Preleukemia in Experimental Leukemogenesis *

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A. Introduction

The long latent period characteristic for both spontaneous and induced murine leukemias may reflect multiple steps in the leukemogenic process. The induction of preleukemia represents the initial phase in leukemogenesis. A sequence of further events (that could differ qualitatively from the initiating factor) is often required for the completion of the neoplastic transformation. The presence of potential leukemia-inducing cells (PLC) among bone marrow cells of mice shortly after treatment with different leukemogenic agents (which represents the preleukemic phase) was demonstrated by an in vivo transplantation bioassay. This method is based on the capacity of lymphoid cells transplanted into histocompatible hosts to give rise to lymphomas of donor origin [1]. A high incidence of PLC was demonstrated among bone marrow cells of intact or thymectomized BL/6 mice exposed to fractionated irradiation or treated with a potent chemical carcinogen. These treatments induced a high incidence of T-cell leukemia in intact mice, whereas thymus removal very markedly decreased the development of the disease [1–3]. The induction of PLC following intrathymic inoculation of two RadLV variants having H-2-associated high or low leukemogenic potential in adult mice has also been demonstrated [4, 5]. The most striking findings involved identification of PLC in mice resistant to overt leukemia development following virus inoculation. For example, a high incidence of PLC (in 80%–100% of the tested mice) was induced in BL/6 mice infected with D-RadLV or in B10S mice treated with A-RadLV, although overt T-cell leukemia incidence following these treatments was 0%–20% [6]. PLC have also been identified among fetal liver cells of 16-day-old AKR embryos as well as among bone marrow cells of intact or thymectomized AKR mice from the age of 14 days onwards [7]. Thus, resistance to leukemia development did not necessarily coincide with resistance to PLC induction.

In contrast to the leukemic cells in the experimental models described above that express the T-cell surface component Thy-1, PLC were shown to have the characteristics of prothymocyts [8, 9]. PLC were also shown to play the role of the immunogen in anti-RadLV-induced leukemia immune responses [6, 8, 10]. Thus, mice having H-2-linked resistance to RadLV leukemogenesis may be sensitized to their PLC, and further PLC differentiation into overt leukemia is suppressed by the PLC-induced antileukemia immune response. While leukemic cells grow progressively in any compatible host, further PLC proliferation and progression to leukemic cells is dependent on certain host conditions [9]. Thus, in T-cell leukemogenesis qualitative differences exist between PLC and leukemic cells.

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The present studies demonstrate the occurrence of a preleukemic phase during radiation-induced AML in SJL/J mice and the presence of dormant PLC in AKR mice following treatment that prevents their characteristic susceptibility to spontaneous T-cell leukemia development.

B. The preleukemic Phase in Radiation-induced Acute Myeloid Leukemia in SJL/J mice

Exposure of 3- to 4-month-old SJL/J mice to a single dose of 300 rads whole body irradiation results in AML development in about 20%–30% [11]. This incidence can be markedly increased by additional co-leukemogenic treatment with corticosteroids alone [12] and with additional cytophosphane treatment (Table 1). A single injection of different corticosteroids as co-leukemogenic treatment yielded about 50% AML; prolongation of the treatment to five weekly injections did not change this AML incidence. But five alternate weekly treatments of prednisone one week and cytophosphan the next week (the whole treatment lasting 10 weeks) increased AML incidence to 92% at a mean latency of 265 days. Neither agent without initial exposure to radiation had a leukemogenic effect. This combined co-leukemogenic treatment therefore seems to promote the proliferation and progression of the radiation preleukemic clone to overt AML.

Cytogenetic studies of AMLs induced in SJL/J mice by exposure to radiation with or without further co-leukemogenic treatment indicated that all tumors were characterized by a deletion of chromosome 2 [13]. This deletion therefore seems to be a necessary step in the radiation-induced tumorigenic process. Five types of chromosome-2 deletion were observed according to variations in size and formation, but each tumor had only one characteristic type of deletion in all cells involved. Control untreated SJL/J mice within the age range of 9–14 months had a normal diploid karyotype irrespective of the presence of spontaneous reticulum cell sarcoma [14], classified recently as B-cell neoplasms [15], observed in the older untreated tested mice.

