Int. J. Radiation Oncology Biol. Phys., Vol 11, pp. 65–69
Printed in the U.S.A. All rights reserved.

0360-3016/85 $03.00 + .00

● *Lymphocytes/Thymomas/TLI*

TARGET CELLS AND THYMUS MICROENVIRONMENT IN THE PATHOGENESIS OF THYMIC LYMPHOMAS IN C57BL/Ka MICE

J. BONIVER, M.D., PH.D.,* M.P. HOUBEN-DEFRESNE, M.D.,† G. GOFFINET, PH.D., P. LENAERTS, M.D.† AND E.H. BETZ, M.D.

Institute of Pathology B.23, Laboratory of Pathological Anatomy, State University of Liège, B-4000 Liège, Belgium

In C57BL/Ka mice, the induction of thymic lymphomas either by inoculation of radiation leukemia virus (RadLV) or by a split dose irradiation requires complex cellular events: 1) Target cells are found among the population of thymic subcapsular blast cells, or, alternatively, of marrow or spleen prothymocytes; 2) Progression of target cells to lymphoma growth requires a multi-step process, which occurs *only* within thymic microenvironment; 3) Target cells are rapidly induced as "preleukemic" cells; 4) After inoculation of RadLV, the initial events occur when target cells are in close association with cells of a specialized component of thymic epithelium, i.e., the so-called "nurse cells"; 5) The leukemogenic agents induce damages to the thymic microenvironment itself; 6) Lymphoma prevention by marrow grafting after irradiation results from mechanisms still unknown which inhibit the progression of "preleukemic" cells to neoplastic growth.

Thymic lymphomas, C57BL/Ka mice, Target cells, Thymic microenvironment, Radiation leukemia virus, Split dose irradiation.

INTRODUCTION

The C57BL/Ka strain of mice is highly susceptible to the experimental induction of thymic lymphomas by ionizing irradiation or by retroviruses, such as the radiation leukemia virus.[20] When young adult C57BL mice are treated with four weekly irradiations of 1.75 Gy, the incidence of tumors reaches more than 90%. The neoplasms appear 4 to 9 months after completion of the irradiation course; they are lymphoblastic lymphomas which first grow within the thymus before spreading to other tissues, eventually giving rise to leukemia.[21] The mechanism by which ionizing radiation induces neoplastic transformation of lymphoid cells is not definitely known. At the present time, the most critical issue is to define the possible role of a retrovirus. Indeed, leukemogenic viruses, such as radiation leukemia virus (RadLV), were isolated from a pool of radiation-induced thymic lymphomas.[29,30] After inoculation into susceptible mice, these viruses induce thymic lymphomas, which are similar, if not identical, to the tumors induced by irradiation.

These thymic lymphomas of the C57BL/Ka mice are attractive models of cancerogenesis. Many investigations are currently being performed, in order to define the intimate mechanisms of action of RadLV or of irradiation on the host cell genome. Another critical problem, which will be the topic of this review, concerns the selective thymotropism of the leukemogenic agents. We will consider here the present status of research on the nature of the target cells and on their progression to neoplastic growth.

Two sets of observations by Henry Kaplan and his coworkers laid the foundation of this research.

First of all, from studies on radiation or RadLV induced lymphomas, Kaplan proposed in 1961[19] that the targets for the leukemogenic factors are the "immature lymphoid cells" with high mitotic activity, which were observed in the outer cortical zone of the thymus regenerating after a leukemogenic split dose irradiation. Similar cells were found in thymuses of newborn or sublethally irradiated adult mice, which are very susceptible to the leukemogenic effects of RadLV.[19]

These studies also emphasized the importance of bone marrow in relation to target cells. Indeed, bone marrow shielding during irradiation strongly decreases the incidence of lymphomas. It was seen that in the thymus of

This work was supported in part by grants from the Fonds de la Recherche Scientifique Médicale (Belgium) and the Centre Anticancéreux près l'Université de Liège.

