

Thymic Nurse Cells: Intraepithelial Thymocyte Sojourn and Its Possible Relevance for the Pathogenesis of AKR lymphomas

B. Kyewski, G. Hunsmann, R. Friedrich, U.-P. Ketelsen, and H. Wekerle

A. Introduction

Undifferentiated lymphoid precursor cells enter the thymus and differentiate there to lymphocyte clones which are diversified, both with regard to their specific antigen receptors as well as to their programmed function in the immune system. Generation of diversity and specialization of T cell subsets, both of which are fascinating examples of cell differentiation, are supposedly the result of interactions between the differentiating lymphoid cells on one side and nonlymphoid thymic stromal cells on the other. It is probable that intercellular interactions imply both communications via soluble mediators as well as direct cell-to-cell contact between the interacting cell partners. Physiologic T cell differentiation is, however, not without hazards. Within the thymus neoplastic transformation of differentiating T cells can occur, and there is evidence that this pathologic change represents an abnormal aberration of certain physiologic developmental events (McGrath and Weissman 1978).

Little is known about the exact mechanisms of physiologic and pathologic intrathymic T cell differentiation, nor do we know much about the localization of the developmental events within the organ. A quite unusual cellular phenomenon, the lymphoepithelial thymic nurse Cell (TNC) complexes, which we recently observed in dissociated rodent thymus populations, may be helpful for further understanding these problems.

TNCs are epithelial cells of enormous size which can be isolated from normal thymuses by differential trypsinization (Wekerle and Ketelsen 1980). They are specialized for incorporating large numbers of functionally intact, actively proliferating thymocytes in their cyto-

plasm. The engulfed thymocytes are completely surrounded by epithelial membranes which display macromolecular specializations presumably involved in creating a special microenvironment. This finding, besides the expression of products of the H-2 subregions K,D,I-A, and I-E/C which are all believed to be centrally engaged in intercellular interactions during development and organization of the immune system, lead to a hypothesis postulating that formation of TNCs represents an intracellular differentiation cycle essentially required for intrathymic T cell maturation (Wekerle et al. 1980).

In this communication we report that TNCs from AKR/J mice are abnormal in several respects. They are of abnormally large size, containing up to 200 thymocytes. They further express retroviral products in their cytoplasm and on their membranes. And, finally, their presence in thymuses negatively correlates with the development of neoplastic thymomas in the adult AKR/J mouse.

B. Results

I. Morphologic Observations

TNCs, freshly isolated from 3-month-old AKR/J mice are abnormally large (Fig. 1). They may reach diameters exceeding 50–70 μm and contain estimated numbers of about 200 thymocytes in their cell bodies. Cytofluorograph light scattering analyses revealed that only the TNC fractions, but not the free thymocytes, are of abnormal sizes. Ultrastructurally, the most striking observation concerns a relatively loose contact formation between engulfed thymocytes and the surrounding in-

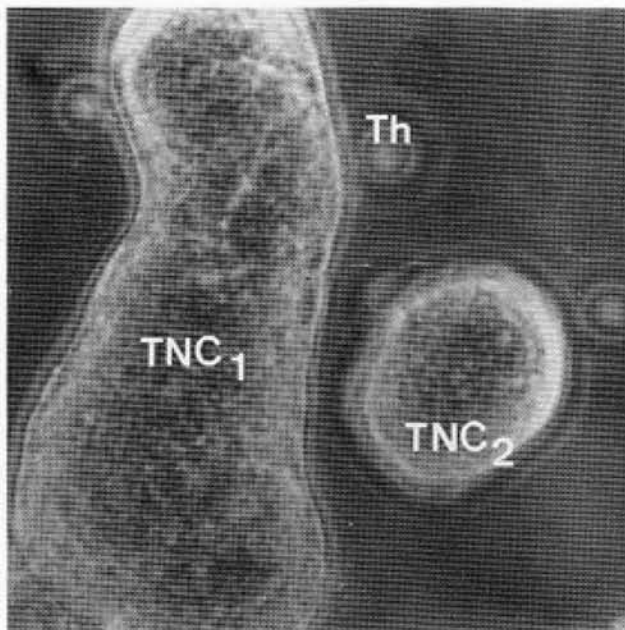


Fig. 1. Giant AKR thymic nurse cell. *Left side* Giant TNC_1 , formed by presumable one epithelial cell containing a high number of lymphoid thymocytes. *Right side*, TNC_2 corresponding to normal size TNCs from nonleukemia prone mouse strains. In the background out of optical plane: free thymocytes (*Th*)

