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Radiation leukemia virus: A marker for the study of intrathymic T cell differentiation

JACQUES BONIVER, CATHERINE DELVENNE, MICHEL JANOWSKI, ROBERT COURTOY & MARIE-PAULE DEFRESNE
Laboratory of Pathological Anatomy and Cytopathology, University Hospital of Liège, Liège and Department of Radiobiology, Nuclear Energy Centre, Mol, Belgium

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Abstract. Radiation Leukemia Virus (RadLV) is a retrovirus, which displays a highly specific thymotropism; after inoculation into young C57 BL/Ka mice, active virus replication is observed only in thymocytes and thymic lymphomas develop in most treated mice. The specific interaction between RadLV and the thymus resides in the particular susceptibility to infection of the most immature cells involved in the intrathymic lymphoid differentiation pathway and of non lymphoid thymic stromal cells. Recent data obtained from 'in situ' hybridization studies for detection of viral transcripts yield more detailed informations on these intriguing interactions.

Abbreviations: mRNA: Messenger RNA; MuLV: Murine Leukemia Virus; RadLV: Radiation Leukemia Virus.

Introduction

Radiation Leukemia Virus is a MuLV type retrovirus, that was initially extracted from radiation-induced thymic lymphomas of the C57BL/Ka mouse strain [1, 2]. After 'in vivo' inoculation, this viral isolate induces a high incidence of thymic lymphomas after a latency period of several months. Interestingly the thymus is the only organ where active virus replication is observed during the preneoplastic latency period [3, 4]. Thus, RadLV is strictly thymotropic, in terms of productive infection and oncogenic potential.

This selective thymotropism suggests a specific interaction between RadLV and the thymus, raising the proposal that the RadLV system can be a model for the study of intrathymic T cell differentiation [5, 6, 7].

The selective interaction between RadLV and thymus is not at the level of susceptibility to infection. In fact, target cells for RadLV are found in

various organs and among several cell types; lymphoid cells or their precursors in bone marrow, fetal liver, lymph node, spleen, and epithelial cells in thymus can be infected by RadLV, as shown by 'in vivo' and 'in vitro' studies [5, 8, 9, 10]. By contrast, active viral replication only occurs in thymocytes [3, 9]. In fact a few weeks after inoculation of the virus, about 90% of thymocytes contain viral antigens, whereas at the same time, only very scarce cells in other organs contain such virus positive cells [3, 4].

Interestingly, when target cells from bone marrow or spleen are infected 'in vitro' and then inoculated into syngeneic animals, virus producing cells are found in high numbers only in the thymus [10, Boniver et al., unpublished observations]. This observation suggests that infected target cells encounter the appropriate conditions for undergoing viral replication only within the thymus. This can be due either to the nature of the target cell and/or to some microenvironmental factors.

Many data argue that target cells are at the earliest level of the T cell differentiation pathway. This view is based on the forementioned observations on the 'in vivo' fate of 'in vitro' RadLV infected bone marrow or spleen cells, and on studies related to the response of the thymus to RadLV.

Decleve et al. [4] clearly showed that after injection of RadLV, the first viral antigen positive cells were scarce lymphoblasts in the thymic outer cortex. Virus replication rapidly spread to the entire cortex. This was confirmed later on by ultrastructural observations [9]. Further studies demonstrated that thymic target cells for productive infections were very scarce (1 in 5000-1000 cells in young adult thymocytes) and displayed the phenotype of cortical lymphoblasts [8, 9]. These data were confirmed in the MCF virus model, whose biological features are closely related to RadLV [11].