Further studies revealed that chromosome-2 deletion represents the radiation-induced initiation of AML. Cells with this deletion were observed among bone marrow cells of SJL/J mice analyzed 4 months following exposure to 300 rads, although the cell donor mice looked normal, both on cytological and on histological examination. About 80% (14/17) of the tested irradiated mice had different levels of cells with deleted chromosome 2 among their bone marrow population (Table 2). It is interesting that among bone marrow cells from several mice tested, two or three clones with different chromosome-2 deletion types were observed [13], probably representing initial polyclonal transformation. In the final AML we always observed one type of chromosome-2 deletion in 100% of leukemic cells, suggesting the derivation of the tumor from a single altered clone. Among a small group of mice treated with both 300 rads and dexamethasone, analysis of bone marrow cells 4 months after treatment revealed cells with the chromosome-2 deletion in all treated mice (9/9). The percentage of cells with the deletion among the 50–70 karyotyped cells of each tested mouse was higher than in mice exposed to 300 rads alone (Table 2). A certain threshold level of cells with the chromosome-2 deletion, beyond 20%, seems to be essential for the actual overt AML development. In mice tested 4 months after receiving 300 rads, 28% (5/17) had more than 20% of deleted cells among those karyotyped versus 55% (5/9) with additional dexamethasone treatment. These levels match the final AML incidence observed in these two treated groups (Table 1).

A common missing segment D–G was observed in the different deletion types of chromosome 2 [13]. The loss of the genetic information located in this region may

Table 1. Induction of AML in SJL/J mice

Leukemogenic treatment[a]	AML incidence	Latency (days)
300 rads	3/16 – 18%	350 ± 22
300 rads + Hydrocortisone acetate (2.5 mg i.p.)	14/30 – 47%	284 ± 28
300 rads + Dexamethazone (0.5 mg s.c.)	16/26 – 60%	250 ± 28
300 rads + Prednisone (1 mg s.c.)	16/32 – 50%	295 ± 21
300 rads + 5 × (Prednisone $\xrightarrow{7\,d}$ cytophosphane) ↓ 1 mg s.c. ↓ 0.5 mg i.p.	12/13 – 92%	265 ± 14
300 rads + 5 × Prednisone	7/14 – 50%	285 ± 42
300 rads + 5 × Cytophosphane	2/10 – 20%	(340;365)
300 rads + 5 × (Prednisone $\xrightarrow{7\,d}$ cytophosphan)	0/18	–

[a] Three month-old female SJL/J mice were exposed to 300 rads whole body irradiation; the leukemogenic treatment was started 1–3 h after radiation.

Table 2. Incidence of chromosome-2 deletion among marrow cells of SJL/L mice, 4 months following exposure to 300 rads and dexamethasone treatment

Treatment of tested mice	Incidence of chromosome-2 deletion in tested mice	Percentage of cells per mouse with deletion 2[a]				
		2%–5%	6%–10%	11%–20%	21%–30%	above 30%
300 rads	14/17 – 82%	6/17 – 35%	1/17 – 6%	2/17 – 11%	2/17 – 11%	3/17 – 17%
300 rads + dexamethasone	9/9 – 100%	0/9 –	2/9 – 22%	2/9 – 22%	1/9 – 11%	3/9 – 44%
SJL/J – 7 months old	0/10 –					

[a] 50–70 cells were karyotyped. The procedure used is described by Trakhtenbrot et al. [13].

be responsible for the generation of AML. The role of gene deletion as a predisposition in tumorigenesis has been extensively studied in connection with neuroblastoma and retinoblastoma development [16, 17]. Several investigators have suggested that loss or inactivation of negative regulatory genes through deletions may affect tumor development by derepressing other critical genes [18, 19]. The c-*abl* gene that has been implicated in the pathogenesis of human CML [20] was mapped by Goff et al. to the mouse chromosome 2 [21]. Perhaps some correlation between the localization of c-*abl* and the breakpoint in the deletion could also be implicated in radiation-induced AML in mice.

C. Preleukemia in AKR Mice

AKR mice are highly susceptible to spontaneous T-cell leukemia, arising predominantly in the thymus of 6- to 12-month-old mice. Leukemia development is associated with the expression of type-C retroviruses since birth, with increased levels of xenotropic virus titers, particularly in the thymus from 5 months onwards and in the formation of the dual tropic recombinant MCF type-C viruses that occur prior to leukemia development [22]. We have previously demonstrated that preleukemic cells (PLC), i.e., those having the potential to develop into T-cell leukemia, were present among bone marrow cells of intact and thymec-