ternal epithelial membranes. The thymocytes were remarkable for their dense, ribosome-rich cytoplasm, active nuclei, and occasional mitoses (Fig. 2).

II. Occurrence of TNCs During Ontogeny

Preliminary investigations of normal mice indicate that TNCs are first detectable on day 16

of gestation. The number of demonstrable TNCs gradually increases with the growth of the organ. They can be found in thymuses at least until the age of 14 months (H. W. and G. A. Luckenbach, in preparation).

Until young adult ages the occurrence of TNCs in AKR/J mice resembles the one in normal strains. It is, however, remarkable that as soon as neoplastic conversion of thymus cells begin to occur, i.e., at the age of 6–8 months, the number of TNCs drastically declines. We never found TNCs in thymuses showing signs of neoplasia. It should furthermore be noted that in transferred thymomas we were unable to demonstrate TNC-like cell complexes.

III. Retroviral Determinants on AKR/J TNCs

The inverse relation between TNC occurrence and thymomagenesis as well as the abnormal TNC structure prompted us to search for products of C-type RNA viruses, a suspected tumorigenic agent. We applied indirect immunofluorescence using specific conventional antisera and fluorescein-labeled anti-immunoglobulin antibodies as markers. The TNCs were investigated subsequent to fixation in paraformaldehyde. We used antisera against Friend leukemia virus gp71, a glycoprotein of the viral coat. This antiserum strongly bound to the membranes of AKR/J as indicated by the bright ring shaped fluorescence (Fig. 3).

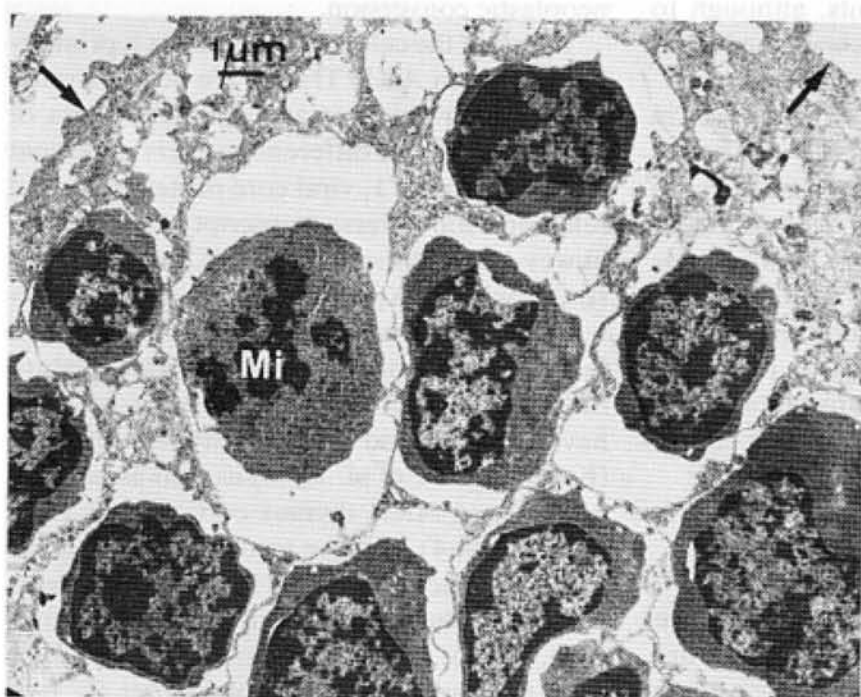


Fig. 2. Ultrastructure of AKR thymic nurse cell. Ultrathin section through TNC isolated from AKR thymus (3 months). *Arrows* mark outer TNC membrane. Note internalized thymocyte in mitosis (*Mi*). Staining: uranyl acetate and lead citrate

