Is Regulation of a Chloride Channel in Lymphocytes Affected in Cystic Fibrosis?

J. H. Chen *et al.* (*1*) report that the cystic fibrosis (CF) molecular defect is expressed by lymphocyte cell lines. However, single-channel patch-clamp experiments in our laboratory suggest that adenosine 3′,5′-monophosphate (cAMP)–dependent openings of chloride channels in lymphocytes, which is the physiological signature used to discriminate between normal and CF-defective cells (*1, 2*), may not always provide an accurate basis for discrimination.

In August 1988, we began, with generous help from Chen *et al.*, a study of chloride channels in lymphocytes. In our studies, carried out with the same Jurkat cell line they used, as well as an additional Jurkat line and a B lymphoblast line (*3*), we confirmed that the channel, with conductance and kinetic properties essentially as described (*1*), was present with apparent densities (assessed by depolarization-induction of channel activity in excised, inside-out patches tested in Ringer with millimolar concentrations of Ca^{2+}) of 31%, which is close to figures previously reported for a similar and possibly identical channel in epithelial cells (*2*). As Chen *et al.* report, the channel is usually silent in the cell-attached mode. Our results diverge from those of Chen *et al.* (*1*) in that we have been unable to induce activity in this channel with 8-bromo–cAMP, with the cAMP-elevating reagents cholera toxin and prostaglandinE_1 (PGE_1), or, in excised patches, with a catalytic subunit of cAMP-dependent protein kinase (*4*). We have tested 371 cells in the cell-attached mode after exposure to one of the above reagents and have seen evidence of activity in only three patches; at least 66 of these have subsequently been shown to have chloride channels by excision and depolarization. In comparison, we saw active channels in 1/30 cell-attached patches that had not been purposely exposed to any cAMP-elevating agent. We saw channel activity at −50 mV clamp potential in 2/51 of patches with kinase present and in 2/82 patches with kinase absent (5 minutes of recording period before depolarization). Subsequent depolarization showed that at least 10 patches in the kinase condition and 13 patches in the no-kinase condition had chloride channels.

During these experiments, we varied many factors that might contribute to the phenomenon of cAMP-dependent regulation, including serum, culture conditions, and the voltage-time series used for clamping, without effect. Since we have not detected differences among these conditions, we are unable to suggest specific testable hypotheses for our negative results. In light of the near perfect activation reported by Chen *et al.* (*1*) and the evidence that an apparently identical channel can be activated with high efficiency in epithelial cells and fibroblasts (*2, 5*), it is possible that an unidentified procedural variable or physical factor renders our cells unresponsive. We do not yet know if this hypothetical factor is specific to lymphocytes tested in our laboratory or is more general. To date we have been unable to find evidence for cAMP-dependent regulation of a similar channel in primary cultures of sweat gland cells, although we have conducted fewer such experiments with those cells, which are less accessible and, for us, more difficult to study in the cell-attached configuration.

The pattern we find for channel regulation in normal lymphocytes is precisely the pattern considered to be definitive for the CF phenotype (*1, 2*). Therefore, although lymphocytes can immediately be used for cloning the chloride channel, we suggest caution in interpreting experiments that compare, at the single-channel level, normal and CF cells.

GRACE HAGIWARA
MAURI KROUSE
ULRIKE MÜLLER
JEFFREY WINE
Cystic Fibrosis Research Laboratory, Stanford University, Stanford, CA 95305-2130

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3. Jurkat E6-1 cells were obtained from J. H. Chen (*1*); we also used Jurkat cells of unknown designation obtained from C. Clayberger, Department of Pediatrics, Stanford University Medical School. Epstein-Barr virus–transformed lymphoblasts were purchased from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository, Coriell Institute for Medical Research, Camden, NJ 08103. The line used was GM00130B (non-CF, 25-year-old male Caucasian).
4. Measurements of cAMP concentrations in lymphocytes in response to cholera toxin and PGE_1 were made by H. Schulman, Stanford University School of Medicine, who also supplied the catalytic subunit of cAMP-dependent protein kinase, which was prepared, assayed, and applied essentially as in (*1*).
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Response: The comment by Hagiwara *et al.* points out the difficulty in studying the chloride channel in epithelial cells and lymphocytes. The channel is unconventional in that it can irreversibly or transiently undergo activation by three different mechanisms, that is, induction by patch excision followed by sustained depolarization, by cAMP-dependent phosphorylation and by some as yet undefined Ca^{2+}-dependent pathway. Given the complexity of the regulatory process and the activation mechanism, it is perhaps not unexpected that we all experience some variability in both channel density and in successful gating of this channel.

At present, we stand by our interpretation of the channel results as reported. The chloride channel in lymphocytes is virtually identical to the channel documented in secretory epithelial cells. On that point there is no disagreement between our two laboratories. The failure of Hagiwara *et al.*, to induce chloride channel gating by cAMP-dependent phosphorylation in sweat gland cells as well as lymphocytes suggests that the same variable may be affecting results in both of their preparations. As we indicated in the note added in proof to our original report, we have found daily fluctuations in channel density. We have yet to determine the specific variable (serum, media, size of patch, and so forth that may be the source of this problem; we welcome comments from other laboratories.

Note added in proof: Recent evidence (*1*) suggests that a cAMP–dependent lymphocyte anion permeability is activated during G1 phase of the cell cycle and that this activation is not detectable in CF-derived lymphocytes. The suggestion that the chloride channel is cell cycle–dependent may account for some of the apparent variability in channel density.

PHYLLIS GARDNER
Department of Medicine,

Stanford University School of Medicine, Stanford, CA 94305
HOWARD SCHULMAN
Department of Pharmacology, Stanford University School of Medicine

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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.

RANDOLPH Y. HAMPTON*
OLIVIER H. MORAND†
Department of Biochemistry, University of Wisconsin, Madison, WI 53706

*Present address: Merck Sharpe & Dohme Research Laboratories, P.O. Box 2000, R80-B11, Rahway, NJ 07065.
†Present address: Department of Metabolic Diseases, F. Hoffmann–La Roche, Ltd., Basel, Switzerland.

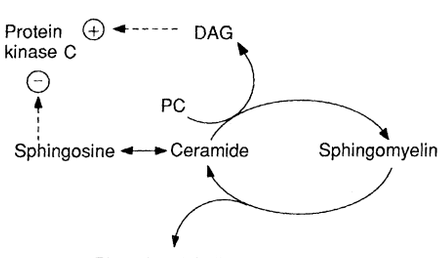

Fig. 1. Proposed dual role of sphingolipid metabolism in PKC regulation.

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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*).

YUSUF A. HANNUN
Department of Medicine, Division of Hematology/Oncology, Duke University Medical Center, Box 3355, Durham, NC 27710

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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