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## Distribution of $\gamma\delta$ T cells in patients with hematological malignancies

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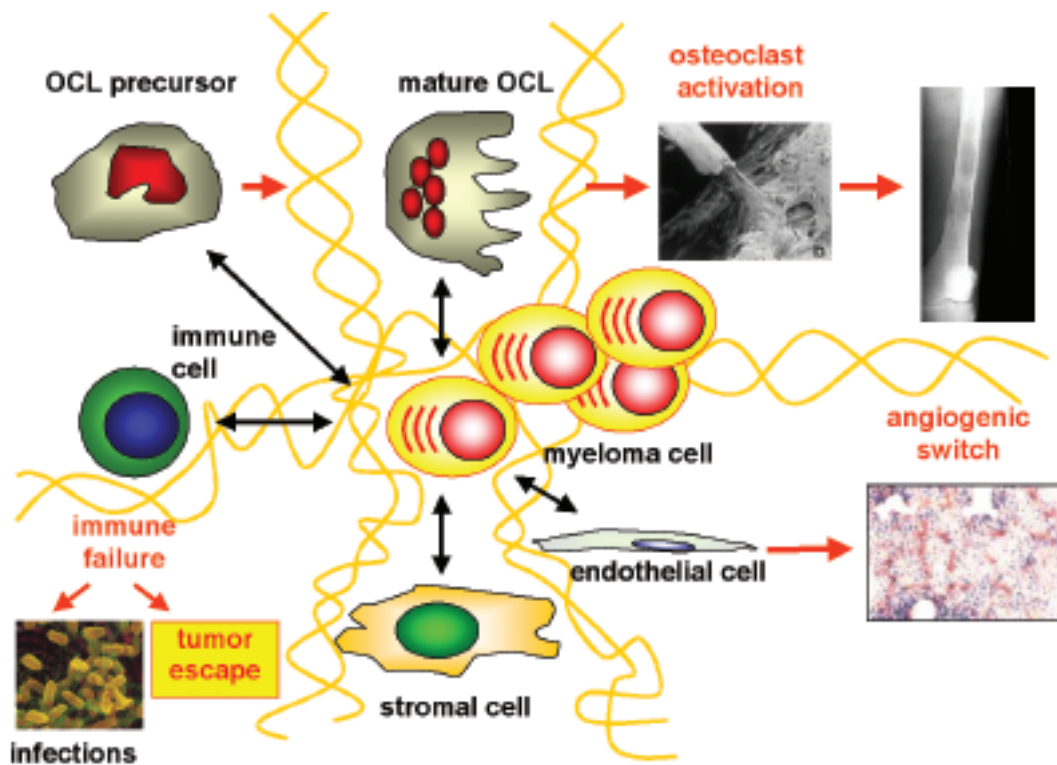
**T**umor progression in multiple myeloma (MM) is due to intrinsic features of myeloma cells and to interactions of myeloma cells with the bone marrow microenvironment. Beside promoting myeloma cell survival and proliferation, these interactions are responsible for: 1) the activation and differentiation 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.<sup>1</sup> Accumulating evidence indicates that innate effector cells such as NK cells, and unconventional T cells such as NKT and  $\gamma\delta$  T cells have a prominent role in tumor surveillance and rejection.<sup>1,2</sup> An important feature of  $\gamma\delta$  T cells is the ability to recognize induced self ligands and to bridge innate and adaptive immune responses. Circulating  $\gamma\delta$  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 V $\gamma$ 9–V $\delta$ 2 and express the CD3<sup>+</sup>CD4<sup>-</sup>CD8<sup>-</sup> phenotype.<sup>3</sup> 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.<sup>4,5</sup> Certain hemopoietic tumor cell lines, such as the Daudi Burkitt's lymphoma and the RPMI 8226 myeloma line are also recognized and lysed by  $\gamma\delta$  T cells *in vitro*.<sup>6,7</sup> A prominent role of  $\gamma\delta$  T cells in the control

of B-cell malignancies has recently been provided in a mouse model of spontaneous B-cell lymphoma.<sup>8</sup>

Based on these data, we have determined the distribution of circulating  $\gamma\delta$  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  $\gamma\delta$  between normal donors and MM patients. Next, we have determined the ability of  $\gamma\delta$  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  $\gamma\delta$  T cells can be activated by NBP because they share the chemical features of nonpeptide compounds naturally recognized by  $\gamma\delta$  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  $\gamma\delta$  T cells,<sup>9</sup> whereas no data were available in MM patients. We have found that 56% of MM patients at diagnosis display a significant expansion of  $\gamma\delta$  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).<sup>10</sup> Representative examples of R and NR patients are shown in Figure 2.

