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## The biological meaning of heterogeneous expression of CD45 on human myeloma cells

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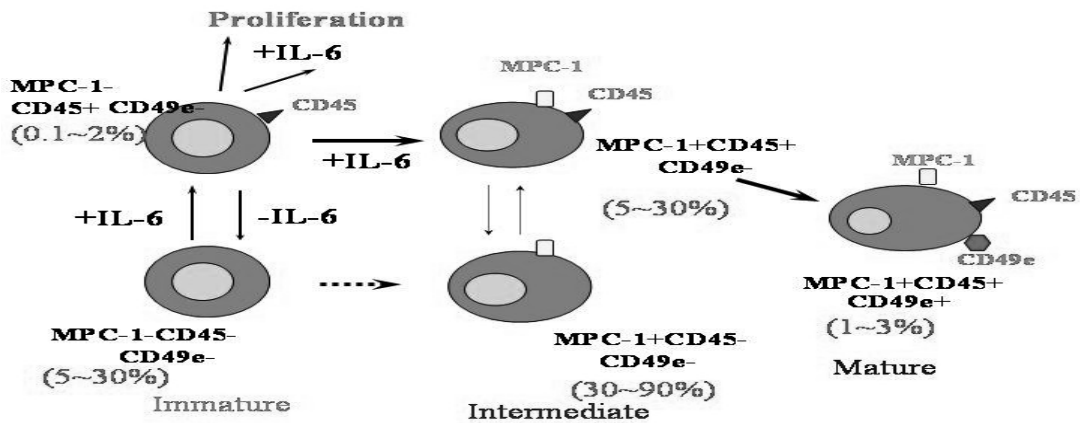
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There is a marked heterogeneity in the morphology, cytogenetics and phenotype of human myeloma cells. Myeloma cells can be classified into at least 5 subpopulations with respect to the expression of adhesion molecules such as MPC-1 and CD49e(VLA-5); MPC-1-CD45+CD49e-, MPC-1-CD45-CD49e- immature myeloma cells, MPC-1+CD45-CD49e-, MPC-1+CD45+CD49e- intermediate myeloma cells and MPC-1+CD45+CD49e+ mature myeloma cells are found in the bone marrow of most myeloma patients, roughly at 0.1~2%, 5-30%, 30-90%, 5-30%, and 1-3%, respectively. Only MPC-1-CD45+CD49e- immature myeloma cells can respond directly to IL-6 to proliferate in primary myeloma cells as well as myeloma cell lines. Also, MPC-1-CD45-CD49e- immature myeloma cells sorted from bone marrow samples as well as CD45- U-266 cell lines can be changed to CD45+ cells by addition of IL-6 in vitro. In both CD45- and CD45+ U-266 cells, STAT3 and MAPK (ERK1/2) can be activated in response to IL-6 equally between them, but src family kinases such as Lyn, Fyn can be activated only in CD45+ U-266 cells. Thus, the activation of the src family kinases associated with CD45 expression is a prerequisite for the proliferation of myeloma cells. In order to clarify the difference of cellular context between CD45- and CD45+ myeloma cells, PCR-based cDNA subtraction

and microarray analysis were performed in the U-266 cells, and the expression of several genes including the VDAC-1 gene was upregulated in CD45+ myeloma cells. Furthermore, sensitivity to stress stimuli between CD45+ and CD45- U-266 cells was also compared. CD45- U-266 cells were markedly more resistant to stress conditions such as serum-free condition. Therefore, we can speculate that in the bone marrow IL-6 can induce proliferation of CD45+ immature cells, but the amount of IL-6 is too low to support CD45+ myeloma cells and loss of CD45 results in no direct response to IL-6 to proliferate but confers resistance to stress condition leading to the longer survival at the limited amount of IL-6.

By using multi-color staining with anti-CD38 antibody, myeloma cells in the bone marrow of the myeloma patients clearly show 5 distinct subpopulations; MPC-1-CD45+CD49e-, MPC-1-CD45-CD49e- immature myeloma cells, MPC-1+CD45-CD49e-, MPC-1+CD45+CD49e- intermediate myeloma cells and MPC-1+CD45+CD49e+ mature myeloma cells. It is well-known, but very intriguing that most myeloma cells do not express CD45, and a limited number of myeloma cells are CD45+. Furthermore, only MPC-1-CD45+CD49e- immature myeloma cells can respond directly to IL-6 to proliferate.