* Chercheur Qualifié du Fonds National de la Recherche Scientifique (Belgium). J. Boniver was a postdoctoral fellow of the Fogarty International Center in Henry S. Kaplan's laboratory at Stanford from October 1977 to September 1979.

† Chercheur du Fonds de la Recherche Scientifique Médicale.
Reprint requests to: J. Boniver.

Accepted for publication 17 August 1984.

thigh shielded animals, rapid repair and maturation to the small lymphocytic stage occurred. These observations led to the conclusion that "the state of differentiation of the lymphoid cells of the host is a significant determination of susceptibility"[19] to leukemogenic agents.

The second set of observations was focused on the importance of thymic microenvironment for lymphoma development in this experimental model. Indeed, thymectomy before or early after leukemogenic treatment totally prevents the development of lymphomas[18,25,32]; however, susceptibility to tumor growth could be restored by grafting thymuses from newborn donors.[22] The tumors arose in the grafts themselves, demonstrating that thymic microenvironment is a preferential site for these neoplasias.

As a consequence, many investigations were devoted to further identify the nature of target cells and the factors of thymic microenvironment which are involved in the lymphomagenic process. We will review separately the data obtained from studies on RadLV and radiation-induced lymphomas.

METHODS AND MATERIALS

RadLV induced lymphomas

After inoculation of RadLV into young C57BL/Ka mice, the first foci of virus producing cells appear in the thymus outer cortex[8] as early as on day 2 following the treatment. Indeed, as demonstrated by electron microscopy,[3] these cells are subcapsular lymphoblasts, which certainly correspond to the "large immature lymphoid cells" proposed by Kaplan[19] as target cells for the leukemogenic virus.

In fact, target cells represent a very small subpopulation of thymocytes: they represent only 0.01 to 0.03% of the total thymocytes.[2] Phenotypic analysis showed that these large cells were of low density and have H-2 and Thy-1 surface antigens.[3] Combined with data on the spread of virus replication,[8] these results led to the conclusion that the target cells comprise a subset of thymocytes, which is at the earliest stage of T cell differentiation and which may be transitional between the prothymocytes and the subcapsular blast cells. Target cells have been also identified in bone marrow[25,31] and spleen[2] as well as in fetal liver.[25] The progeny of these cells gave rise to an active virus replication and to lymphomas only in thymus bearing mice.[25] These observations suggest that extrathymically located T cell precursors such as prothymocytes can also act as target cells. Similar results were reported later by Cloyd[7] for the characterization of thymic target cells for a MCF virus in AKR mice.

To sustain virus replication and to progress to neoplastic transformation, target cells have to interact specifically with non lymphoid stromal cells of the thymic microenvironment. Indeed, the first virus producing lymphoblasts are preferentially bound to special lymphoepithelial complexes of the outer cortex, i.e., the so-called "thymic nurse cells" or TNCs.[17] This observation suggests that early virus replication is restricted to a well defined stage of T cell differentiation, which may be under the control of a subset of cortical epithelial cells. Indeed, as shown by several recent studies, nurse cells appear to be a site for interaction of immature lymphocytes and thymic epithelium.[17,27,38] It has been proposed, although not yet demonstrated, that cell contacts within these complexes induce the developing thymocyte to undergo some critical differentiation step.[17,27,38]

During the weeks following inoculation, virus replication spreads to the entire thymocyte population of the cortex.[3,8] At this time, the thymus is slightly smaller than normal, but does not show any significant histological abnormalities.

However, an important functional disturbance can be identified in these thymuses. Indeed, they contain cells which are able to give rise to a lymphoma after inoculation into a histocompatible thymus bearing host.[10] Such cells were initially described in several models by Haran Ghera[11,12] and designated as "*preleukemic cells*". Interestingly enough, the first preleukemic cells, which are detected as early as 2 days after virus inoculation, are selectively located in "thymic nurse cells", in the same way as the first virus producing lymphoblasts.[16] These data strongly suggest that preleukemic cells directly derive from the subset of thymocytes which sustain productive infection with RadLV, and thus from the target cells themselves.

