Ann. Parasitol. Hum. Comp., 1993, *68 :* n° 2, 67-69.

Mémoire.

Key-words: Entamoeba histolytica. Isoenzyme pattern. Virulence marker.

Mots-clés : Entamoeba histolytica. Profils enzymatiques. Marqueurs de virulence.

CHANGE OF ISOENZYME PATTERN DURING LONG-TERM POLYXENIC CULTIVATION OF *ENTAMOEBA HISTOLYTICA*

N. VRCHOTOVÁ, O. DITRICH, M. GIBODA

SUMMARY

Isoenzymes of phosphoglucomutase and hexokinase were repeatedly evaluated using starch gel electrophoresis in polyxenic cultures of *Entamoeba histolytica*. In two out of 18 strains spontaneous changes of isoenzyme patterns were recorded. While originally they were categorized into virulent group of zymodemes, following isoenzyme analysis classified them as non-virulent. The relation between virulence and isoenzyme pattern is questionable.

RÉSUMÉ : **Changement de la formule isoenzymatique d'*Entamoeba histolytica* pendant la culture polyxénique prolongée.**

Les isoenzymes PGM et HK ont été évaluées sur plusieurs générations par électrophorèse sur gel d'amidon de cultures polyxéniques de *Entamoeba histolytica*. Deux souches sur 18 présentent un changement de profil isoenzymatique. A l'origine ces souches ont été classées dans le groupe virulent selon les zymodèmes, alors que les souches correspondantes des autres générations sont classées comme non-virulentes. La relation entre la virulence et le profil isoenzymatique est remise en question.

INTRODUCTION

The electrophoretic isoenzyme pattern of *Entamoeba histolytica* was considered to be reliable virulence marker for a considerable period. Sargeaunt (1985) divided *E. histolytica* strains into virulent (isolates from patients with symptomatic amoebiasis) and non-virulent (isolates from cyst-carriers) groups and categorized their isoenzyme patterns. Out of four enzymes originally evaluated (malic enzyme, glucose phosphate isomerase, hexokinase and phosphoglucomutase) only the last two were showed to be significant. Virulent strains were characterized the following isoenzymes: β isoenzyme and the same time lack α isoenzyme of phosphoglucomutase (PGM), β and δ isoenzymes of hexokinase (HK).

The objectives of present work were to estimate the influence of long-term polyxenic cultivation to the isoenzyme pattern.

MATERIALS AND METHODS

In the strain collection of *Entamoeba histolytica*, altogether 18 strains were polyxenically cultivated on Dobell-Leidlaw medium *(Table I)*. The first 14 isolates originated from cyst carriers coming to Czechoslovakia from various geographical areas in tropics and subtropics. The last four strains were obtained from the strain collection of the Martsinovsky Institute of Tropical Medicine and Parasitology, after long-term polyxenic cultivation in Pavlovova's medium. More detailed information about the origin of strains and about their virulence evaluated using *in vivo* intracaecal inoculation of laboratory rats were presented formerly (Giboda *et al.*, 1990).

To analyze isoenzyme patterns, thin-layer starch gel electrophoresis was carried out after Wraxall and Culliford (1968) and Sargeaunt *et al.* (1978). The isoenzyme electrophoretic mobility of PGM and HK was observed.

RESULTS

Results of repeated isoenzyme analysis of all strains are presented in *Table I.* In most strains, isoenzyme pattern remained constant during several months of polyxenic cultivation.

The change of isoenzyme pattern was recorded in 2 strains. Although the strain designated 515 was isolated from cyst-carrier, the patient had specific antibodies in this serum against *E. histolytica*. One month after the isolation, the strain was found non-virulent by intracaecal inoculation of rats, but the original isoenzyme analyses indicated that it belongs to a virulent zymodeme (β isoenzyme of PGM and β and δ isoenzymes of HK). Repeated analyses 7 and 8 months after the isolation indicated, that the isoenzyme pattern changed: we found no β, but α isoenzyme of PGM, and no β and δ, but α and γ isoenzymes of

Institute of Parasitology, Academy of Sciences of Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic.

Accepté le : 15 décembre 1992.

