[haematologica reports]
2006;2(3):32-34

Distribution of γδ T cells in patients with hematological malignancies

MARIANI S
PANTALEONI F
PEOLA S
HWANG SY
CASTELLA B
MATTA G
FOGLIETTA M
FIORE F
COSCIA MA
BOCCADORO M
MASSAIA M

Divisione di Ematologia dell'Università di Torino and Laboratorio di Ematologia Oncologica, Centro di Ricerca in Medicina Sperimentale, Turin, Italy

Tumor progression in multiple myeloma (MM) is due to intrinsic features of myeloma cells and to interactions of myeloma cells with the bone marrow microenvironment. Beside promoting myeloma cell survival and proliferation, these interactions are responsible for: 1) the activation and differentiation of osteoclast precursors into mature osteoclasts, yielding to bone resorption and lytic lesions, 2) the angiogenic switch sustained by endothelial cells and other stromal cells, via paracrine and autocrine loops, involving multiple VEGF/VEGF-R and other pathways; 3) a progressive immune failure that is responsible for the enhanced susceptibility to infections of MM patients and the escape of myeloma cells to host immune surveillance. This scenario is schematically illustrated in Figure 1.

In recent years, the immune surveillance hypothesis has been revitalized by the input of experimental, epidemiological, and clinical data.[1] Accumulating evidence indicates that innate effector cells such as NK cells, and unconventional T cells such as NKT and γδ T cells have a prominent role in tumor surveillance and rejection.[1,2] An important feature of γδ T cells is the ability to recognize induced self ligands and to bridge innate and adaptive immune responses. Circulating γδ T cells constitute about 1-5% of peripheral blood T cells. More than 70% of them use the same T-cell receptor (TCR) V region pair Vγ9-Vδ2 and express the $CD3^+$ $CD4^-$ $CD8^-$ phenotype.[3] This TCR enables them to recognize families of unprocessed nonpeptide compounds of low molecular weight (100-600 Da) with conserved patterns, comprising natural phosphoesters derived from mycobacteria and other pathogens, referred to as phosphoantigens, and to a lesser extent several ubiquitous metabolites such as alkylamines from plant extracts.[4,5] Certain hemopoietic tumor cell lines, such as the Daudi Burkitt's lymphoma and the RPMI 8226 myeloma line are also recognized and lysed by γδ T cells *in vitro*.[6,7] A prominent role of γδ T cells in the control of B-cell malignancies has recently been provided in a mouse model of spontaneous B-cell lymphoma.[8]

Based on these data, we have determined the distribution of circulating γδ T cells in the peripheral blood of 44 MM patients at diagnosis and 20 age-matched normal donors. No differences were detected in the percentages or total counts of γδ between normal donors and MM patients. Next, we have determined the ability of γδ T cells to proliferate to zoledronic acid (Zol). Zol is the most potent aminobisphosphonate (NBP) available for clinical use. NBP are commonly used in MM and other cancer patients to effectively prevent osteoclast activation and related skeletal events. Interestingly, it has previously been reported that γδ T cells can be activated by NBP because they share the chemical features of nonpeptide compounds naturally recognized by γδ T cells. Alternative mechanisms have also been proposed, like the ability of NBP to inhibit the mevalonate pathway in antigen-presenting cells. In normal donors, Zol displays a more potent ability than other NBP to expand and activate γδ T cells,[9] whereas no data were available in MM patients. We have found that 56% of MM patients at diagnosis display a significant expansion of γδ T cells after 7 days stimulation with Zol and low doses IL-2. These patients have been defined as responders (R), whereas MM patients who did not show any proliferative expansion have been defined as non-responders (NR).[10] Representative examples of R and NR patients are shown in Figure 2.

γδ T cells can be divided into subsets according to their phenotype, proliferative capacity and effector functions. Naive and memory γδ T cells (both $CD27^+$) display high proliferative capacity, but low effector functions, whereas effector and late effector γδ T cells (both $CD27^-$) display the opposite pattern.[11] $CD27^+$ γδ T cells were the major subset in R patients, whereas $CD27^-$ γδ T cells predominated in NR patients. Further analysis showed that the major subset

