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Modelling of Malignant Lymphoma in Rabbits, Using Oncogenic Viruses of Non-Human Primates

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The discovery of lymphoid B-lymphotropic herpesvirus-producing cell lines from M. arctoides peripheral lymphocytes (MAL-1) producing their own lymphotropic virus HVMA was reported earlier [1]. Subsequently, two more M. arctoides virus-producing lymphoid cell lines (MAL-2, MAL-3) were discovered in our laboratory. The cells of some baboon lymphoid cultures, parallel with B-lymphotropic herpesvirus, produced C-type retrovirus antigenically similar to human T-lymphotropic virus (HTLV)-I. The cells of MAL-1, MAL-2 and MAL-3 cultures also produced B-lymphotropic herpesvirus, and small amounts of retrovirus particles were revealed (Fig. 1). Furthermore, it was reported that a number of Old World monkeys, including M. arctoides, can be the carriers of simian T-lymphotropic virus (STLV) related to HTLV-I [3].

One of our research aims was to a achieve a simplified modelling of virus-associated malignant lymphoma, which we have been investigating in primates. Tests in some laboratory animals, including rabbits, on their sensitivity to B-lymphotropic herpesviruses of baboons (HVP) failed to give positive results. At the same time, some reports appeared on the transformation of rabbit lymphocytes in vitro caused by HTLV-I [2].

Six young grey rabbits bred in Sukhumi (each weighing 500–600 g) were inoculated

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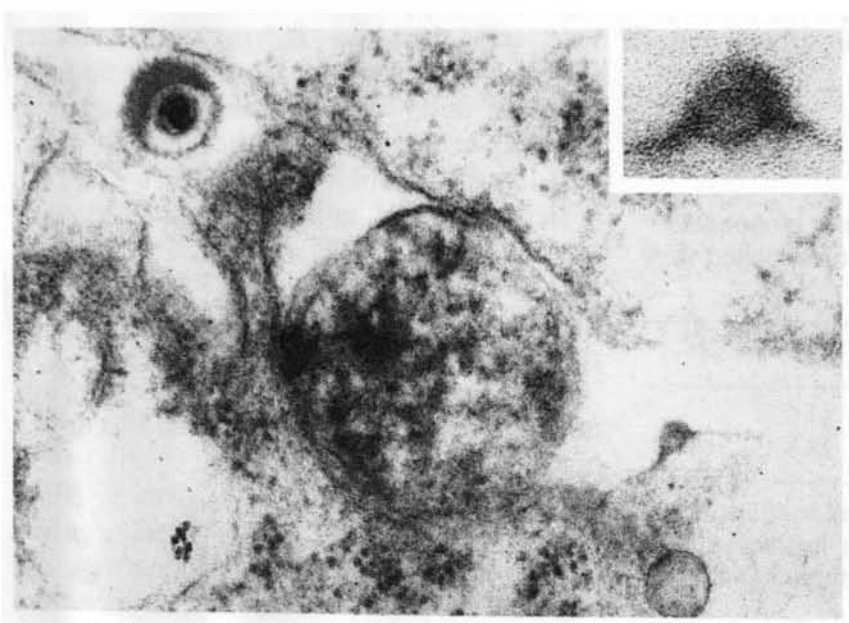

Fig. 1. Type-C and herpesvirus particles in tissue culture MAL-3 cells; × 60 000 and × 200 000

