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Letter to the Editor

Correspondence re: R. J. Spiegel, I. T. Magrath, and J. A. Shutta. Role of Cytoplasmic Lipids in Altering Diphenylhexatriene Fluorescence Polarization in Malignant Cells. Cancer Res., *41:* 452–458, 1981.

On the Lipid Fluidity of Malignant Lymphoid Cell Membranes

In a recent report, Spiegel *et al.* (3) studied the diphenylhexatriene fluorescence polarization (*P* values) of whole human lymphoblasts and diverse malignant lymphoid cells and their plasma membranes. The authors noted that cytoplasmic lipids accumulate in lymphoid neoplasms and thus decrease the *P* values of these cells, and they concluded that fluorescence polarization measurement on whole cells is an unreliable method to monitor the fluidity of the plasma membrane.

The same conclusion has been drawn *inter alia* by the present authors (5, 7) who showed that cytoplasmic lipid droplets accumulating in mouse lymphoma cells as a function of time after transplantation significantly decreased the *P* values of these cells. The ascites lymphoma cell strains had been obtained by i.p. transplantation of primary thymic lymphomas arisen spontaneously in male GR/A mice.

Moreover, in all cell systems studied (7), the endomembranes contributed to the *P* values resulting in lower *P* values of whole cells than of the corresponding plasma membranes. For this reason, fluorescence polarization measurements done on whole cells, whatever their type, were in general deemed unsuitable for the above-mentioned purpose.

Secondly, Spiegel *et al.*, (3) found no significant differences in the *P* values of plasma membranes isolated from the various neoplastic lymphoid cells or cell lines and plasma membranes from normal lymphocytes. In contrast, the *P* values of the plasma membranes of the GR/A lymphoma cells were significantly lower than those of normal thymocyte plasma membranes (7). However, it has also been shown (1, 2, 4, 5) that (*a*) lymphoma plasma membranes with significantly decreased *P* values and cholesterol/phospholipid molar ratios arise only after 2 or more i.p. transplantations of the primary tumors following selection of cells that are capable of growth in the ascites form, (*b*) this effect may have an immunological basis and may be related to the nonlethal exfoliation of plasma membrane vesicles with high *P* values from the ascites lymphoma cells, and (c) the low *P* values of ascites lymphoma plasma membranes may be normalized to the values of thymocyte plasma membranes if the lymphoma cells are grown in a solid tumor either s.c. or on the peritoneal wall. Growth at other sites, *i.e.*, in spleen or lymph nodes, may differentially affect the cholesterol/phospholipid molar ratio of the lymphoma cellular membranes,[1] which is the major determinant of fluidity (6). In conclusion, membrane fluidity in this lymphoid tumor cell system is a complex and dynamic property depending on the conditions in which the cells grow.

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[1] W. J. Van Blitterswijk, unpublished results.

The letter of Van Blitterswijk *et al.* raises a number of important issues regarding the methodology and premises underlying current investigations of membrane abnormalities in cancer. Their studies support our observations that cytoplasmic lipids and endomembranes are frequently responsible for the findings observed when whole-cell probe techniques are used to determine membrane events.

More importantly, their observations also reflect our concern that studies of membranes in various *in vitro* or *in vivo* tumor models warrant careful review for experimental artifacts. Plasma membrane lipids and intracellular lipids appear to be readily altered by both external and internal stimuli. As reflected in Van Blitterswijk's letter, our data, and numerous other re-

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ports (2, 3, 6, 9, 10), the composition of the external environment (*e.g.*, nutrient media, ascites, or local tissues), the time of observation after transplantation or subculturing of tumor cells into a new environment, and the effects of selection producing sampling errors may all be important. A thorough understanding of the techniques of membrane isolation and purification is also critical for interpreting studies in this field. We found that slight contamination of the membrane "band" isolated on a sucrose gradient could drastically affect observations. If disparate isolation techniques are used by different investigators, their studies may not be measuring the same "membrane." This may account for the present conflict in reported results. Our study and others (4, 6) have failed to demonstrate significant differences in the fluorescence polarization (*P* value) of isolated membranes of malignant or transformed cells and their normal counterparts. However, well-executed studies by Van Blitterswijk's group and others (7) have in fact demonstrated significant membrane differences. Another recent study has shown *P* value alterations in certain malignant cells without a corresponding difference in cholesterol/phospholipid ratios (5). The reason for these different findings is unclear but may reflect experimental technique or true differences in the cell populations being studied. From the currently published literature, it can be stated that the regular occurrence of membrane alterations in malignant cells has not been clearly demonstrated, and its real contribution to the carcinogenic process in any human cancer remains only hypothetical.

It should also be noted that the techniques utilized to measure membrane fluorescence polarization yield only an average measurement that does not account for membrane heterogeneity, probe lifetime variability, or the interaction of the probe with other molecules. For these reasons, the conversion of fluorescent probe or electron spin resonance probe parameters into measures of membrane "microviscosity" is a gross oversimplification and unwarranted. Extrapolation of these empiric measurements to statements about membrane physics and cell surface characteristics must be regarded at this time as largely speculative.

Finally, it should be stated that despite these caveats, this area remains of great interest and has tremendous potential in a number of areas of tumor biology and perhaps cancer therapeutics. Among the many areas of active investigation in this field, some of which are alluded to by Van Blitterswijk, a few others also seem particularly promising and tantalizing in their implications. For example, it has recently been shown that strains of L1210 murine leukemia cells sensitive or resistant to Adriamycin differ in their measured plasma membrane *P* values.[1] Other recent studies have indicated that lipid alterations may affect drug transport or sensitivity to chemotherapeutic agents (1, 8). These types of studies may herald future directions in the study of membrane alterations in cancer and underline the importance of refining the current techniques used to assess the composition and dynamics of the plasma membrane.

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[1] A. Ramu, personal communication.

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