The growth of cell culture-attenuated rinderpest virus in bovine lymphoblasts with B cell, CD4 and CD8 alpha/beta T cell and gamma/delta T cell phenotypes

P. B. Rossiter, K. A. J. Herniman, I. D. Gumm and W. I. Morrison

Kenya Agricultural Research Institute, National Veterinary Research Centre, Muguga, PO Box 32, Kikuyu and International Laboratory for Research on Animal Diseases, PO Box 30709, Nairobi, Kenya

Cloned bovine lymphoblastoid cell lines, transformed by the protozoan parasite Theileria parva were infected with cell culture-attenuated rinderpest virus vaccine. The virus grew readily in lymphoid B cells, CD4 and CD8 alpha/beta T cells and gamma/delta T cells producing new infectivity, viral antigens, c.p.e and total cell death. There did not appear to be a predilection for any particular phenotype of lymphoblast. The results imply that if the vaccine causes immunosuppression, it could do so through a variety of mechanisms.

Although technically feasible, adequate control of rinderpest still eludes many African and Asian countries. As a result major international campaigns have been inaugurated to improve this control and, hopefully, to eradicate the disease from both continents. During the early stages the campaigns will try to increase the levels of herd immunity in affected areas through vaccination of susceptible cattle. At present the vaccine most widely accepted for this purpose is the cell culture-attenuated variant of the Kabete ‘O’ strain of rinderpest virus (RV) (Plowright & Ferris, 1962) and its derivatives (Mariner et al., 1990). This vaccine causes a subclinical infection that induces long-lasting titres of neutralizing antibody and clinical immunity. However, RV, including the vaccine strain, grows in lymphoid tissues in vivo (Plowright, 1964; Taylor & Plowright, 1965) and there is debate as to whether the vaccine may cause immunosuppression, which frequently follows disease caused by virulent strains (Scott, 1964). Some studies have reported that vaccine increases the clinical severity of concurrent infections and interferes with immunizations in which it is combined with other infectious agents (Kathuria et al., 1976; Jeggo et al., 1987). In investigating this problem further it would be helpful to know which phenotypes of lymphocyte RV is capable of infecting. Such information would provide insight into the pathogenesis of the disease and might indicate potential mechanisms of immunosuppression.

RV grows readily in mitogen-stimulated bovine lymphoblasts and in continuously growing lines of bovine lymphoblasts (Rossiter & Wardley, 1985; Rossiter et al., 1988). Recently, it has been possible to develop cloned cell lines of bovine lymphoblasts with known phenotype (Baldwin et al., 1988) transformed by the protozoan parasite Theileria parva. Therefore a study was made to determine which phenotypes of transformed bovine lymphoblast would support RV growth.

Cell culture-attenuated vaccine virus (Plowright & Ferris, 1962) which had been passaged 98 times in bovine kidney cells was used throughout the study.

Cloned cell lines transformed by T. parva were established from purified populations of lymphocytes as described previously (Baldwin et al., 1988). Briefly, lymphocyte subpopulations were purified from peripheral blood mononuclear cells by flow cytometry, infected with T. parva sporozoites and cultivated in microtitre plates at limiting dilution. Six cloned cell lines were derived from one animal, D409: two of these were B cell lines (B1 and B2), two were gamma/delta T cells (N1 and N2) identified by the monoclonal antibody IL-2 (Clevers et al., 1990); one was a CD4+ alpha/beta T cell line (T1) and one was a CD8+ alpha/beta T cell line (T4). The B cells were derived from surface IgM+ cells; following infection one clone continued to express IgM whereas the other was negative. The phenotypes and main immunological functions of the cell lines are summarized in Table 1.

Lymphoblasts were pelleted by gentle centrifugation in loose-capped polypropylene tubes (Sterilin) and infected by resuspension in 1 ml of virus containing 10⁴ to 10⁶ TCID₅₀. After 1 h at 37 °C they were washed...
four times in 2 to 3 ml of RPMI-1640 medium. They were then resuspended in 4 ml of the same medium supplemented with antibiotics, 20% foetal bovine serum, 2 mM-l-glutamine and 5 x 10^{-5} M-2-mercaptoethanol and cultured at 37 °C in 5% CO_2 in air. At the start of culture 0-30 ml aliquots of cells and clarified medium were removed from the cultures and then daily for up to 8 days. The volume of culture fluid was made back up to 4 ml with new growth medium after every 3 days. Replicate cytocentrifuge smears were made from infected cells and the clarified medium was stored at -70 °C for subsequent virus infectivity assay.

