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HETEROTRANSFORMATION OF TRYPANOSOMA CRUZI AS MEDIATED BY A FUNGUS

HETEROTRANSFORMATION OF *TRYPANOSOMA CRUZI* AS MEDIATED BY A FUNGUS*

Introduction

Although transformation has been demonstrated with some frequency in bacteria, this phenomenon has been shown in protozoans upon only four occasions: Greenberg and Trembley (1954) and Greenberg (1956) noted a transfer of pyrimethamine resistance between a resistant and a sensitive strain of *Plasmodium gallinaceum*, and Inoki and Matsushiro (1960) have shown DNA mediated transformation of pararosaniline resistance in *Trypanosoma gambiense*. Honigberg and Read (1960) and Honigberg and Livingston (1968) noted an increase of pathogenicity of an avirulent *Trichomonas gallinae* upon exposure to a cell-free homogenate of a virulent strain and also showed similar results by mixing virulent DNA with the avirulent strain. Recently, Lehmann (1970) noted that cultures of *Trypanosoma cruzi* grown with the fungus *Candida sp.* possessed, cytochemically, more dehydrogenases than axenic controls; after animal passage and subsequent culture, the same enzyme complement as seen in mixed cultures prevailed. Aside from the above, Yoeli *et al.* indicated a transfer of drug-resistance between mixed species of recent malaria involving *P. berghei* and *P. vinckei*.

Materials and Methods:

Three strains of cultural *Trypanosoma cruzi* were employed; they were maintained in Tobies medium (1950) or in Brain-Heart Infusion Blood agar at a room temperature of 23-26°C. The two strains involved in the first part of the study were Bull, isolated in Costa Rica by Dr. Rodrigo Zeledon from a naturally infected dog, and H-38, from Costa Rica, from a human proved positive by xenidiagnosis with *Triatoma dimidiata*, the gut contents of which were injected into a mouse. The haemoflagellate used in the second portion of the work was

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T. cruzi H-134; this human derived strain was isolated by xenodiagnosis and subsequently inoculated into a rat from which it was subcultured. The initial strain of fungus used was Candida sp. as identified by Dr. Fernando Montero-G.

Dehydrogenases were searched for by the cytochemical method of Barka and Anderson (1963) and the incubation medium consisted of either phosphate buffer (pH 7.0) or phosphate buffer (pH 7.8). The following represents the working medium:

Buffer	2.5 ml.
Substrate stock (1 M)	1.0 ml.
DPN or TPN (5 mgm. per ml.)	1.0 ml.
$MgCl_2$, 0.05 M	1.0 ml.
Nitro BT (5 mgm. per ml.)	2.5 ml.
Distilled water	1.0 ml.

"Just" air-dried smears of 7-12 day cultures were placed in moist chamber petri dishes and incubated for one and twenty hours after which they were rinsed in distilled water, air dried and observed; results for both times were identical. The dehydrogenases studied were: alpha-keto-glutaric, fumaric, glucose-6-phosphate, isocitric, lactic, malic, oxalosuccinic, pyruvic and succinic - wet smears fixed with acetone, ethanol, formacin,or methanol were generally negative.

Candida sp. to be used for preparing cell-free homogenates and for nucleic acid extraction were grown for 4-6 days in flasks containing 100-125 ml of Sabaroud Maltose broth. The organisms were concentrated by centrifuging, re-suspended in 0.01M Na Citrate and disintegrated in an ice bath by means of a Sonifier Cell Disruptor. Material used in cell free homogenate work was pre-filtered through a 0.45 Micron grid membrane and sterilized by passing through an UF fritted glass filter. DNA and RNA were prepared by the methods described by Mandel and Honigberg (1964) and Clark (1964); the products were sterilized by filtration through a fritted glass filter.

