[CANCER RESEARCH 63, 3019–3020, June 15, 2003]

Advances in Brief

Delayed *in Vivo* Disease Progression Is Associated with High Proportions of CD45$^+$ Myeloma Cells in the 5T2MM Murine Model[1]

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Abstract

Both CD45$^+$ and CD45$^-$ multiple myeloma (MM) cells are observed in the bone marrow (BM) of MM patients; however, their impact on the outcome of the disease is unknown. Most (92%) of the mice injected with murine 5T2MM cells develop myeloma in 10–12 weeks and show hind leg paralysis at the end phase of the disease. In the end stage 5T2MM cells are predominantly CD45$^-$, in analogy to the common human situation. Herein we report that 8% of the mice have a delayed tumor progression (14–24 weeks) with a complete different feature in the end stage of the disease. These animals had typically a bowed back and never got paralyzed. The MM cells in the BM of these mice were predominantly CD45$^+$.

These data indicate that CD45 subsets are associated with the final outcome of myeloma disease.

Introduction

MM[3] is a plasma cell malignancy with the BM as the main affected organ (reviewed in Ref. 1). The majority of the MM cells in patients have a CD45$^-$ phenotype. The common leukocyte antigen, CD45, is a transmembrane protein tyrosine phosphatase expressed on all of the B cells. During maturation toward plasma cells its expression is gradually lost, and fully matured plasma cells are CD45$^-$ (2). Although a heterogeneous CD45 expression pattern on human MM cells in the BM has been recognized by several groups (3–5), the role of the CD45$^+$ and CD45$^-$ MM cells in myeloma pathogenesis is unclear. Our group uses the 5T2MM mouse model to investigate the *in vivo* roles of CD45 subsets in myeloma biology. In analogy to the human situation, the 5T2MM cells have a heterogeneous CD45 expression (6). Compared with the CD45$^-$ 5T2MM cells, CD45$^+$ 5T2MM cells express higher levels of the insulin-like growth factor I receptor (6), which is involved in their chemotaxis to the BM (7). CD45$^+$ 5T2MM cells also express higher levels of proteases involved in their invasion, including matrix metalloproteinase-9 and urokinase type plasminogen activator receptor (8). Moreover, CD45$^+$ 5T2MM cells have enhanced *in vitro* chemotaxis toward the BM microenvironment and have higher *in vitro* invasive capacities (8). In line with these findings, *in vivo* experiments indicated a higher homing capacity of CD45$^+$ MM cells to the BM (6). Despite these phenotypic differences in the expression of key molecules involved in BM homing and the functional differences in the BM migratory capacities, the impact of CD45 heterogeneity on the outcome of myeloma disease is unknown. In this work we provide *in vivo* data indicating that mice bearing high proportions of CD45$^+$ 5T2MM show distinct clinical features and have a delayed disease progression compared with animals with predominantly CD45$^-$ MM cells.

Materials and Methods

5T2 Myeloma Model. 5T2 myeloma cells originate from spontaneously developed myeloma in aged C57BL/KaLwRijHsd mice (9, 10). The model was initiated and is continued by i.v. injection of diseased BM cells into young (6–10 weeks old) syngenic recipients (Harlan, Horst, the Netherlands) as described previously (7). 5T2MM cells have a heterogeneous CD45 expression (6) and are a model for the most common form of human myeloma (11).

Flow Cytometry and Serum Paraprotein Quantification. BM cells were flushed out from femora and mononuclear cells (BMNC) were isolated by centrifugation of the samples on Lympholyte M (Cedarlane, Hornby, Ontario, Canada). Tumor load was assessed by staining of the BMNC with anti-5T2MM idiotype-specific antibodies (12). Rat antimouse IgG1-PE (Becton Dickinson, San Jose, CA) was used as secondary reagent. CD45 expression on the 5T2MM cells was analyzed by double staining with 5T2MM idiotype and with CD45-FITC (clone AMS4508; Biosource International, Camarillo, CA). Mice were bled before killing, and serum paraprotein levels were quantified by electrophoresis (12).