The selective location within nurse cells is only transient. Indeed, about one month after virus inoculation, preleukemic cells can be detected in thymocyte cell suspension that do not contain any nurse cells.[16] At that stage, however, they still require the thymic microenvironment for their progression to frank neoplasia. The acquisition of autonomy occurs later on, during the second trimester following RadLV infection. Then, cells that are able to give rise to a tumor in thymectomized hosts are detected in thymuses which are not yet grossly tumoral.[10]

Interestingly, these thymus-independent (pre)leukemic cells found in non-tumoral thymuses are not identical to the neoplastic cells growing in lymphomatous tumors. Transplantation experiments have clearly shown that the first ones give rise to a lymphoma into a recipient after a latent period of about 200 days,[10,16] whereas the second ones yield a tumor after less than 30 days (Houben-Defresne and Boniver, unpublished data, December 1983).

Such studies demonstrate the multistep evolution of target cells to neoplastic growth: the first preleukemic cells are thymus dependent and located in TNCs; later on, they are still thymus dependent although they are no longer associated with TNCs; autonomous thymic independent cells then arise and finally give rise to fast-growing lymphoma cells.

The preleukemic cells have not yet been isolated, nor characterized phenotypically. An attempt was made by Goffinet *et al.*[10] to correlate the level of alkaline phosphatase activity in the thymus and the time course of appearance of preleukemic cells. In the C57BL/Ka model, alkaline phosphatase appears as a membrane marker of thymocytes at a certain level of differentiation and of lymphoma cells. The enzyme is found on a large number of embryonic thymocytes[28] and in a small subset of adult thymic lymphoblasts, which are selectively located in nurse cells.[10] After RadLV inoculation, the intra TNCs virus producing lymphoblasts are also APase positive (Houben-Defresne and Boniver, unpublished data, June 1983) and the frequency in the preleukemic thymus does not increase until autonomous, thymus independent (pre)leukemic cells are found.[10] These results suggest that the APase positive thymocytes do play a role in lymphomagenesis. However, monoclonal antibodies are needed to isolate such cells and to study further the role of the enzyme in physiological and pathological conditions.

Besides the thymus, bone marrow also contains preleukemic cells after RadLV inoculation. Previous studies by Haran Ghera *et al.*[13,14] had clearly shown that in several models, such as in AKR or in C57BL/6 mice after irradiation or inoculation of the D-RadLV variant, preleukemic cells belong to the population of bone marrow prothymocytes. We do not believe that this is the case in C57BL/Ka leukemogenesis. In this strain, after RadLV inoculation, preleukemic cells were detected after two days in the thymus and only after 7–15 days in the bone marrow. In the numerous experiments that we performed, there was *always* a 5–10 days time interval between preleukemic cells appearance in thymus versus bone marrow.[16] This time course suggests that the preleukemic cells found in C57BL/Ka bone marrow are post-thymic T cells and derive from migrating thymocytes with preleukemic capacities. This view has recently been confirmed by experiments in which marrow preleukemic cells were destroyed by a treatment with anti-Thy-1 antibody and complement (Houben-Defresne, unpublished data, January 1984).

Our observations clearly demonstrate that "nurse cells" are a part of the thymic microenvironment required for the transformation of target cells into preleukemic cells. One can postulate that the interaction between thymocytes and epithelial cells within these complexes is involved in the preneoplastic process. It is possible that, for acquiring preleukemic capacities, infected thymocytes have to reach a certain differentiation stage more or less similar to that occurring in the physiological process. Alternatively, or perhaps simultaneously, an alteration of the epithelial cell itself, for example as a result of viral infection, may lead to abnormal interactions with thymocytes, resulting in wrong differentiation signals. This latter possibility is supported by the fact that the epithelial component of nurse cells is also infected by RadLV[17]; moreover, alterations of thymic epithelium have been demonstrated in AKR spontaneous and Moloney virus induced lymphomas.[15,26,40]

Other components of thymic microenvironment account for the thymus dependency of preleukemic cells. Indeed, several weeks after inoculation of RadLV, preleukemic cells are found *outside* of nurse cells, but they can grow only within the thymus. The involved factors have still to be analyzed; they may be related to other stromal cells, such as macrophages or interdigitating dendritic cells, or to some thymocyte subpopulations. The recent availability of monoclonal antibodies, which recognize various subsets of thymus non lymphoid cells, will certainly be of great help in the further study of this important question.

