NEOPLASTIC CELLS, through their various stages of growth and differentiation, develop a certain imperviousness to the various environmental, immunologic, toxicologic, and pharmacologic onslaughts to which they are subjected. A general example of this problem is expression of multidrug resistance wherein the activity of a glycoprotein pump responsible for efflux of toxins from the cell is increased, causing relative cross-resistance to a broad spectrum of compounds including anthracyclines, taxanes, and vinca alkaloids.\(^1\) Expression of multidrug resistance at the time of diagnosis is associated with a substantially worse outcome in patients with acute myeloid leukemia,\(^2\) and increased MDR gene expression accounts in part for the progressively diminishing responsiveness of patients at the time of relapse as they are subjected to repeated cycles of drug combinations.

The recent success of using all-trans retinoic acid (RA) in acute promyelocytic leukemia (APL)\(^6\) has engendered interest not only to the novelty of the drug but also to its apparent mechanism of action whereby cells develop an increasingly mature phenotype before their eventual elimination.\(^5\) As reported by both French and American groups,\(^8,10\) the approach of using all-trans RA for remission induction followed by anthracycline-based consolidation has already achieved a significant increase in both relapse-free and overall survival in patients with APL. However, this treatment sequence was less inspired than impelled by early observations that despite near universal success in inducing remission, antileukemic responses induced by all-trans RA were brief in duration. Moreover, relapses that have occurred while taking the drug have absolutely precluded another response. A final paradox was that leukemic cells taken from relapsing patients appeared to retain sensitivity in vitro despite clinical resistance. In APL at least, retinoid resistance has seemed to develop quite regularly and rapidly.

Retinoids are critical regulators of numerous physiologic actions including visual response, morphogenesis, and various aspects of cellular differentiation,\(^11\) and their involvement is quite ancient from an evolutionary standpoint. Thus, it should come as no surprise that nature has evolved several means of dealing with an excess of these naturally occurring substances. A brief review of retinoid pharmacology suggests several possible areas whereby this process may arise\(^12\) (Fig 1). All-trans RA normally circulates in plasma in nanomolar concentrations, and it traverses the cell membrane by passive diffusion across a concentration gradient. However, any contribution of this plasma component to normal physiology seems unlikely because under homeostatic conditions, cells probably derive whatever retinoic acid they require by intracellular conversion from preformed retinol. Whatever its source, cytoplasmic all-trans RA can be bound to cellular retinoic acid binding proteins; in myeloid cells, the predominant form is CRABP-II. While originally thought to perform a largely sequestory function, recent data suggest that such binding facilitates presentation of the retinoid to oxidative enzymes that catalyze conversion to inactive metabolites.\(^13,14\) A second catalytic pathway may involve an interaction with lipid hydroperoxides that generate free oxygen radicals that also result in oxidative inactivation.\(^15,16\) Only unbound retinoic acid that diffuses through the nuclear membrane is potentially effective in transducing a response. In the nucleus, all-trans RA binds to one of several retinoic acid receptors (RARs), members of the steroid/thyroid superfamily of nuclear receptors\(^17\); to date, \(\alpha, \beta,\) and \(\gamma\) subtypes have been characterized. When activated by their ligand, RARs form dimers that then bind to specific DNA segments known as retinoic acid response elements (RAREs). Translation of mRNAs then control the downstream activation of retinoid response genes that ultimately effect the retinoid response. The translocation in APL disrupts the gene encoding RAR-\(\alpha\) which is located on chromosome 17, causing its fusion to the PML gene on chromosome 15.\(^18-20\) RAR-\(\alpha\) has been directly implicated in granulocytoid maturation and differentiation,\(^21\) and transfection of mutant PML/RAR-\(\alpha\) confers relative retinoid resistance to HL60 cells in culture.\(^22\)

With that background, there are several obvious regions where retinoid resistance might arise, with the important caveat that such resistance must necessarily be relative rather than absolute. (It is difficult to envision a scenario of complete insensitivity to an essential regulatory molecule that normally functions in so many critical pathways.) In APL, one obvious explanation is the generation of further mutations in RAR-\(\alpha\). The utility of retinoids in causing differentiation of human leukemia was first suggested by Breitman et al\(^23,24\) using HL60, a promyelocytic leukemia cell line that was subsequently shown not to carry the 15;17 translocation.\(^25\) Several groups have now isolated mutant HL60 subclones that are resistant to all-trans RA, and in at least one of these lines, an RAR-\(\alpha\) mutation has been identified.\(^26\) (Other groups have reported isolation of RA-resistant subclones of NB4 cells, a new line that stably carries the 15;17 translocation;\(^27\) however, the mechanism[s] involved have not yet been elucidated.) Nonetheless, it seems un

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Supported in part by Grants No. FD-R-000764 from the Orphan Products Division, Food and Drug Administration, CA-57645 from the National Cancer Institute, and EDT-47 from the American Cancer Society.

