

REPORT NO. IAEA-R-1414-F

TITLE

Attempts to adapt *Theileria parva*, the causative organism of East Coast fever of cattle to small laboratory animals, (part of a coordinated programme on isotopes and radiation in animal parasitology and immunology)

FINAL REPORT FOR THE PERIOD

15 October 1973 - 14 October 1974

AUTHOR(S)

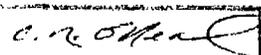
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CERTIFIED BY: 

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Final Report

- a. i) Contract number 1414/RB
ii) Title of Project "Attempts to adapt Theileria parva, the causative organism of East Coast fever of cattle, to small laboratory animals".
iii) Institute - East African Veterinary Research Organization.
iv) Chief scientific investigator - A.D. Irvin
v) Period covered - October 1973 to October 1974.

b. Description of work carried out:-

i) Irradiation of athymic nude mice

Groups of homozygous Nu Nu mice, heterozygous Nu mice and Swiss mice were irradiated at 400, 600, 800 and 1000 rads on a single occasion. All mice in the 1000 rad groups died. The other mice all survived a 50 day experimental period. There was no difference in the susceptibility of Nu Nu mice to the effects of irradiation when compared with heterozygotes. Swiss mice were slightly more resistant than either of the other two groups.

ii) Susceptibility of athymic nude mice to the inoculation of T. parva-infected bovine tissue culture

T. parva-infected bovine lymphoid cells grown in culture, were inoculated into 6 groups of mice:-

1. Nu Nu irradiated at 800 rads
2. Nu Nu non-irradiated
3. Nu irradiated at 800 rads
4. Nu non-irradiated
5. Swiss irradiated at 800 rads
6. Swiss non-irradiated

Tumour-like masses, similar to those described earlier (Irvin, Brown, Kanhai, Kimber and Crawford, 1974) were detected in mice from groups 1, 2, 3 and 5. Those in group 1 mice continued to grow throughout the 50-day experimental period, reaching a final mass of about 25% of the total body weight. Parasitized bovine lymphoid cells were detected in large numbers in these masses, and also on impression smears of body organs. There was no evidence that mouse cells became invaded by the parasite.

Tumour-like masses in mice from groups 2, 3 and 5 reached their maximum size about day 14, and all had regressed by day 35.

Irradiated Nu Nu mice were shown in this, and subsequent experiments, to be unable to reject inoculated T. parva-infected cells. These cells, when inoculated by the sub-cutaneous route, were massively invasive and eventually killed the mice. On intra-peritoneal inoculation however, cells failed to become established. This was subsequently shown to be as a result of macrophage activity which had been unaffected by irradiation. This contrasts with previous work (Irvin et al, in preparation) when good 'take' of cells was achieved following inoculation of cells into irradiated, neonatally thymectomised Swiss mice. 'Take' in these mice was thought to have occurred because of the incomplete development of the peritoneal macrophage system in neonatal mice.

In order to develop a system for passaging T. parva-infected bovine lymphoid cells in mice, which might lead to parasite attenuation or infection of mouse cells, the intra peritoneal route of inoculation is preferable since cells grow in suspension and are easy to harvest.

iii) Heterotransplantation of T. parva-infected cells from cattle to mice

Lymph nodes were collected from a steer which died of experimentally-induced East Coast fever. The nodes were treated to obtain suspensions of parasitized cells which were inoculated subcutaneously into irradiated Nu Nu mice. Tumour-like masses containing parasitized cells developed in all mice. These masses behaved similarly to those induced by inoculation of cells transformed and cultured in vitro.

Cells from the tumour-like masses were successfully passaged 6 times in irradiated Nu Nu mice, in which they survived for a total of over 4 months without being transferred to culture or cattle.

iv) Inoculation of irradiated mice with irradiated tissue culture

In earlier work (Irvin, Brown and Stagg, 1974) it was recorded that irradiation of T. parva-infected bovine lymphoid cells could result in an increased production of microschizonts.

Culture irradiated at 800 rads was inoculated into groups of Swiss mice which had been irradiated, splenectomised or splenectomised and irradiated. Theilerial piroplasms in mouse erythrocytes were detected in small numbers in two irradiated mice.