Statistical Analysis. Student's *t* test was used for statistical analysis. $P \leq 0.05$ were considered as significant.

Results and Discussion

The majority (92%) of naïve mice injected with 5T2MM cells are terminally diseased at 10–12 weeks after tumor inoculation. Typically, these animals have paralyzed hind legs as illustrated in Fig. 1*A*. Top panels of Fig. 2 illustrate that the BM of these mice contain predominantly CD45$^-$ MM cells, in analogy with the common human situation. Myeloma cells isolated from these mice are used for experiments and for the continuation of the model. Thus, the dot plots in the top panel of Fig. 2 are also representative for the phenotype of the 5T2MM cells inoculated in the naïve mice. The remaining, nondiseased mice (~8% of the animals) are usually euthanasized. However, in this study, these mice were followed until signs of overt disease were observed. The mice had a delayed tumor take and were terminally diseased at 14–24 weeks after tumor injection. These mice had completely different clinical features (Fig. 1*B*) than the early (normal) diseased animals (Fig. 1*A*). These animals typically had a bowed back and never got paralyzed, in contrast with the normal diseased mice. These clear differences in take time and disease appearance lead us to determine the differentiation status of the MM cells in these mice. Dot plots in the bottom panels of Fig. 2 illustrate that the myeloma cells had a predominant CD45$^+$ phenotype. In Table 1 the take time, tumor load, serum paraprotein concentration and percentage of CD45$^+$ 5T2MM cells between the two groups are compared. Besides the differences in take time and percentage of CD45 5T2MM cells, mice with a delayed take had higher tumor load and paraprotein levels, most probably attributable to the longer duration of the disease.

Our data are in line with very recent observations of Moreau *et al.* (13) that high levels of CD45$^-$ MM cells in myeloma patients are associated with poor clinical prognosis. As already mentioned, CD45$^+$ 5T2MM cells are the predominant *in vivo* BM homing clone

Received 12/9/02; accepted 4/23/03.

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[1] Supported by OZR-VUB, FWO, Belgische Federatie tegen Kanker and International Myeloma Foundation (IMF) Ashley Barit Research Grant (to K. A.). K. A. and K. V. are postdoctoral fellows of the Fund for Scientific Research-Flanders (Belgium; FWO-Vlaanderen).

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[3] The abbreviations used are: MM, multiple myeloma; BM, bone marrow.