Accordingly, one might propose that susceptibility to RadLV is probably confined to cells, which are at a stage of differentiation between the multipotential stem cell and the outer cortex lymphoblast, with active replication most likely starting in this latter population. If so, the compiled data undoubtedly leave a gap since it is presently thought that immigrating thymic precursors penetrate the thymus at the cortico-medullary junction and in the deep cortex, where they interact with stromal macrophages before moving towards the outer cortex and then undergoing the very active production of immature cortical thymocytes [12, 13]. Going back to Decleve's study [4], it is clear that nothing was observed before the detection of virus producing cells in the outer cortex. This might be due to an insufficient technology since, in 1975, immunofluorescence was the only available technique.

Results and discussion

As described below, our data yield new information on this question. Recently in situ hybridization studies were performed in our laboratory. ^{35}S labeled DNA probes of a RadLV/VL3 clone [14] were used for detecting RadLV messenger RNAs (mRNAs) on frozen sections of thymus collected at various time intervals after inoculation of the virus. The first evidence of RadLV transcription appeared during the first 24 hours in the medulla and in the cortico-medullary junction. The cells with mRNA were both lymphoid and non lymphoid, forming sorts of complexes evoking the thymocytes rosettes, which are commonly isolated from the thymus by various procedures [13]. Some positive cells were isolated and obviously non lymphoid. It was only 24–48 hours later, when viral mRNA was found in the thymic outer cortex: again these mRNAs were detected in both lymphoid and non lymphoid cells, evoking the nurse cells. Some free positive cells were also seen in the deep cortex. After several days, more and more cortical lymphoid cells were positive, whereas the numbers of viral mRNA containing cells did not increase in the medulla.

The data provide further clue that susceptibility to productive infection by RadLV is restricted to the earlier cells of thymic lymphopoiesis, i.e. most likely at the level of the so called 'intrathymic thymocyte precursors'. These precursors might be productively infected at the cortico-medullary junction and then migrate towards the outer cortex. This problem should be tested by more sophisticated technical study in which the expression of CD4 and CD8 antigens would be determined in the first cells which express viral mRNA after RadLV injection.

This study, as well as our previous ultrastructural investigations [9, 15], suggests also that thymic non lymphoid stromal cells take part in the initiation of viral production. By 'in situ' hybridization, we have observed viral mRNA very early (12-24 hours) after RadLV injection in non lymphoid cells, putatively macrophages, of the medulla or of the cortico-medullary junction. Some time later (36-60 hours), non lymphoid stromal cells of the outer cortex were also positive for viral replication. These complexes most likely correspond to the thymic nurse cells, where virus budding particles were observed by electron microscopy at the same time after RadLV injection [15].

Stroma cells, which are thought to act as inductive microenvironment for immature T cells, are thus also productively infected by RadLV, as well as their companion thymocytes. The significance of this observation has still to

be analyzed, but one can speculate on the possible facilitation of viral replication under these circumstances.

A final interesting set of observations suggests that a part of the T cell differentiation pathway is not involved in RadLV spread. Indeed, several weeks after virus injection, more than 90% of cortical thymocytes show evidence of virus production at the viral mRNA (the present work) or protein levels [4], whereas still very few medullary thymocytes are positive. The interpretation of such results is not easy, since there is still a lot of controversy about the possible filiation of medullary-versus cortical thymocytes. Either cortical cells migrating towards the medulla switch off the process of viral replication, or the virus producing cortical thymocytes are not the precursors of medullary lymphoid cells. This question should be solved by further studies.

Altogether, the presently available data strongly suggest that the thymotropism of RadLV is related to its specific capacities to replicate along the intrathymic pathway of immature lymphoid cell production, from the incoming precursor in the medulla to the small immature thymocyte of the deep cortex, through successive steps of migration, proliferation and differentiation. The molecular basis of this selectivity is still poorly known. It might be related to the inductive signals originating in non lymphoid stroma cells of the thymic microenvironment. This experimental system clearly indicates how pathological models can be helpful to study the cellular events occurring in the thymus.

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Address for correspondence: Dr J. Boniver, Laboratory of Pathological Anatomy and Cytopathology, University Hospital of Liège (B35) B-4000 Liège, Belgium.