Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 45 (1988)
Heft: 2

Artikel: In vitro culture of "Trypanosoma brucei gambiense" isolated from human cerebro-spinal fluid : short communication

Autor: Kronenberger, P. / Miezan, B.

DOI: https://doi.org/10.5169/seals-314071

Acta Tropica 45, 181–182 (1988)

Projet de Recherches Cliniques sur la Trypanosomiase (PRCT), B.P 1425, Daloa, Ivory Coast

In vitro culture of *Trypanosoma brucei gambiense* isolated from human cerebro-spinal fluid

Short communication

P. KRONENBERGER, B. MIEZAN

T. b. gambiense infections in humans are known for their very low parasitemias which are often undetectable with current techniques. Consequently, inoculation into mammalian hosts has been the only method available to study the parasite. However, the possible existence of selection mechanisms within these hosts which may alter the biological characteristics of the trypanosome, brings into question the validity of our present knowledge of this parasite.

The development of in vitro culture systems for bloodstream form trypanosomes (Hirumi et al., 1977) has only partially resolved this problem as existing in vitro techniques for *T. b. gambiense* still rely on mouse passaged trypanosomes to initiate a culture (Brun et al., 1981; Baltz et al., 1985; Mhando et al., 1986). However, Jenni and Brun (1982) and Brun and Jenni (1983) were able, on one occasion, to isolate *T. b. gambiense* by the direct transfer of blood or cerebro-spinal fluid from a patient into culture.

The present communication describes an in vitro culture system for *T. b. gambiense* isolated directly from human cerebro-spinal fluid (CSF).

Microtus montanus embryonic fibroblasts (MEF) (Brun et al., 1981) were grown in 80% HEPES buffered Eagles Minimal Essential Medium (EMEM, from Gibco), 10% heatinactivated normal human serum and 10% fetal calf serum (Gibco). For trypanosome culture, the medium was supplemented with 2-mercaptoethanol (Sigma), Na-pyruvate (Gibco), thymidine (Sigma) and hypoxanthine (Sigma) according to Baltz et al. (1985). Kanamycine (Bristol Labs) and Nystatine (Gibco) were initially added to the medium at concentrations up to 400 µg/ml and 400 U/ml, respectively. This medium is referred to as BEMEM FCS/NHS.

5 ml of CSF were collected aseptically from patients by lumbar puncture. To estimate trypanosome numbers, half of the sample was centrifuged twice and counted. The other half of the sample was used for direct inoculation in vitro.

The CSF was diluted to 50% with medium BEMEM FCS/NHS and transferred to a 24-well plate containing the confluent MEF monolayer; the wells were filled to the top. The plates were incubated at 37 °C in 4% CO_2/96% air. The next day, when trypanosomes were seen on the feeder layer, 90% of the CSF/medium mixture was discarded and the supernatant adjusted to 1 ml/well with fresh medium. The culture was left untouched until the trypanosome density reached 10^4/ml in the supernatant, which took about 2 to 3 weeks.

Using the system described, it was possible to isolate and grow *T. b. gambiense* from CSF of sleeping sickness patients, starting with low parasite numbers. The results of 22 trials are given in Table 1 which shows that the system was successful in about half of the cases. Trypanosomes could be grown to 10^6 parasites/ml. The population doubling time varied between 18 and 24 h.

Preliminary results suggest that the method is equally suited for trypanosomes isolated from lymph node fluid: 3 out of 6 such isolates were cultured successfully. Culture of bloodstream trypanosomes was also attempted, but with little success. Apparently, the obligatory separation of the few trypanosomes from whole blood, by mini anion exchange column or by differential centrifugation, reduces their ability to grow.

Correspondence: P. Kronenberger, Hamburg Tropical Institute, Liberian Research Unit, P. O. Box 538, Bong Mining Cy, Monrovia, Liberia

Table 1. Isolation trials from CSF

Patient (1)	Tryps (2)	CSF		Mouse (3)	In vitro		Mouse Infect. Test (4)
		Protein (mg%)	Cells (/mm^3)		Initial growth	Reached 10^4/ml	
897/2	3	52	388	+	+	+	+
1004R	80	91	110	–	+	+	+
1049	2	64	298	–	+	+	–
1051	2	48	266	ND	+	+	+
1052	1	50	202	–	–	–	ND
1053	0	57	504	–	–	–	ND
1057	1	33	122	–	+	–	ND
1058	10	50	188	–	+	–	ND
1059	2	29	12	ND	+	–	ND
1060	5	44	140	ND	–	–	ND
1030R	4	52	224	+	+	+	+
614RR	1	87	116	ND	+	–	ND
1062	5	71	372	ND	+	+	ND
1040R	1	61	460	–	+	+	ND
1076	0	28	6	ND	–	–	ND
1077	0	21	6	ND	–	–	ND
1078	1	107	764	ND	+	–	ND
1079	2	64	284	–	+	–	ND
1080	5	56	406	+	+	–	ND
1081	50	39	30	–	+	+	ND
1085	1	78	1260	–	+	–	ND
1086	20	75	1236	–	+	+	ND

ND = Not done; (1) "R" is a code for relapse after an Arsobal treatment, "RR": 2nd relapse. (2) Estimate of the number of trypanosomes in the inoculum. (3) Human isolates which infected *Mastomys natalensis*. (4) Infectivity of the in vitro grown trypanosomes to *M. natalensis*.

Experiments are underway to investigate the resistance of trypanosomes to Melarsoprol (Arsobal, Mel B), the only commercially available drug to cure late stage sleeping sickness in humans, using in vitro isolated CSF-trypanosomes from untreated patients and from patients who relapsed after an Arsobal treatment.

Acknowledgments. We thank Dr. P. de Raadt, P. Cattand, Dr. F. Doua, Dr. D. Mehlitz, Dr. L. Jenni, Dr. T. Baltz, Dr. I. Maudlin and P. Galbreath for helpful discussions and the staff of the P. R. C. T. Clinic and Laboratory in Daloa for their technical assistance. This work was supported by the World Health Organization and the Ministry of Public Health, Ivory Coast.

Baltz T., Baltz D., Giroud Ch., Crockett J.: Cultivation in a semi-defined medium of animal infective forms of *Trypanosoma brucei, T. equiperdum, T. evansi, T. rhodesiense* and *T. gambiense*. EMBO Journal *4*, 1273–1277 (1985).

Brun R., Jenni L.: In vivo and in vitro production of *Trypanosoma b. gambiense*. Proceedings of Symposium on the Diagnosis of African sleeping sickness due to *T. gambiense*, ed. by P. J. Crooy, p. 33–35. Smith Kline-RIT, Antwerp 1983.

Brun R., Jenni L., Schönenberger M., Schell K.-F.: In vitro cultivation of *Trypanosoma brucei, T. rhodesiense* and *T. gambiense*. J. Protozool. *28*, 470–479 (1981).

Hirumi H., Doyle J. J., Hirumi K.: African trypanosomes: cultivation of animal-infective *Trypanosoma brucei* in vitro. Science *196*, 992–994 (1977).

Jenni L., Brun R.: A new in vitro test for human serum resistance of *Trypanosoma (T.) brucei*. Acta trop. (Basel) *39*, 281–284 (1982).

Mhando P. J., Yanagi T., Nakazawa S., Fukuma T., Kanbara H.: In vitro cultivation of bloodstream forms of *Trypanosoma brucei gambiense* with fibroblasts from various organs of the mouse. Trop. Med. *28*, 45–53 (1986).