This represents the first time that T. parva has been shown capable of infecting non-bovid cells.

v) Adaptation and possible attenuation of Theileria parva-infected cells grown in irradiated mice

Theileria parva-infected bovine lymphoid cells were taken from 8 cattle immediately after death from East Coast fever. Cells were inoculated into groups of irradiated Swiss and athymic nude mice. Cells became established in one group of Swiss mice and 2 groups of athymic mice. Development of cells in mice only occurred if cells concurrently established in culture; when establishment in culture was delayed, cells failed to develop in mice. Cells from one of the isolates in athymic mice were passaged 6 times through further mice. On inoculation of these mouse-passaged cells into cattle, the animals underwent mild reactions and subsequently resisted a lethal ECF challenge. This suggests that T. parva-infected cells may have become attenuated on passage through mice resulting in a potential East Coast fever immunization procedure.

vi) Comparative growth of bovine lymphosarcoma cells and Theileria parva-infected lymphoid cells in athymic (nude) mice

Twelve athymic (nude) mice were irradiated at 700 rads and 12 similar mice were untreated. Four mice from each group were inoculated subcutaneously with T. parva-infected bovine lymphoid cells; 4 from each group with bovine lymphosarcoma cells; and 4 from each group with normal bovine cells.

Tumours developed in all the irradiated mice inoculated with T. parva-infected cells or lymphosarcoma cells, but not in any other groups. Tumours in these 2 groups grew progressively: those of T. parva-infected cells were extensively invasive and killed the mice, whereas the lymphosarcoma cells were not invasive, no deaths resulted, but mice were killed on humane grounds when the tumours became so large that they hampered movement and inhibited feeding and drinking.

These findings contrast with the natural conditions in cattle: lymphosarcoma being a malignant neoplasia, and T. parva infection (East Coast fever) a non-malignant condition with no evidence of tumour formation.

SUMMARY

Title "Attempts to adapt Theileria parva, the causative organism of East Coast fever of cattle, to small laboratory animals".

Institute East African Veterinary Research Organization

Chief Investigator A.D. Irvin

Period of Contract October 1973 to October 1974

When T. parva-infected bovine lymphoid cells, grown in culture, were inoculated subcutaneously into whole body irradiated Swiss mice, tumour-like masses developed at the site of inoculation. These were composed almost entirely of parasitized bovine cells, and no evidence of mouse cell invasion could be detected. Some vascular spread of bovine cells was noted.

Rejection of these masses began after about 14 days and was usually complete by 50 days. Increasingly improved "take" of parasitized bovine cells was achieved using, respectively: irradiated, neonatal Swiss mice; irradiated, thymectomised, neonatal Swiss mice; and irradiated congenitally athymic (nude) mice. In this last group of mice there was continued growth of cells at the site of inoculation resulting in very large-tumour-like masses. Vascular spread of parasitized cells was extensive, and death of mice, in many cases, occurred as a result of the invasive behaviour of cells. Mouse erythrocytes, infected with piroplasms, were occasionally seen.

When T. parva-infected cells were taken from a fatal case of East Coast fever, and inoculated into irradiated athymic mice, cell growth was similar to that observed in mice inoculated with culture-derived cells. Parasitized bovine cells were passaged six times in athymic mice, and after 136 days, cells still appeared morphologically normal.

Cells which had undergone passage in mice were inoculated into susceptible cattle. The cattle underwent mild reactions and resisted subsequent challenge.

The work carried out suggests that irradiated athymic mice may be a useful laboratory model for East Coast fever research, for example, in chemotherapeutic and immunization studies. Further adaptation of T. parva should also be possible so that the parasite can be grown in more conventional mice.

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- Irvin, A.D., Brown, C.G.D., Kanhai, G.K., Kimber, C.D. & Crawford, J.G. (1974)
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Submitted for publication

- Irvin, A.D., Brown, C.G.D., Kanhai, G.K. & Stagg, D.A.
Comparative growth of bovine lymphosarcoma cells and Theileria parva-infected lymphoid cells in athymic (nude) mice.
- Irvin, A.D., Brown, C.G.D., Stagg, D.A., Kanhai, G.K., Kimber, C.D. & Radley, D.E.
Adaptation and possible attenuation of Theileria parva-infected cells grown in irradiated mice.