Smears were stained by May-Grünwald-Giemsa for cytological examination to reveal cell morphology, viral c.p.e. and mitosis (a measure of cell growth). Duplicates were fixed in acetone and examined by direct immunofluorescence for RV antigens (Rossiter & Jessett, 1982a). Thin sections of selected cultures were also examined by electron microscopy following fixation in a mixture of glutaraldehyde, paraformaldehyde and picric acid. Production of new infectious virus was assayed by microtitration in either BK or Vero cells (Rossiter & Jessett, 1982b) with all harvests from individual cell lines being titrated at one time.

Cytology revealed a high proportion of mitotic figures in all cultures at the start of the growth curves, and these remained evident in all cultures until they were destroyed by the virus or the experiment was finished. Uninfected alpha/beta T lymphoblasts are shown in Fig. 1(a). Viral c.p.e. could be detected in all infected cultures within 48 to 72 h, and isolated cells with c.p.e. were occasionally seen as early as 24 h post-infection (p.i.). In the two B cell lines the c.p.e. was initially seen as a discrete, eosinophilic, intracytoplasmic inclusion which appeared to increase in size as the culture progressed (Fig 1b). Pyknosis was common in lymphoblasts with large inclusions, and such cells then appeared to develop karyorrhexis and eventually to lyse. Syncytia were not seen in B cell cultures. In contrast, the c.p.e. in the CD4^+ and CD8^+ alpha/beta T cell cultures was characterized by the development of large, sometimes massive, syncytia with dark blue-staining cytoplasm, a core of many nuclei and large amorphous, eosinophilic, intracytoplasmic inclusions (Fig 1c). These syncytia predominated in the CD4^+ and CD8^+ cell cultures and individual cells with inclusions were only occasionally seen. The gamma/delta T cell cultures developed a c.p.e. essentially similar to B cells although isolated small syncytia were sometimes seen. Intranuclear inclusions were not seen in any cultures.

By inverted microscopy healthy lymphoblasts usually appeared spherical, although sometimes pleomorphic, and highly refractile. They grew in suspension as small or large clumps although a proportion of B and gamma/delta T cells attached to the culture surface without flattening. Infected B and gamma/delta T lymphoblasts appeared as dull, granular, shrunken cells which increased in proportion until no refractile cells were seen. In CD4^+ and CD8^+ alpha/beta T cell lines the syncytia were easily visible as huge balloons either floating on their own or protruding from clumps of healthy cells. They did not persist and rapidly disappeared from cultures in which they had been numerous, leaving a suspension of disintegrated cell debris.

Immunofluorescence revealed viral antigens, particularly in the cytoplasm, of up to 5% of cells 48 h p.i. and the proportion progressively increased to 100% of viable cells by 72 to 96 h p.i.

New infectious virus was produced in all of the cultures. The growth curves (Fig. 2) showed that new virus could be detected 1 to 2 days p.i. Highest titres of new infectivity were detected 3 days p.i. after which they declined rapidly, possibly because the cultures tended to become acidic after 3 to 4 days. Comparison of the growth curves (Fig. 2) showed that the shape of the curve was similar in all six cell lines and the range of maximum virus yields on day 3 was from 10^{4.4} to 10^{6.5} TCID_{50}/ml. Amongst the lines the highest titres were recorded in the N2 and B1 lines with decreasingly lower titres in T1, B2, T4 and N1 cells.

Electron microscopy revealed typical paramyxovirus-like nucleoprotein material in the cytoplasm of infected cells and syncytia. These results show that, in vitro, RV can infect, multiply in, and destroy lymphoid cells with a wide range of phenotypes including B cells, CD4^+ or CD8^+ alpha/
Fig. 1. (a) Healthy alpha/beta T lymphoblasts not infected with RV. Each cell contains \textit{T. parva} schizonts (open arrowheads). B cell and gamma/delta T cells are morphologically indistinguishable from these. Cells are stained with May-Gr"unwald-Giemsa. Bar represents 20 \textmu m. (b) B lymphoblasts infected with RV vaccine. Arrows point to intracytoplasmic inclusions; open arrowheads point to \textit{T. parva} schizonts. Cells were stained with May-Gr"unwald-Giemsa. Bar represents 20 \textmu m. (c) T lymphoblasts infected with RV vaccine. One large syncytium (solid arrowhead) amidst uninfected cells. Arrow indicates intracytoplasmic inclusion; open arrowhead points to accumulation of \textit{T. parva} schizonts inside the cluster of nuclei. Cells were stained with May-Gr"unwald-Giemsa. Bar represents 20 \textmu m.