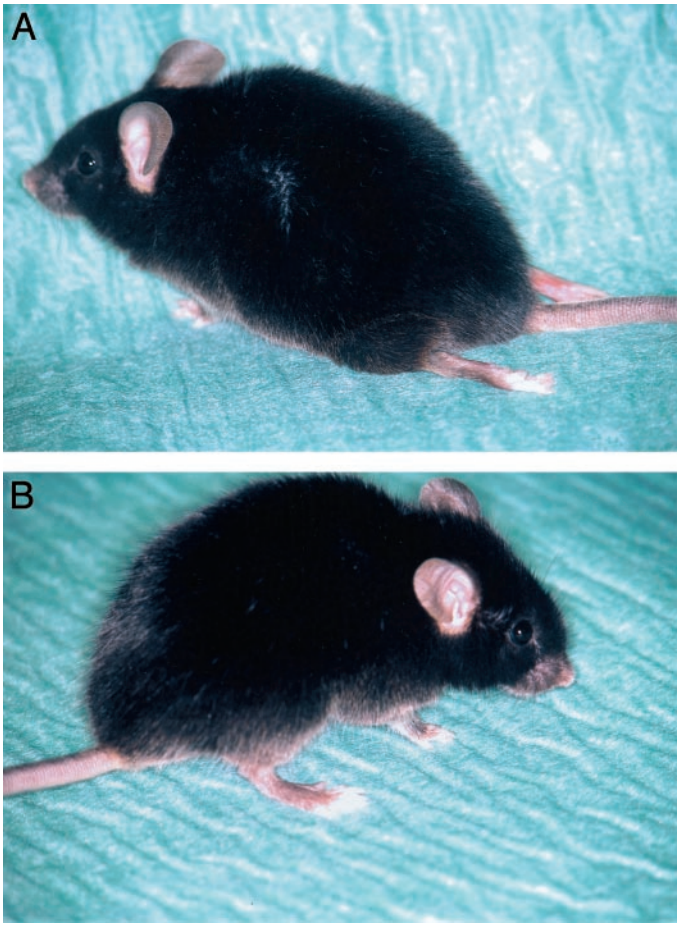

Fig. 1. *A*, normal take (10–12 weeks). The majority of 5T2MM inoculation mice (92%) were terminally diseased between 10 and 12 weeks after i.v. tumor injection. Typically, these mice develop hind leg paralysis. *B*, delayed take (14–24 weeks). A minority (8%) of the animals injected with 5T2MM cells were terminally diseased between 14 and 24 weeks after tumor inoculation. These mice had typically a bowed back and never got paralyzed, in contrast with the normal diseased mice.

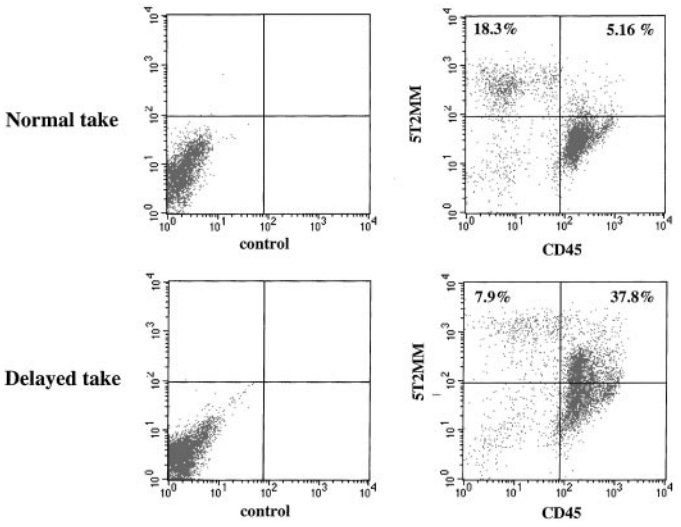

Fig. 2. CD45 expression profile on 5T2MM cells isolated from mice with normal and delayed take. BM mononuclear cells from terminally diseased mice were stained for 5T2MM-idiotype and CD45 expression for flow cytometry. Percentages $CD45^-$ 5T2MM cells (*top left quadrant*) and $CD45^+$ 5T2MM cells (*top right quadrant*) in the total BM mononuclear population are indicated. *Dot plots* from 1 mouse representative of 6 in each category are illustrated.

Table 1 *Comparison between mice with normal and delayed take*

	Terminally diseased mice[a]		
	Normal take	Delayed take	$P <$
Take time (days)	74.0 ± 3.3	132.1 ± 34.8	0.002
Tumor load (%)[b]	26.2 ± 5.5	60.8 ± 26.6	0.01
Paraprotein (g/dl)	1.0 ± 0.2	2.4 ± 1.9	0.09
% $CD45^+$ MM cells	23.1 ± 11.1	86.1 ± 5.8	0.0001

[a] Mean ± SD values of six terminally diseased mice in each group are shown.
[b] Tumor load indicates the % of MM cells in the BM mononuclear population.

(6). In a recent work we demonstrated that after homing the $CD45^+$ 5T2MM cells retain this phenotype in the early disease stages and gradually differentiate into $CD45^-$ MM cells during the disease progression (14). In the end stage of the disease the majority of the MM cells have obtained a $CD45^-$ phenotype. Apparently this differentiation does not occur in a minority (8%) of the mice. The mechanisms underlying these observations are not understood currently. However, the data underline the prognostic value of CD45 expression and are in keeping with our finding of high *in vivo* proliferative capacity of $CD45^-$ 5TMM cells compared with $CD45^+$ MM cells (6).

In conclusion, in previous works we demonstrated that $CD45^+$ and $CD45^-$ 5T2MM cells have different chemotactic, invasive, proliferative, and BM homing capacities (6, 8). The data in this work strongly indicate that CD45 subsets of the 5T2MM cells are also associated with the final outcome of myeloma disease, suggesting that these subsets may have crucial roles in myeloma biology.

