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Cellular events in radiation-induced lymphomagenesis*

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Fractionated whole-body irradiation induces thymic lymphomas in most of treated C57Bl/Ka mice. The cellular events occurring during the latency period consist of the emergence of preleukaemic cells and of marked alterations to the T-cell lineage and the microenvironment within the thymus. The proportions of the various thymocyte subsets are modified, suggesting a blockage in the normal differentiation process. Thymic epithelial cells are functionally modified, leading to decreased interactions with immature thymocytes. Interestingly, bone marrow grafting early after irradiation, which inhibits the development of lymphomas, induces the disappearance of preleukaemic cells from the thymus, whereas thymocyte subpopulations and thymic epithelium are restored. Interferon γ and tumor necrosis factor α also prevent the onset of lymphomas. Studies on the effect of bone marrow transplantation and cytokine inoculation in split-dose irradiated mice should allow characterization of the factors that modulate the progression of preleukaemic cells towards the neoplastic state.

1. Introduction

The haemopoietic system is a preferential target for the oncogenic effects of radiation as illustrated by many observations in humans and in experimental animals (Kato and Schull 1982, Tubiana 1983, Upton 1984, Mole 1986).

Among the models that have been widely studied is the induction of thymic lymphomas by split-dose whole-body ionizing irradiation in mice (Kaplan 1967). Thymic lymphomas can also occur spontaneously in some mouse strains (Gross 1951) or are induced by viruses (Gross 1951, Lieberman and Kaplan 1959, Gross 1970) or chemicals (Doell and Carnes 1962) in other strains. These lymphomas can be compared to the rather rare lymphoblastic lymphomas that occur in young adult humans (Lennert 1978), the aetiology of which has not yet been defined.

2. An experimental model

In the classical Kaplan protocol (Kaplan and Brown 1952), 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 1 month of age. More than 90 per cent of irradiated mice develop a thymic lymphoblastic lymphoma between the 4th and the 12th month of age. Later, neoplastic cells invade lymph nodes, spleen, liver and bone marrow leading to leukaemia (Kaplan 1967).

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A similar lymphoproliferative disorder is induced by inoculating a retrovirus (RadLV) in 1-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 (Leiberman and Kaplan 1959).

3. The questions

The cell biologists have focused their interest on the following questions.

1. What are the cell populations involved in the neoplastic process?
2. What are the factors involved in the promotion of the target cells towards lymphoma transformation?

4. The target cells

As mentioned above, irradiation or RadLV induce thymic lymphoblastic lymphomas. The neoplastic cells are lymphoblasts, which display heterogeneous phenotypes: most of them bear Thy-1 antigen, a universal marker of T lymphocytes in mice; they are heterogeneous in terms of CD4 or CD8 antigens, which are the differentiation markers of various subpopulations of T cells; they are not bona-fide representatives of any defined thymocyte subpopulations, and thus their phenotype does not allow speculation on the origin of the target cells (Hooghe and Boniver 1985, Greimers *et al.* 1986, Rongy *et al.* unpublished observations). It should be noted from such data that these targets are related to the cells which can give rise to such lymphoblasts, i.e. that marrow prothymocytes, intrathymic thymocyte precursors or cortical lymphoblasts may be sensitive to the induction of neoplastic transformation.

Many investigations have been devoted to the identification of the target cells. In the late 50s, Kaplan and Brown (1957) emphasized the morphological parallelism between neoplastic lymphoblasts and the lymphoblasts that accumulate clustered in the outer cortex of the thymus during the regeneration following X-irradiation and in the preleukemic thymuses. Studies in RadLV inoculated animals or *in vitro* yielded further information in this direction, since early after virus infection the first virus-producing cells were identified as lymphoblasts of the subcapsular zone (Declève *et al.* 1975, Boniver *et al.* 1981a). More recent studies by using *in situ* hybridization indicate that cells at the corticomedullary junction show evidence of viral replication a few hours before those of the outer cortex (Boniver *et al.* 1989a).

Interestingly, the early virus-producing cells display the same topography and morphology as the most immature cell of the intrathymic T cell differentiation pathway (Kyewisky 1987, Penit 1988). Other data also clearly indicate that bone marrow prothymocytes can be targets, at least for RadLV (Lieberman and Kaplan 1976). This suggests that susceptibility to the lymphomagenic agents is preferential at the earlier stage of pre- or intrathymic thymic lymphopoiesis. The molecular substrate of this peculiar susceptibility is unknown.

5. The preleukaemia cells

During the months following irradiation or virus inoculation the animals behave normally and look healthy. The thymus is slightly smaller than normal, and when examined by morphological means it does not show any sign of tumour growth. Nevertheless, a new subclass of thymocytes emerges as early as 1–2 days after

treatment, and remains within the thymus until mice develop lymphomas: these cells have acquired preneoplastic capacities, i.e. they are capable of inducing thymic lymphomas after transfer into histocompatible recipients (Boniver *et al.* 1981b). Interestingly, these cells do not behave like lymphoma cells: the latter can grow after inoculation in normal or thymectomized recipients, whereas the former lead to lymphomas only within thymus-bearing mice. Thus, 'preleukaemic' cells, as designated by Haran Ghera several years ago, are thymus-dependent for progression towards neoplastic growth (Haran Ghera 1978, Boniver *et al.* 1981b). Preleukaemic cells can be considered as 'initiated' cells, which must receive promotional influences for giving rise to fully transformed cancerous cells.