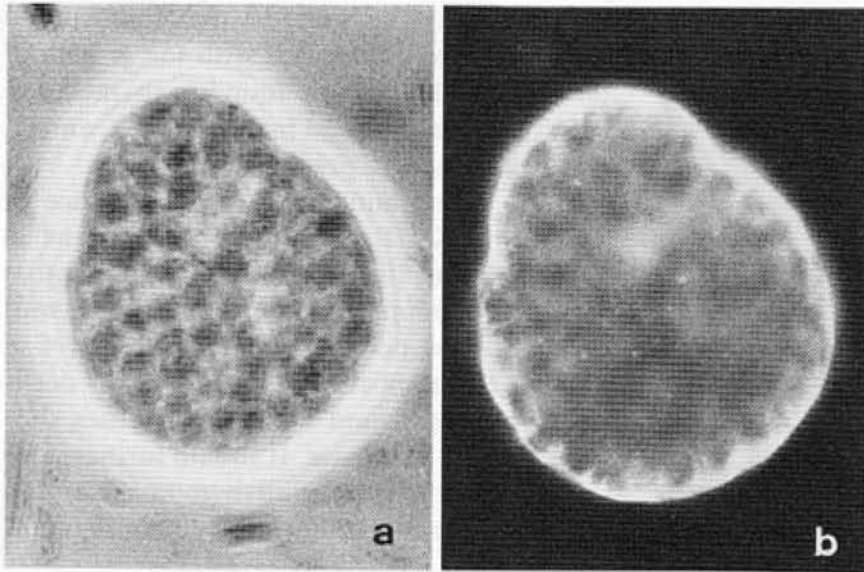


Fig. 3. Expression of retroviral gp71 on an AKR thymic nurse cell. Immunofluorescence demonstration using rabbit anti-FLV gp71 antibodies as markers and FITC-conjugated goat anti-rabbit immunoglobulin antibodies as label. TNCs were isolated from thymuses of 2-month-old AKR/J mice and fixed with 3.7% paraformaldehyde before staining. **a** Phase contrast; **b** Epifluorescence illumination.

The binding was virus specific for several reasons. First, preimmune normal serum did not stain the cells. Second, the activity could be absorbed by virus infected mouse 3T3 fibroblasts but not by uninfected control cells. Third, the serum bound to transformed Friend leukemia cells but not to normal mouse lymphocytes. Except for retroviral coat gp71, AKR/J TNCs expressed Friend leukemia virus (FLV) core protein p30 determinants in their cytoplasm. This binding was also immunospecific as revealed by specificity controls similar to the ones used for gp71 determination.

Although expression of viral determinants on AKR/J cells was unequivocal, it should be noted that TNCs from mouse strains without thymoma predilection also unexpectedly expressed retroviral determinants, although to a somewhat lower degree. This was true for the other strains tested, C3H/f and C57BL/6.

To confirm the presence of RNA viruses in AKR/J TNCs, we cultured TNCs along with other thymic cell fractions and screened the culture supernatants for viral reverse transcriptase activity. We found that viral enzyme activity was demonstrable in TNC cultures from young mice, but not in cultures of single thymocytes (Fig. 4). Conversely, in preleukemic cell cultures reverse transcriptase was produced mainly in cultures of smaller cells.

C. Discussion

The key findings reported in this paper are that TNCs from young adult AKR/J mice are morphologically changed, that they are no

longer demonstrable in neoplastic thymomas, and that AKR TNCs express viral products in relative high dosages.

It is known that typical pathologic changes of thymic structure precede thymomagenesis. It is well documented that starting from 4 months of age the cortical areas begin to involute, first focally and later in a generalized pattern (Arnesen 1958; Metcalf 1966; Siegler and Rich 1963).

