EXPRESSON OF MULTIDRUG RESISTANCE GENE (MDR1) IN NORMAL HEMATOPOIETIC CELLS

To the Editor:

In the December 1, 1992 issue of Blood, two reports focused on the expression of the multidrug resistance gene (MDR1) in normal peripheral blood leukocytes and in normal marrow progenitors. In these studies, MDR1 expression was investigated by quantitating specific MDR1-mRNA with a 32-cycle polymerase chain reaction (PCR) assay and by evaluating the MDR1 gene product, a 170-Kd transmembrane glycoprotein (P-gp), with flow cytometry using monoclonal antibodies (MoAb) that were directed against intracytoplasmic or extracytoplasmic P-gp domains, referred to as C219 and UIC2, respectively. Because it is believed that P-gp is a drug efflux pump, cell efflux of fluorescent dyes, including Rhodamine-123, that the amount C219 and DOX was lower than determined, with and without Verapamil, cyclosporine-A, and other agents that are known to inhibit P-gp function. Both studies showed that P-gp was expressed in several peripheral blood lymphocyte subsets. Moreover, Drach et al. found that blood lymphocytes as well as CD10+ marrow cells and CD34+ myeloid progenitors carried a substantial amount of MDR1-mRNA that was as high as and even higher than the amount expressed in a doxorubicin-resistant mutant cell line. In normal lymphocytes and in marrow progenitors, Rhodamine-123 and DOX retention was shown to be significantly suppressed by chemical inhibitors as well as by the P-gp-directed UIC2 MoAb. It was not clear why Rhodamine-123 efflux and inhibition was higher in a mutant MDR cell line (DOX 6) than in lymphocytes and in myeloid progenitors, although the amount of MDR1-mRNA was significantly lower in the former than in the latter.

Because these data are very relevant to a number of important questions about the role of P-gp and MDR in normal and in malignant hemopoiesis and in treatment of cancer, leukemia, and lymphoma, we call attention to some prior findings and to some technical points that raise doubts regarding the "overexpression" of P-gp in normal blood and marrow cells, as it appeared in these reports.

In 1991, we investigated P-gp expression in normal cells and tissues using C219 and MRK-16 (an MoAb directed against P-gp extracytoplasmic domains) and a very sensitive immunocytochemical method, namely the alkaline phosphatase antialkaline phosphatase technique. We found that P-gp was detectable in blood and marrow myeloid cells and in peripheral blood lymphocytes (but not in lymphnodes, spleen, and Peyer's patches), but always at a low to very low level, in contrast to positive MDR controls and P-gp-overexpressing normal tissues such as liver and kidney. Marie et al., using PCR or in situ hybridization, found that some marrow cells carried detectable amount of MDR1-mRNA, but that the amount was several times lower than positive controls and that the gene product (P-gp) was barely detectable. In 1992, we systematically investigated P-gp expression in peripheral blood normal leukocytes by immunocytochemistry with MRK16, by flow cytometry with MRK-16, C219, and a third MoAb (JSB-1), and by Northern blotting of MDR1-mRNA. We found that P-gp was always expressed in the majority of lymphocytes, but we confirmed that the expression was always lower than or as low as that of negative controls (parental, non-MDR cell lines). For example, the mean fluorescence index with MRK-16 was 3 to 5 for normal lymphocytes, was 3 to 4 for negative controls (parental, non-MDR cell lines), and was 16 to 34 for positive controls (CEM VLB and LOVO DX MDR cell lines). In agreement with these data, Tiirikainen et al. found that less than 1% of normal lymphocytes reacted significantly with the MoAb JSB-1. Moreover, we found that MDR1-mRNA was below the range of detection or within the range of negative controls in all tested samples, a result also found by Holmes et al. Based on Drach et al., one would expect that in peripheral blood lymphocytes a consistent amount of MDR1-mRNA is detectable without resorting to PCR amplification. Concerning the functional effects of P-gp expression in lymphocytes and in normal hematopoietic cells, it is worth remembering that Cumber et al. showed that, in 10 normal subjects, cyclosporine-A had little effect (1% to 12%) on increasing intracellular lymphocyte daunorubicin fluorescence. Chao et al. found that cyclosporine-A and verapamil did not increase the toxicity of VP16 213 to normal colony-forming unit granulocyte-macrophage (CFU-GM). Tong et al. showed that P-gp-directed immunotoxins or MoAb were toxic for MDR cells but not for human normal progenitors (CFU-granulocyte, erythrocyte, monocyte, megakaryocyte, burst-forming unit-erythroid, and CFU-GM).

An exhaustive discussion of all the above observations would lead far beyond the limits of a letter. All data, including our own, points toward the expression of the MDR1 gene in normal blood lymphocytes and in marrow progenitors. We also found that tissue macrophages and, to a lesser extent, blood monocytes and granulocytes express the MDR1 gene. The critical point is likely to be the degree of the expression, and not merely expression itself. It would be an important achievement if Drach et al. and Chaudhary et al. could confirm their findings by immunocytochemistry and by progenitor in vitro assays, and could allow a comparison of their flow cytometry data of normal cells with flow cytometry data of their negative and positive controls. Present disagreement and inconsistencies could depend in part on controls, reagents, and methods, as recently reviewed and stressed by Haber.

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REFERENCES

4. Pileri SA, Sabattini E, Falini B, Tazzari PL, Gherlinzoni F,
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10. Chao NJ, Aihara M, Blume KG, Sikic BI: Modulation of etoposide (VP-16) cytotoxicity by verapamil or cyclosporine in multidrug-resistant human leukemic cell lines and normal bone marrow. Exp Hematol 18:1193, 1990


RESPONSE

In response to our report on mdr1 expression in subsets of normal hematopoietic cells,1 Baccarani et al raise some technical points regarding the measurement of mdr1. They point out that findings obtained with different methods may not always be consistent, which explains in part some discrepancies between results reported by them2,3 and others.4,5 However, we cannot identify inconsistencies in our own results when different methods of mdr1 detection were compared. Results clearly show that normal CD8+ lymphocytes and CD56+ natural killer cells express higher levels of mdr1 mRNA than DOX6 cells, which is in agreement with the Rhodamine-123 efflux studies (51.1% decrease of verapamil-blocked Rhodamine-123 efflux for CD8+ cells, 76.1% for CD56+ cells, and 34.5% for DOX6 cells; Fig 5 in Drach et al). In unseparated normal blood cells, mdr1 expression was readily detected by polymerase chain reaction. Less sensitive techniques such as Northern blotting may result in false negativity.

We agree with Baccarani et al that quantitation is an important issue in standardizing mdr1 measurements. However, it is not clear why Baccarani et al state that “P-glycoprotein was always expressed in the majority of lymphocytes,” as their “positive” fluorescence index (3 to 5) was indistinguishable from that of “negative” cells (3 to 4). Apparently, cells with different levels of background fluorescence were compared and appropriate isotypic controls are lacking.

Our results, as well as those of Chaudhary et al,4,5 provide evidence for constitutive expression of mdr1 in normal blood and bone marrow subpopulations. In our published reports,1,6 we do not postulate “overexpression.” This term should be reserved for cell lines and malignant cells that exhibit higher mdr1 levels than their normal counterparts.

REFERENCES


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Expression of multidrug resistance gene (MDR1) in normal hematopoietic cells [letter; comment]

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