regulatory T cells (Treg) (Figure 1A), in addition to total $CD3^-/CD56^+$ NK cells, $CD14^+$ monocytes and DC subsets (e.g. circulating $HLA\text{-}DR^+/CD16^+$ tissue macrophages, $HLA\text{-}DR^+/CD123^+$ p-DC and $HLA\text{-}DR^+/CD33^+$ m-DC) (Figure 2A), as well as total $CD19^+$ B-cells and their maturation-associated subsets in PB - immature ($CD10^+$, $CD27^-$, $CD38^+$), naïve ($CD10^-$, $CD27^-$, $CD38^-$) and memory ($CD10^-$, $CD27^+$, $CD38^-$) B-lymphocytes plus plasmablasts/plasma cells ($CD27^{++}$, $CD38^{++}$, $CD10^-$) - and in BM - B-cell precursors ($CD19^+$, $CD38^+$, $CD45^{lo}$), naïve plus memory ($CD19^+/CD38^-/CD45^+$) B-lymphocytes and normal plasma cells ($CD138^+/CD38^+/CD19^+/CD45^+/CD56^-$) - (Figure 3A), as described previously.[17,19-21] In all cases, data were acquired in a FACSCanto II flow cytometer (BDB) using the FACSDiva software (version 6.1, BDB) for ≥ 10^6 leukocytes/tube using the EuroFlow instrument setup data acquisition standard operating procedures.[22] INFINICYT software (Cytognos SL, Salamanca, Spain) was used for the data analysis.

Statistical methods

The number of PB cell populations was recorded as absolute number of cells/μL while BM cell populations were described as percentages of all BM nucleated cells, after correcting for the levels of plasma cell infiltration. The median and mean values (and their standard deviation) and range were calculated for all the parameters. The Mann-Whitney U test was used to evaluate the statistical significance of differences observed between groups. The SPSS software package (version 15.0, SPSS, Inc., Chicago, IL, USA), was used for all statistical analyses.

Results

Distribution of distinct populations of effector cells and regulatory T cells in peripheral blood and bone marrow

The $CD4^+/CD8^+$ ratio was significantly lower, both in the PB and BM, of LTDC-MM patients than in healthy adults ($P \leq 0.0001$), patients with MGUS ($P \leq 0.05$) or symptomatic MM patients ($P \leq 0.008$) (Figure 4A). This was mainly due to an increase in the number of $CD8^+$ T cells in LTDC-MM, MGUS and symptomatic MM patients with respect to healthy adults, the level of $CD4^+$ T-cells remaining unaltered. LTDC-MM patients also showed increased numbers of $CD56^{lo}$ NK-cells in PB *versus* healthy adults (P=0.01) (Figure 4B). The number of total T-lymphocytes and the $TCR\gamma\delta^+$ T cells was similar among groups (*data not shown*). In addition, the number of Treg cells in the BM of LTDC-MM patients was within the normal range (similar to that found in healthy adults) but significantly lower than that in symptomatic MM patients (P=0.04) (Figure 1B).

Distribution of B-cell populations in peripheral blood and bone marrow

The total PB and BM B-cell counts from LTDC-MM patients were within the normal range (similar to those found in healthy adults), but significantly higher than those observed in the PB of MGUS patients (P=0.04) and in the PB and BM of symptomatic MM patients (P=0.004 and P=0.005, respectively) (Figure 3B). A similar pattern was also found for the distribution of B-cell precursors in the BM of LTDC-MM patients *versus* symptomatic MM patients (P=0.002) (Figure 3C). In depth analysis of the different subsets of PB B cells (immature, naïve and memory B-lymphocytes plus plasmablasts/plasma cells) showed that the pre-germinal center cell subsets (e.g. immature and naïve B-lymphocytes) were present in LTDC-MM patients at similar values to those in healthy adults, while increased with respect to those in MGUS patients (P=0.01 and P=0.006, respectively) and symptomatic MM patients (P=0.01 and P=0.0001, respectively). Conversely, no differences were detected in PB for memory B cells or plasmablasts/plasma cells among the three groups (Figure 3C). Despite this, the number of terminally differentiated normal BM plasma cells was significantly increased in LTDC-MM *versus* symptomatic MM patients (P=0.002), with values similar to those in MGUS patients (P=0.6), but still reduced compared to those in healthy adults (P=0.03).

