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CELL FUSION, USING SENDAI VIRUS, TO EFFECT INTER-SPECIES TRANSFER OF A CELL-ASSOCIATED PARASITE (*THEILERIA PARVA*)

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Abstract—IRVIN A. D., BROWN C. G. D., KANHAI G. K., STAGG D. A. and ROWE L. W. 1974. Cell fusion, using Sendai virus, to effect inter-species transfer of a cell-associated parasite (*Theileria parva*). *International Journal for Parasitology* **4**: 519–521. Baby hamster kidney (BHK) cells were fused with *Theileria parva*-infected bovine lymphoid cells, using u.v. light-inactivated Sendai virus. The resultant hamster/bovine heterokaryons were shown to be infected with *T. parva*. In some cases parasites were detected in cells which apparently contained only BHK nuclei.

INDEX KEY WORDS: Cell fusion; heterokaryons; baby hamster kidney cells; *Theileria parva*; Sendai virus; bovine lymphoid cells; autoradiography; tritiated thymidine.

INTRODUCTION

THE PRODUCTION of interspecific heterokaryons by virus-induced cell fusion was first performed by Harris & Watkins (1965). Since that time the technique has been widely used in a variety of fields but its application in the field of parasitology has not so far been exploited. We have used the technique to effect inter-species transfer of an intra-cellular host specific parasite (*Theileria parva*) the causative organism of East Coast fever of cattle.

MATERIALS AND METHODS

T. parva-infected bovine lymphoid cells (C2 strain) were grown in suspension in modified Eagles MEM with Earles balanced salt solution supplemented with 20% foetal calf serum (Malmquist, Nyindo & Brown, 1970). Baby hamster kidney cells (BHK 21 clone 13) were maintained as monolayers in identical medium, and prior to fusion were subjected to a 24-h pulse of 0·25 μCi/ml of thymidine-6-H^3 (specific activity 5 Ci/mmol). Autoradiography revealed that 98 per cent of cells were thus labelled. Sendai virus was grown in embryonated hens' eggs incubated at 35°C using the method of Steplewski & Koprowski (1970). Virus was inactivated with u.v. light and haemagglutination titres determined as described by Harris, Watkins, Ford & Schoefl (1966). Cell fusion was carried out in Leighton tubes with flying coverslips using standard techniques (Harris *et al.*, 1966; Steplewski & Koprowski, 1970).

Sendai virus was used at 8000 HAU per 10^7 total cells. Virus was added to a cold C2 cell suspension and, after 15 min, the virus/C2 mixture was added to established monolayers of BHK cells in Leighton tubes. The cell ratio was approximately 10 C2 to 1 BHK and the total cell number per tube was 10^7 in 2 ml of medium. One-h after fusion, supernates were discarded and monolayers washed, trypsinised and diluted 1 to 8 in fresh medium. Two-ml aliquots were then seeded into clean tubes. Two-h later, and subsequently every 24 h for 5 days, coverslips from pairs of Leighton tubes were removed, washed twice in normal saline, fixed in methanol, stained in Giemsa and mounted cell surface uppermost on a microscope slide. Coverslips were examined microscopically and notable fields recorded and photographed. The coverslips were then decolorised in methanol, extracted in 5% trichloroacetic acid at 4°C for 30 min and treated for autoradiography as previously described (Irvin, Brown, Boarer, Crawford & Kanhai, 1973) using a 6-day exposure period. The fields previously recorded were re-photographed.

RESULTS

In the coverslip preparations examined 24 h after cell fusion, 15·0 per cent of cells were multinucleate and of these 6·3 per cent were

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heterokaryons containing theilerial macroschizonts (Fig. 1). In all preparations the two types of nuclei in heterokaryons were readily differentiated by autoradiography (Fig. 2). In some cases the C2 nuclei were apparently rejected from the heterokaryons but the macroschizonts, which continued to develop, were less readily rejected and many cells showed only BHK nuclei with macroschizonts distributed throughout the cytoplasm. In some cells, macroschizonts began to transform towards microschizonts and in one series of experiments microschizonts were seen in 3-day heterokaryons.