tomized young mice [7]. Thus, the occurrence of PLC in young AKR mice was shown to be thymus independent, but their progression to overt T-cell leukemia development was dependent on specific host conditions, including presence of the thymus. These findings suggested that the thymus provides a suitable environment for the promotion of PLC, contributing to the progression and differentiation of bone marrow-derived immortalized or preleukemic thymic precursors (prothymocytes) into T-cell leukemia. The promotion and progression of PLC into overt T-cell leukemia in AKR mice seems to be dependent on the delayed formation of the dual tropic virus (DTV) established in the thymus of 5- to 6-month-old AKR mice [22]. Indeed, recently we demonstrated that interference with DTV formation by injection of a viral isolate from a B-cell leukemia of AKR origin (designated 24-666) into the thymus of 14-day-old AKR mice very markedly reduced T-cell leukemia development. We observed a 10%–20% incidence at a mean latent period of 385 days. Some of these treated mice (10%–26%) developed B-cell leukemia beyond 400 days of age [24]. Since we suggested that the initial site of PLC occurrence in AKR mice is among bone marrow cells (and thymus independent), it seemed obvious that these virus 24-666-treated mice should be carriers of PLC. We therefore tested the leukemogenic potential of lymphoid cells from 12-month-old AKR mice infected when 14 days old with virus 24-666, using the transplantation bioassay method (transferring cells i.v. into [AKR × DBA/2]F1 mice). The results obtained (Table 3) clearly indicate that prevention of T-cell leukemia development by virus 24-666 does not affect the high incidence of PLC present among cells in the thymus, bone marrow, and spleen. The majority of the tumors originating from AKR donor cells had the characteristics of B-cell leukemias. Thus, dormant PLC were shown to be present in intact AKR mice that lacked DTV in their thymus.

Thymus removal in 1- to 3-month-old AKR mice markedly reduces the spontaneous tumor incidence (10%–20% extrathymic lymphoid tumors which appear late in life). The presence of PLC in old thymectomized AKR mice was previously demonstrated [25]. These PLC could be triggered to develop into B-cell leukemias after transplantation into syngeneic or hybrid (AKR × DBA/2)F1 intact or thymectomized recipients [25]. The majority of the leukemias expressed high levels of IgM, κ light chain, Ia molecules FcR, the Ly-1 marker, and the TL.4 antigen. Almost all thymectomized AKR mice were found to harbor PLC throughout their life span. These thymectomized AKR mice therefore represent an endogenous, spontaneously occurring preleukemic dormant state. Host inhibitory factors seem to contribute to the proliferation arrest of PLC in thymectomized mice, since only by transfer of lymphoid cells of old donor mice to young cell recipients could PLC proliferate and give rise to overt B-cell leukemia development. It is plausible to assume that prolonged thymectomy in AKR mice causes changes in regulatory mediators, including deficiency of T-cell factors necessary for B-cell growth turnover. It was therefore interesting to test whether grafting of a newborn AKR thymus subcutaneously into 10-month-old thymectomized AKR mice could affect the leukemia incidence in these thymus graft recipients. Since thymectomy prevents DTV formation, we also tested the possible effect of infection with DTV (from T-cell leukemia origin) or the virus 24-666 (isolated from a B-cell leukemia) on leukemia development and breakdown of PLC dormancy. Ten-month-old thymectomized AKR mice received a single i.v. injection of these virus isolates. The results obtained are summarized in Table 4. Indeed, the incidence of B-cell leukemia was markedly increased by these different experimental manipulations (66%–84% versus 18% in the untreated thymectomized control mice). Thus, PLC can be prompted to develop

Table 3. Leukemogenic potential of lymphoid cells from 12-month-old AKR mice infected when 14 days old with virus 24-666

Cells tested	Leukemia incidence	T-cell leukemia	B-cell leukemia	Null cell leukemia
Thymus	12/14 – 85% (86 ± 24)	3/14 – 21%	6/14 – 43%	3/14 – 21%
Bone marrow	12/14 – 85% (80 ± 16)	0/14 –	10/14 – 71%	2/14 – 14%
Spleen	10/13 – 77% (92 ± 27)	1/13 – 8%	7/13 – 54%	2/13 – 15%

Thymocytes, bone marrow cells, and spleen cells were injected i.v. from each individual mouse to (AKR × DBA/2)F1 recipients (1:1 transfer). The AKR donor origin of the developing leukemias and the tumor characteristics were done as previously described [25].

Table 4. Termination of PLC dormancy

Treatment[a]	B-Leukemia incidence	T-Leukemia incidence
–	5/28 – 18% (500 ± 20)	2/28 – 7% (385;430)
n.b. thymus s.c.	16/24 – 66% (480 ± 45)	2/24 – 8% (350;516)
DTV × 1 (i.v.)	22/26 – 84% (460 ± 74)	0/26
24-666 × 1 (i.v.)	21/26 – 80% (512 ± 58)	0/26

[a] AKR females thymectomized when 45–55 days old were further treated when 10 months old; both lobes of a syngeneic newborn (n.b.) thymus were grafted subcutaneously. The origin and preparation of the injected viruses are described in [7] and [24].

into overt lymphoma in the thymectomized mice by different factors, their mode of action being still an enigma.