TABLE I. — *Repeated isoenzyme analyses of polyxenic* Entamoeba histolytica *strains.*

No	STRAIN	ORIGIN	PERIOD	PGM		HK			
			(month)	α	ß	α	ß	γ	δ
1	MGA	cyst	5	+	−	+	−	+	−
		carrier	6	+	−	+	−	+	−
			17	+	−	+	−	+	−
2	MGB	cyst	5	+	−	+	−	+	−
		carrier	6	+	−	+	−	+	−
			12	+	−	+	−	+	−
3	MGC	cyst	14	+	−	+	−	+	−
		carrier	17	+	−	+	−	+	−
4	MGG	cyst	1	+	−	+	−	+	−
		carrier	5	+	−	+	−	+	−
5	MGH	cyst	1	+	−	+	−	+	−
		carrier	5	+	−	+	−	+	−
6	MGI	cyst	5	+	−	+	−	+	−
		carrier	8	+	−	+	−	+	−
			11	+	−	+	−	+	−
7	V	cyst	1	+	−	+	−	+	−
		carrier	2	+	−	+	−	+	−
			8	+	−	+	−	+	−
8	PA	cyst	3	+	−	+	−	+	−
		carrier	5	+	−	+	−	+	−
9	FH	cyst	10	+	−	+	−	+	−
		carrier	11	+	−	+	−	+	−
			14	+	−	+	−	+	−
10	276	cyst	1	+	−	+	−	+	−
		carrier	6	+	−	+	−	+	−
			8	+	−	+	−	+	−
11	815	cyst	2	+	−	+	−	+	−
		carrier	6	+	−	+	−	+	−
12	1245	cyst	5	+	−	+	−	+	−
		carrier	8	+	−	+	−	+	−
			10	+	−	+	−	+	−
13	37	cyst	3	+	−	+	−	+	−
		carrier	5	+	−	+	−	+	−
14	515	cyst	1	−	+	−	+	−	+
		carrier	7	+	−	+	−	+	−
			8	+	−	+	−	+	−
15	A	amoebic	456	−	+	−	+	−	+
		dysentery	460	−	+	−	+	−	+
16	K	amoebic	444	−	+	−	+	−	+
		dysentery	448	−	+	−	+	−	+
17	SM	amoebic	252	−	+	−	+	−	+
		dysentery	256	−	+	−	+	−	+
18	T	amoebic	264	−	+	−	+	−	+
		dysentery	268	+	−	+	−	+	−
			277	+	−	+	−	+	−

Period: The interval between the isolation and isoenzyme analysis. +/−: presence/absence of the isoenzyme.

HK. This pattern corresponded to non-virulent zymodeme. At interval during the 6 month period rat virulence test of this strain was proved to be non-virulent.

From one animal tested, the strain *E. histolytica* designated R515 was reisolated. It was maintained in the same way as the parent culture. Isoenzyme profile of R515 strain after 2 months cultivation coresponded to the same non-virulent zymodemes as the parental strain.

We observed the similar changes in other polyxenically cultivated stock strain T. At the time of isolation it was highly virulent in the rat, but during continual cultivation the virulence of this strain slowly decreased (Gordeeva L. M. personal communication). In our rat experiments 22 years after isolation the strain was proved to be non-virulent (Giboda *et al.*, 1990). Isoenzyme analysis indicated that strain T belongs to virulent zymodeme (presence of β isoenzyme of PGM and β and δ isoenzymes of HK). The strains was continuously maintained in our laboratory and was re-tested 4 and 9 months later. The results of both of these later tests indicated that the isoenzyme pattern changed (the presence of α isoenzyme of PGM and α and isoenzymes of HK) and corresponded to non-virulent zymodeme.