If thymic microenvironment is required for preleukemic cells to progress, it is itself modified during this leukemogenic process. We have shown that none, or very few, nurse cells can be isolated from the second month after RadLV inoculation onwards.[16] It is very likely, although not demonstrated, that the epithelial cells themselves do not disappear from the thymus. The lack of nurse cell recovery means that the complexes between lymphocytes and epithelial cells do not form any longer. Thus, a qualitative and/or a quantitative defect of either the lymphoid or the epithelial component of the nurse cell is altered as a consequence of the leukemogenic treatment. As several studies on nurse cells indicate that these complexes contain early thymocytes, one may postulate that the nurse cell depletion is due to a RadLV induced defect of T cell precursors; however, recent studies (Boniver and Houben-Defresne, unpublished data, March 1984) suggest that this is not the case. It is thus more likely that alterations of the epithelial cells is the most important pathogenic factor. Ultrastructural studies have not shown epithelial cell necrosis.[3] Thus, the defect could be only qualitative. The previously mentioned virus infection of epithelial cells, which results in virus glycoprotein production on the membrane, could disturb the interactions with thymocytes. Further studies are needed to clarify this problem.

The above described interactions between the target cells and the thymic microenvironment indicate the importance of the mechanisms which control the cell proliferation and differentiation, in this model of leukemogenesis as well as in any model of carcinogenesis. Let us recall the general thought that alterations of these mechanisms are involved in the progression and promotion of "initiated" preneoplastic cells. If target cells for RadLV belong to a subset of immature T cells and require thymus microenvironment to become a lymphoma, there are yet a few exceptions to this general rule that have been described in particular experimental conditions. First, spleen lymphomas developed in athymic nude mice after intracranial inoculation of RadLV.[37] Next, antigen specific immunocompetent T

cell lymphomas were obtained by *in vitro* infection of spleen cells from antigen-primed mice. After inoculation into congenic host, donor type tumors developed and were established as cell lines which displayed antigen specific helper,[9,39] suppressor[36] or cytotoxic (Schaaf Lafontaine and Boniver, unpublished data, March 1984) activities. It is thus possible that under stressed conditions, RadLV escapes to its selective tropism for immature thymocytes and thymus microenvironment.

Radiation-induced thymic lymphomas

After a split dose whole-body irradiation, target cells obviously derive from the regenerating population of thymic subcapsular blast cells. During the first two weeks following the last fraction of the X rays, there is a proliferation of lymphoblastic cells,[5,23] morphologically similar to those which first sustain virus replication after inoculation of RadLV.[3]

As previously suggested by Kaplan,[19] radiation-induced alterations of the marrow thymus interactions certainly account for the particular pattern of thymus repopulation after fractionated irradiation. Indeed, the pool of bone marrow prothymocytes is impaired by the course of irradiation: the activity of terminal deoxynucleotidyl transferase (TdT), which is normally present in prothymocytes, and the capacity of bone marrow cells to repopulate the thymus of sublethally irradiated mice, disappear almost completely during the first two months following the last dose of X rays.[4,35] On the other hand, after bone marrow grafting, which is known to restore prothymocyte capacities,[4,35] thymus repopulation follows a normal pattern and there is no accumulation of cortical blast cells, which rapidly mature in small lymphocytes[5,19] whereas lymphoma growth is prevented.[24] Taken together, these data suggest that after fractionated irradiation the thymus has to repopulate by itself, by the proliferation of surviving thymocytes and without any efficient supply of marrow precursors.