Distribution of circulating tissue macrophages and dendritic cells in peripheral blood and bone marrow

The overall DC counts in PB and BM were similar in LTDC-MM, MGUS, and symptomatic MM patients and in healthy adults. However, a more detailed analysis of the distinct subsets of DC and tissue macrophages showed a significantly different composition of this cell compartment in LTDC-MM *versus* symptomatic MM patients (Figure 2B,C). LTDC-MM patients had higher percentages of p-DC (P=0.05) in both PB and BM, as well as of m-DC (P=0.01) in PB; by contrast, the number of tissue macrophages was decreased in both the BM (P=0.06) and PB (P=0.02) of these patients. Overall, these differences were associated with a recovery of the normal distribution of PB DC and tissue macrophages in LTDC-MM patients towards levels similar to those found in healthy adults (P>0.05). Nevertheless, differences were still noted between LTDC-MM patients and healthy adults for BM tissue macrophages (P=0.03) and p-DC (P=0.03).

Distribution of lymphoid cells and dendritic cells in multiple myeloma patients with long-term disease control according to depth of response and duration of remission

Persistent minimal residual disease (MRD) was detected by highly sensitive multiparametric flow cytometry in the BM of 20/28 (70%) LTDC-MM patients, while the other eight cases (30%) were MRD-negative with a sensitivity of 10^{-5}. These eight patients were considered as being in immunophenotypic complete remission, while the first 20 MRD-positive patients were considered as showing an MGUS-profile by immunophenotyping, due to the coexistence of normal and residual myelomatous plasma cells. Interestingly, when we compared the distribution of T, B, NK and DC in these two groups of LTDC-MM, no significant differences were observed. The only relevant finding was a significantly higher number of normal plasma cells, both in the BM (P=0.01) and PB (P=0.04) of MRD-negative *versus* MRD-positive patients. In addition, we stratified LTDC-MM patients into those achieving a complete response (n=11) *versus* those with a near complete response or partial response (MGUS-profile; n=17). As for

the former comparison, no major differences were found in the distribution of PB T, B, NK and DC between the groups (Table 2).

Finally, we investigated whether the duration of remission in LTDC-MM patients was associated with specific changes in the immune system, by comparing patients with ≥ 10 years disease control (n=7) *versus* patients with 5-10 years follow-up (n=21). Our results showed that the former cohort had higher total T-cell counts in the PB (P=0.004), including both the $CD4^+$ (P=0.001) and $CD8^+$ (P=0.008) subsets, naïve B cells (P=0.04), and m-DC (P=0.03) (Table 2).

