Membrane Remodeling During Phagocytosis in Chronic Myelogenous Leukemia Cells

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Introduction

First, I would like to thank Dr. Neth and the organizers for making this meeting possible. For those of us who have come a long distance, the trip has clearly been educationally profitable and the hospitality of Dr. Neth and his delightful teams of wagon drivers have certainly made this a memorable personal experience as well.

This evening I would like to tell you very briefly the results of some experiments we conducted with chronic myelogenous leukemia cells following phagocytosis. The experiments were initially undertaken in the hope that we could detect some difference in the biochemical behavior of leukemic cells and normal cells which might further our understanding of the former or suggest some therapeutic approaches. At the very beginning, I must tell you that we found no differences between the leukemic and normal cells. However, in both cells we did note some unusual changes in the lipid composition of sub-cellular membrane constituents following phagocytosis of considerable biologic interest. These changes may offer some possibilities for therapeutic manipulation of abnormal phagocytic cells.

Since the lipid composition of biological membranes is a major determinant of their barrier qualities and their permeability characteristics, and since during phagocytosis there is a major architectual rearrangement of membrane constituents in the phagocytic cell, we sought to determine the lipid composition of various permeability barriers within the phagocytic cell both before and after phagocytosis. Although at the time, we were not aware of any therapeutic implications of such potential differences, recent observations of Dr. Tulkens in Brussels suggest that such changes may modulate the effects of some so-called "lysosomotropic" drugs currently utilized in leukemia chemotherapy.

Methods

The basic procedures followed are outlined in Figure 1. In brief, we collected heparin anti-coagulated blood from untreated human patients with chronic myelogenous leukemia. We then separated the CML granulocytes from the red cells and platelets by gravity sedimentation and gentle saline washing. Throughout the isolation procedure, plastic containers and pipettes were used to minimize white cell aggregation. The isolated washed cells were then incubated with an excess of opsonized polystyrene beads so that a large amount of phagocytosis occurred with-

Schema for isolation and lipid analysis of whole cells, Lysosomal Membranes, Plasma Membranes, and Phagosomal Membranes of CML Granulocytes

