

Cellular Aspects of the Pathogenesis of Radiation - Induced Thymic Lymphomas in C57 BL Mice (Review)

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Abstract. Radiation - induced thymic lymphomas in C57BL/Ka mice are interesting models for studying the successive steps of carcinogenesis. Irradiation initiates «preleukemic» cells, which are promoted to become neoplastic. Studies in mice in which lymphoma development is inhibited by a bone marrow transplantation after irradiation suggest that radiation - induced alterations to the T cell lineage, and particularly to thymic microenvironment, are critical for the promotion of preleukemic cells. It is proposed that the lack of physiological differentiation signals within the thymus, as a result of irradiation, allows these cells to escape the normal controls of thymocyte production and pushes them towards neoplastic transformation. A disturbance in the production of cytokines may be involved, since exogenous cytokines, such as Interferon gamma or Tumor Necrosis Factor α , can inhibit radiation - induced lymphomagenesis, reproducing the effects of bone marrow transplantation. The model is thus suitable for studying the mechanisms of carcinogenesis and designing biological manipulation devoted to cancer prevention in individuals who have been exposed to oncogenic agents.

The mechanisms of carcinogenesis are highly complex and involve successive steps: in normal tissues, cells are hit by oncogenic agents which *initiate* them to become neoplastic. However, to acquire the fully neoplastic phenotype, these «preneoplastic» initiated cells have to be *promoted* by endogenous or exogenous factors, which can act along the latency period. This multiple step process eventually results in the induction of multiple genetic alterations, some of which concern oncogenes, and in the permanent disturbances in cell proliferation.

Much information is presently available on the role of

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oncogenes in the cancerous cell, as well as on growth factors and their specific receptors. However, we are still poorly informed on the intimate cellular mechanisms leading to the establishment of the cancerous stage, and particularly on the events related to the promotion phase, resulting in the conversion to final neoplastic growth.

Among the experimental models allowing such studies, the induction of thymic lymphomas in mice by X rays or by retroviruses has proved to be highly interesting. We shall summarize here the most recent available data on this field.

The experimental model

The induced tumors are lymphoblastic lymphomas displaying heterogeneous phenotypes (1, 2, 3). In the classical Kaplan's protocol (4), female C57 BL/Ka mice are irradiated with four sublethal whole body X ray irradiations of 1.75 Gy, applied at weekly intervals, starting at one month of age. More than 90% of irradiated mice develop a thymic lymphoblastic lymphoma between the 4th and the 12th month of age. Later on, neoplastic cells invade lymph nodes, spleen, liver and bone marrow leading to leukemia (5).

A similar lymphoproliferative disorder is induced by inoculating a retrovirus (RadLV) in one month - old mice of the same C57 BL/Ka strain. This virus was initially extracted from radiation - induced thymic lymphomas of C57 BL/Ka mice (6).

Initiation

Many studies have been designed in order to identify the target cells susceptible to the leukemogenic agents. In the late 50s, Kaplan and Brown (7) observed that neoplastic lymphoblasts were morphologically similar to lymphoblasts which accumulate clustered in the outer cortex of the regenerating thymus after X irradiation and in the preleukemic thymuses. Furthermore, in the RadLV model, early after virus infection, the first virus producing cells were identified as lymphoblasts of the subcapsular zone (8, 9). More recent studies using *in situ* hybridization indicate that cells at the

cortico - medullary junction show evidence of viral replication a few hours before those of the outer cortex (10). Interestingly, the early virus producing cells display the same topography and morphology as the most immature cells of the intrathymic T cell differentiation pathway (11, 12), properties *i.e.* the thymocyte precursors. Bone marrow prothymocytes can be also targets, as least for RadLV (13). The earlier stage of pre - or intrathymic lymphopoiesis is thus particularly susceptible to lymphomagenic agents. The molecular substrate of this susceptibility is still unknown.

After irradiation or virus inoculation, the animals behave normally and look healthy for several months. The thymus does not show morphological signs of tumor growth. Nevertheless, a new subset of thymocytes emerges very early after treatment and remains within the thymus until the onset of lymphomas. These new cells have acquired preneoplastic properties, *i.e.* they are capable of inducing thymic lymphomas after transfer into histocompatible recipients (14). Interestingly, these preneoplastic cells are different from lymphoma cells: whereas tumor cells can grow after inoculation in normal or thymectomized mice, they lead to lymphomas only within thymus - bearing mice. Thus they are thymus - dependent for progression towards neoplastic growth (14, 15, 16). These preleukemic cells, as designated by Haran Ghera several years ago, are «initiated» target cells.

