Formal Discussion on Resistance to Purine Antagonists in Experimental Leukemia Systems

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A formal discussion, such as I have been scheduled to give, commonly takes the form of a presentation of the discussant's own work, with or without relation to the papers discussed. I find myself in somewhat the same position previously admitted by Dr. Skipper; my work in this field had already been published (3, 4, 7) or was in press (5) when I received Dr. Brockman's paper. I will therefore expand only slightly on the résumé of it that Dr. Brockman has already given and use this opportunity to expound on a few things that I think need more consideration in studies of resistance to drugs.

At the outset of my studies on the nucleotide pyrophosphorylase enzymes in man it seemed relatively simple to apply the well-polished methods developed for mouse cell studies to leukocytes from patients before and after therapeutic trials of 6-mercaptopurine (6-MP). The first stumbling block was the abundance of the critical inosinic acid pyrophosphorylase in human red blood cells. This was overcome by developing the technic of dextran sedimentation augmented by hypotonic osmotic shock to lyse residual red cells in making the human leukocyte isolations (6). Unfortunately, this technic yields no leukocytes when applied to even a large volume of blood if the white cell count is below about 4000/cu mm. This limitation, added to the usual problems of any clinical research effort in patients with leukemia, rapidly led to a revision of the design of the study. We began assaying the enzymes in the blood of any leukemia patient we could get who was off all anti-leukemic drug therapy for at least a few days and had an adequate leukocyte count with a high proportion of unequivocally leukemic cells as measured by the percentage of blasts. The rate of accession of cases was discouragingly slow, but over the course of 27 months 26 acceptable cases were studied. Since only 1 patient with a low enzyme level had been encountered, it seemed unnecessary to continue, and a retrospective classification of these cases into those sensitive and those resistant to 6-MP was made.

For this purpose we elected to establish 2 subclasses of resistant cases. The most rigid, which seemed resistant beyond any question, required that the patient show no fall in his leukocyte count or blast percentage following treatment with 90 mg of 6-MP mg/sq m for 60 days or 180 mg/sq m for 30 days. The enzyme assay must also have been done within 3 weeks of termination of therapy. By these "rigid" criteria only 6 of our patients qualified as resistant. The 2nd and less definitive category of resistant patients included 9 more cases that failed to meet all the criteria for the "rigidly resistant" group but that were considered by our clinicians to be failures on adequate 6-MP therapy. Of all 15 resistant patients only a single patient, who happened to be in the "rigidly resistant" group, showed any loss of inosinic-guanylic pyrophosphorylase. His loss was nearly complete and was confirmed by duplicate analyses on each of 2 successive days.

Chart 1 shows the values of the nucleotide pyrophosphorylase activities found for the 4 purine substrates as measured on the soluble protein fractions prepared from the leukocytes of patients with the various types of leukemia and of normal persons. The figures plotted are averages by type of disease for our total patient experience, excluding the 1 patient with apparent enzyme loss. The individual values of enzyme activity for the 6 "rigidly resistant" patients are shown in Chart 2, and the 1 patient with the low values is seen to stand out. His adenylic acid pyrophosphorylase activity in the same crude enzyme preparation was inordinately high and certainly vouched for the absence of any inhibitor or incidental inactivation of this preparation.

Although this series is small, it strongly suggests that loss of inosinic-guanylic pyrophosphorylase activity is a rather uncommon finding in human leukemia that is resistant to 6-MP. Our humans appear to differ in this respect from the majority of 6-MP-resistant mouse tumors, cultured cell lines, and microorganisms. Some other mechanism must account for the resistance to 6-MP in most human leukemia. A number of possible mechanisms suggest themselves. Permeability problems, causing failure of 6-MP to enter the cell, have been considered and were mentioned by Dr. Brockman with respect to several mouse and culture cell lines. I explored this possibility in resistant L1210 cells at about the same time that Dr. Brockman was finding his pyrophosphorylase losses (2, 3). Fortunately for our friendship, we did not wind up with 2 explanations of L1210 resistance to 6-MP. Although permeability was not the answer in this case, I would like to see more experimental tests for impaired permeability in resistant cells. If a few well-documented examples could be demonstrated, it would provide impetus for similar testing on human leukemic cells.

