Haematology and Blood Transfusion Vol. 26
Modern Trends in Human Leukemia IV
Edited by Neth, Gallo, Graf, Mannweiler, Winkler
© Springer-Verlag Berlin Heidelberg 1981

Radiation-Induced Murine Leukemias and Endogenous Retroviruses: The Time Course of Viral Expression

V. Erfle, R. Hehlmann, H. Schetters, J. Schmidt, and A. Luz

A. Introduction

Ionizing radiation is well known to be a potent inducer of leukemias in animals and man (for review see UNSCEAR report 19). The mouse has been most frequently chosen to elucidate the factors and mechanisms necessary for leukemia development by irradiation. In the mouse the association of radiation-induced lymphoblastic leukemias with retroviruses has been detected which has led to the concept of an etiologic involvement of endogenous retroviruses in the induction of this disease. This concept implies the activation by radiation of endogenous, genetically inherited retrovirus sequences which acquire oncogenic properties and then transform their specific target cells (Kaplan 1977). This concept has been supported by the isolation of leukemogenic viruses from radiation-induced lymphoblastic leukemias (Gross 1958; Lieberman and Kaplan 1959). But recent work from several laboratories has brought contradicting results. At the moment there is no sufficient experimental evidence to decide whether endogenous retroviruses play a major role in the induction of radiation-induced leukemias.

Our approach to this problem was the evaluation of the time course of retrovirus expression and of the tissue distribution of viral expression. For this purpose the content of viruses and of viral proteins was determined in spleen, bone marrow, thymus, and lymph nodes of irradiated C57Bl/6 mice from the time of irradiation to the time of tumor development. We report here the radiation-induced changes of viral expression prior to and at the time of appearance of lymphoblastic leukemia and discuss their possible contribution to tumor development.

B. Materials and Methods

I. Leukemia Induction

Specific pathogen-free female C57Bl/6 mice of our own colony have been submitted at 4 weeks of age to 4×175 rads whole-body irradiation in weekly intervals (Caesium 137 source, 30 rads/min). Separate groups of irradiated and control animals were observed daily for gross signs of leukemia.

II. Tissue Preparation

Irradiated and control mice were sacrificed monthly. Spleen, thymus, bone marrow, and inguinal and mesenteric lymph nodes were aseptically removed. Single cell preparations for cocultivation studies have been prepared with one part of the tissue samples. The remaining part was homogenized and then frozen at $-70°C$ for the determination of viral proteins.

III. Virus Assays

Single cell suspensions of 1×10^6 cells were cocultivated with indicator cells [C3H 10 T 1/2 for N-tropic ecotropic virus and mink lung cells (CCL 64) for xenotropic virus] which had been seeded 24 h earlier in 5 cm culture dishes at a cell number of 2×10^5. Virus growth was tested after the first and the fifth passage by immunoperoxidase staining with antimurine leukemia virus (MuLV), anti-p30-serum (Nexø 1977), and by the XC plaque test. MuLV p30 content was determined by the ELISA technique according to Schetters et al. (1980).

IV. Viral Antibodies

The antibodies against eco- and xenotropic MuLV have been determined by the ELISA method with sucrose density gradient purified viruses as antigen.

C. Results

The fractionated whole-body irradiation of our C57Bl/6 mice with a dose of 4×175 rads resulted in a total incidence of lymphoblastic leukemia of 34% (17/50) within 12 months after irradiation. The first tumor appeared in month 5 and the majority of the animals came down with leukemia in month 6, 7, and 8.

The expression of endogenous retroviruses in the leukemia latency period has been evaluated by testing the appearance of infectious ecotropic virus (which infects mouse cells), of infectious xenotropic virus (which does not infect mouse cells), and of the major viral core protein p30 in spleen, thymus, bone marrow and lymph nodes. Ecotropic virus could be isolated very rarely. Only one or two animals per month in the irradiated and the control groups were found to express ecotropic virus in one of the organs tested. The majority of the isolations occurred in months 6 to 9, and there were no major differences between the treated mice and the controls during the time of observation. In contrast, xenotropic viruses could be isolated in much higher frequency and most frequently in irradiated animals. In some months all animals in the irradiated group were found to harbour xenotropic virus in bone marrow and spleen. The peak of xenotropic virus expression was during the time period from month 5 to 10. More animals in the irradiated groups than in the control groups showed the occurence of xenotropic virus in spleen (45% to 28%), thymus (21% to 4%), and bone marrow (52% to 24%).

Xenotropic virus yield in lymph nodes was equally low in irradiated and control animals (7% to 7%). The data on infectious virus expression in the time from months 5–10 are shown in Table 1.

Virus expression in the leukemic animals was without a regular pattern. One animal out of eight was found to have ecotropic and xenotropic virus in all of the four organs tested, two animals expressed xenotropic virus in all organs, and one mouse was found to have ecotropic virus only in the spleen. In four leukemic animals no infectious retroviruses could be detected.