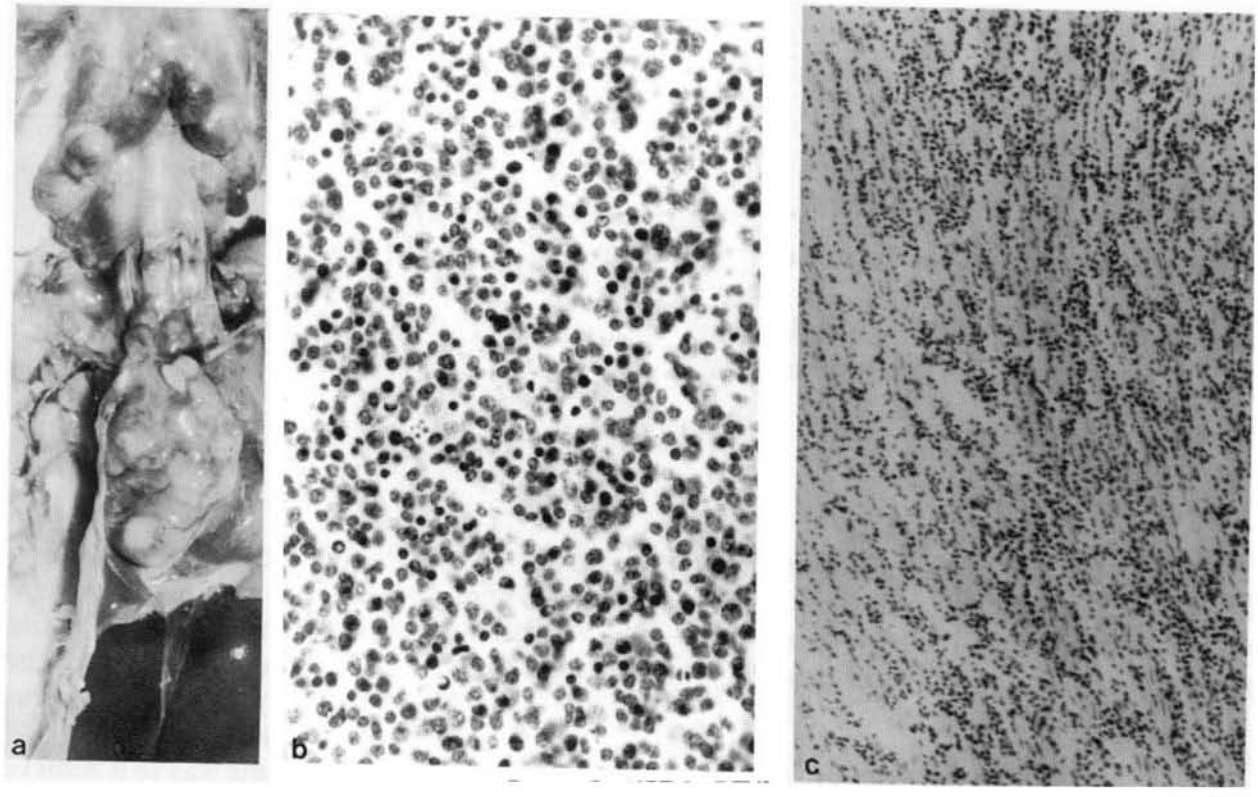

Fig. 2. a The neck lymph nodes, thymus and heart involvement in induced rabbit malignant lymphoma. Proliferation of malignant lymphoma cells in lymph node (**b**) and heart (**c**). Haematoxylin and eosin, × 500 (**b**) and × 160 (**c**)

intramuscularly with M. arctoides cell cultures (MAL-1, MAL-2, MAL-3). Inoculation led to the development of malignant lymphomas in all six rabbits. The first signs of the process were revealed 20–25 days after inoculation, and after 35–40 days the lymphoma acquired a generalized character. The sites mostly affected were popliteal lymph nodes, pelvic, mesenteric and neck lymph nodes as well as spleen, kidneys (rarely), liver, thymus, skin and bone marrow (Fig. 2). The injection of tumour materials from one animal into three others was successful in one case. We have also succeeded in causing generalized lymphoma by injection of cell-free filtrated supernatant of MAL-3 culture. Cultivation of the tumour cells of one of the rabbits led to the establishment of suspension lymphoid cell culture (RT-I), which now has 18 passages. Growing factor supplement was not needed. Culture cells had no B-lymphoid markers (Table 1) and have so far not revealed the presence of B- or T-lymphotropic viruses. Tumour cells, including those being cultivated, possess rabbit karyotype.

Table 1. Testing of tumour and mononuclear peripheral blood cells of a rabbit inoculated with MAL-2 culture cells

Source of cells	SIg^+ cells (%)[a]
Tumour	14.0
Mononuclear from blood	28.8

[a] Cells were tested in a direct immunofluorescent test with the use of fluorescein isothiocyanate-conjugated swine anti-rabbit immunoglobulins (Dakopatts, Denmark).

In six control animals, inoculated with materials of MAL-1, MAL-2 and MAL-3 cultures heated at 56 °C for 1 h, a 4-month observation has revealed no tumour occurrence.

The sera of a rabbit inoculated with homogenate of rabbit lymphoma (induced by a mixture of MAL-2 and MAL-3 cells) were tested against Epstein-Barr virus (EBV)-, HTLV-I and STLV-I positive and negative target cells before and after inoculation, using an indirect immunofluorescence test

Table 2. Reactivity of first passage rabbit sera against EBV/HVP and HTLV/STLV-I positive/negative cells in an indirect immunofluorescence test[a]

Target cells / Rabbit sera	Human B cells		Human T cells		Baboon B cells	Rabbit cells
	EBV-VCA/EA− HTLV-I− (Raji)	EBV-VCA/EA+ HTLV-I− (P3HR-I)	HTLV-I+ EBV− (C9I-PL)	HTLV-I− EBV− (H9)	HVP+ STLV-I+ BEV+ (594S-F9)	? ? (OK-I)
Before inoculation	−	−	−	−	−	−
After inoculation day 36	−	+[d]	++[c]	+[c]	+[b]	++[c]
After inoculation day 60	−	++[b]	+[c]	±[c]	++[b]	+[c]

[a] Rabbit was inoculated by homogenate of tumour induced by a mixture of MAL-2 and MAL-3 in another rabbit.
[b] Brilliant cytoplasmic fluorescence, low percentage of positive cells (5%–10%).
[c] Faint cytoplasmic fluorescence in practically all cells.
[d] Intensity of fluorescence

(Table 2). The preinoculation serum was negative against all types of target cells. The reactivity pattern of the postinoculation sera suggested the presence of antibodies against EBV/HVP antigens and against cell antigen(s) shared by human T cells and cultured rabbit tumour cells during lymphoma development.

The results of the present research show high oncogenicity for rabbits inoculated with M. arctoides lymphoid cell cultures. It is possible that the occurrence of tumours in rabbits after the injection of MAL-1, MAL-2 and MAL-3 cultures is associated with simultaneous expression of EBV-like B-lymphotropic herpesvirus and perhaps C-type retrovirus.

References

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