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NOTES

Obligate Parasitism of Trachoma Agent: Lack of Trachoma Development in Ethidium Bromide-Treated Cells

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Trachoma agent failed to develop in eukaryotic cells pretreated with ethidium bromide.

Trachoma agent is a prokaryotic obligate parasite of eukaryotic cells [Y. Becker, *in* A. Zuckerman and D. Weiss (ed.), Dynamic aspects of the host-parasite relationship in infectious diseases, Academic Press, Inc., New York, *in press*], but the reason for the obligate parasitism is not yet known. Recent studies from our laboratory (7; Sarov and Becker, Excerpta Med., *in press*) indicated that the elementary bodies of trachoma agent, which are the infectious stage in the agent's growth cycle, contain deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerase molecules. The synthesis of trachoma RNA within the trachoma elementary bodies can be stimulated by incubation in vitro in the presence of the four nucleoside triphosphates. Under these conditions, polyribonucleotide chains with a molecular weight of about 10^6 daltons were synthesized. This finding suggested that trachoma elementary bodies might depend on the host cell for the supply of the four nucleoside triphosphates for the initiation of RNA synthesis and the developmental cycle. This prompted the idea that the cell mitochondria might play a vital role in the initiation of trachoma development. This notion was strengthened by further findings (1), e.g., that the alkaloid emetine (4, 5), which completely and irreversibly inhibits peptide synthesis on cytoplasmic, but not on mitochondrial (6), polyribosomes, did not interfere with the development of trachoma agent. It was therefore of interest to determine the effect of ethidium bromide, which forms reversible complexes with DNA (8) and inhibits the synthesis of nucleic acids in mitochondria (9), on the development of trachoma agent. Trachoma agent failed to develop in cells treated with ethidium bromide prior to, or during, infection.

The T'ang strain of trachoma agent was serially propagated in FL human amnion cell cultures (3). Ethidium bromide, 1 μg/ml, purchased from Sigma Chemical Co., St. Louis, Mo., was added to the FL cultures either prior to or after trachoma infection, and the infected cultures were incubated at 37 C for 48 hr. The yield of trachoma elementary body progeny in untreated and eithidium bromide-treated cells was determined by the plaque assay developed by Bernkopf (2).

As shown in Table 1, at low concentrations ethidium bromide only partially affected trachoma development. Increase in drug concentration to 1 μg/ml inhibited the formation of the trachoma progeny by 99.9%, whereas 2 μg of ethidium bromide per ml completely prevented the development of any trachoma infectious progeny. The stages in the developmental cycle of trachoma agent which were sensitive to ethidium bromide were studied by adding the drug (2 μg/ml) at different time intervals after infection and determining the trachoma yield at 48 hr postinfection. It was found that, when ethidium bromide was added during the initial 6 hr after infection, the development of trachoma agent was completely prevented. Addition of the drug after this stage markedly inhibited trachoma development but still allowed the formation of a small yield of infectious trachoma progeny (Table 2). Addition of ethidium bromide to the infected cells up to 23 hr after infection resulted in an inhibitory effect ranging from 99.9%, when added at 10 hr after infection, to 74%, when added at 24 hr after trachoma infection.

TABLE 1. *Effect of ethidium bromide on the synthesis of trachoma elementary bodies progeny*

Drug concn (μg/ml)	Trachoma yield (plaque-forming units/ml)	Per cent inhibition
0	4.0×10^7	0
0.10	1.4×10^7	0
0.25	1.6×10^7	0
0.50	0.7×10^7	54
0.75	1.0×10^6	93
1.00	2.5×10^4	99.9
2.00	0	100

TABLE 2. *Effect of ethidium bromide on trachoma development when added at different time intervals after infection*

Inhibitor added at (hr after infection)	Trachoma yield (plaque-forming units/ml)	Per cent inhibition
0	0	100.0
3	0	100.0
6	0	100.0
10	5.0×10^3	99.9
14	2.0×10^5	99.7
17	3.5×10^6	95.0
23	2.1×10^7	74.0
29	7.3×10^7	9.0
48 (control)	8.0×10^7	0.0

Addition of ethidium bromide to the infected cultures later than 23 hr had almost no inhibitory effect on the development of trachoma progeny. These results demonstrated that the initial 24-hr period of the trachoma developmental cycle is the stage sensitive to ethidium bromide treatment. The highest sensitivity to ethidium bromide was displayed during the early 10 hr after infection. This finding also indicated that the initiation of trachoma development in the eukaryotic cells depends on processes sensitive to ethidium bromide treatment.

