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

REFERENCES AND NOTES

1. J. H. Chen, H. Schulman, P. Gardner, *Science* **243**, 657 (1989).
2. R. A. Frizzell, G. Rechkemmer, R. L. Shoemaker, *ibid.* **233**, 558 (1986); M. J. Welsh and C. M. Liedtke, *Nature* **322**, 467 (1986); R. A. Schoumacher *et al.*, *ibid.* **330**, 752 (1987); M. Li *et al.*, *ibid.* **331**, 358 (1988).
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*).
5. C. E. Bear, *FEBS Lett.* **237**, 145 (1988).
6. Supported by NIH grants DK 39659-01 and HL 42368 and by the Cystic Fibrosis Research, Inc., and the Cystic Fibrosis Foundation. We thank C. Ward and T. Law for culturing the cells and for experimenting with culture conditions, H. Schulman for providing the kinase and running assays of kinase activities, J. Chen and C. Clayberger for providing cells, and J. Chen, H. Schulman, P. Gardner, R. Aldrich, S. Garber, and R. Tsien for comments.

15 February 1989; revised 16 March 1989; accepted 15 September 1989

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,