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"THYMIC NURSE CELLS" CONTAIN THE FIRST VIRUS PRODUCING CELLS AFTER INOCULATION OF THE RADIATION LEUKEMIA VIRUS IN C57BL/Ka MICE

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INTRODUCTION

In mice, the development of thymic lymphomas either spontaneously, or after a treatment by irradiation chemicals or retroviruses, raises the question of the interactions between putative oncogenic factors and the normal pathway of T cell differentiation. Lymphomas induced by inoculation of the Radiation Leukemia Virus (RadLV)+ are good experimental models for studying such interactions. The injection of this virus into C57BL/Ka mice results in specific modifications, i.e. overt virus replication and eventual lymphoma development, which are selectively located within the thymus (review: Kaplan, 1977).

The identification of the differentiation characteristics in the "target cells" for productive infection by RadLV and for neoplastic transformation is critical for understanding the mechanism of leukemogenesis.

Previous studies have shown that target cells for productive infection by RadLV are very scarce in the thymus (1 in 50000 cells) and display a phenotype compatible with that of transitional forms between bone marrow prothymocytes and most of thymus subcapsular cells (Decleve et al., 1977, Boniver et al., 1980 and submitted). Therefore, target cells could correspond to those "immature thymocytes" that have been postulated by Wekerle et al. (1980) to sojourn within particular lymphoepithelial complexes, called "thymic nurse cells." The present work was undertaken to demonstrate this hypothesis.

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RESULTS

"Thymic Nurse Cells" are large epithelial cells which can be recovered after enzyme digestion of the thymus and repeated 1 g sedimentation of the resulting cell suspension (Wekerle and Ketelsen, 1980). Each young adult thymus contains 15000-20000 TNC's. Many lymphoid cells (9±1.4 per TNC) are located within vagination of the epithelial cell cytoplasm; 20% of them are blast cells, similar to those described in the normal thymus outer cortex (Boniver et al., 1979). With scanning electron microscope, TNC's look like spheres, whose surface displays relief corresponding to TNC lymphoid cells (Fig. 1a). Generally, the external membrane of the TNC is continuous; in about 10% of TNCs, there are communications between the extra-cellular medium and the TNC lymphoid cells (Fig. 1b).

Most of such TNC's derive from the outer cortex of the thymus. Thymuses were dipped into Fluorescein Isothiocyanate as described by Scollay et al (1980). Such a treatment led to a fluorescent staining of the 8-12 most external cell layers. The cell suspension obtained by enzyme dissociation contained 5-10% of fluorescent cells. About 55-70% of the TNC's recovered after repeated sedimentation were fluorescent, demonstrating that they belong to the 8-12% most external cell layers.

Membrane immunofluorescence studies using monoclonal antibodies confirmed the cortical origin of TNC's. Indeed, the expression of thy-1, Lyt-1 and Lyt-2 antigens in lymphoid cells released from TNC's was shown similar to that of the major cortical thymocyte population.

The following experiments were undertaken to look for the presence of virus producing cells in TNC's after inoculation of the RadLV into C57BL/Ka mice. Indeed, on the 2nd and 4th days after the inoculation, virus budding particles could be observed only in very scarce blast cells selectively located within the subcapsular zone (Boniver et al., submitted). TNC's were isolated from such thymuses and examined with the electron microscope. They looked smaller than in the normal thymuses. Moreover, they contained a smaller number of lymphoid cells (4.1±1.4 per TNC); 30-35% of them were blast cells. Virus particles were seen budding from the membranes of lymphoid blast cells located in these TNC's (Fig. 2).

Next, a quantitative study was devoted to determine whether these virus-producing cells were selectively located in TNC's. For this purpose, C57BL/Ka mice were inoculated with RadLV-VL3 and sacrificed 2 days later. Thymuses were removed, dissociated with enzymes and submitted to repeated sedimentation. The frequency of virus producing cells in the various fractions obtained along this