References

1. Kuehl, W. M., and Bergsagel, P. L. Multiple myeloma: evolving genetic events and host interactions. Nat. Rev. Cancer., *2:* 175–187, 2002.
2. Jensen, G. S., Poppema, S., Mant, M. J., and Pilarski, L. M. Transition in CD45 isoform expression during differentiation of normal and abnormal B cells. Int. Immunol., *1:* 229–236, 1989.
3. Hata, H., Xiao, H., Petrucci, M. T., Woodliff, J., Chang, R., and Epstein, J. Interleukin-6 gene expression in multiple myeloma: a characteristic of immature tumor cells. Blood, *81:* 3357–3364, 1993.
4. Joshua, D., Petersen, A., Brown, R., Pope, B., Snowdon, L., and Gibson, J. The labelling index of primitive plasma cells determines the clinical behaviour of patients with myelomatosis. Br. J. Haematol., *94:* 76–81, 1996.
5. Ishikawa, H., Tsuyama, N., Abroun, S., Liu, S., Li, F. J., Taniguchi, O., and Kawano, M. M. Requirements of src family kinase activity associated with CD45 for myeloma cell proliferation by interleukin-6. Blood, *99:* 2172–2178, 2002.
6. Asosingh, K., De Raeve, H., Croucher, P., Goes, E., Van Riet, I., Van Camp, B., and Vanderkerken, K. *In vivo* homing and differentiation characteristics of mature (CD45-) and immature (CD45+) 5T multiple myeloma cells. Exp. Hematol., *29:* 77–84, 2001.
7. Vanderkerken, K., Asosingh, K., Braet, F., Van Riet, I., and Van Camp, B. Insulin-like growth factor-1 acts as a chemoattractant factor for 5T2 multiple myeloma cells. Blood, *93:* 235–241, 1999.
8. Asosingh, K., Menu, E., Van valckenborgh, E., Vande Broek, I., Van Riet, I., Van Camp, B., and Vanderkerken, K. Mechanisms involved in the differential bone marrow homing of CD45 subsets in 5T murine model of myeloma. Clin. Exp. Metastasis, *19:* 583–591, 2002.
9. Radl, J., De Glopper, E. D., Schuit, H. R., and Zurcher, C. Idiopathic paraproteinemia. II. Transplantation of the paraprotein- producing clone from old to young C57BL/KaLwRij mice. J Immunol., *122:* 609–613, 1979.
10. Radl, J., Croese, J. W., Zurcher, C., Van den Enden-Vieveen, M. H., and de Leeuw, A. M. Animal model of human disease. Multiple myeloma. Am. J. Pathol., *132:* 593–597, 1988.
11. Asosingh, K., Radl, J., Van Riet, I., Van Camp, B., and Vanderkerken, K. The 5TMM series: a useful *in vivo* mouse model of human multiple myeloma. Hematol. J., *1:* 351–356, 2000.
12. Vanderkerken, K., De Raeve, H., Goes, E., Van Meirvenne, S., Radl, J., Van Riet, I., Thielemans, K., and Van Camp, B. Organ involvement and phenotypic adhesion profile of 5T2 and 5T33 myeloma cells in the C57BL/KaLwRij mouse. Br. J. Cancer, *76:* 451–460, 1997.
13. Moreau, P., Robillard, N., Avet-Loiseau, H., Pineau, D., Morineau, N., Milpied, N., Mahe, B., and Bataille, R. Lack of CD45 expression has a strong prognostic value in patients with multiple myeloma (MM) receiving high-dose therapy (HDT). Blood, *11:* 810a, 2002.
14. Asosingh, K., De Raeve, H., Van Riet, I., Van Camp, B., and Vanderkerken, K. Multiple myeloma tumor progression in the 5T2 murine model is a multi-stage and dynamic process of differentiation, proliferation, invasion and apoptosis. Blood, *101:* 3136–3141, 2003.

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Cancer Res 2003;63:3019-3020.