Eur J Cancer Clin Oncol, Vol. 18, No. 11, pp. 1181–1185, 1982.
Printed in Great Britain.

0277-5379/82/111181-05$03.00/0
© 1982 Pergamon Press Ltd.

Host Endocrine Status Mediates Oncogenesis: Leukemia Virus-induced Carcinomas and Reticulum Cell Sarcomas in Acyclic or Normal Mice

WALTER PIERPAOLI*† and ASHER MESHORER‡

*Institute for Integrative Biomedical Research, Ebmatingen, Switzerland and ‡Animal Breeding Center, The Weizman Institute of Science, Rehovot, Israel

Abstract—*A highly selective leukemogenic virus obtained from thymic lymphosarcomas (LS) of C57BL/6 mice was inoculated intraperitoneally into normal or gonadectomized C57BL/6 male mice and into normal or masculinized, acyclic, Charles River (CR) outbred female mice. This virus induced early onset of LS in both normal and gonadectomized C57BL/6 mice. The same virus induced a relatively late onset of many mammary adenocarcinomas in the normal CR mice and ovary adenocarcinomas and systemic neoplasms (LS and reticulum cell neoplasms) in the acyclic females. Topography, histopathology and the average latent period of the tumors were completely different between the normal and the acyclic females. This is consistent with the idea that a leukemia virus can induce tumors other than leukemia and that permanent alterations of the physiological hormonal cyclicity plays a major role in determining whether or on which target cells the ubiquitous virus will exert its carcinogenic action.*

INTRODUCTION

ATTENTION has recently been focused on the prominent role of specific, permanent, congenital or induced neuroendocrine derangements as a conditioning cause for genesis of tumors in general and of systemic neoplastic processes in particular [1–3]. Specific, hereditary, strain-linked hormonal alterations have been recognized in mice which develop spontaneous or induced leukemia (lymphosarcoma, LS) or reticulosarcoma (reticulum cell neoplasia, RCN) [1–3]. Conversely, inhibition or depression of adenohypophyseal function has led to a drastic reduction of irradiation or chemical carcinogen-induced leukemia [2]. The experimental development of these concepts aimed at preventing, reproducing or mimicking those leukemia-linked hormonal derangements in spontaneous LS or RCN-resistant or susceptible mice and thus to influencing both onset and development of those neoplastic processes [3]. The final idea emerging from all these experimental observations was that the host environment conditions and modulates the expression and potential carcinogenicity of viruses, and that the same viruses can act on or transform *different* target cells [1–3]. In other words, the expression of carcinogenicity and the affinity of viruses for targets and cells depends also on the unique species, strain, sex and age-dependent host environment and not exclusively on the intrinsic nature of leukemogenic viruses. This is also true in so far as the immune capacity and resistance of the host is also largely linked and dependent on its genetically inherited neuroendocrine build-up [4]. That the activation of oncogenic viruses and expression of tumor virus genomes depend on hormones has been demonstrated by other models [5, 6].

We report here on experiments showing that inoculation of the same leukemia virus preparation obtained from X-ray-induced thymomas (LS) in C57BL/6 mice into normal female mice of a different strain (Charles River,

Accepted 15 June 1982.
†Correspondence to: Dr. W. Pierpaoli, Institute for Integrative Biomedical Research, Lohwisstrasse 50, 8123 Ebmatingen, Switzerland.

CR) or into masculinized CR females (rendered acyclic by neonatal injection of testosterone) results in the development of tumors *other* than LS. These tumors originate in organs whose functions have been permanently altered by the endocrine manipulation (e.g. the ovary and lymphatic tissues) or in organs (e.g. the mammary gland) which simply represent the privileged target for the 'leukemia' virus in the new, normal host.

MATERIALS AND METHODS

Animals

Outbred, Charles River (CR) albino mice, originally purchased from Wander AG, Bern, were bred and maintained under conventional conditions in our animal quarters. Young adult inbred C57BL/6 mice were a gift from the Animal Farm of Hoffman La Roche AG, Füllinsdorf, Switzerland.

Masculinization procedure

For induction of acyclic sterility and permanent virilization of females, newborn CR mice were injected s.c. on the day of birth with 1 mg testosterone propionate in peanut oil. Controls were injected with oil only. The methods for and the consequences of permanent masculinization have been described at length [7–9].

Inoculation of virus

The radiation leukemia virus used in these studies was a gift from Professor N. Haran-Ghera, The Weizman Institute of Science, Rehovot, Israel. This virus was originally obtained from bone marrow of irradiated, non-leukemic C57BL/6 mice and kept by serial passage line *in vivo* in C57BL/6 mice [10]. The highly leukemogenic virus variant used was made from thymic lymphomas induced in adult C57BL/6 mice by intrathymic inoculation of a virus (called V-136) capable of inducing lymphomas without further co-leukemogenic treatment (irradiation). This virus, when injected intrathymically into normal C57BL/6 mice, yielded a very high leukemia incidence (80–100%) with an average latent period (ALP) of 70–100 days. The procedure of virus preparation has been previously described [10]. Quantities of 0.2 ml virus V-136 were injected i.p. into the normal or masculinized female CR mice at one month of age or into normal or gonadectomized C57BL/6 male mice.

Orchidectomy

Gonadectomy was performed by conventional surgical procedure in prepubertal C57BL/6 male mice (one-month-old animals).