Thus, in ontogeny of AKR mice the TNC abnormalities described here appear to be the first demonstrable changes. Since TNCs seem to be located in the thymic cortex (unpublished observations), it is probable that the preleukemic loss of TNCs in AKR thymuses is related to the cortical involution, which precedes neoplastic conversion.

Which of the cellular components is primarily changed in AKR TNCs? We demonstrated retroviral products in the epithelial parts of TNCs, using three different markers: viral coat glycoprotein gp71, viral core protein p30, and released viral reverse transcriptase. It should, however, be stressed that TNCs from mouse strains without a particular leukemia susceptibility express viral determinants as well. In fact, virus content seems to be a normal feature of thymic epithelial cells, as of virus particles have been found in EM studies of fetal (Koppenheffer et al. 1978) as well as of adult normal and AKR thymus cortical cells (DeHarven 1964). Similar to tissues of the genitourinary tract, where gp71-like determinants have been shown in copious amounts (Lerner et al. 1977), virus expression on AKR TNCs does not seem to be pathologic per se. It is

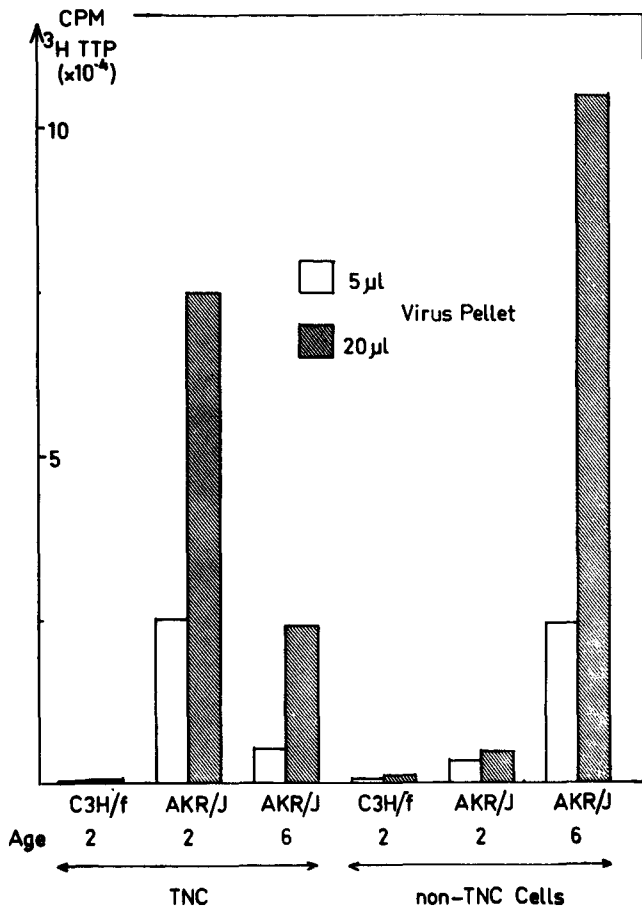


Fig. 4. Biosynthesis of reverse transcriptase in short term cultures of AKR TNC and small thymus cell populations. TNC and single thymocyte fractions of 2-month-old C3H/f and AKR/J and of preleukemic 6-month-old AKR/J mice were cultured for 4 days. Culture supernatants were harvested repeatedly and sedimentable material ("virus pellet") was assayed for reverse transcriptase activity (Novak et al. 1979)

possible, though not yet proven, that a property of the thymocytes is the basis for the changes. AKR TNCs contain enormous numbers of thymocytes, which are, as their "normal" counterparts, morphologically intact and actively proliferate within the TNCs (Wekerle and Ketelsen 1980). AKR intra-TNC thymocytes, however, often show a very loose contact between their surface membranes and the surrounding epithelial caveolar membranes. At present it is not possible to decide whether the abnormal cell numbers are due to an enhanced intra-TNC proliferation or to an increased recruitment of immigrating thymocytes.

Are the changes of AKR TNCs related to neoplastic thymocyte conversion? Lacking functional data, any consideration of this problem must be highly speculative. Yet, some

observations on AKR thymocyte properties make such speculations quite attractive.