The possible pathways in T- and B-cell leukemogenesis in AKR mice are summarized in Fig. 1. From birth, AKR mice carry "immortalized" PLC, identified among the bone marrow prothymocytes. In intact AKR mice the progression of PLC into overt T-cell lymphoma is dependent on the delayed formation of DTV in the thymus of 5- to 6-month old mice [23]. Thymus subpopulation changes due to DTV formation [26] may trigger stem cell migration, including PLC, into the "injured" thymus, and thereby provide the microenvironment for further PLC differentiation into T-cell leukemia. The capacity of DTV to impair certain T-cell functions [23] could also interfere with immune surveillance triggered by the immunogenic capacity of PLC and thereby contribute to the progression of PLC into overt T-cell leukemia. Interference with DTV formation in the thymus by a competitive virus (24-666) or by thymus removal abrogates the environment required for the transition of PLC to T-cell leukemia. These mice are carriers of PLC that can be triggered, by different experimental manipulations, to develop into B-cell leukemias. Different inhibitory factors may contribute to the arrest of PLC proliferation in these hosts. Most of the B lymphomas that develop express the Ly-1 antigen [25]. Since Ly-1$^+$ B cells were shown to secrete IgM autoantibodies [27], they may affect regulatory factors necessary for the proliferation of PLC into autonomous B-leukemic cells. Removal of PLC into young recipients might prevent the interaction of the autoantibodies with PLC and thereby facilitate their

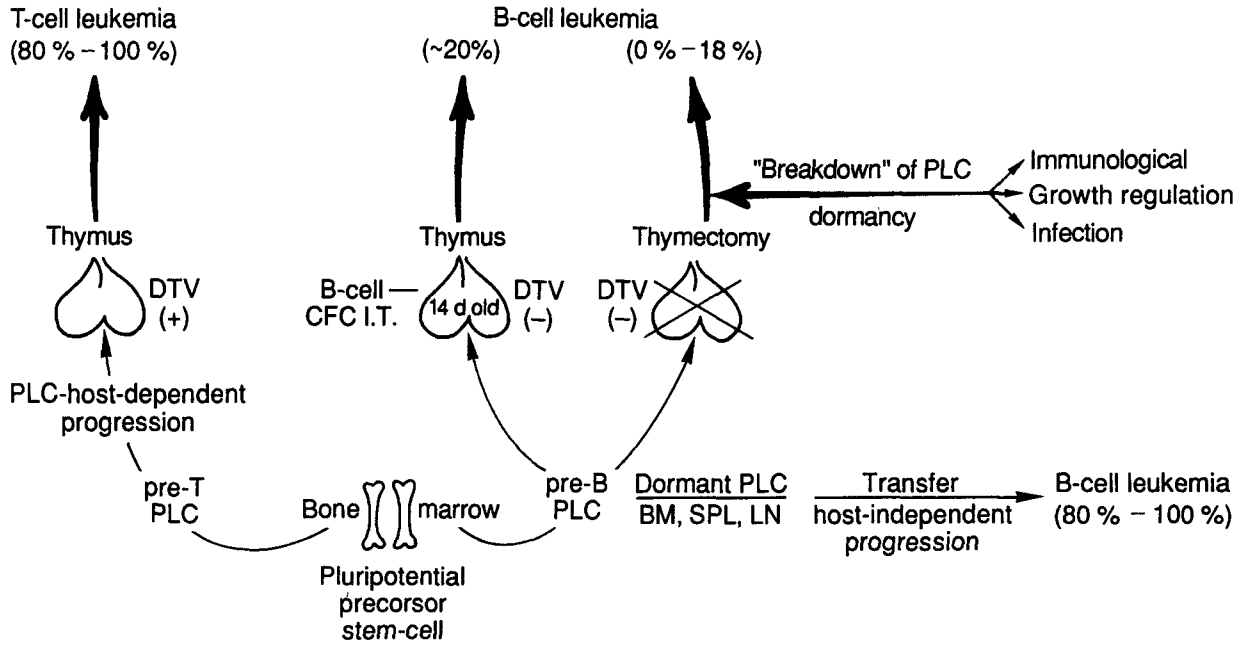

Fig. 1. T- and B-cell leukemogenesis in AKR/J mice

progression into B-cell leukemias. Breakdown of PLC dormancy in thymectomized mice by thymus grafts might act by providing the lacking hematopoietic growth factors. The viral infection might perhaps also act via lytic effects on certain lymphoid populations, thereby affecting immune integrity or regulatory factors mediated by these cells.

One obvious problem is whether PLC in intact and thymectomized AKR mice represent a pluripotential hematopoietic precursor cell sharing pre-T and pre-B properties or, alternatively, whether two different lymphoid progenitors restricted to T- or B-cell pathways are involved in T- and B-cell leukemia development in AKR mice. Our findings that B-cell leukemias of AKR origin express the T-cell antigen TL.4, found exclusively on T-cell leukemias, including the early-occurring ones in AKR mice, as well as on some lymphoid cells of 1- to 20-day-old AKR mice [25, 28], may suggest that a common progenitory $TL.4^+$ cell might serve as a precursor for both T- and B-cell leukemia in AKR mice.

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