With increasing age the control animals developed increasing antibody titers against ecotropic and xenotropic virus. The mean antibody titers in the irradiated animals followed the same pattern except in the 2 months after the last whole-body irradiation. In these 2 months, the irradiated mice exhibited markedly higher antibody titers than the control animals. In the 1st month after the last irradiation all irradiated animals showed higher antibody titers than the control mice, whereas in the following month some of the treated mice had titers like the controls (Fig. 1).

The viral p30 protein content in spleen, thymus, bone marrow, and lymph nodes did not exhibit major differences between irradiated and control animals during the observation period. The mean p30 values rose from initially 5–10 ng/mg protein in month 1 and 2 to values of about 200 ng/mg protein in spleen, thymus, and bone marrow and 500 ng/mg in lymph nodes in month 6 and 7. Thereafter the p30 content declined in treated and untreated animals to less than 100 ng/mg protein in month 9 and 10. A significant difference of p30 content has been observed between thymus of leukemic animals (mean value 210 ng/mg

Table 1. Expression of infectious retroviruses during months 5–10 (age) in C57Bl/6 mice after whole-body irradiation

	Ecotropic				Xenotropic			
	Spleen	Thymus	Bone marrow	Lymph-nodes	Spleen	Thymus	Bone marrow	Lymph-nodes
Irradiated Animals	19% 5/26 [a]	8% 2/26	12% 3/25	12% 3/25	45% 13/29	21% 6/29	52% 15/29[b]	7% 2/28
Controls	16% 4/25	4% 1/25	4% 1/25	20% 5/25	28% 8/29	4% 1/25	24% 7/29[b]	7% 2/29

[a] Number of animals expressing virus/number of animals tested
[b] $p<0.05$

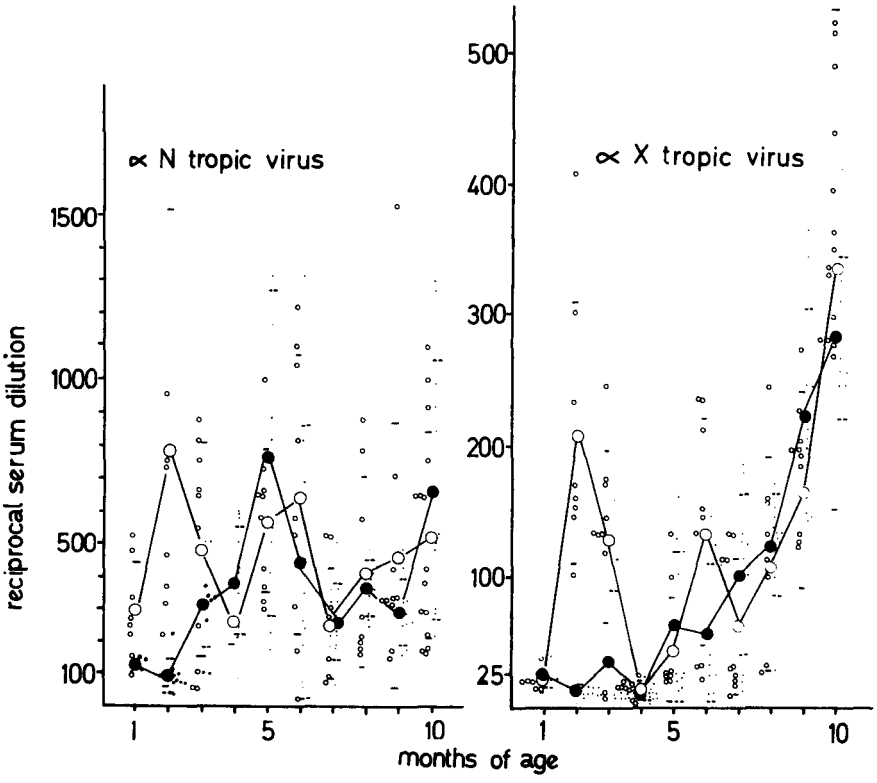

Fig. 1. Antiviral antibodies against ecotropic and xenotropic endogenous retrovirus in C57B1/6 mice after whole-body irradiation. The mean antibody titer of 10 animals per month together with the single values is indicated. ○, irradiated animals; ●, control animals

protein) and that of age-matched control animals (mean value 30 ng/mg protein).

D. Discussion

Our main findings in favor of radiation-induced changes of retrovirus expression in irradiated mice are (1) the early increase of antiecotropic and antixenotropic virus antibody titers and (2) the increased expression of xenotropic virus in spleen, thymus, and bone marrow later on during the time of leukemia appearance. Our results do not indicate a major role for ecotropic viruses in the development of radiation-induced murine leukemias (Ellis et al. 1980; Haas 1977; Sankar-Mistry and Jolicoeur 1980). This is consistent with the fact that radiation-induced leukemias can also be induced in mouse strains such as the NZB mouse which do not harbor the genome for the production of infectious ecotropic MuLV (Harvey et al. 1979).

