Response:

Is there any evidence supporting a critical role of ectopic PI-9 expression in tumor immune escape?

The authors appreciate the interest of Bots et al in our recent paper regarding the role of PI-9 in CTL- and NK-cell-mediated lysis of lymphoma cells. The hypothesis that the granzyme B inhibitor PI-9 expressed in certain human malignancies may prevent their clearance by the immune system has been controversial for many years. We think, however, that the argumentation of Bots et al is debatable. The study by Classen et al, which Bots et al cite as not supporting our results, has investigated leukemic blasts from 2 patients only. The notion that “cytotoxic lymphocytes isolated from peripheral blood contain less granzymes than most cytotoxic lymphocyte lines,” referring to the study by Sayers et al, is partially true for granzyme M, but not for granzyme B, which is the only granzyme inhibited by PI-9. Sayers et al clearly show that the 2 major granzymes A and B are highly expressed in freshly isolated CD8+ T cells at a level similar to cytotoxic lymphocyte lines. In their own study, Medema et al have actually shown that indeed, transfection of SPI-6 (mouse PI-9) into tumor cells conveyed a level of protection from granzyme B–induced apoptosis, but did not inhibit CTL-induced cytolysis. This finding is in accordance with various more recent studies showing that other granzymes with strong antitumor activity can substitute granzyme B in vitro and in vivo, as reviewed by Lieberman. In the 2 studies mentioned by Bots et al, which linked PI-9 expression in tumor tissues to clinical outcome, there was no evidence of a clinically effective immune response to tumor in which granzyme B resistance might play a role.

In our opinion, the most convincing in vivo studies regarding the potential role of granzyme B and perforin in cancer are those in knock-out mice reported by Trapani’s group. Lymphoma cells grew efficiently in perforin-deficient mice, whereas granzyme A– and/or B–deficient mice rejected large tumor doses as avidly as wild-type mice, indicating that granzyme B was completely dispensable in lymphoma eradication. These studies also clearly show a strong correlation between cytolytic activity of granzyme B knockout CTLs in vitro and in vivo. Taken together, the studies by the groups of Trapani and Lieberman are in line with our studies in human lymphoma cells as they provide strong evidence that granzyme B is not critical for antitumor effector functions in vitro and in vivo. We therefore believe that the results from the murine knock-out models as well as our human in vitro experiments justify the conclusion considering lymphoma to be sensitive to perforin-dependent pathways despite PI-9 expression. Of course, we fully agree with Bots et al that the final answer regarding the role of PI-9 in immune rejection of human tumors can only be provided in vivo, which would require clinical studies based on immunotherapy with proven clinical efficacy.

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References

9. Becton et al report the results of a trial in children with acute myeloid leukemia (AML) who were randomized to receive consolidation therapy with or without cyclosporine-A (CsA). The authors measured P-glycoprotein (P-gp) expression in vitro by flow cytometry, using MRK16 antibody staining. P-gp positivity was defined as more than 5% of cells staining MRK16 positive, which

To the editor:

Does modulation of P-glycoprotein have clinical relevance in pediatric acute myeloid leukemia?

Becton et al report the results of a trial in children with acute myeloid leukemia (AML) who were randomized to receive consolidation therapy with or without cyclosporine-A (CsA). The authors measured P-glycoprotein (P-gp) expression in vitro by flow cytometry, using MRK16 antibody staining. P-gp positivity was defined as more than 5% of cells staining MRK16 positive, which
was found in 14% of samples. However, only 1.7% of samples showed P-gp positivity using a more traditional cut-off level of 20%.

We studied P-gp expression using MRK16 (5 μg/mL) as described before in 58 untreated pediatric AML samples (median, 88% blasts; range, 69%–98%). Applying a 20% cut-off level, 56 of 58 samples stained P-gp positive (median 67% of blasts were MRK16 positive; range, 20%–94%). However, the fluorescence index (FI; the ratio of fluorescence of specific antibody divided by isotype matched control) varied from 1.11 to 7.26 (median, 2.05), which is 1.6-fold lower than for pediatric acute lymphoblastic leukemia (ALL) (median, 3.36), a disease which is generally considered to have low P-gp activity. Moreover, in vitro daunorubicin resistance did not differ between AML samples, with FIs above or below the median. No differences in the percentages of MRK16-positive AML blasts or FIs at diagnosis were found between 15 patients who relapsed versus 28 patients who remained in remission (median, 59% MRK16-positive cells and FI 1.99 vs 59% and 1.96, respectively). All patients received intensive AML–Berlin-Frankfurt-Muenster (BFM)–based chemotherapy.