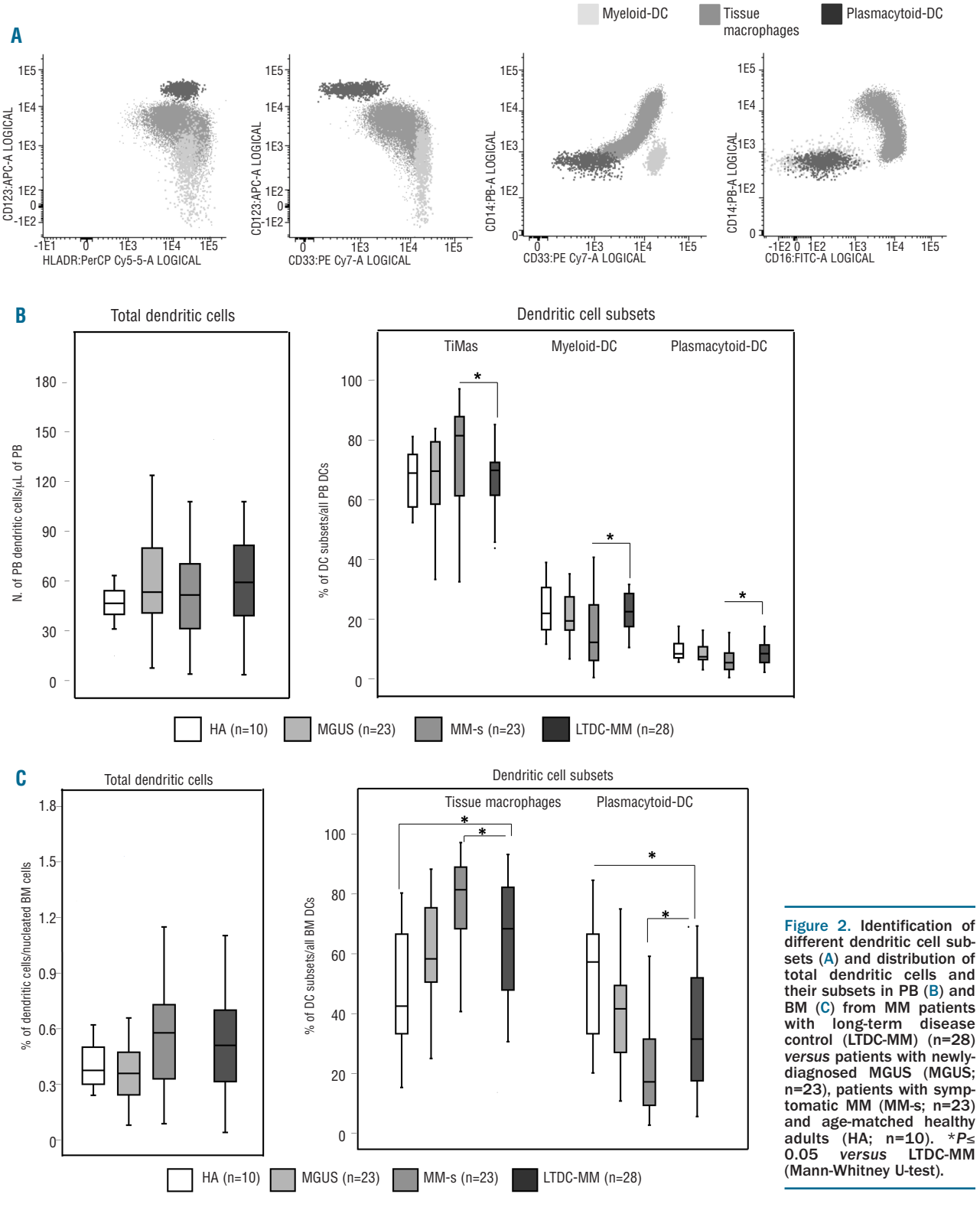

Figure 2. Identification of different dendritic cell subsets (A) and distribution of total dendritic cells and their subsets in PB (B) and BM (C) from MM patients with long-term disease control (LTDC-MM) (n=28) *versus* patients with newly-diagnosed MGUS (MGUS; n=23), patients with symptomatic MM (MM-s; n=23) and age-matched healthy adults (HA; n=10). *$P \leq 0.05$ *versus* LTDC-MM (Mann-Whitney U-test).

Discussion

Despite the fact that until recently MM was considered incurable, the introduction of high dose therapy/ autologous stem cell transplantation and novel drugs has made it possible for a small fraction of patients to attain long-term (≥5 years) disease control even in the absence of a complete response, after reverting to having an MGUS-like profile. The underlying mechanisms leading to disease suppression in these patients are largely unknown, although immune surveillance has been hypothesized to play a critical role.