beta T cells and gamma/delta T cells. This extends the findings of Rossiter \& Wardley (1985) who reported significantly higher virus growth in whole blood lymphoblast cultures enriched for T cells compared with T-depleted cultures. However, the multiplication of RV is dependent on host cell growth, and these earlier experiments used chemical mitogens specific for T or polyphenotopic lymphocyte populations but did not use a
mitogen specific for B lymphocytes. Thus, since blastogenesis was not measured, the response of the B cells in that study may have been considerably less than the very high levels seen in the continuous cell lines used in this study. In the same way, variations in the mitotic and metabolic rates of the individual cell lines might explain the relatively small differences recorded here in the growth of RV in the different cell lines. Overall, it was not possible to indicate a significant preference of the virus for any particular phenotype. This has been confirmed by other experiments at this laboratory in which RV has been grown in at least 12 other lymphoblastoid lines of different phenotypes. This is in keeping with results obtained with other morbilliviruses including measles virus which has been shown to grow in a variety of lymphocyte subsets (Joseph et al., 1975).

The reason for the marked difference between the type of viral c.p.e. seen in alpha/beta T and B or gamma/delta T lymphoblasts is unknown. Syncytia are characteristic of RV c.p.e. in monolayer cultures of epithelial and fibroblastic origin and can be seen in histological sections of epithelial, sub-epithelial and lymphoid tissues from clinically affected animals (Maurer et al., 1956; Thiery, 1956). The first pathology that is usually seen in the lymph nodes of infected cattle is necrosis within germinal centres (Thiery, 1956; Khera, 1958; Tajima & Ushijima, 1971), which are regarded as B lymphocyte-dependent areas, and which may become completely devoid of lymphoblasts. Subsequently, viral c.p.e. and necrosis appear in the rest of the cortex, paracortex and medulla. Khera (1958) observed syncytia only in the paracortex of lymph nodes, which is populated predominantly with alpha/beta T cells, and not in the medulla or germinal centres. RV grows only in blasting lymphocytes (Rossiter & Wardley, 1985) which are proportionally much more prevalent in germinal follicles than in the rest of the cortex and paracortex. One possible explanation, therefore, of early events in the pathogenesis is that, initially, the virus can grow only in blasting B lymphocytes in the germinal centres after which it spreads to infect T cells blasting in response to the virus and other blastogenic stimuli, and in which it causes syncytia as well as necrosis.

An immunodominant site that binds to major histocompatibility complex class 2 receptors on human and mouse T lymphocytes has been described on the fusion protein of measles virus (Partidos & Steward, 1990). It would be interesting to determine whether similar specific binding with the fusion protein from RV was involved in the development reported here of syncytia in alpha/beta T but not gamma/delta T or B lymphocytes.

The ease with which the ballooning syncytia could be seen in cultures of infected alpha/beta T cells has lent itself to diagnosis. The cells have proved highly susceptible to a wide range of RV strains and related viruses, and have significant advantages over BK or Vero cells in terms of rapidity and their sensitivity for virus isolation from diagnostic sample materials (Rossiter et al., 1992).

Lymphoblastoid cells transformed by *T. parva* have not been shown to have different lymphoblast cell surface receptors from those on uninfected lymphoblasts transformed by mitogens or growth factors. When treated with parasiticidal drugs that completely destroy the parasite, the cell lines remain viable but lose their ability to multiply indefinitely. They are, thus, comparatively unaltered from lymphoblasts that are not infected with *T. parva* and it seems reasonable to extrapolate these results to *in vivo* infection. In cattle, therefore, attenuated RV may grow in most if not all lymphoblasts including T cells with helper, cytotoxic or suppressor functions, B cells and gamma/delta T cells. Thus the vaccine has the potential, theoretically at least, to cause immunosuppression through a variety of mechanisms. Whether or not it does so, however, requires further study of experimental infections and clinical field trials.

This paper is published with the permission of the Director, National Veterinary Research Centre, Kenya Agricultural Research Institute, Muguga. P. B. Rossiter and K. A. J. Herniman (deceased) were employed by the Overseas Development Administration, London which funded this work.
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(Received 1 July 1992; Accepted 13 October 1992)