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0006-4971/93/8207-00S 1$3.00/0

Blood, Vol 82, No 7 (October 1), 1993: pp 1949-1953

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likely that further acquired RAR-α mutations can explain the clinical problem of retinoid resistance. First, clinical resistance appears almost universally and it develops quite rapidly. Once established, it has proven unexpectedly long-lived; however, most groups have reported that patients with APL who have relapsed from a remission induced by all-trans RA but who have not taken the drug for a number of months can achieve a second remission induced by all-trans RA. Second, the described RAR-α mutations in human leukemic cell lines have arisen only under exceptionally artificial circumstances with the pressure of continuous culture and subculture at high retinoid acid concentrations (usually ≥10^{-4} mol/L). By contrast, the usual clinical dose of all-trans RA achieves a peak plasma concentration >10^{-6} mol/L only transiently (for 1 to 2 hours) which disappears with a half-life of 40 minutes. For a genetic mutation theory to hold, one also cannot postulate that RAR-α mutations occur generally but rather only in APL cells (perhaps because the previous translocation had increased the likelihood of spontaneous mutation). Moreover, the mutation must either occur rapidly (to account for the brief remission duration) or naturally (leading to a preferential growth advantage of cells that carry the additional defect). Finally, the mutation cannot be stably carried because it does not appear that clinically induced resistance is permanent. The combination of these unlikely postulates strongly suggests that other mechanisms are operative.

The unlikelihood of further mutations has yielded the current leading hypothesis, namely that pharmacologic alterations in the metabolism of all-trans RA are responsible for clinical retinoid resistance. At once, this hypothesis suggests changes that might occur rapidly and universally, and that might be inducible in the presence of drug but yet reversible at some point after drug withdrawal. We had previously shown that continuous treatment of patients with APL results in a progressive diminution of plasma drug concentrations and speculated that this decrease might eventually in levels that were below some critical threshold that would initiate cytodifferentiation. This induced decrease is now known to be neither disease-specific to APL, nor even species-specific, however, the mechanisms of the induced decrease and the magnitude of its contribution to clinical resistance are not entirely clear.

Clinically, systemic treatment with all-trans RA markedly increases expression of cytoplasmic retinoic acid binding proteins in leukemic cells. In this issue of BLOOD, Delva et al report similar results but with two important distinctions: first, they show that in the same patient CRABP-II expression is increased at the time of relapse compared with that patient’s pretreatment levels; second they show that while cells from relapsing and clinically resistant patients retain partial sensitivity to the cytodifferentiation actions of all-trans RA, a clear shift to the right has occurred in the concentration-effect curve. Sensitivity was retained at a favorite (albeit suprapharmacologic) concentration of 10^{-6} mol/L, but minimal effects were observed at 10^{-7} mol/L. In leukemic cells, an increased amount of CRABP might rapidly bind an excess of cytoplasmic all-trans RA, thus preventing its transport to the nucleus and facilitating its enzymatic catabolism. However, there are some problems with accepting this phenomenon as a sole explanation for resistance. Intuitively, one would expect that the binding capacity of this protein in APL cells would be rapidly saturable with continuous dosing and that the excess would then freely spill into the nucleus. That objection is overcome by noting that increased CRABP expression does not occur in leukemic cells in isolation, but rather as a generalized process in many tissues. Previous work had shown that topical applications of all-trans markedly increases CRABP expression in skin. Thus, one could envision the whole body as a “retinoid sponge” that might rapidly absorb the circulating retinoid from plasma. The work of Delva et al suggest an explanation that is complimentary to the previously described reduction in extracellular drug levels that nonetheless yields the same effect, namely a reduced intranuclear concentration of the ligand.

Some recent developments have increased the complexity of this picture. The pharmacology of all-trans RA has now been examined in patients with a variety of diseases and in normal subjects. It appears that there are extraordinarily wide differences in constitutive rates of retinoid catabolism among individuals, and that these differences are readily distinguished by administering test doses of all-trans RA. We have pursued these tests in more than 80 subjects (and R. Warrell, et al, unpublished data), and it is clear that differences in catabolic rates occur without prior exposure
to exogenous retinoids. In other words, some individuals achieve very low plasma drug levels even with their first dose, and in many cases, this accelerated rate also correlates with reduced plasma concentrations of endogenous retinoids.\textsuperscript{31} There may well be both genetic and environmental explanations for such metabolic differences, but it is clear that clinical pharmacologic studies in small numbers of individuals can be quite misleading. It is tempting to speculate that alterations in such rates create susceptibility to diseases that might be reversed by retinoid treatment, or that individuals with a constitutively rapid rate of clearance might be those who would most benefit from retinoid treatment (albeit in diseases other than APL). An increase in cytochrome P450 enzyme activity may account in part for these differences, because nonspecific inhibitors of these enzymes such as ketoconazole and liarozole significantly reduce both the constitutive and inducible patterns of accelerated catabolism,\textsuperscript{31,40,41} a finding that suggests they are both a manifestation of the same process. In addition, Muindi and Young\textsuperscript{16} have shown that increases in lipid hydroperoxides (the alternate pathway in Fig 1) also can accelerate the oxidation of all-trans RA, and that this process may also be inducible.

