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Letters to the Editor

CYSTIC FIBROSIS CILIARY INHIBITOR

Letter to the Editor

Don R. Barnett and Barbara H. Bowman
Department of Human Biological Chemistry and Genetics
University of Texas Medical Branch
Galveston, Texas 77550

We wish to correct a statement regarding our work (4) mentioned by Beratis et al. (3), in Pediatric Research. In a review of the background of isolation of the cystic fibrosis (CF) ciliary inhibiting protein these authors state "Bowman et al., by using DEAE-cellulose chromatography identified, in media from fibroblast cultures derived from patients with CF, a chromatographic fraction that inhibits the ciliary activity of oyster gill tissues. Gel filtration of the fraction which contained the ciliary inhibitor separated three protein peaks which corresponded to molecular weights of 140,000-160,000, 20,000 and 13,000 or less, but ciliary inhibitory activity could not be detected in any of these molecular weight species." The actual statement in our paper was "The three molecular weight species were detected by radioactivity and were in concentrations too low to test on oyster cilia." In recently published data from our laboratory, the molecular weight of the cystic fibrosis ciliary inhibitor was found to be in the range of 4,500-10,000 and was estimated to be approximately 7,000 (2,6).

The ciliary inhibitor which we have identified may or may not be the same small molecular weight factor observed by Beratis et al. (3). It is very encouraging, however, that the fractions from media of cultured cystic fibrosis fibroblasts causing inhibition of ciliary movement in two ciliary systems have been found to be in the same molecular weight range by investigators in three different laboratories (2,3,6,7,10).

The fact that amniotic cells from fetuses at high risk for the CF gene, as well as cells derived from heterozygous individuals, produce the ciliary inhibitor (3,4,5,7,9) suggests a close relation to the genetic defect and necessitates further identification and characterization of the responsible molecule. There has been one instance of conflicting results. Conover, et al. (8), have reported that the ciliary inhibitor from fibroblast media has properties common to the complement component C3a, and have suggested that the inhibitor is C3a anaphylatoxin. Our recent findings, however, demonstrate that there is no indication of immunological cross-reactivity between our preparation of the ciliary inhibitor and an authentic sample of C3a using a monospecific antibody to C3a. In addition, our samples do not demonstrate anaphylatoxin activity when tested in the guinea pig ileum smooth muscle system (1).

References

1. Barnett, D.R., Hugli, T.E., and Bowman, B.H.: Immunological studies on the cystic fibrosis ciliary inhibitor from cultured fibroblasts. (In preparation).
2. Barnett, D.R., Kurosky, A., Bowman, B.H., Hutchison, H.T., Schmoyer, I., and Carson, S.D.: Cystic Fibrosis: Molecular weight estimation of the ciliary inhibitor. Tex. Rep. Biol. Med. 31:703 (1973).
3. Beratis, N.G., Conover, J.H., Conod, E.J., Bonfort, R.J., and Hirschhorn, K.: Studies on ciliary dyskinesia factor in cystic fibrosis. III. Skin fibroblasts and cultured amniotic fluid cells. Pediat. Res. 7:958 (1973).
4. Bowman, B.H., Barnett, D.R., Matalon, R., Danes, B.S., and Bearn, A.G.: Cystic fibrosis: Fractionation of fibroblast media demonstrating ciliary inhibition. Proc. Nat. Acad. Sci. USA, 70:548 (1973).
5. Bowman, B.H., Lockhart, L.L., Herzberg, V.L., Barnett, D.R., Armstrong, D., and Kramer, J.: Cystic fibrosis: Synthesis of ciliary inhibitor by amniotic cells. Clinical Genetics 4:461 (1973).
6. Bowman, B.H., Schmoyer, I., Barnett, D.R., Kurosky, A., and Hutchison, T.: Cystic fibrosis: Biochemical characterization of the ciliary inhibitor. Amer. J. Hum. Genet. 25:16A (1973).
7. Conover, J.H., Beratis, N.G., Conod, E.J., Ainbender, E., and Hirschhorn, K.: Studies on ciliary dyskinesia factor in cystic fibrosis. II. Short term leukocyte cultures and long term lymphoid lines. Pediat. Res. 7:224 (1973).
8. Conover, J.H., Conod, E.J., and Hirschhorn, K.: Ciliary dyskinesia factor: A component of complement. Amer. J. Hum. Genet. 25:21A (1973).
9. Danes, B.S., and Bearn, A.G.: Oyster ciliary inhibitation by cystic fibrosis culture medium. J. Exp. Med. 136:1313 (1972).
10. Danes, B.S., Litwin, S.D., Hutteroth, T.H., Cleve, H., and Bearn, A.G.: Characterization of cystic fibrosis factor and its interaction with human immunoglobulin. J. Exp. Med. 137:1538 (1973).