Nuclear fusion was seen in a number of parasitized cells. In most cases these cells appeared to be

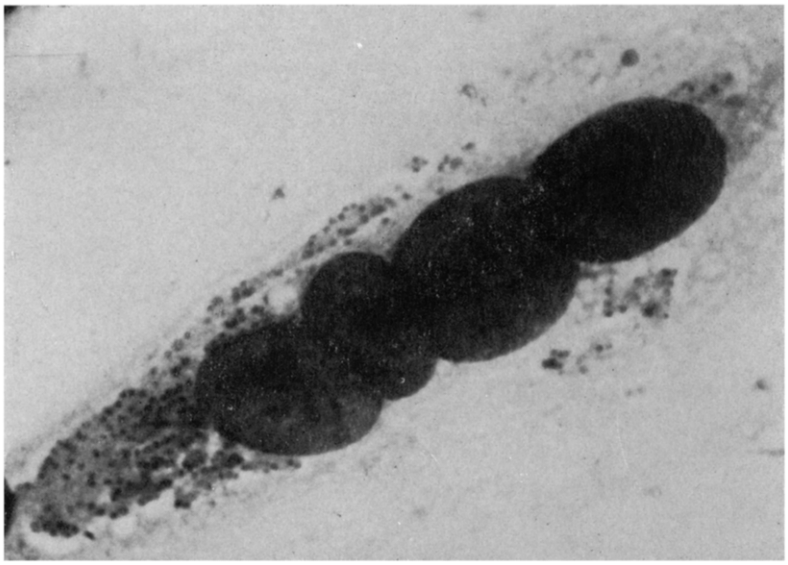

FIG. 1. A multinucleate cell containing macroschizonts of *T. parva*, 48 h after fusion. Giemsa stain × 1400.

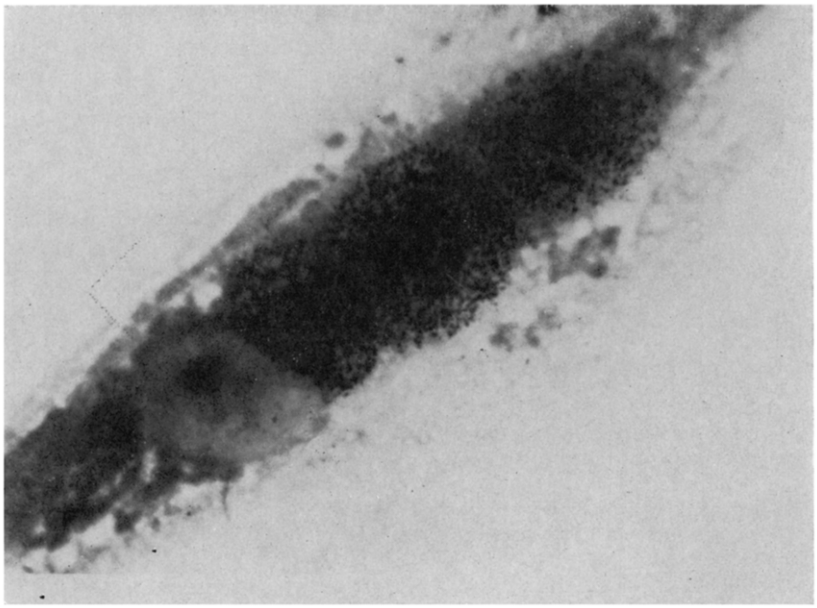

FIG. 2. The same cell as Fig. 1 after autoradiography, showing that the cell is a heterokaryon containing 3 BHK nuclei labelled with tritiated thymidine and one unlabelled C2 nucleus. × 1400.

BHK homosynkaryons. Heterosynkaryon formation could not be confirmed.

DISCUSSION

The work described demonstrates the feasibility of transferring a cell-dependent parasite to the cells of a different species. So far it has not been possible to maintain parasitized cultures beyond 7 days, but attempts are currently in progress to select out parasitized hybrid and BHK cells and to grow them in pure culture. In addition, preliminary studies with other cell lines have shown that mouse heart cells will also fuse with C2 cells. If selective growth can be achieved, and a non-bovine cell line parasitized with *T. parva* is obtained, the production of an attenuated parasite strain for immunisation of cattle and the development of a small laboratory model become real possibilities. Furthermore it seems likely that cell fusion techniques may be applicable in inducing transfer of other intra-cellular parasites, such as *Plasmodium*, to cells of different species.

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