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Sphingomyelin Synthase and PKC Activation

In their review of sphingolipids as regulators of cell function, Y. A. Hannun and R. M. Bell (*1*) discuss the hypothesis that some of the reactions involved in sphingolipid metabolism may regulate protein kinase C (PKC) activity through the generation or removal of PKC-inhibiting metabolites. An important addition to the pathways considered in (*1*) is the reaction catalyzed by phosphatidylcholine:ceramide phosphocholine transferase (sphingomyelin synthase). This enzyme transfers the phosphorylcholine head group from the phospholipid phosphatidylcholine (PC) to ceramide, yielding sphingomyelin and diacylglycerol (DAG) (Fig. 1). Since DAG is a known activator of PKC, the action of sphingomyelin synthase allows for a mechanism by which metabolism of sphingolipids can result in stimulation of PKC (through production of DAG).

The sphingomyelin synthase reaction is the major route of sphingomyelin synthesis in a number of biological systems, and a substantial fraction of the enzyme activity appears to be associated with the plasma membrane (*2*). Hence, the high activity, products, and location of sphingomyelin synthase are all consistent with a possible role in PKC regulation. Two examples of how sphingomyelin synthase might physiologically affect PKC activity are as follows. (i) The conversion of sphingosine to sphingomyelin, by acylation and subsequent sphingomyelin synthase–catalyzed head group transfer, would cause the net loss of a PKC inhibitor (sphingosine) and the gain of a PKC activator (DAG) (Fig. 1). Such bimodal regulation might result in steep activation curves and "on-off" stimulation of PKC. This two-step pathway should also be considered when one interprets data from long-term experiments (more than 6 hours) with pharmacological concentrations of sphingosine, where significant metabolism is known to have occurred (*1*). (ii) The sequential action of sphingomyelin synthase (*2*) and neutral- or acid-sphingomyelinase (*3*) yields a two-step cycle in which ceramide is consumed and regenerated with the concomitant conversion of PC into phosphorylcholine and DAG (Fig. 1). In some instances, DAG may stimulate sphingomyelinase activity (*4*) or sphingomyelin synthase activity (*5*). If these observations are generally true, then the cycle could amplify a transient increase in DAG caused by receptor-mediated events by generating more DAG from the abundant stores of PC. A growing conviction that PC is a source of DAG in numerous signaling events (*6*) warrants further consideration of this cycle.

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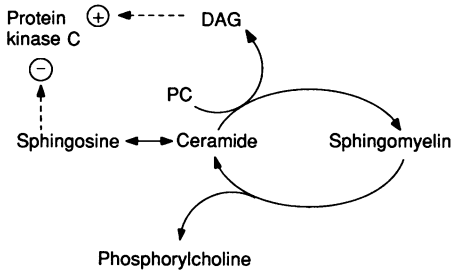

Fig. 1. Proposed dual role of sphingolipid metabolism in PKC regulation.

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Response: The "transferase" pathway of sphingomyelin synthesis is of proven significance for sphingomyelin. Its physiologic role, however, is at present unknown. The comment by Hampton and Morand raises the interesting possibility that sphingomyelin and diacylglycerol production are simultaneously controlled. A number of investigators have looked into this question [for example, (*1*)], and they have not found the "transferase" pathway to be physiologically regulated. However, since the field of physiologic studies of sphingolipid turnover is in its infancy, all options should be evaluated and merit scientific discussion.

Note added in proof: Recent studies on sphingomyelin turnover have defined metabolic pathways for regulated sphingomyelin hydrolysis and regeneration (*2*).

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Rab 12 kD

C. E. Brinckerhoff *et al.* report the "Autocrine induction of collagenase by serum amyloid A–like and β_2-microglobulin–like proteins" (*1*). These authors have found that stimulation of rabbit fibroblasts with agents such as phorbol myristate acetate induces production of "autocrine proteins that, by themselves, can act on the fibroblasts to stimulate collagenase production." Isolation and NH_2-terminal amino acid sequencing of autocrine proteins of 14 kD and 12 kD revealed on "computer searching of the data base" respective homologies with human serum amyloid A and human β_2-microglobulin.