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N.G.Beratis, J.H.Conover, E.J.Conod, R.J.Bonforte, K.Hirschhorn
Department of Pediatrics, Division of Medical Genetics, Mount Sinai School of Medicine of C.U.N.Y., New York, N. Y. 10029

Drs. Barnett and Bowman in their letter imply that we have incorrectly interpreted their results. We do not agree with this statement and are, on the other hand, gratified that this exchange of letters will help to clarify the issue and that their recent findings reported in this letter and elsewhere (1,2) are in accord with our findings regarding the molecular weight of the ciliary dyskinesia factor reported originally in our paper (3).

Bowman et al.(4) by using DEAE-cellulose chromatography have reported the fractionation of the culture medium of normal, heterozygous and homozygous CF skin fibroblasts into two protein peaks. The major peak was found to inhibit the ciliary activity of oyster gill tissue, but the minor peak consisting of low molecular weight material was lost during dialysis and, therefore, was not examined for ciliary inhibition. Likewise, two radioactive peaks, a major and a minor, were identified in medium of fibroblasts maintained in radioactive leucine. The major peak also inhibited the oyster ciliary activity, while the minor peak was lost during dialysis. Gel filtration of DEAE-cellulose fractions on Sephadex G-200 separated three radioactive peaks with approximate molecular weights of 150,000, 20,000 and 13,000 or lower. Whether or not their inability to detect ciliary inhibitory activity in these 3 fractions was due to a concentration problem or to other factors in no way makes our statement that "ciliary inhibitory activity could not be detected" incorrect. Alternative explanations could include loss of activity during gel filtration or irreversible adsorption of this small molecule to the Sephadex G-200 used in their column. We hope that this matter is satisfactorily clarified.

With reference to the last paragraph of Barnett and Bowman's letter, we find their results very interesting and only wish to indicate that we have only suggested that this molecule may be C3a (5) and that we still feel that this molecule is related to the complement-kinin system.

References

1. Barnett, D.R., Kurosky, A., Bowman, B.H., Hutchison, H.T., Schmoyer, I., and Carson, S.D.: Cystic Fibrosis: Molecular weight estimation of the ciliary inhibitor. Tex.Rep.Biol. Med. 31:703 (1973).
2. Bowman, B.H., Schmoyer, I., Barnett, D.R., Kurosky, A., and Hutchison, T.: Cystic fibrosis: Biochemical characterization of the ciliary inhibitor. Amer.J.Hum.Genet. 25:16A (1973).
3. Beratis, N.G., Conover, J.H., Conod, E.J., Bonforte, R.J., and Hirschhorn, K.: Studies on ciliary dyskinesia factor in cystic fibrosis. III. Skin fibroblasts and cultured amniotic fluid cells. Pediat.Res. 7:958 (1973).
4. Bowman, B.H., Barnett, D.R., Matalon, R., Danes, B.S., and Bearn, A.G.: Cystic fibrosis: Fractionation of fibroblast media demonstrating ciliary inhibition. Proc.Nat.Acad.Sci. USA, 70:548 (1973).
5. Conover, J.H., Conod, E.J., and Hirschhorn, K. Studies on ciliary dyskinesia factor in cystic fibrosis. IV. Its possible identification as anaphylatoxin (C3a)-IgG complex. Life Sciences 14:253 (1974).

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