Hence, in contrast to Becton et al, we usually found weak MRK16 expression in most AML cells. No likely explanation other than technical issues can be provided for these differences. This stresses the need to assess P-gp status by different techniques, including the use of more than 1 antibody as well as functional assays, in line with published consensus recommendations. Efflux experiments were performed by Becton et al, but results were not provided. That integration of antibody and functional data is possible was shown in a recent clinical trial with PSC-833 in adult AML.

As Becton et al found no benefit for the use of CsA, we do not understand their final remark that “reversing chemotherapy resistance mediated by MDR1/P-gp remains a reasonable question for future pediatric AML trials.” This statement is not supported by their own data, which showed that (1) P-gp expression was only found in a minority of children with AML; (2) P-gp positivity did not confer poor outcome in the non-CsA–treated arm; and (3) the addition of CsA did not improve outcome. In addition, other studies of pediatric AML do not provide evidence for P-gp overexpression. Based on the available data, we would rather state that future pediatric AML trials should not be focused on P-gp reversal. This is in line with the notion that P-gp activity increases with age, which, together with the relatively high frequency of good-risk cytogenetics, may, at least in part, explain the good prognosis of pediatric AML when compared with adults with AML.

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C.M.Z. analyzed the data and wrote the letter. M.L.d.B. supervised the laboratory work performed by K.M.K. and A.H.L.; K.H., D.R., and U.C. were responsible as trial coordinators for the clinical data; and G.J.L.K. and R.P. chair the respective departments and laboratories where this work was performed.

References


Response:

Clinical relevance of P-gp expression in pediatric myeloid leukemia

Zwaan and colleagues raise an important point in relation to methodologic differences in assessing MDR1. The Rotterdam group used a method to detect total P-gp surface as well as intracellular protein; this required fixation and permeabilization of cells. We selected a method that was used in prior studies of AML to detect surface expression of P-gp in viable cells. It should be noted that when POG 9421 was activated, some of the methodologic details were still evolving. Thus, we made a midstudy correction to use phycoerythrin (PE) rather than fluorescein isothiocyanate (FITC) staining for MRK16 and staining at room temperature versus at 4°C to improve surface staining efficiency. We also used a second antibody, 4E3, and a well-studied functional efflux assay. Our results with the MRK16 and 4E3 antibodies were virtually identical with regard to percentage of positive cells and staining intensity. The MRK16/isotype ratio ranged from 0.49 to 6.17, with a mean of 1.09; functional efflux ranged from 0.57 to 5.90, with a mean of 1.76, showing a direct correlation of increasing efflux with increasing surface expression of P-gp. As noted by Zwaan et al, we also failed to see a direct correlation of surface P-gp expression and increasing inhibitory concentration at 50% (IC50) values for daunorubicin (data not shown). We have not done an extensive correlation of MDR1 expression in ALL blasts versus AML blasts, but preliminary comparisons agree with Zwaan et al that surface P-gp expression is higher in T-cell acute lymphoblastic leukemia (T-ALL) than AML.

Thus, the differences in frequency of P-gp expression levels noted by us and Zwaan et al are due to methodologic differences—we measured surface expression only, whereas Zwaan et al detected only P-gp staining. Regardless of methodology and contrary to expectation, we both agree that increasing P-gp
expression did not correlate with in vitro drug resistance in AML. While this raises questions regarding the clinical value of MDR1 modulation strategies, our results suggest a possible non–P-gp–mediated effect of cyclosporine, as also noted by List et al.5 Since cure rates are still quite low in certain subsets of AML, we feel that that it is appropriate to explore the mechanisms by which cyclosporine appears to be beneficial. More specific P-gp inhibitors may also be a better test of this concept.

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