In our studies on patients we used soluble cell protein fractions *in vitro*. This is an arbitrary system and might be relatively insensitive to an alteration of the enzyme that significantly reduced its activity *in vivo*. Conceivably, some type of inhibition of the enzyme activity may obtain *in vivo* and be inoperative under our test conditions. I confess to having used the enzyme preparation technic of assay only because I could not get intact cells to show

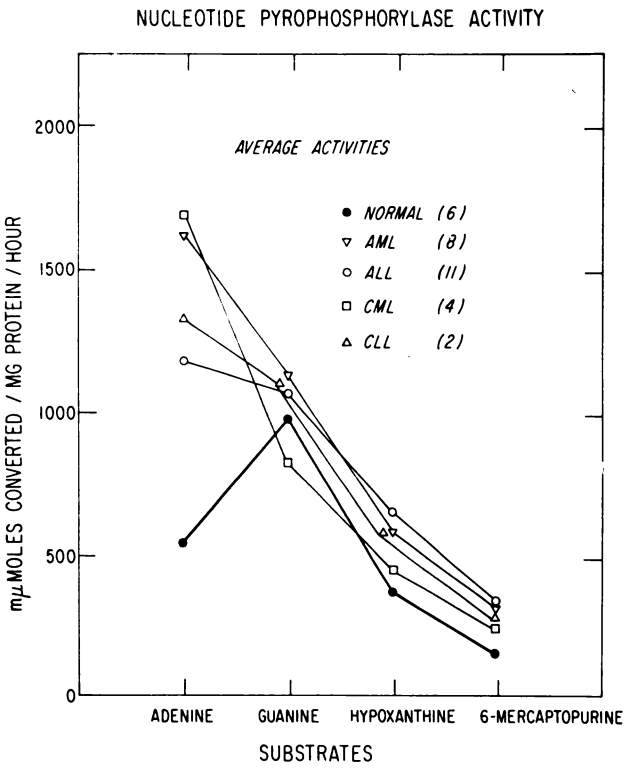

CHART 1.—Average nucleotide pyrophosphorylase activity with respect to 4 different purine substrates as determined on soluble protein fractions from leukocytes from normal humans and from patients with acute myelogenous leukemia (AML), acute lymphatic leukemia (ALL), chronic myelogenous leukemia (CML), and chronic lymphatic leukemia (CLL). The lines simply connect the values for different substrates for the same type of patient. The numbers in parentheses are the number of subjects represented in the average for the particular disease.

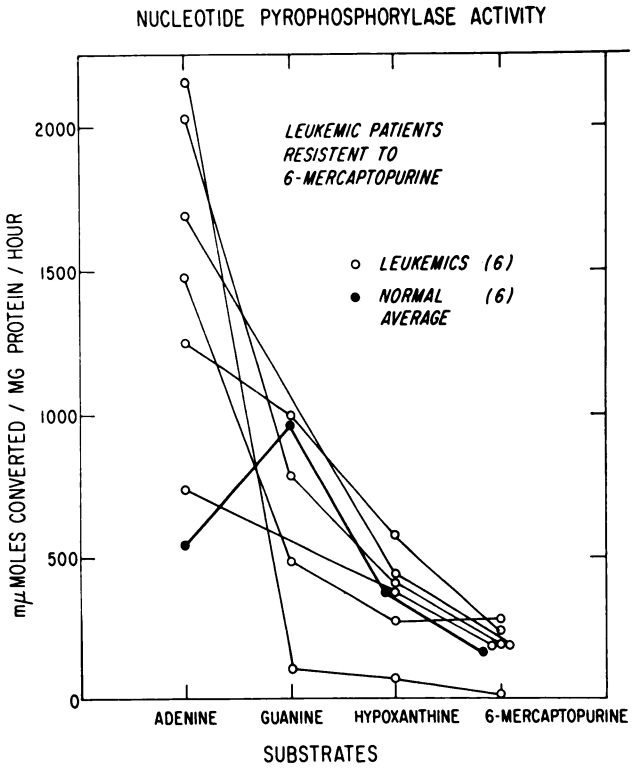

CHART 2.—Nucleotide pyrophosphorylase activity with respect to 4 different purine substrates as determined on soluble protein fractions from leukocytes from 6 patients with leukemia resistant to 6-mercaptopurine. The average values for 6 normal humans are plotted as a heavy line for reference. One patient of the 6 in the "rigidly resistant" group shows nearly complete loss of activity toward guanine, hypoxanthine, and 6-mercaptopurine; this was in the presence of high adenylic acid pyrophosphorylase activity in the same enzyme preparation.

enough nucleotide pyrophosphorylase activity to permit accurate assay of the partial losses that I had expected to encounter. The human cell studies are hampered by the limitations on the amount of cells available and the rather common unavailability of an identical 2nd crop from the same source to confirm or extend findings, even a few days following an initial study.

Having already completed my report on what I have done and having begun to expound on things to be done, I would like to continue in this vein. It seems desirable to place emphasis on really elucidating other mechanisms of resistance in any biologic system available. When we can grow human leukemic cells in culture in bulk and continuously, we may hope, or probably even expect, to generate drug-resistant sublines. Such material would seem ideal. While working toward this, more experiments and less speculation should be done on the mouse and culture cell lines that present unexplained drug resistance. Lack of permeability or active transport should be tested for in these lines. Failure to retain or bind a drug following exposure may be a factor, as has recently seemed to be the case with methylglyoxal bis(guanylhydrazone) in resistant as compared with sensitive lines of L1210 (1). Tests should be made for enhanced degradation of the drug and for increased concentrations of competitive analogous normal metabolites.

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