No definitive information is presently available on the cell surface phenotype of these preleukaemic cells, or on their morphology, or on their localization within the thymus. Some recent data suggest that they belong to the most immature cells of the thymus (Rongy *et al.*, unpublished observations) and that they do not display any significant proliferative capacity during the preleukaemic latency period. They would constitute a polyclonal population (Ben-David *et al.* 1988), albeit lymphoma cells which are oligo- or monoclonal (Astier-Gin *et al.*, unpublished observations).

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

6. The thymus microenvironment

As stated above, the 'initiated' preleukaemic cells can undero 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.

Many studies in the AKR model indicate the role of thymic stroma for the induction of thymic lymphomas; in fact, normal lymphocytes from mice with no spontaneous tendency to lymphoma development become lymphomatous when they can grow within the thymic stroma of a preleukaemic animal (Zielinski *et al.* 1982). In that system it is believed that thymic stromal cells sustain the emergence of oncogenic recombinant viruses, able to infect lymphocytes, and modify the pattern of differentiation of lymphocytes.

In the C57BL/Ka system we have shown that a subpopulation of epithelial cells is severely damaged by fractionated irradiation as well as by RadLV (Houben-Defresne *et al.* 1983, Defresne *et al.* 1986a, b). They are thymic nurse cells, which are epithelial cells of the outer cortex, closely intermingled with maturing thymocytes. Under normal conditions these cells are involved in the differentiation process of early thymocytes. In preleukaemic mice these epithelial cells lose the capacity to interact with early thymocytes and to create complexes with them. As a result, nurse cells can no longer be detected in the thymus of preleukaemic animals.

The fact that these alterations of thymic nurse cells appear in all conditions which lead to lymphoma in the thymus argues in favour of their role in thymic lymphoma development. Observations on the effects of bone marrow grafting in irradiated animals are compatible with this hypothesis (see below).

7. The T cell lineage

Besides the alterations to the thymus microenvironment, the preleukaemic mice show important modifications in the T lymphocyte population and in their precursors. Observations in irradiated mice are particularly interesting in this

respect. The pool of T cell precursors (prothymocytes) in bone marrow is strongly depleted during the whole preleukaemic period (Pazmino *et al.* 1978, Boniver *et al.* 1981b, Van Bekkum *et al.* 1984).

Simultaneously, marked modifications of thymocyte subclasses are observed, and a subpopulation of abnormal thymocyte precursors emerges in the thymus (Rongy *et al.* unpublished observations). Whether these modifications are the results of the direct effects of irradiation on stem cells, or are related to the aforementioned changes of thymic microenvironment, still poorly defined.

8. The effects of bone marrow grafting in 4 × 1·75 Gy irradiated mice

Kaplan and colleagues (1953) clearly demonstrated that bone marrow shielding during irradiation or transplantation of normal bone marrow cells early after the last irradiation of 1·75 Gy inhibited the development of lymphomas, the incidence of which dropped from 90 per cent to less than 10 per cent. Interestingly, these protective treatments do not inhibit the emergence of preleukaemic cells in the thymus, but these preneoplastic cells do not persist for more than the first 6 weeks following irradiation (Defresne *et al.* 1986a). Thus, bone marrow transplantation does not prevent the induction of preleukaemic cells by irradiation, but induces their disappearance.

This biological manipulation is interesting since it should allow the factors which contribute to the progression of preleukaemia cells to lymphoma to be defined. Among the numerous studies which were devoted to this subject, it was shown that bone marrow transplantation restores the phenotype and the functions of prothymoctyes (Pazmino *et al.* 1978, Rongy *et al.* unpublished observations), thymocyte subpopulations (Rongy *et al.* unpublished observations), thymic epithelial nurse cells (Defresne *et al.* 1968a, b) and spleen NK cells (Noël *et al.* 1985). Recent studies suggest that the recovered nurse cells might be mostly important (Humblet *et al.* 1989). How this can lead to the elimination of preleukaemic cells is still unknown. It is clear that bone marrow transplantation provides the thymus with normal precursors of thymocytes and of stromal cells of the histiocytic lineage. Consequently, the local production of some cytokines might be restored, which could then act on thymic epithelium. In the fully reconstituted thymus the preleukaemic cells would not find the appropriate conditions for progression to thymic lymphoma.

Recent data from our group indicate the role of cytokines in this system: the injection of TNFα or IFNγ into irradiated animals inhibits the onset of thymic lymphomas (Boniver *et al.* 1989b). Interestingly these cytokines act on epithelial nurse cells by increasing their capacity to interact with early thymocytes (Defresne *et al.* unpublished observations).

9. A hypothesis

Fractionated irradiation induces preleukaemic cells among early thymocytes through a still poorly known mechanism (see the paper by Janowski *et al.* in this issue, pp. 00–00). These cells, albeit normal thymocytes, display the capacity to persist in the thymus for several months until they become neoplastic. During this latency period, preleukaemic cells do not proliferate actively, either because they are in a dormant stage or because they undergo asymmetrical division. Their progression to malignancy is promoted by modifications in T cell precursors and the thymic microenvironment, which are induced by fractionated irradiation.

Under these conditions several normal signals of differentiation are lacking. The reason why preleukaemic cells are converted into lymphoma cells is still undefined, but might be due to a novel genomic alteration occurring randomly in the unstable and preleukaemic initiated cell population.

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