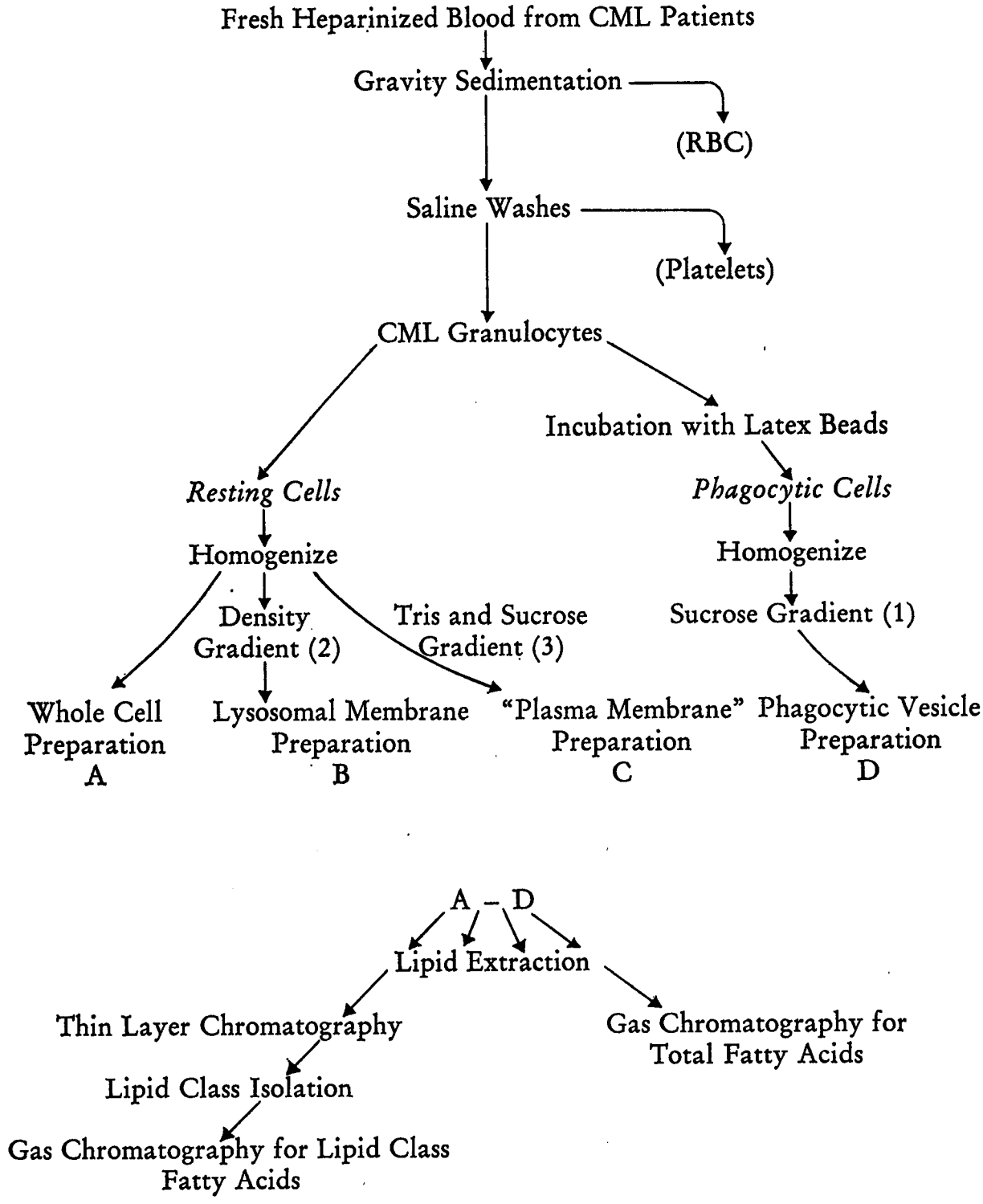

Fig. 1

in approximately 15 minutes. The reaction was stopped by the addition of excess cold saline and the cells were then homogenized in order to liberate the latex beads which were now coated with a phagocytic membrane which was probably derived from a combination of lysosomal membranes and plasma membranes which had fused to form the phagocytic vacuole following particle ingestion. These membranes were then isolated by density centrifugation following the method of Wetzel and Korn (1) while resting cells were similarly homogenized and fractionated to obtain both primary lysosomal membranes and plasma membranes by the methods of Cohn and Hirsch; and Warren, Glick and Nass, respectively (2, 3). The resultant membrane preparations were then extracted for lipid analysis with chloroform and methanol following the method of Folch, Lees, and Sloane Stanley (4) with the modification of adding of one miligram per 100 cc's of Butyrated Hydroxy Toluene as anti-oxidant to all of the solvents. The liquid extracts were then assayed by thin layer chromatography using the method of Skipski, Peterson, and Barclay (5) and by a total lipid phosphorus assay (6). The lipid classes were isolated from the thin-layer plates by elution and analyzed for fatty acids by gas chromatography utilizing methods published elsewhere (7). An "unsaturation index" (U.I.) was calculated for each sample as the sum of the number of double bonds in each fatty acid species multiplied by its mole percentage as determined by the gas chromatography. This index served as a gross estimate of the "fluidity" of the membranes analyzed and also probably reflects their flexibility and permeability.

Results

A summary of the overall results is presented in Table I. Here the "unsaturation index" of the lipids in the whole cells used as starting material is compared to the unsaturation indices of both lysosomes and plasma membranes isolated from those cells prior to phagocytosis, as well as phagocytic vesicles isolated following phagocytosis. Plasma membranes are presented in quotation marks in this table because the product derived by this technique, although the best available, is still subject to some question in terms of purity. In terms of overall unsaturation indices,

Table I: Unsaturation Indices and Representative Fatty Acids of Total Phospholipids in Various Fractions of Chronic Myelogenous Leukemia Granulocytes*

	Whole Cells	Granules	"Plasma Membrane"	Phagocytic Vesicles
U. I.**	110 ± 8	124 ± 4	100 ± 8	65 ± 9
20:4	14 ± 2	17 ± 1	11 ± 2	5 ± 1
16:0	20 ± 3	16 ± 1	28 ± 6	31 ± 6

* Tables I and II modified from Smolen & Shohet (6) with permission of the Journal of Clinical Investigation. ± figures = 1 S. D.