Results

I. Results of the initial work (Lehmann, 1970)

In the uncontaminated condition, both Bull and H-$_3$8 showed only one dehydrogenase each - isocitric dehydrogenase at pH 7·0 for Bull strain and lactic dehydrogenase at pH 7·0 for H-$_3$8. After 10-14 day exposure to Candida sp. Bull displayed lactic dehydrogenase at both pH 7·0 and 7·8 and isocitric dehydrogenase at pH 7·0 and 7·8; strain H-$_3$8, under the same conditions, was positive for lactic dehydrogenase at both pH levels and for isocitric dehydrogenase at pH 7·0. Following animal passage and subculture the now axenic fungus-exposed strains showed identical enzyme patterns as those in the Candida-trypanosome association; the patterns were maintained through five subcultures of 10 days each which marked the termination of this particular phase of the work.

II. Results of the second phase

Dehydrogenases of Candida sp., T. cruzi, and T. cruzi in association with Candida.

Candida sp. alone shows positive reactions for 7 of the 9 dehydrogenases at both pH levels (7.0 and 7.8). Alpha-keto-glutaric and malic dehydrogenases were the exception.

T. cruzi grown axenically is positive for but three of the nine enzymes at both pH levels; these are glucose-6-phosphate, isocitric and lactic dehydrogenases. When, however, T. cruzi H-138 is grown in the presence of Candida six of the nine dehydrogenases are present; those lacking are alpha-keto-glutaric, fumaric and malic. Fumaric dehydrogenase, a constituent enzyme of Candida sp., is not transferred to T. cruzi in this particular circumstance.

T. cruzi cultured with Candida cell free homogenate

Flagellates grown in the presence of Candida cell extract, and with cell extract treated with RNA-ase show enzymes identical to those found in Candida including fumaric dehydrogenase. When the homogenate is exposed to DNA-ase the trypanosomes display only those enzymes found in the wild, or normal, strain of parasite.

T. cruzi cultured with DNA and RNA extracted from Candida.

Trypanosomes grown with Candida DNA show all of those enzymes normally associated with the fungus. Exposure of the DNA to DNA-ase reverses the action and the flagellates are positive for only those three enzymes expected in normal, axenic cultures.

Fungus RNA, either alone or RNA-ase treated, imparts no additional ehzymes to T. cruzi. Only those reported from the wild strain are to be seen.

Passage of the transformed strain through a vertebrate.

Thirty day DNA transformed culture material was injected into three laboratory mice. Four weeks later the mice were sacrificed and cultures were made in Brain-Heart Infusion medium.

Cultures from two of the three mice were positive and enzyme analyses of 9-12 day cultures were made. The results showed that the enzyme complement of the organisms was identical to that of the DNA transformed cultures.

Normal cultures were treated as above. Cytochemical examination indicated that the flagellates possessed the same dehydrogenases as previously noted in the wild strain.

Discussion

It seems evident that the incidents reported above constitute an example of true transformation; actually, because of rather wide taxonomic disparity between the involved organisms, the term heterotransformation might well be employed. Those facts substantiating the case are the action of fungal cell free homogenate and DNA in imposing in the trypanosomes exactly the same dehydrogenases noted in the fungus; likewise, the negating effect of DNA-ase upon these entities further substantiates the notion of transformation. The heredible nature of this phenomenon is shown by the fact that the transformed characters remain stable following animal passage and subsequent cultivation.

Summary - with reference to the second portion of the investigation.

One human, cultural strain of Trypanosoma cruzi cytochemically displays three dehydrogenases at two pH levels: Glucose-6-phosphate, isocitric and lactic dehydrogenases. When cultured in the presence of a fungus, Candida sp., the flagellate acquires an additional four enzymes which are among the normal complement of the fungus; only one fungal enzyme does not appear in the trypanosome, fumaric dehydrogenase.

T. cruzi cultured in the presence of either Candida DNA or cell free homogenate can be shown to possess the identical enzyme content of the fungus and following animal passage it was found that the characters remained stable. Candida RNA has no influence on enzyme modification nor does DNA or cell homogenate treated with DNA-ase. Transformation appears to be the mechanism but it is a unique form of transformation in that the organisms are not closely related and, in fact, are members of different Kingdoms.

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