In this exploratory study, we analyzed the distribution of the most representative populations of different func-

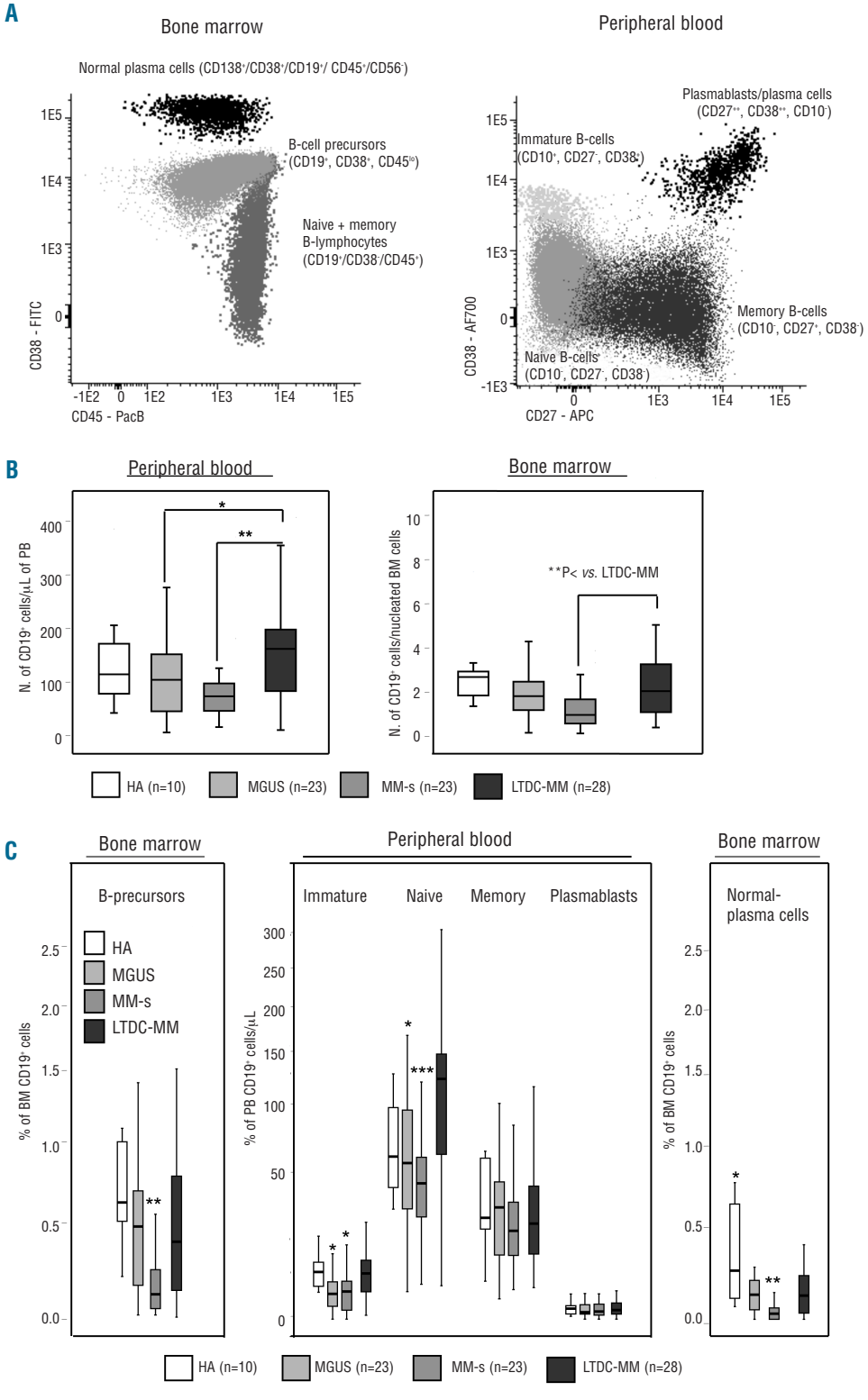

Figure 3. Immunophenotypic identification of the different B-cell subsets in bone marrow and peripheral blood (A), and the distribution of total CD19+ B-cells (B) and their subsets (panel C) in MM patients with long-term disease control (LTDC-MM) (n=28) *versus* patients with newly-diagnosed MGUS (MGUS; n=23), patients with symptomatic MM (MM-s; n=23) and age-matched healthy adults (HA; n=10). *$P \leq 0.05$, **$P \leq 0.005$, ***$P \leq 0.001$ *versus* LTDC-MM (Mann-Whitney U-test).