DISCUSSION

Reports by various authors testing virulence of strains and clones of this organism suggest that cultures of *E. histolytica* are in fact heterogenous population containing both virulent and non-virulent representatives (Orozco *et al.*, 1985). Furthermore, it was demonstrated that the isoenzyme pattern of this organism can be changed after change of cultivation conditions and bacterial association (Mirelman *et al.*, 1986*a*; Mirelman *et al.*, 1986*b*; Mirelman, 1987). In that case changes were significant, since according to the zymodeme strain classification changed from non-virulent into virulent. In some cases strain of *E. histolytica* possessing virulent zymogram has been isolated from cyst-carriers (Gittler and Mirelman, 1986; Giboda *et al.*, 1990). Changes of isoenzyme pattern were activated experimentally by infecting animals with two different virulent clones of different virulent zymodemes; the zymodeme pattern of recovered virulent organism was different from that of both challenged clones (Sargeaunt *et al.*, 1988). The results of some experiments revealed the steadiness of the isoenzyme pattern after restoring of virulence using cholesterol feeding or repeated animal passage (Vinayak and Chugh, 1985; Clarck *et al.*, 1992) consider on the basis of their isoenzyme (HK) evaluation and on the basis of examination using small subunit of the ribosomal RNA, the pathogenic and nonpathogenic forms of *E. histolytica* as being distinct species.

Our results support the opinion that the isoenzyme pattern of *E. histolytica* may be influenced by culture conditions. In both cases, the change was observed in both enzymes tested simultaneously, what indicates that the change was not caused by a single mutation.

The correlation between isoenzyme pattern and virulence is not so evident as were stated originally.

Acknowledgments. — The authors are indebted to Dr GORDEEVA, Martsinovski Institute Trop. Med. Parasitol, Moscow for the providing of collection strains and Dr VEREŠOVÁ and Mrs. STARCZEWSKA for the technical assistance.

REFERENCES

Clark C. G., Cunnick C. C., Diamond L. S. : *Entamoeba histolytica:* Is conversion of « nonpathogenic » amebae to the « pathogenic » form a real phenomenon? *Exp. Parasitol.*, 1992, *74,* 307-314.

Giboda M., Ditrich O., Vrchotová N., Gutvirth J., Noll P., Štěrba J. : *Entamoeba histolytica*—the virulence of the imported strains. *Folia Parasitol.,* 1990, *37,* 9-19.

Gitler C., Mirelman D. : Factors contributing to the pathogenic behavior of *Entamoeba histolytica. Am. Rev. Microbiol.,* 1986, *40,* 237-261.

Mirelman D. : Effect of culture conditions and bacterial associates on the zymodemes of *Entamoeba histolytica. Parasitol. Today,* 1987, *3,* 37-40.

Mirelman D., Bracha R., Chayen A., Aust-Kettis A., Diamond L. S. : *Entamoeba histolytica:* Effect of growth conditions and bacterial associates on isoenzyme patterns and virulence. *Exp. Parasitol.,* 1986, *62,* 142-148.

Mirelman D., Bracha R., Wexler A., Chayen A. : Changes in isoenzyme patterns of a cloned culture of nonpathogenic *Entamoeba histolytica* during axenization. *Infect. Immunity,* 1986, *54,* 827-832.

Orozco E., Suárez M. E., Sanchez T. : Differences in adhesion, phagocytosis and virulence of clones from *Entamoeba histolytica,* strain HM1:IMSS. *Int. J. Parasitol.,* 1985, *15,* 655-660.

Sargeaunt P. G. : Zymodemes expressing possible genetic exchange in *Entamoeba histolytica. Trans. Roy. Soc. Trop. Med. Hyg.,* 1985, *79,* 86-89.

Sargeaunt P. G., Jackson T. F. H. G., Wiffen S. R., Bhojnani R. : Biological evidence of genetic exchange in *Entamoeba histolytica. Trans. Roy. Soc. Trop. Med. Hyg.,* 1988, *82,* 862-867.

Sargeaunt P. G., Williams J. E., Greene J. D. : The differentiation of invasive and non-invasive *Entamoeba histolytica* by isoenzyme electrophoresis. *Trans. Roy. Soc. Trop. Med. Hyg.,* 1978, *72,* 519-521.

Vinayak V. K., Chugh S. : Electrophoretic isoenzymes of isolates of *Entamoeba histolytica* before and after revival of their virulence. *Indian J. Med. Res.,* 1985, *81,* 372-377.

Wraxall B. G. D., Culliford B. J. : A thin—layer starch—gel method for enzyme typing of blood strains. *J. Forensic. Sci. Soc.,* 1968, *8,* 81-82.