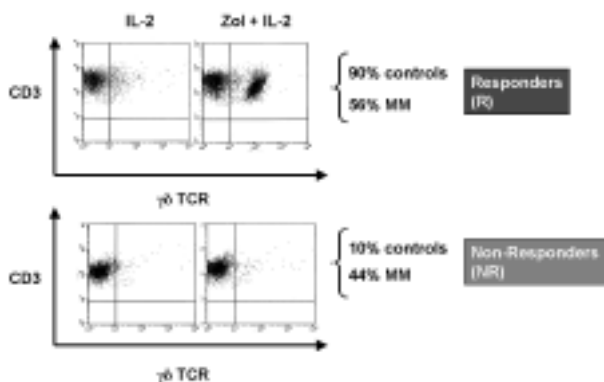
$\gamma\delta$  T cells can be divided into subsets according to their phenotype, proliferative capacity and effector functions. Naive and memory  $\gamma\delta$  T cells (both CD27<sup>+</sup>) display high proliferative capacity, but low effector functions, whereas effector and late effector  $\gamma\delta$  T cells (both CD27<sup>-</sup>) display the opposite pattern.<sup>11</sup> CD27<sup>+</sup>  $\gamma\delta$  T cells were the major subset in R patients, whereas CD27<sup>-</sup>  $\gamma\delta$  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<sup>-</sup> CD27<sup>+</sup>), with the highest proliferative capacity among  $\gamma\delta$  T cells. Contrariwise, the major subset in NR consisted of late effector cells (CD45RA<sup>+</sup> CD27<sup>-</sup>), which have the highest effector activity.

Thus, R and NR patients are characterized by a very different distribution of  $\gamma\delta$  T cells, with the former equipped with all major subsets, whereas the latter lacking the naive and central memory  $\gamma\delta$  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.

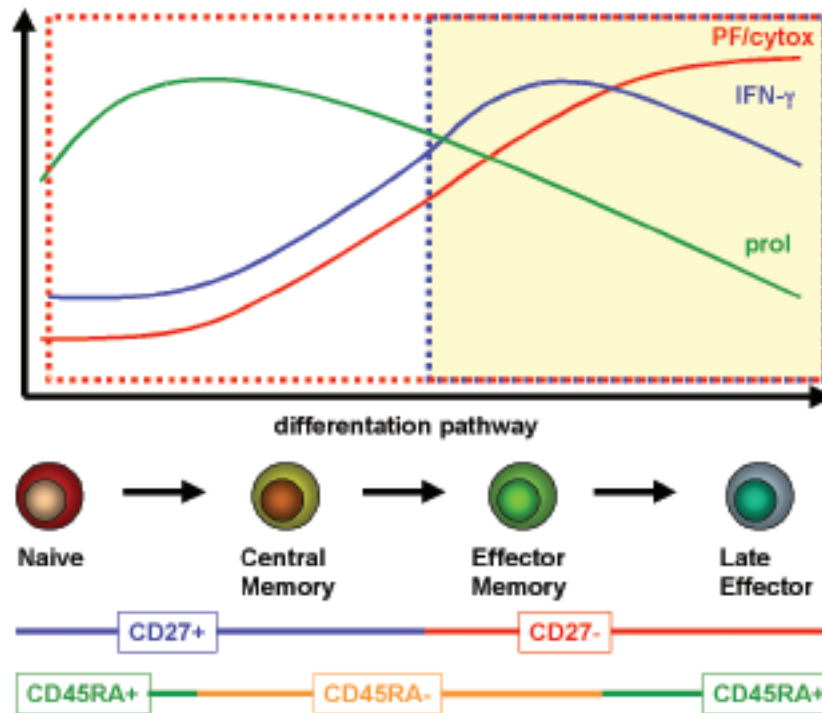


**Figure 2.** Representative examples of MM patients with or without proliferative expansion of peripheral  $\gamma\delta$  T cells after 7 days stimulation with Zol and IL-2.

The composition of  $\gamma\delta$  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  $\gamma\delta$  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  $\gamma\delta$  T cells in the peripheral blood of CLL patients at diagnosis. We have found that, as a percentage,  $\gamma\delta$  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  $\gamma\delta$  T-cell subsets of NR MM patients, namely the strong reduction of naive and central memory  $\gamma\delta$  T cells.



**Figure 3.** The peripheral blood  $\gamma\delta$  T cells of R patients (dotted red line area) contains all subsets, whereas  $\gamma\delta$  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  $\gamma\delta$  T cells and what are the tumor antigens responsible for the exhaustion of naive and central memory  $\gamma\delta$  T cells in CLL. Attention is focused on MICA, MICB and other self induced stress antigens.

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