## Subpopulations of Myeloma cells in the Bone Marrow



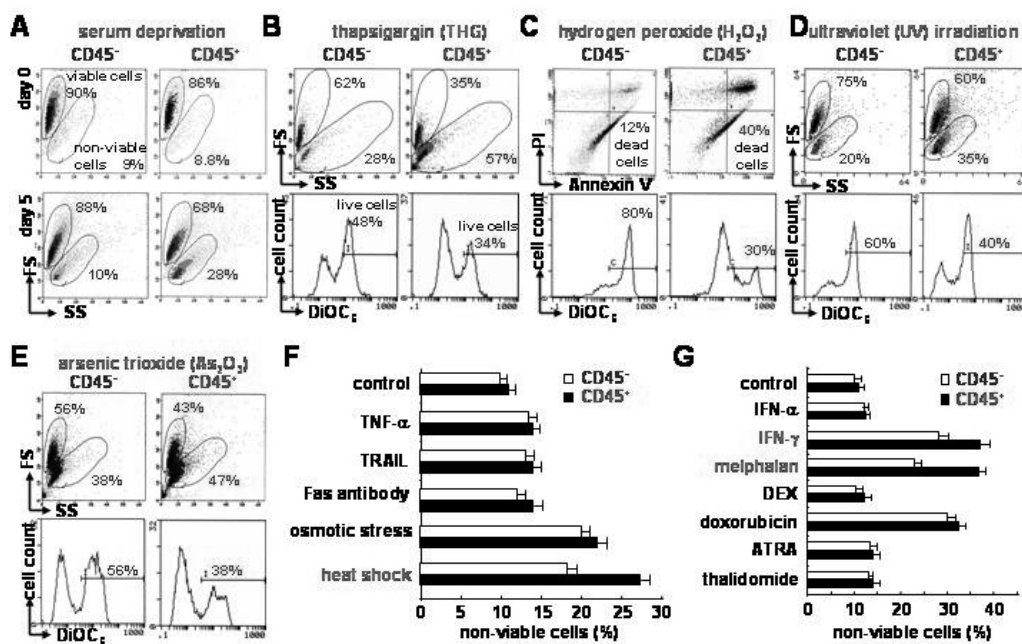
Immature myeloma cells, especially, CD45+ immature myeloma cells increase in the bone marrow along with relapse or progression of overt myelomas.

Stimulation of IL-6 activates STAT3 and ERK1/2 following activation of JAKs via gp130, so we examined whether both pathways were differentially activated in CD45+ and CD45- U266 cell lines by IL-6 stimulation. IL-6 induced the phosphorylation of both STAT3 and ERK1/2 in CD45- U266 cells as well as CD45+ U266 cells, but elevated activations of Lyn and Fyn kinases were found in CD45+ but not CD45- U266 cells

From PCR-based cDNA subtraction assay

and microarray screening, we found that the expression of VDAC(voltage dependent anion channel)-1 gene was increased in CD45+ U266 cells. CD45+ U266 cells are more sensitive to these stress stimulations such as H<sub>2</sub>O<sub>2</sub> and thapsigargin treatment through more increased release of cytochrome C and augmented activity of caspase 3 and 9. Furthermore, the transfectants of VDAC-1 gene showed more sensitive to these stress stimulations than mock transfectants in U-266 cells. Also, a PTPase inhibitor, vanadate, or specific src family kinase inhibitor, PP2, suppressed these stress-induced apoptosis of CD45+ U-266 cells, but not of CD45- U-266 cells.

## CD45+ U-266 cells are more sensitive to various apoptotic stimuli than CD45- cells



Therefore, we suppose that CD45+ immature myeloma cells can proliferate with the increased amount of IL-6 in the bone marrow of human myelomas, but the amount of increased IL-6 is not usually enough for the expanded immature myeloma cells to proliferate further and survive. CD45+ immature cells might be converted into CD45- myeloma cells, for CD45- cells are more resistant to stress conditions, escaping from apoptosis. The expression of CD45 molecule on the myeloma cells could contribute to the expansion of population size of myeloma cells along with the amount of IL-6. Further investigation is needed to clarify the biological relevance of increased expression of VDAC-1 gene in CD45+ immature myeloma cells.

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