Very little is known about the nature of these preleukemic cells, either of their morphology or of their localization within the thymus. Recently, we showed that they probably belong to the most immature cells of the thymus (3). Furthermore, it is obvious that they do not display any significant proliferating capacity during the preleukemic latency period. They would constitute a polyclonal population (17) whereas lymphoma cells are oligo - or monoclonal (Astier - Gin *et al*, personal communication).

Whether preleukemic cells bear any gene alteration, *i.e.* at the level of proto - oncogenes, is presently unknown.

Promotion

The «initiated» preleukemic cells undergo their progression towards lymphoma transformation only within the thymus.

This raises the question of the substrate of this thymus dependency and of the possible «promoting» effects of thymus microenvironment on the initiated cells.

A subpopulation of epithelial cells is deeply damaged by fractionated irradiation as well as by RadLV (18, 19). Thymic epithelial nurse cells, which are closely intermingled with immature thymocytes in the subcapsular zone, lose the capacity to interact with early thymocytes in preleukemic thymuses. Consequently, very few, if any, lymphoepithelial nurse cells are still detected in the thymus of preleukemic animals.

These alterations to thymic nurse cells appear in all conditions which lead to lymphoma in the thymus (19, 20).

Their role in thymic lymphoma development is therefore highly probable.

Important alterations to early T cell lineage are also observed in preleukemic animals. The pool of T cell precursors (prothymocytes) in bone marrow is strongly depleted during the whole preleukemic period after fractionated irradiation (14, 21, 22). The proportion of the various thymocyte subpopulations is modified and a subclass of abnormal thymocytes precursors appears within the thymus (3). These modifications to early T cells are undoubtedly related to the injury to marrow prothymocytes and to thymic microenvironment. The mechanisms by which irradiation induces all these alterations are still poorly known.

Bone marrow shielding during irradiation or a graft of normal bone marrow cells early after the last irradiation of 1.75 Gy inhibits the development of lymphomas, since the incidence of tumors drops from 90% to less than 10% (22, 23). Interestingly, preleukemic cells are still detected in the thymus, but only for the first 6 weeks following irradiation (24). Thus, when bone marrow transplantation is performed, preleukemic cells are still induced by irradiation, but they are later on eliminated.

Bone marrow transplantation has many effects in the 4 × 1.75 Gy irradiation mice; the phenotype and the functions of prothymocytes (21, Rongy *et al* in preparation), thymocyte subpopulations (3) thymic epithelial nurse cells (24) and spleen NK cells (25) are restored. The reconstitution of nurse cells might be the most important (26). The mechanisms responsible for the disappearance of preleukemic cells are still unknown. In fact, bone marrow transplantation provides the thymus with normal precursors of thymocytes and of stromal cells of the histiocytic lineage. One of the consequences of this restoration might be the reconstitution of the local production of soluble factors, *i.e.* cytokines, which would then act on thymic epithelium and restore its functions. As a result, the preleukemic cells would not find the appropriate conditions for progression to thymic lymphoma. In support of this working hypothesis, we have recently demonstrated the role of cytokines in this system: the injection of TNF α or IFN into irradiated animals decreases the incidence of thymic lymphomas significantly (26). These cytokines are also capable of acting on epithelial nurse cells by increasing their capacities of interacting with early thymocytes (27).

Hypothesis

Fractionated irradiation *initiates* preleukemic cells among early thymocytes. The mechanism involved is still poorly known. The preleukemic cells remain in the thymus for several months. During this period, they do not accumulate, because they are in a «dormant» stage or divide asymmetrically. Their transformation into malignant cells is *promoted* by the RadLV - or radiation - induced modifications in the T cell lineage and the thymic microenvironment. Consequently,

several cell - cell signals which are normally required for differentiation are missing. The conversion of preleukemic cells into lymphoma cells might be due to novel genomic alterations which would occur randomly in the initiated cell population.

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