tional compartments of the immune system in MM patients achieving long-term disease control. Similarly to what has been previously described for symptomatic MM,[12,23-25] effector CD8$^+$ T and NK cells were also found to be increased in LTDC-MM. Recruitment of cytotoxic cells into the tumor microenvironment could reflect a host immune surveillance mechanism to control tumor growth, which would be inefficient in newly-diagnosed symptomatic patients. In this regard, an expansion of Treg cells potentially mediating immune suppression may be crucial for myelomatous plasma cells to evade immune surveillance, and the main obstacle for successful immunotherapy (e.g. idiotypic vaccination in MM).[26,27] Interestingly, our study showed that LTDC-MM patients have lower numbers of Treg cells than do symptomatic MM patients, particularly in the BM. Despite a large body of evidence that points to increased numbers and a functionally effective status of Treg cells in both hematologic and non-hematologic malignancies,[28] the results reported in MM are controversial, which is probably related to the different methodologies and techniques used to identify the cells.[29-31] In our study, Treg cells were identified based on their unique CD4$^+$, CD25^{hi}, CD127$^{-/lo}$ phenotype (Figure 1A), which has been shown to be directly correlated to the major CD4$^+$, FoxP3$^+$ Treg subset.[32,33] It can, therefore, be hypothesized that in LTDC-MM patients, the reduction of