As in the case of RadLV induced lymphomas, there is a 3 to 9 month latent period between the completion of the leukemogenic irradiation and the appearance of grossly detectable thymic lymphomas. During this period the thymus remains normal macroscopically, or is even smaller than in the controls.[5,23] No significant histological changes can be observed.[5] Cell surface studies demonstrated changes in the level of membrane Thy-1 and H-2 antigens,[6] whereas intracellular TdT activity was strikingly decreased.[35]

Here again, the most important consequence of X ray treatment is the induction of "preleukemic" cells. In irradiated C57BL/Ka mice, they appear first in the thymus, and are detected in bone marrow later on.[4] As after inoculation of RadLV, radiation induced preleukemic cells require thymus microenvironment for giving rise to lymphomas. However, these preleukemic cells have not been found preferentially associated with any stromal component of the thymus, such as nurse cells (Houben-Defresne and Boniver, unpublished data, March 1984). The factors of microenvironment interfering with target cells and preleukemic cells in irradiated mice have still to be defined.

The leukemogenic course of irradiation induces alterations of thymic microenvironment. Indeed, activation of endogenous ecotropic retroviruses in epithelial cells has been reported.[1] Moreover, just as after RadLV inoculation, nurse cells disappear from the irradiated thymus (Houben-Defresne and Boniver, unpublished data, March 1984), as a result of alterations of the marrow prothymocyte population and of thymic microenvironment ([4], Houben-Defresne and Boniver, unpublished data, March 1984).

Bone marrow grafting after irradiation restores the nurse cell population and also facilitates thymus regeneration and prevents the development of lymphomas. Even more interesting is the observation that under such conditions, preleukemic cells are found in thymus and bone marrow, where they appear as early as in the unprotected animals. However, their presence is only transient since no preleukemic cells can be detected from the second post irradiation month onwards (Houbden-Defresne and Boniver, unpublished data, March 1984). Thus, preleukemic cells induction is not inhibited by bone marrow grafting but the progression to neoplasia is blocked. The mechanism of this blockade has yet not been demonstrated. Since marrow derived cells actively repopulate the irradiated thymuses,[4] there could be some kind of "competition" between regenerating thymocytes and preleukemic cells leading to the eradication of these cells. Another mechanism refers to the observation that marrow grafting restores the activity of NK cells in irradiated animals.[34] The recovered NK cells could act on preleukemic cells and destroy them. Marrow grafting may also prevent preleukemic progression by restoring some components of thymic microenvironment. It is known that thymic macrophages and interdigitating dendritic cells are destroyed by high doses of irradiation.[33] Split dose irradiation may have the same effect and contribute to lymphoma growth. Further studies are needed to test these hypotheses.

REFERENCES

1. Boniver, J., Decleve, A., Dailey, M.O., Honsik, C., Lieberman, M., Kaplan, H.S.: Macrophage and lymphocyte depleted thymus reticulo-epithelial cell cultures: Establishment and functional influence on T lymphocyte maturation, C-type virus expression and lymphomatous transformation *in vitro*. *Thymus* **2:** 193–213, 1981.
2. Boniver, J., Decleve, A., Finn, O.J., Honsik, C., Lieberman, M., Kaplan, H.S.: Detection of infectious centers in C57BL/Ka lymphoid cell population infected *in vitro* by the radiation leukemia virus. *Cancer Res.* **40:** 544–549, 1980.
3. Boniver, J., Decleve, A., Honsik, C., Lieberman, M., Kaplan, H.S.: Phenotypic characterization of thymus target cells susceptible to productive infection by the radiation leukemia virus. *J. Nat. Cancer Inst.* **67:** 1139–1151, 1981.