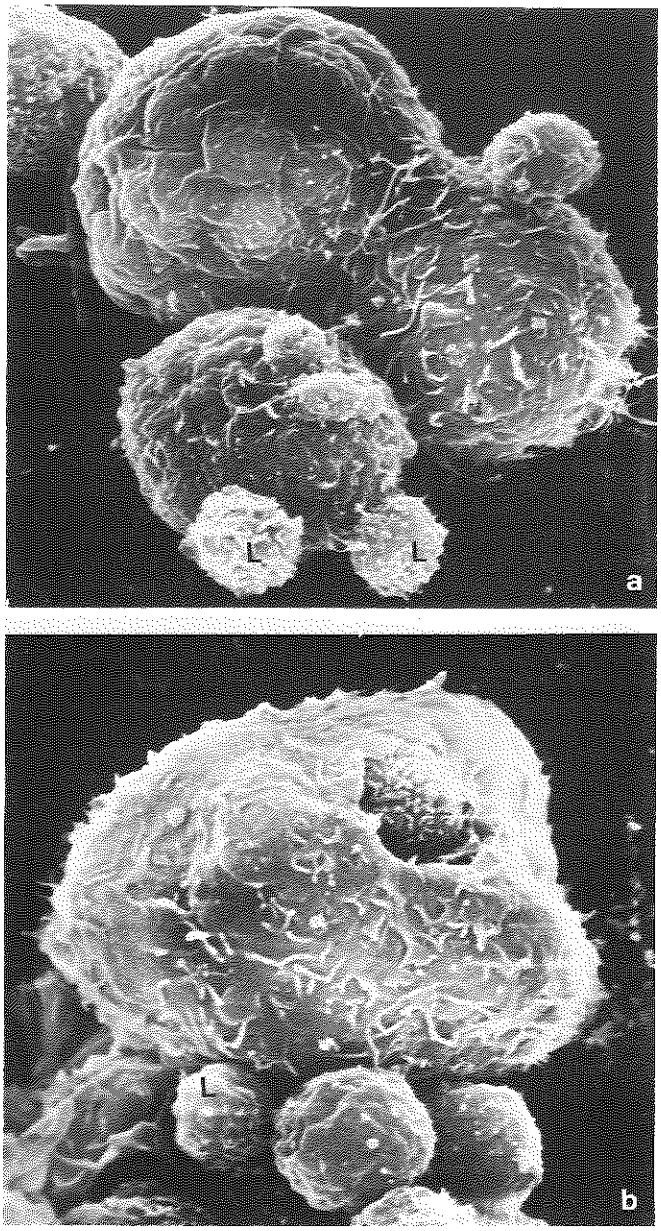

FIG. 1. Scanning electron microscopy of thymic nurse cells. a) x 3900; b) x 4500. L = free lymphoid cells.

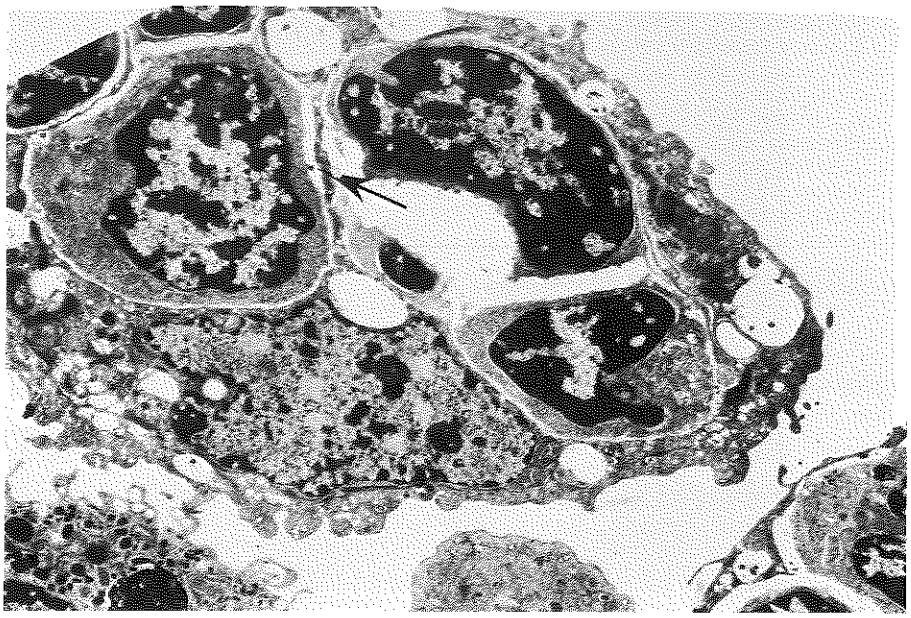

FIG. 2. Thymic Nurse Cell on day 4 after intrathymic inoculation of RadLV. It contains a small number of lymphoid cells. A viral particle is budding from the membrane of a blast cell (x 2000).

procedure and in the lymphoid cells released from RNC's was determined by using a sensitive infectious center assay (Boniver et al., 1980). In the whole thymocyte populations, only 1 in 30000 cells could act as infectious centers. At the opposite, the frequency of virus producing cells was 300 times higher in lymphoid cells released from RNCs (1 in 100) (Houben-Defresne et al., submitted).

DISCUSSION

The data clearly indicate that TNC's are a selective site for virus replication to occur in thymus lymphoid cells after inoculation of RadLV. Further studies are now in progress to determine whether target cells themselves are yet located within TNC's at the time of infection. Interestingly, preliminary results on thymus repopulation in bone marrow-grafted 400 R-treated mice suggest that some (if not all) of the first donor type cells appear in TNC's before spreading in the whole thymus cortex (Boniver and Houben-Defresne, unpublished data).

Taken together, the data obtained so far support the hypotheses that the "TNC" lymphoepithelial complexes contain a subpopulation of immature lymphocytes (Wekerle et al., 1980), which can act as specific targets for productive infection by RadLV (Boniver et al., submitted). It is proposed that the interactions between RadLV and immature lymphoid cells within Thymic Nurse Cells could be critical for the initiation of the leukemogenic process and its selective localization within the thymus.

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