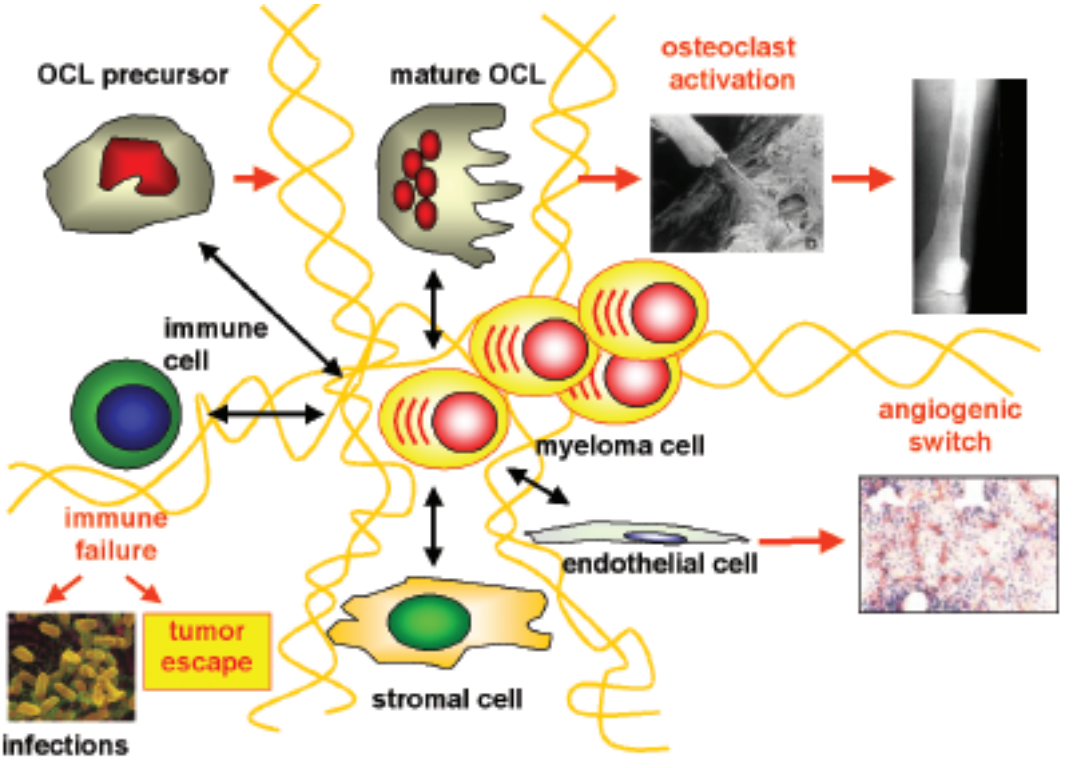

Figure 1. Interactions promoting tumor cell survival in MM.

in R consisted of memory cells (CD45RA$^-$ CD27$^+$), with the highest proliferative capacity among γδ T cells. Contrariwise, the major subset in NR consisted of late effector cells (CD45RA$^+$ CD27$^-$), which have the highest effector activity.

Thus, R and NR patients are characterized by a very different distribution of γδ T cells, with the former equipped with all major subsets, whereas the latter lacking the naive and central memory γδ T-cell subsets. Thus, one can predict that R patients can mount both proliferative and cytotoxic responses, whereas NR patients are able only to mount cytotoxic responses.

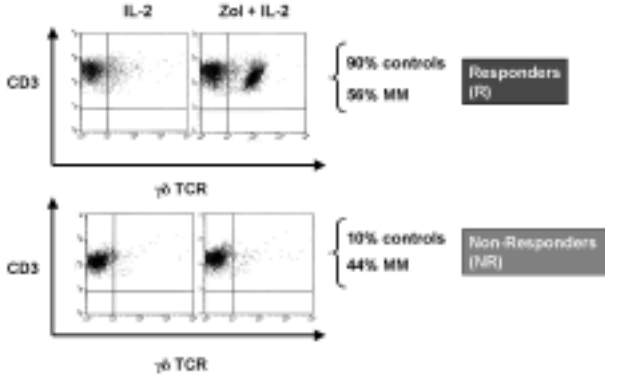

Figure 2. Representative examples of MM patients with or without proliferative expansion of peripheral γδ T cells after 7 days stimulation with Zol and IL-2.

The composition of γδ T-cell subsets in R and NR patients are illustrated in Figure 3.

Indeed, we have shown that both R and NR patients can exert cytotoxic activity against myeloma cell lines or primary myeloma cells upon activation with Zol and IL-2. As expected, the generation of cytotoxic activity in R patients is associated with proliferation, whereas in NR there is cytotoxicity without proliferation. Studies are in progress to determine the prognostic significance, if any, of these unbalanced distribution of γδ T-cell subsets in MM patients.

Chronic lymphocytic leukemia is a B-cell lymphoproliferative disorders sharing many characteristic with MM. Indeed, recent data indicate that interactions with *nurse* cells in the tumor microenvironment are important to promote the growth and survival of tumor cells, as in MM. Moreover, some of the new drugs that are very effective in MM, like lenalidomide and others targeting stromal cells other than myeloma cells, are also very effective in CLL. We have therefore investigated the distribution of γδ T cells in the peripheral blood of CLL patients at diagnosis. We have found that, as a percentage, γδ T cells are decreased in CLL, whereas they are increased as total counts. Thirty-five percent of CLL patient at diagnosis are NR and they share the same abnormal distribution of γδ T-cell subsets of NR MM patients, namely the strong reduction of naive and central memory γδ T cells.