** Unsaturation Index and Sum of Mole % FA Times # Double Bonds/FA.

phatocytic vesicles have a considerably lower unsaturation index than any of the other fractions. This includes particularly both the granule and plasma membrane fractions which are felt to be the precursors of the phagocytic vesicle membranes. When representative polyunsaturated and fully saturated fatty acids are also examined in the same table, it can be seen that there is a consistent reduction in polyunsaturated fatty acid and an increase in saturated fatty acid in the phagocytic vesicles. Similar changes are noted in Table II which presents lipid analyses for the phosphatidylcholine and phospatidylethanolamine lipid sub-classes; again, the data are presented for whole cells prior to phagocytosis and phagocytic vacuoles following phagocytosis. This data may be somewhat more significant than the whole lipid analyses in that the specific lipid sub-classes, which might be expected to enter into metabolic rearrangements following phagocytosis, were particularly analyzed. Again, the phagocytic vesicules have considerably reduced unsaturation indices in both lipid classes in comparison to whole cells, and consistent changes are found when representative fatty acids are examined.

Table II: Unsaturation Indices and Representative Fatty Acids of Separated Phospholipids in Various Fractions of Chronic Myelogenous Leukemia Granulocytes

	Phosphatidylcholine		Phosphatidylethanolamine	
	Whole Cells	Phagocytic Vesicles	Whole Cells	Phagocytic Vesicles
U. I.	70	49	140	122
20:4	35	26	24	98
16:0	33	52	12	43

Discussion

Figure 2 outlines our current understanding of the phagocytic process in terms of the disposition of membrane constituents. Here it can be seen that the phagocytic membrane is composed of elements of both the primary lysosomal membrane and the plasma membrane. Unfortunately, we do not know the percentage of each antecedent constituent and the diagram drawn here is not meant to imply any quantitative accuracy. Nevertheless, it can be seen from Tables I and II that the final lipid composition of the phagocytic vacuole membrane is considerably more saturated than that of either of its parent membranes, so that simple mixing of membrane constituents, in any proportion, can not explain the observed composition of the phagocytic membrane.

Two possible mechanisms for this change in membrane lipid saturation are also outlined in Figure 2. The first is a hydrogen peroxide mediated attack on the unsaturated fatty acid bonds of the lipids making up this membrane. Karnovsky and Sbarra showed long ago that the oxidative burst produced during phagocytosis is related to the generation of hydrogen peroxide within the ingesting granulocyte (8). Many subsequent investigators including most prominately Dr.