4. Boniver, J., Decleve, A., Lieberman, M., Honsik, C., Travis, M., Kaplan, H.S.: Marrow thymus interactions during radiation leukemogenesis in C57BL/Ka mice. *Cancer Res.* **41:** 390–391, 1981.
5. Boniver, J., Simar, L.J., Courtoy, R., Betz, E.H.: Quantitative analysis of thymus lymphoid cells during murine radioleukemogenesis. *Cancer Res.* **38:** 52–58, 1978.
6. Chazan, R., Haran Ghera, N.: The role of thymus subpopulation in "T" leukemia development. *Cell. Immunol.* **23:** 356–375, 1976.
7. Cloyd, M.W.: Characterization of target cells for MCF virus in AKR mice. *Cell* **32:** 217–225, 1983.
8. Decleve, A., Travis, M., Weissman, I.L., Lieberman, M., Kaplan, H.S.: Focal infection and transformation "*in situ*" of thymus cell subclasses by a thymotropic murine leukemia virus. *Cancer Res.* **35:** 3585–3595, 1975.
9. Finn, O.J., Boniver, J., Kaplan, H.S.: Induction, establishment *in vitro* and characterization of functional, antigen-specific, carrier-primed murine T lymphomas. *Proc. Nat. Acad. Sci. U.S.A.* **76:** 4033–4037, 1979.
10. Goffinet, G., Houben-Defresne, M.P., Boniver, J.: Correlation of alkaline phosphatase activity to normal T cell differentiation and to radiation leukemia virus induced preleukemic cells in the C57BL mouse thymus. *Cancer Res.* **43:** 5416–5426, 1983.
11. Haran Ghera, N.: Dependent and autonomous phases during leukemogenesis. *Leukemia Res.* **2:** 11–21, 1978.
12. Haran Ghera, N.: Spontaneous and induced preleukemic cells in C57BL/6 mice. Brief communication. *J. Nat. Cancer Inst.* **60:** 707–710, 1978.
13. Haran Ghera, N.: Potential preleukemic cells among bone marrow cells of young AKR/J mice. *Proc. Nat. Acad. Sci.* **77:** 2923–2926, 1980.
14. Haran Ghera, N., Rubio, N., Leef, F., Goldstein, G.: Characteristics of preleukemia cells induced in mice. *Cell Immunol.* **37:** 308–314, 1978.
15. Heine, U.I., Krueger, G.R.F., Karpinski, A., Munoz, E., Krueger, M.B.: Quantitative light and electron microscopic changes in thymic reticular epithelial cells during Moloney-Virus induced lymphoma development. *J. Cancer Res. Clin. Oncol.* **106:** 102–111, 1983.
16. Houben-Defresne, M.P., Boniver, J.: Thymic nurse cells account for the thymus dependency of preleukemic cells in mice after inoculation of radiation leukemia virus. *Leukemia Res.* **7:** 575–580, 1983.
17. Houben-Defresne, M.P., Varlet, A., Goffinet, G., Boniver, J.: Thymic nurse cells are the first site of virus replication after inoculation of the radiation leukemia virus. *Leukemia Res.* **6:** 231–241, 1982.
18. Kaplan, H.S.: Influence of thymectomy, splenectomy and gonadectomy on incidence of radiation induced lymphoid tumors in strain C57 black mice. *J. Nat. Cancer Inst.* **11:** 83–90, 1950.
19. Kaplan, H.S.: The role of cell differentiation as determinant of susceptibility to virus carcinogenesis. *Cancer Res.* **21:** 981–983, 1961.
20. Kaplan, H.S.: On the natural history of the murine leukemias: presidential address. *Cancer Res.* **27:** 1325–1340, 1967.
21. Kaplan, H.S.: Leukemia and lymphoma in experimental and domestic animals. *Ser. Haematol.* **7:** 94–163, 1974.
22. Kaplan, H.S., Brown, M.B.: Development of lymphoid tumors in non-irradiated thymic grafts in thymectomized irradiated mouse. *Science* **119:** 439–446, 1954.
23. Kaplan, H.S., Brown, M.B.: Radiation injury and regeneration in lymphoid tissues. In *The Leukemias: Etiology, Pathophysiology and Treatment,* J.W. Rebuck, F.H. Bethell and R.W. Monto (Eds.). New York, London, Academic Press. 1957, pp. 163–175.