Our results may suggest some function of xenotropic viruses in the process of radiation-induced murine leukemogenesis. However, since xenotropic murine retroviruses normally do not infect mouse cells, an induction of lymphoblastic leucemia in these mice via an activation of infectious xenotropic viruses seems not to be a plausible mechanism. Also the infection of mouse thymocytes with xenotropic viruses by coinfection with ecotropic virus (Declève et al. 1977) appears to be unlikely because of the lack of expression of ecotropic virus in these mice.

One possible conclusion would be that endogenous retroviruses are not involved in the induction of murine radiation-induced leukemias. An alternative would be that these viruses take part in the disease in the following way: Endogenous retroviruses are discussed to have a physiologic role in differentiation and in the immune system (McGrath and Weissman 1978; Moroni and Schumann 1977). Changes in cell surface structures of lymphocytes may disturbe the complex cell to cell interactions which regulate normal lymphocyte kinetics. The appearance of humoral antibodies against such (viral) cell surface structures could be a factor in the disturbance of normal regulation. The induction of antiviral antibodies by radiation might be a first step toward unlimited growth, since all the irradiated animals developed antibodies, whereas only 34% of the animals became leukemic.

Such antibody-mediated effects can be demonstrated by the induction of leukemia in thymectomized AKR mice by antibodies against leukemic cells reactive also with retrovirus antigens (Kohn et al. 1977). Experiments defining the biologic activity of the radiation-

induced antibodies as well as experiments evaluating different viral parameters in time course experiments with individual animals exposed to radiation doses that induce about 50% leukemia incidence should throw more light on this problem.

Acknowledgments

This work was performed in association with EURATOM (Contract No. 218-76-1 BIO D) and was supported in part by the Deutsche Forschungsgemeinschaft (SFB 51). The expert technical assistance of Ms. S. Schulte-Overberg, S. Hartnagel, E. Jatho, L. Ireland, E. de Fries, and L. Strubel is gratefully acknowledged.

References

Declève A, Lieberman M, Ihle JH, Kaplan HS (1977) Biological and serological characterization of the C-type RNA viruses isolated from the C57Bl/Ka strains of mice. III. Characterization of the isolates and their interactions in vitro and in vivo. In: Duplan JF (ed) Radiation-induced leukemogenesis and related viruses. Elsevier/North Holland Biomedical Press, Amsterdam Oxford New York, pp 247–264 – Ellis RW, Stockert E, Fleissner E (1980) Association of endogenous retroviruses with radiation-induced leukemias of BALB/c mice. J Virol 33:652–660 – Gross L (1958) Attempt to recover filterable agent from X-ray-induced leukemia. Acta Haematol (Basel) 19:353–361 – Haas M (1977) Transient virus expression during murine leukemia induction by X-irradiation. J Natl Cancer Inst 58:251 – Harvey JJ, Tuffrey M, Holmes HC, East J (1979) Absence of ecotropic or recombinant murine leukaemia virus in preleukaemic and leukaemic X-irradiated NZB mice. Int J Cancer 24:373–376 – Kaplan HS (1977) Interaction between radiation and viruses in the induction of murine thymic lymphomas and lymphatic leukemias. In: Duplan JF (ed) Radiation-induced leukemogenesis and related viruses. Elsevier/North Holland Biomedical Press, Amsterdam Oxford New York, pp 1–18 – Kohn RR, Scoitto CG, Hensse S (1977) Age-dependent leukemia produced in thymectomized AKR mice by antiserum to leukemic cells. Exp Gerontol 13:181–187 – Lieberman M, Kaplan HS (1959) Leukemogenic activity of filtrates from radiation-induced lymphoid tumors of mice. Science 130:387–388 – McGrath MS, Weissman IL (1978) In: Clarkson B, Marks P, Till J (eds) Normal and neoplastic hematopoietic cell differentiation. Cold Spring Harbor Laboratory, Cold Spring Harbor, pp 577–589 – Moroni C, Schumann G (1977) Are endogenous C-type viruses involved in the immune system? Nature 269:600–601 – Nexø BA (1977) A plaque assay for murine leukemia virus using enzymecoupled antibodies. Virology 77:849–852 – Sankar-Mistry P, Jolicoeur P (1980) Frequent isolation of ecotropic murine leukemia virus after X-ray irradiation of C57Bl/6 mice and establishment of producer lymphoid cell lines from radiation-induced lymphomas. J Virol 35:270–275 – Schetters H, Hehlmann R, Erfle V, Ramanarayanan M (1980) The detection and quantification of C-type viral proteins in tissues and sera with an enzyme immunoassay. Infect Immun 29:972–980