To demonstrate that ethidium bromide affects the host cell mitochondria rather than the developing trachoma elementary bodies, the FL cells were treated with ethidium bromide (10 μg/ml) for different intervals prior to trachoma infection. As shown in Table 3, pretreatment of FL cells for 3 hr, followed by removal of the excess of the drug, rendered the cells insensitive to trachoma infection. Pretreatment of FL cells with ethidium bromide for shorter time periods permitted some, though considerably reduced, development of trachoma agent. The ethidium bromide-treated FL cells regained their ability to support trachoma development after reincubation for a 24-hr period after removal of the drug.

TABLE 3. *Effect of ethidium bromide on trachoma development when added to cells prior to infection*

Ethidium bromide[a] added at (hr prior to infection)	Trachoma yield (plaque-forming units/ml)	Per cent inhibition
0 (control	2.8×10^7	—
1	2.2×10^6	70
2	1.6×10^5	97
3	0	100
4	0	100
5	0	100

[a] Ethidium bromide was added to the FL cell cultures at a concentration of 10 μg/ml at the times indicated. Prior to infection, the cultures were washed with several changes of phosphate buffer, inoculated with trachoma agent, and further incubated at 37 C. At 48 hr after infection the cultures were harvested, and the yield of infectious elementary bodies was determined.

The major finding in the present study is the demonstration that the development of trachoma agent is sensitive to ethidium bromide treatment during the initial stages of development. Since it was found that ethidium bromide selectively inhibits mitochondrial RNA synthesis (9), it is possible to assume that in the absence of active mitochondria trachoma elementary bodies fail to develop. However, it is also possible that pretreatment of the host cells with ethidium bromide allowed the retention of a sufficient concentration of the drug to inhibit the development of trachoma elementary bodies. The ability of trachoma agent to develop in emetine-treated cells (B. Gutter and Y. Becker, *submitted for publication*) in the absence of host protein (1, 4) and nucleic acid synthesis (4, 5; Z. Gilead and Y. Becker, Eur. J. Biochem., *in press*), but in the presence of active mitochondria, might indicate the importance of the latter for trachoma development. However, further studies are needed to elucidate the mechanism of ethidium bromide inhibition of trachoma agent development.

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LITERATURE CITED

1. Becker, Y., and Y. Asher. 1972. Synthesis of trachoma agent proteins in emetine-treated cells. J. Bacteriol. 109:966–970.
2. Bernkopf, H. 1967. A plaque test for TRIC agents. Amer. J. Ophthalmol. 63:1206–1207.
3. Bernkopf, H., P. Mashiah, and Y. Becker. 1962. Correlation between morphological and biochemical changes and the appearance of infectivity in FL cell cultures infected with trachoma agent. Ann. N.Y. Acad. Sci. 98:62–81.

4. Grollman, A. P. 1966. Structural basis for inhibition of protein synthesis by emetine and cycloheximide based on an analogy between ipecac alkaloids and glutarimide antibiotics. Proc. Nat. Acad. Sci. U.S.A. 56:1867–1874.
5. Grollman, A. P. 1968. Inhibitors of protein biosynthesis. Effects of emetine on protein and nucleic acid biosynthesis in HeLa cells. J. Biol. Chem. 243:4089–4094.
6. Perlman, S., and S. Penman. 1970. Mitochondrial protein synthesis resistant to emetine and response to RNA synthesis. Biochem. Biophys. Res. Commun. 40:941–948.
7. Sarov, I., and Y. Becker. 1971. Deoxyribonucleic acid-dependent ribonucleic acid polymerase activity in purified trachoma elementary bodies: effect of sodium chloride on ribonucleic acid transcription. J. Bacteriol. 107:593–599.
8. Tomchik, R., and M. G. Mandel. 1967. Action of ethidium bromide on growth of herpes virus in cell cultures. Nature (London) 215:87–88.
9. Zylber, E., C. Vesco, and S. Penman. 1969. Selective inhibition of the synthesis of mitochondria-associated RNA by ethidium bromide. J. Mol. Biol. 44:195–204.