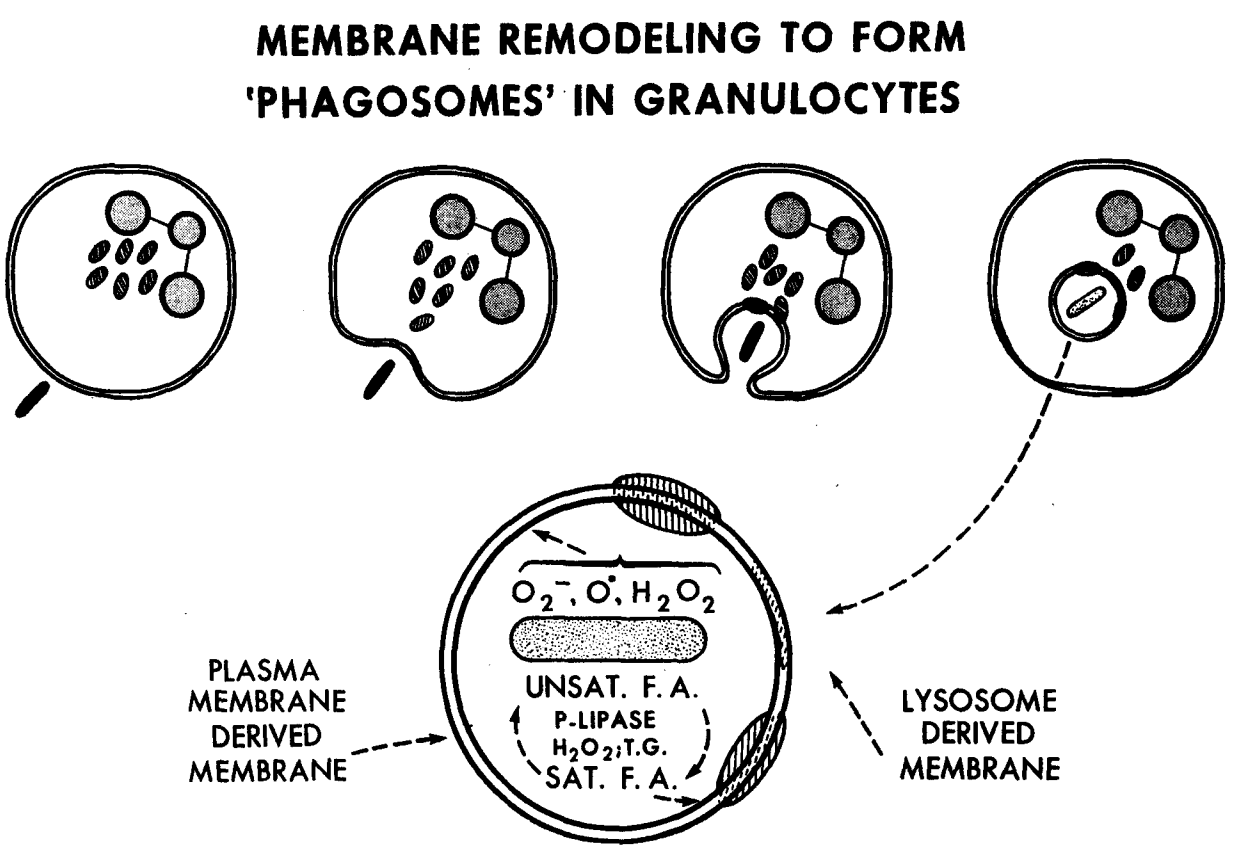

Fig. 2: A schematic diagram representing changes in lipid membrane constituents of granulocytes during phagocytosis. The plasma membrane invaginates and fuses with lysosomal membrane elements to form the eventual phagosomal membrane. This new membrane is thus derived from two parent membranes. However, the final concentration of saturated fatty acids in the phagosomal membrane is greater than that of either the parent membranes. It is assumed that either peroxidation of the phagosomal membrane through the action of hydrogen peroxide or superoxide, or the selective reacylation of that membrane through the activity of a phospholipase or a reacylation system, is responsible for this remodeling.

Klebanoff (9) have suggested that this hydrogen peroxide may be crucial in mediating the subsequent killing of the phagocytized bacteria. Hydrogen peroxide either directly, or indirectly by one of its unstable precursors, superoxide, is well known to attack double bonds of unsaturated fatty acids; and if liberated in close proximity to the newly formed phagocytic membrane, might well be responsible for much of its changed unsaturation index. Alternatively, or perhaps additionally, a phospholipase-reacylase enzyme cycle has been described in white cells (10) which, also perhaps under the influence of hydrogen peroxide, may preferentially attack unsaturated fatty acids on phospholipids replacing them with saturated fatty acids derived from triglycerides (11, 12).

Whether one or both of these mechanisms is operative, it seems clear that the permeability of the phagocytic vesicle may be quite different from that of other membranes within the cell. One might argue that this would be of considerable importance physiologically in that activated lysosomal products would be retained in a contained space surrounding the ingested foreign particle. This would facilitate

the rapid destruction of the ingested bacteria on the one hand while protecting the cell from auto-digestion on the other. I should hasten to add that direct measurements of phagocytic vacuole membrane permeability for large enzyme molecules have not been made, and that this line of reasoning is dependent upon assumptions concerning the influence of the unsaturation index. Moreover, it is much more likely that small co-factor permeability would be influenced than gross enzymatic permeability by these changes. Nevertheless, a dynamic compartmentalization of digestive capacity of the cell following phagocytosis is strongly suggested by these experiments.

A possibility of therapeutically exploring these phenomena has been suggested by the recent studies of Dr. Tulkens and this is the reason I wish to bring this data to the attention of this audience this evening. Dr. Tulkens in Dr. DeDuve's laboratory has shown that certain antibiotics permeate lysosomes quite readily and then apparently become trapped there, perhaps in part due to changes in their ionic state induced by the acidic environment (13). Daunomycin and Adriamycin are included within this classification of drugs. Dr. Tulkens also feels that the Daunomycin probably enters the cell by "piggyback endocytosis" a process somewhat analogous to the phagocytosis process we have studied with larger latex granules. It may be that the therapeutic effectiveness of these drugs, which is probably dependent upon nuclear penetration, is limited by this preferential sequestration in the lysosomes. It is not inconceivable that interference with the remodeling process which has been described here, by the addition of an anti-oxidant group to the chemotherapeutic molecules, would reduce lysosomal sequestration and increase the biologic effectiveness of those agents.

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