24. Kaplan, H.S., Brown, M.B., Paull, J.: Influence of bone marrow injection on involution and neoplasia of mouse thymus after systemic irradiation. *J. Nat. Cancer Inst.* **14:** 303–316, 1953.
25. Kaplan, H.S., Lieberman, M.: The role of lymphoid and haemopoietic target cells in viral lymphomagenesis of C57BL/Ka mice. II. Neoplastic transformation of bone marrow derived cells in the thymic microenvironment. *Blood Cells* **2:** 301–317, 1976.
26. Krueger, G.R.F., Karpinski, A., Heine, U.I., Koch, B.: Differentiation block of prethymic lymphocytes during Moloney virus induced lymphoma development associated with a thymic epithelial defect. *J. Cancer Res. Clin. Oncol.* **106:** 153–157, 1983.
27. Kyewski, B.A., Kaplan, H.S.: Lymphoepithelial interactions in the mouse thymus: Phenotypic and kinetic studies on thymic nurse cells. *J. Immunology* **128:** 2287–2294, 1982.
28. Lagerlof, B.A., Kaplan, H.S.: Specificity of the relationship between thymic alkaline phosphatase activity and lymphoma development in strain C57BL mice. *J. Nat. Cancer Inst.* **38:** 437–458, 1967.
29. Latarjet, R., Duplan, J.F.: Experiment and discussion on leukemogenesis by cell free extracts of radiation induced leukemia in mice. *Int. J. Rad. Biol.* **5:** 339–344, 1962.
30. Lieberman, M., Kaplan, H.S.: Leukemogenic activity from radiation-induced tumors of mice. *Science* **130:** 387–388, 1959.
31. Lieberman, M., Kaplan, H.S.: Lymphoid tumor induction by mouse thymocytes infected *in vitro* with Radiation Leukemia Virus. *J. Nat. Cancer Inst. Monogr.* **22:** 549–554, 1966.
32. Lieberman, M., Kaplan, H.S.: The role of lymphoid and hematopoietic target cells in viral lymphomagenesis of C57BL/Ka mice. I. Susceptibility to viral replication. *Blood Cells* **2:** 291–299, 1976.
33. Longo, D.L., Davis, M.L.: Early appearance of donor-type antigen-presenting cells in the thymuses of 1200 R radiation-induced bone marrow chimeras correlates with self-recognition of donor I region gene product. *J. Immunol.* **130:** 2525–2527, 1983.
34. Parkinson, D.R., Brightman, R.P., Waksal, S.D.: Altered natural killer cell biology in C57BL/6 mice after leukemogenic split-dose irradiation. *J. Immunol.* **126:** 1460–1464, 1981.
35. Pazmino, N.H., Mc Ewan, R., Ihle, J.N.: Radiation leukemia in C57BL/6 mice. II. Correlation of altered expression of terminal deoxynucleotidyl-transferase to induction of leukemia. *J. Exp. Med.* **148:** 1338–1350, 1978.
36. Ricciardi-Castagnoli, P., Doria, G., Adorini, L.: Production of antigen-specific suppressive T cell factor by radiation leukemia virus transformed suppressor T cells. *Proc. Nat. Acad. Sci. U.S.A.* **78:** 3804–3807, 1981.
37. Ricciardi-Castagnoli, P., Lieberman, M., Finn, O.J., Kaplan, H.S.: T cell lymphoma induction by radiation leukemia virus in athymic nude mice. *J. Exp. Med.* **148:** 1292–1310, 1978.
38. Wekerle, H., Ketelsen, U.P., Ernst, M.: Thymic nurse cells. Lymphoepithelial cell complexes in murine thymuses: morphological and serological characterization. *J. Exp. Med.* **151:** 925–944, 1980.
39. Yefenof, E., Azar, Y., Eidelsztein, P., Ben Sasson, S.Z.: Immortalization of antigen specific helper T cell lines by transformation with the radiation leukemia virus (RadLV). *J. Immunol.* **128:** 625–628, 1982.
40. Zielinski, C.C., Waksal, S.D., Datta, S.K.: Thymic epithelium is programmed to induce preleukemic changes in retrovirus expression and thymocyte differentiation in leukemia susceptible mice. Studies on bone marrow and thymic chimeras. *J. Immunol.* **129:** 882–889, 1982.