Weissman et al. found that few normal but a high proportion of preneoplastic and neoplastic thymocytes express receptors for retroviruses (McGrath and Weissman 1979). As has been suggested for Epstein-Barr viruses in the case of human B lymphocytes (Schwarz 1980), binding of viruses to thymic T cells in AKR thymuses is thought to trigger proliferation (McGrath and Weissman 1979). Accepting this possibility, does the AKR abnormality reside in an atypical thymocyte response pattern, and does recognition of TNC membrane-expressed viral products lead to excessive mitotic activity? Irrespective of whether this proliferation is sufficient for subsequent transformation or whether it "only" provides some of the requirements for cancerogenic cytogenetic changes to occur (Klein 1979), we believe that the study of AKR TNC components will offer an approach to reinvestigate the role of lympho-epithelial interactions, which has been a matter of debate (Haas et al. 1977; Peled and Haran-Ghera 1978; Waksal et al. 1976).

Acknowledgments

We thank Mrs. Maria Döll for her excellent technical assistance and Mrs. Rosemary Schneider for typing this manuscript. This work was supported by grants from the Deutsche Forschungsgemeinschaft.

References

- Arnesen K (1958) Preleukemic and early leukemic changes in the thymus of mice. *Acta Pathol Microbiol Scand [A]* 43:350-364
- DeHarven E (1964) Virus particles in the thymus of conventional and germfree mice. *J Exp Med* 120:857-868
- Haas M, Sher T, Smolinsky S (1977) Leukemogenesis in vitro induced by thymus epithelial reticulum cells transmitting murine leukemia viruses. *Cancer Res* 37:1800-1807
- Klein G (1979) Lymphoma development in mice and humans: Diversity of initiation is followed by convergent cytogenetic evolution. *Proc Natl Acad Sci USA*: 2442-2446
- Koppenheffer TL, Philips JH, Vankin GL (1978) C-type virus-lymphocyte interactions in the developing mouse thymus. *Am J Anat* 153:165-170
- Lerner RA, Wilson CB, DelVillano CB, McConahey PJ, Dixon FJ (1977) Endogeneous oncoviral expression in adult and fetal mice: Quantitative, histologic and physiologic studies of the major viral glycoprotein

pg70. J Exp Med 143:151–166 – McGrath MS, Weissman IL (1978) A receptor-mediated model of viral leukemogenesis: Hypothesis and experiments. In: Clarkson B, Marks PA, Till JE (eds) Differentiation of normal and neoplastic hematopoietic cells. Cold Spring Harbor Laboratory, New York, pp 577–589 – McGrath MS, Weissman IL (1979), AKR leukemogenesis: Identification and biological significance of thymic lymphoma receptors for AKR retroviruses. Cell 17:65–75 – Metcalf D (1966) Histologic and transplantation studies on preleukemic thymus of the AKR mouse. J Natl Cancer Inst 37:425–442 – Novak U, Friedrich R, Mölling K (1979) Elongation of DNA complementary to 5' and of the avian sarcoma virus genome by the virion associated RNA dependent DNA polymerase. J Virol 30:438–452 – Peled A, Haran-Ghera N (1978)

Lack of transformation of murine thymocytes by thymic epithelium. Nature 274:266–269 – Schwarz RS (1980) Epstein-Barr virus-oncogen or mitogen? (editorial). N Engl J Med 302:1307–1308 – Siegler R, Rich MA (1963) Unilateral histogenesis of AKR thymic lymphoma. Cancer Res 23:1774–1781 – Waksal SD, Smolinsky S, Cohen IR, Feldman M (1976) Transformation of thymocytes by thymus epithelium derived from AKR mice. Nature 263:512–514 – Wekerle H, Ketelsen U-P (1980) Thymic nurse cells – Ia-bearing epithelium involved in T-lymphocyte differentiation? Nature 283:402–404 – Wekerle H, Ketelsen U-P, Ernst M (1980) Thymic nurse cells. Lymphoepithelial cell complexes in murine thymuses: Morphological and serological characterization. J Exp Med 151:925–944