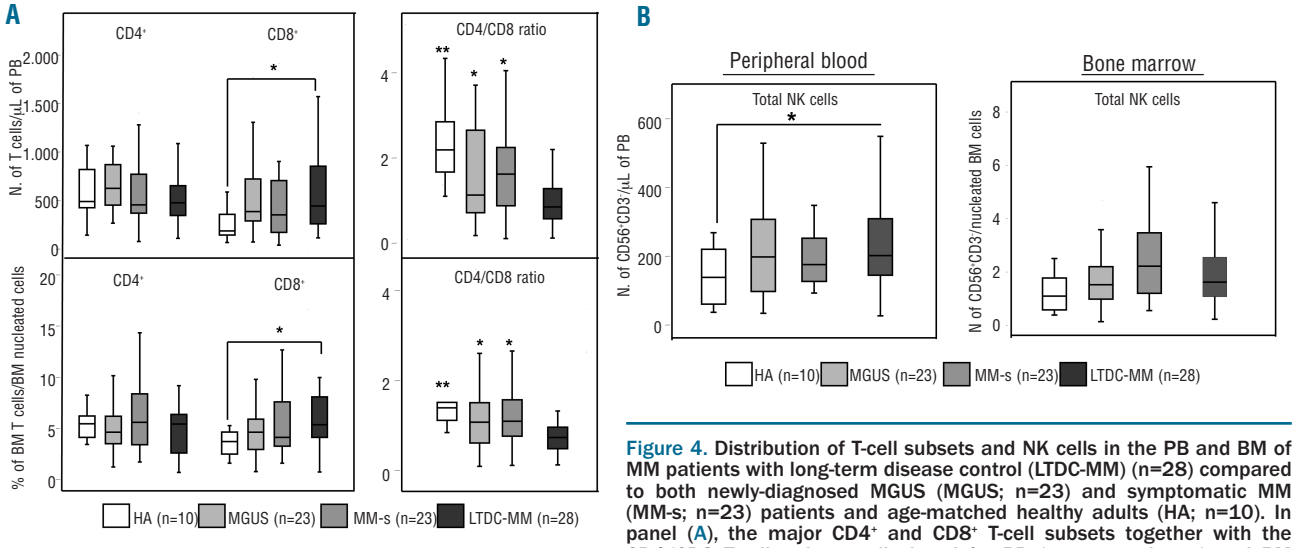

Figure 4. Distribution of T-cell subsets and NK cells in the PB and BM of MM patients with long-term disease control (LTDC-MM) (n=28) compared to both newly-diagnosed MGUS (MGUS; n=23) and symptomatic MM (MM-s; n=23) patients and age-matched healthy adults (HA; n=10). In panel (A), the major CD4$^+$ and CD8$^+$ T-cell subsets together with the CD4/CD8 T-cell ratio are displayed for PB (upper quadrants) and BM samples (lower quadrants). Panel (B) shows the distribution of NK cells in PB (right) and BM (left) of HA, and patients with MGUS, MM-s, or LTDC-MM is displayed. *$P \leq 0.05$ and **$P \leq 0.001$ *vs.* LTDC-MM (Mann-Whitney U-test).

Table 1. Distribution of different T-, B-, NK- and DC cell populations in the PB and BM of MM patients with long-term disease control (LTDC-MM) (n=28) *versus* age-matched healthy adults (HA) (n=10) and newly diagnosed MGUS (n=23) and symptomatic-multiple myeloma (MM-s) (n=23) patients.

	HA PB	MGUS	MM-s PB	LTDC-MM	HA BM	MGUS BM	MM-s	LTDC-MM
T cells	864±433	1271±565	1108±637	1266±979	9±3	11±6	12±7	12±6
CD4$^+$	574±284	642±227	593±343	519±245	5±2	6±3	7±4	5±2
CD8$^+$	252±161*	544±423	464±416	688±774	3±1*	5±4	6±3	7±4
T- γδ	26±19	43±41	34±27	41±45	0.32±.27	0.45±.3	0.33±.22	0.4±.27
Treg	41±16	47±18	42±26	37±16	0.5±.16	0.48±.29	0.57±.33	0.40±.24
NK cells	141±84	249±204	227±141	230±134	1.2±.7	2.1±2	2.7±2	2.2±2
B cells	142±101	107±70*	87±73**	164±104	3±2	2±1	1.2±1**	3±2
B precursors	-	-	-	-	0.7±.3	0.6±.6	0.2±.2**	0.7±1.1
Immature	8±5	4±3*	4±4*	9±7	-	-	-	-
Naïve	95±95	66±44*	50±40***	119±78	-	-	-	-
Memory	37±22	35±28	31±38	34±29	-	-	-	-
Normal-PC	2±2	1.4±2	1.3±1.4	1.2±1	0.32±.25*	0.17±.20*	0.04±.05**	0.19±.28
DC	45±14	68±43	56±40	59±27	0.50±.37	0.39±.25	0.6±.42	0.52±.28
m-DC	10±5	16±16	9±10**	13±8	-	-	-	-
TiMas	31±12	46±39	42±38	40±22	0.28±.35	0.26±.23	0.55±.51	0.39±.28
p-DC	4±1	6±4	5±7	5±3	0.21±.06*	0.13±.06	0.12±.10	0.15±.10

*Peripheral blood results are expressed as mean number of cells/ µL ± one standard deviation; bone marrow numbers reflect percentages of cells from all nucleated bone marrow cells ± one standard deviation. *P≤ 0.05, ** P≤0.005, ***P≤0.001 vs. LTDC-MM respectively (Mann-Whitney U-test). PC: plasma cells; TiMas: tissue macrophages.*

Table 2. Distribution of different T-, B-, NK- and DC cell populations in the PB of MM patients with long-term disease control (LTDC-MM) in continuous complete response (n=11) *versus* LTDC-MM with an MGUS-like profile (n=17) and in (LTDC-MM) patients with more than 10 years of follow-up (n=7) *versus* other LTDC-MM patients (n=21).