There is obviously a tremendous redundancy inherent in these systems that act in concert to buffer intracytoplasmic retinoid concentrations, but their net effect is the same: modulation of the effective intranuclear retinoid concentration before ligand binding and receptor activation. A key question is whether this knowledge can be usefully exploited to contravene the problem of acquired retinoid resistance in APL or other diseases for which such treatment may be useful. Several possible strategies are outlined in Table 1. If excess CRABP is the major problem as suggested by Delva et al,\textsuperscript{38} then use of a retinoid that does not bind CRABP yet still generates the retinoid response may represent a useful approach. In fact, however, one such compound has been readily available for some time: 13-cis retinoic acid. Unfortunately, this agent does not appear to have substantial clinical activity,\textsuperscript{42,43} and in vitro it seems to require at least a log higher concentration than all-trans RA to differentiate freshly aspirated human APL cells in short-term culture.\textsuperscript{42} Intermittent treatment rather than the current practice of continuous daily dosing represents another possible strategy. Adamson et al\textsuperscript{44} recently showed that a drug-induced elevation in CRABP levels decreased within 2 weeks of stopping therapy in a small number of patients. Delva et al\textsuperscript{38} suggest that relapsing APL patients might be tested for such levels using reverse transcription and quantitative polymerase chain reaction analysis to indicate potential clinical responsiveness. However, two problems with this strategy are that retinoid resistance is probably not mediated by increased CRABP alone, and that clinical resistance after withdrawal from all-trans RA dosing extends considerably beyond the 2-week point (longer than 1 year in several of our patients). Nonetheless, anecdotal evidence from several patients with APL suggest that maximal cytodifferentiating effects may be achieved within the first 2 weeks of treatment, and that additional therapy during induction may be superfluous. This important lead (ie, the shortest duration of therapy rather than the lowest possible dose) should be explored further.

Several strategies have sought to overcome the induced decrease in plasma drug levels. The simplest approach would be to administer a larger quantity of drug by the intravenous route. This approach has been constrained to date by the lack of a suitable pharmaceutical formulation; however, a liposomally encapsulated form of all-trans RA has recently become available,\textsuperscript{45} and while probably impractical for extended therapy it should provide a critical test of the pharmacologic theory of acquired resistance. Another approach is to use pharmacologic inhibitors of cytochrome P450 oxidative enzymes. While short-term treatment with both ketoconazole and liarozole can attenuate the induced increase in catabolic rates,\textsuperscript{31,40,41} these combinations have not yet been shown to sustain high plasma levels on an extended basis. A combination with interferon-\(\alpha\) is theoretically appealing both for a possible additive effect of the two agents as well as the activity of interferon to affect the P450 pathway.\textsuperscript{36,47} Although one report clearly suggested reversal of acquired resistance,\textsuperscript{48} another report has shown acceleration of leukemia by this approach.\textsuperscript{49} Combinations of putatively differentiating agents surely represent a fruitful area for clinical testing but not necessarily for the purpose of overcoming retinoid resistance. It was originally believed that lower doses of all-trans RA might decrease induced resistance and avoid other undesirable effects such as excessive leukocytosis and the "retinoic acid syndrome"\textsuperscript{50}; however, this has not proven to be the case.\textsuperscript{51} Finally, it must be recognized that none of the aforementioned strategies may prove successful; therefore, drug discovery using novel screening systems such as NB4 or fresh human leukemic cells in short-term culture may represent the most promising approach. Although HL60 has provided

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<tr>
<td><strong>Strategy</strong></td>
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<tr>
<td>Liposomal encapsulation</td>
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<td>Cytochrome P450 enzyme inhibitors (ketoconazole, liarozole, etc)</td>
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<td>Low-dose retinoid treatment</td>
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tremendous insights into leukemogenesis and myeloid differentiation, as a predictive model for human neoplasia it has proven somewhat less than representative. For APL, the availability of systems that stably carry the relevant chromosomal translocation should provide a considerably more informative result. The first agents that should be tested include alternative retinoids, such as 9-cis retinoic acid, an agent that binds to both RARs and retinoid “X” receptors (RXRs). Other possibilities include agents that selectively activate specific retinoid receptors such as RAR-β, RAR-γ, or the RXRs.

The problem of retinoid resistance in APL extends well beyond this particular disease and applies to the prospects for “differentiation therapy” as a method of cancer treatment. Single agents such as retinoids may affect neoplastic cells at only a specific point in their life cycle. Myeloid cells appear to require all-trans RA at the promyelocyte stage and will arrest there if there is a block; however, leukemic progenitors at an earlier stage may well be insensitive, but at that stage they retain the capacity for self-renewal. If this is a general case, then treatment on an indefinite basis will be required absent some other intervention (eg, use of a cytotoxic drug or generation of an immune response). If so, then resistance to the agent must absolutely not occur, otherwise the clinical response will inevitably prove short-lived, as in acute promyelocytic leukemia. Blood 72:567, 1988


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Retinoid resistance in acute promyelocytic leukemia: new mechanisms, strategies, and implications [editorial; comment]

RP Jr Warrell