In the case of the "β_2-microglobulin-like" protein, additional insight is obtained by more extensive examination of the literature. The "Complete amino acid sequence of rabbit β_2-microglobulin" reported by Kindt and colleagues in 1979 (*2*) is identical at 16 of 18 positions determined for the Rab 12-kD autocrine protein. Furthermore, the residues at the two discrepant positions, 6 and 10, may also be identical. Deamidation of acid amides is a recognized problem in protein sequencing and could have resulted in assignment of aspartic acid at position 6 of Rab 12 kD (*1*) compared to asparagine in rabbit β_2-m (*2*). The other apparent difference is at position 10, which is tyrosine in

rabbit β_2-m (*2*) and tryptophan in Rab 12 kD (*1*). The possibility that the assignment of tryptophan results from a typographical error in figure 2 of Brinckerhoff *et al.* (*1*) is suggested by the misquoted assignment in that figure of tryptophan, instead of tyrosine, at the same position of human β_2-m, the only sequence to which Rab 12 kD is compared.

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Malaria Red Cell Cytoadherence

In their report on the identification of a malaria sequestration receptor, Ockenhouse *et al.* (*1*) allude to in vitro correlates used to study cytoadherence of *Plasmodium falciparum*–infected erythrocytes. They do not discuss the ex vivo perfusion of the rat mesentery microcirculation, the only method that detects adherence to venular endothelium and the only method with which investigators have studied the cytoadherence phenomenon under flow conditions and physiological wall shear stress (*2*). The venules are the site of true in vivo cytoadherence and microcirculatory occlusion. This approach deserves at least as much attention as cytoadherence to melanoma cells, which are actually not very relevant to in vivo conditions. With the use of the microcirculatory assay, we have found that both soluble thrombospondin (TSP) and antibodies to thrombospondin have a profound inhibitory effect on cytoadherence of *P. falciparum*–infected red cells to venular endothelium (*3*). However, reversibility with these agents has not been attempted, so it is misleading to say (as the authors do) that "neither TSP nor antibodies to TSP have been reported to reverse cytoadherence." Nevertheless, the fact that cytoadherence is blocked with these agents is sufficient evidence that TSP is an important factor in the cytoadherence process. Moreover, Ockenhouse *et al.* compare activity of soluble glycoprotein IV (GPIV) to that of TSP in inhibition assays on a weight basis. This comparison is unwise when the molecular weights differ by a factor of 4 to 5 (TSP is a 450-kD protein, as compared with 88 kD for GPIV); what is really needed is an estimate of relative affinities. This can best be obtained by a comparison in the same system under the same conditions of ionic composition and strength and, of course, equimolar concentrations. Until this comparison is made, one should have an open mind about the relative affinities of GPIV and TSP. Finally, recent studies have shown that TSP and GPIV bind to each other (*4*), hence demonstrating that identifying a receptor does not exclude other proteins (such as TSP) from playing a "binding" role between endothelium receptors and the red cells. It is likely, therefore, that a final picture of malaria red cell cytoadherence will involve a scheme in which both proteins (and perhaps others) play an important role.

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Response: Roth *et al.* object to the fact that we did not cite their work in our paper. We are unaware of any data that enable one to determine whether an ex vivo rat mesoappendix model more closely approximates in vivo sequestration of human parasitized erythrocytes than does the in vitro binding of parasitized erythrocytes to melanoma cells or cultured human endothelial cells. We described the results of in vitro assays because these are the assays used in our laboratory. We encourage investigators who use other assays to confirm, refute, or expand upon our findings using their models.

With specific reference to thrombospondin (TSP), the molecular basis of its interactions with bound infected erythrocytes (IRBC) and with the leukocyte differentiation antigen CD36 is unknown, and there is not an unambiguous single explanation for all of the available data. However, it is clear from previously published reports (*1*) that TSP alone is insufficient to support cytoadherence to cells in vitro. Preliminary data from our laboratory (*2*) suggest that CD36 and TSP bind to independent ligand(s) on IRBC. We have been unable to perform studies comparing the affinity of CD36 with that of TSP because, although we have confirmed that immobilized TSP will bind IRBC, we have been unable, using the methods described in our paper, to detect binding of iodinated TSP to IRBC.

We, like Roth *et al.*, continue to have open minds regarding the relative importance of CD36, thrombospondin, intercellular adhesion molecule 1 (*3*), and other receptors in cytoadherence and sequestration.

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