Peripheral blood cell population	LTDC-MM		LTDC-MM	
	CR (n=11)	MGUS-like (n=17)	<5-10 years (n=21)	≥10 years (n=7)
T cells	1429±1372	1161±640	1054±1044**	1756±562
CD4+	504±274	529±232	437±214**	747±199
CD8+	861±1125	575±430	561±848**	940 ±434
Treg	36±17	38±15	35±15	45±18
NK cells	256±151	212±122	246±123	212±156
B cells	136±100	181±106	130±83**	230±109
Immature	6±5*	11±7	7.6±7	11±6.8
Naïve	101±84	131±73	95±64**	162±63
m-DC	12±7	13±8	11±5**	19±11

Results expressed as mean number of cells/μL ± one standard deviation. *P≤ *0.05* versus *LTDC-MM in complete response and LTDC-MM with an MGUS profile;* **P≤ *0.05* versus *LTDC-MM < 5-10 years follow-up and LTDC-MM ≥10 years follow-up, respectively. (Mann-Whitney U-test).*

Treg in the BM microenvironment could promote a stronger cytotoxic effect of the expanded $CD8^+$ T- and NK-lymphocytes, favoring local immune surveillance. If this hypothesis holds true, a recovery of B-cell response and antibody production should also be seen in LTDC-MM patients.

Disrupted B-cell lymphopoiesis and antibody secretion (e.g. immune paresis), with compromised production and BM homing of normal plasma cells, is a hallmark of symptomatic MM.[11,34-36] In LTDC-MM, we observed a replenishment of early B-cell subsets in the BM (B-cell precursors), translating into increased numbers of circulating immature and naïve B-cells in PB, and a recovery of end-stage normal plasma cells in the BM. Recovery of B-cell production in the BM could be related to decreased competition between clonal plasma cells and normal plasma cells (as well as B-cell precursors), for a limited number of SDF-1-associated BM niches.[36] In line with this hypothesis, we found that circulating PB plasmablasts/plasma cells from LTDC-MM patients displayed lower expression of the CXCR4 surface chemokine receptor than those from patients with symptomatic MM, similar to the expression in healthy adults (*data not shown*); these observations could indicate that newborn plasmablasts/plasma cells can more easily migrate and home to the appropriate BM niches. Collectively, these findings would support the hypothesis that the regeneration of B-cell precursors in the BM could drive the shift towards increased numbers of newly generated B cells in PB, which would also eventually increase the homing and number of normal plasma cells in the BM. This B-cell replenishment loop occurring after therapy[36] and particularly its long-term maintenance could be a singular feature of LTDC-MM. Further investigations about the specific mechanisms involved in the maintained B-cell recovery are, therefore, required.

We also investigated the DC compartment in both the BM and PB of LTDC-MM patients. Overall, our results showed that LTDC-MM patients recovered normal counts of different subsets of DC in PB; however, a reduction in p-DC together with an increase in tissue circulating macrophages was still noted (*versus* healthy adults), but such alterations were much less pronounced than in patients with symptomatic MM. It is noteworthy that the partial normalization of DC counts and the distribution of these cells described here for LTDC-MM patients are similar to those reported by Kovarova *et al.*[37] in MM patients who remained in remission for 6 months after autologous stem cell transplantation.

Since patients who are relapse-free for ≥10 years are considered to be operationally cured,[4] we further investigated the immune profile of this specific subgroup of LTDC-MM patients. Interestingly, these patients had significantly increased numbers of $CD8^+$ and $CD4^+$ T cells, naïve B cells and m-DC, not only compared to LTDC-MM patients with 5-10 years follow-up, but also compared to healthy adults. These findings suggest that the immune profile of LTDC-MM patients does not reflect a simple overall recovered immune status identical to that of healthy adults of similar age, but rather an unique "immune signature".

In summary, our results indicate that LTDC-MM patients have a constellation of unique immune changes, consisting of increased numbers of effector cytotoxic $CD8^+$ T-lymphocytes and NK cells, B cells, normal plasma cells and also DC, with simultaneous reduction in Treg cells potentially favoring both the action of cytotoxic cells, and the recovery of B-cell production and homing of normal plasma cells into the BM. Altogether, these unique features of patients with LTDC-MM suggest that the patients have improved immune surveillance, which deserves further investigations to dissect the specific underlying molecular and functional mechanisms involved.

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Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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