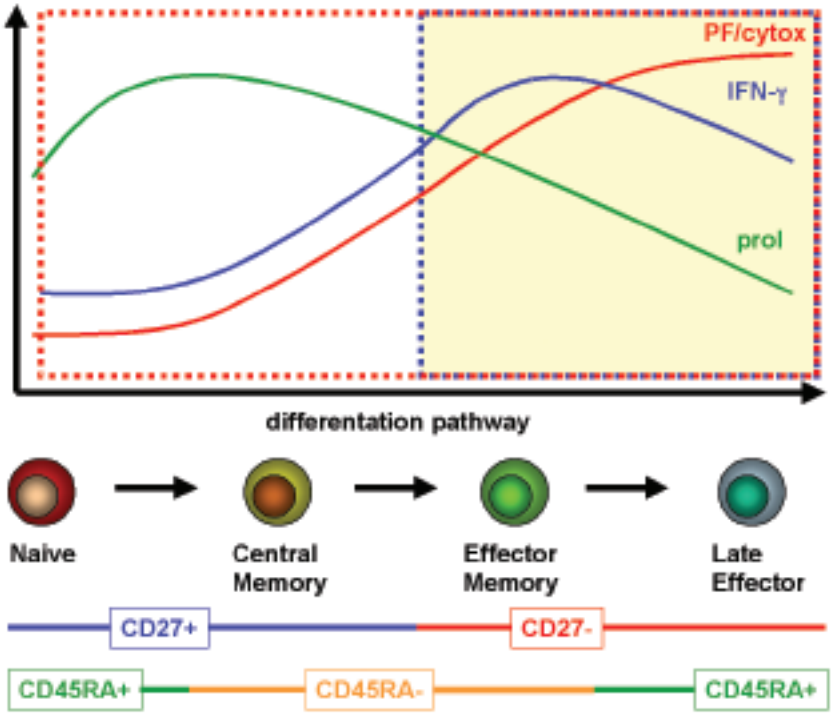

Figure 3. The peripheral blood γδ T cells of R patients (dotted red line area) contains all subsets, whereas γδ T cells of NR patients (dotted blue line shaded area) mainly contain effector and late effector subsets.

Interestingly, the R or NR status is very closely associated with the IgVH mutational status: almost all NR patients are unmutated, whereas the opposite is true for R patients that are uniformly mutated. Studies are in progress to determine the prognostic relevance of this unbalanced distribution of γδ T cells and what are the tumor antigens responsible for the exhaustion of naive and central memory γδ T cells in CLL Attention is focused on MICA, MICB and other self induced stress antigens.

References

1. Smyth MJ, Godfrey DI, Trapani JA. A fresh look at tumor immunosurveillance and immunotherapy. Nat Immunol 2001; 2: 293-9.
2. Diefenbach A, Raulet DH. The innate immune response to tumors and its role in the induction of T-cell immunity. Immunol Rev 2002;188: 9-21.
3. Hinz T, Wesch D, Halary F, Marx S, Choudhary A, Arden B, et al. Identification of the complete expressed human TCR V gamma repertoire by flow cytometry. Int Immunol 1997; 9:1065-72.
4. Tanaka Y, Morita CT, Tanaka Y, Nieves E, Brenner MB, Bloom BR. Natural and synthetic non-peptide antigens recognized by human gamma delta T cells. Nature 1995;375:155-8.
5. Bukowski JF, Morita CT, Brenner MB. Human gamma delta T cells recognize alkylamines derived from microbes, edible plants, and tea: implications for innate immunity. Immunity 1999;11:57-65.
6. Davodeau F, Peyrat MA, Hallet MM, Gaschet J, Houde I, Vivien R, et al. Close correlation between Daudi and mycobacterial antigen recognition by human gamma delta T cells and expression of V9JPC1 gamma/V2DJC delta-encoded T cell receptors. J Immunol 1993;151:1214-23.
7. Selin LK, Stewart S, Shen C, Mao HQ, Wilkins JA. Reactivity of gamma delta T cells induced by the tumour cell line RPMI 8226: functional heterogeneity of clonal populations and role of GroEL heat shock proteins. Scand J Immunol 1992;36:107-17.
8. Street SE, Hayakawa Y, Zhan Y, Lew AM, MacGregor D, Jamieson AM, et al. Innate immune surveillance of spontaneous B cell lymphomas by natural killer cells and gammadelta T cells. J Exp Med 2004;199: 879-84.
9. Thompson K, Rogers M. Statins prevent bisphosphonate-induced γ,δ-T-cell proliferation and activation *in vitro*. J Bone Miner Res 2004, 19:278-88.
10. Mariani S, Muraro M, Pantaleoni F, Fiore F, Nuschak B, Peola S, et al. Effector γδ T cells and tumor cells as immune targets of zoledronic acid in multiple myeloma. Leukemia 2005;18:139-45.
11. Dieli F, Poccia F, Lipp M, Sireci G, Caccamo N, Di Sano C, et al. Differentiation of effector/memory Vδ2 T cells and migratory routes in lymph nodes or inflammatory sites. J Exp Med 2003;198:391-7.