Onset of tumors

The animals were controlled singly by visual inspection and palpation of mesenteric, axillary and inguinal lymph nodes, spleen and other abdominal organs once weekly. Dispnea and frequent or abnormal respiration were the first signs of intrathoracic (thymic or pulmonary) neoplasia, combined with loss of weight and general deterioration. When the presence of tumors was determined, the animals were sacrificed and dissected for examination and diagnosis. Animals dying spontaneously were autopsied when the death was recent (1–4 hr) or discarded from the experiments.

Light microscopy

In all the cases reported, the diagnosis was confirmed or done by histopathological analysis. The tissues were fixed in Bouin's fluid, embedded in paraffin and stained with hematoxylineosin.

RESULTS

Onset of leukemia (lymphosarcoma or thymoma, LS) in normal or orchidectomized C57BL/6 male mice

As shown in Table 1, the intraperitoneal (i.p.) inoculation of 0.2 ml viral preparation (V-136) into normal or orchidectomized C57BL/6 mice resulted in the early development of LS in almost all the normal or orchidectomized mice. No significant difference was evident in incidence and average latent period (Table 1).

Onset of tumors in normal or virilized CR female mice

Inoculation of the leukemia virus preparation into normal cyclic or masculinized, acyclic Charles River mice resulted in development of tumors which differed completely, both histopathologically and topographically, within the two groups of normal or virilized females. The normal cyclic females developed mostly mammary tumors (20 out of 40 = 50% of the mice, with an average latent period of 18 months) 3 lung and 1 ovary adenocarcinoma and 10 systemic neoplastic diseases (4 LS, 1 myeloid leukemia, 3 reticulum cells sarcomas type B and 2 type A, Table 2). Histopathologically and topographically the tumors were completely different. The tumor polymorphism was much more pronounced in the non-cyclic, virilized females inoculated with V-136. Ten animals developed ovary adenocarcinoma with

Table 1. Leukaemogenesis in normal or orchidectomized C57BL/6 male mice inoculated with leukemia virus†*

Normal	ALP	Orchidectomized	ALP
18/20(90%)	7 ± 3	14/15(93%)	7 ± 3

ALP: average latent period (months ± S.D.).
*Gonadectomy was performed at a prepubertal age (one-month-old mice).
†Viral preparation (V-136) (0.2 ml) was inoculated i.p. at 40 days of age.

precocious lung metastasis (10 out of 47 = 21% of the mice, with an average latent period of 20 months; Table 2), a few mammary carcinomas (6 out of 47 = 13%), carcinomas of other organs (lungs, uterus, bladder) and two chondroblastomas (Table 2). Nineteen mice (19 out of 47 = 40%) developed systemic neoplasia (7 LS, 11 reticulum cell neoplasia type B and 1 type A) and the average latent period was different when compared to that of females of the normal cyclic group (LS, 20 instead of 14 months; reticulum cell neoplasis type B, 20 instead of 26 months). In addition to the different ALP, tumor topography, histopathology and the greater polymorphism, several virilized females developed simultaneously multiple types of tumors, generally carcinomas *and* reticulosarcomas. Masculinized females which were not inoculated with the virus developed only a few reticulum cell neoplasms and carcinomas but no ovary tumors (Table 2). Only 1% of normal, untreated, virgin CR female mice developed spontaneous mammary adenocarcinomas under our maintenance conditions.

DISCUSSION

The findings emerging from the experiments reported above show that inoculation of a selected, highly leukemogenic viral preparation originally obtained from thymomas of C57BL/6 mice resulted, as expected, in early development of disseminated lymphosarcomas (LS) when the virus was injected into the same strain of mice (C57BL/6) from which the virus originated. On the contrary, the same virus

*Table 2. Onset of tumors in masculinized female Charles River mice and in masculinized or normal mice inoculated with leukemia virus**

	Normal virus-injected (%) $n = 40$	ALP	Masculinized virus-injected (%) $n = 47$	ALP	Masculinized (%) $n = 25$
Adenocarcinoma	16† (40)	18 ± 3	3† (6.5)	16 ± 4	1(4) ALP 25
Leyomyosarcoma	3† (7.5)		3† (6.5)		
Squamous cell carcinoma	1† (2.5)				
Adenocarcinoma (Fallopian tubae)	1 (2.5)	20			
Adenocarcinoma (ovaries)	1 (2.5)	22	10 (21)	20 ± 3	
Adenocarcinoma (lungs)	3 (7.5)	24 ± 6	2 (4.2)	18–10	
Adenocarcinoma (uterus)			1 (2.1)	19	2(8) ALP 19–20
Carcinoma (bladder)			1 (2.1)	14	
Fibrosarcoma (subcutaneous)			2 (4.2)	17–16	
Phaeochromocytoma			1 (2.1)	17	
Chondroblastoma			2 (4.2)	16–19	
Myeloid leukemia	1 (2.5)	20			
Lymphosarcoma	4 (10)	14 ± 11	7 (15)	20 ± 7	
Reticulum cell sarcoma type B	3 (7.5)	26 ± 4	11 (23)	20 ± 4	1(4) ALP 23
Reticulum cell sarcoma type A	2 (5)	18–21	1 (2.1)	21	2(8) ALP 19–22
Negative, doubtful or no diagnosis	8		9		

ALP: average latent period (months ± S.D.).
*Leukemia virus (0.2 ml) was inoculated i.p. at one month of age.
†ALP has been calculated together.

preparation promoted the late onset of histomorphologically and topographically different tumors when injected into a strain with a genetically different background (e.g. immunological capacity and neuroendocrine regulation). In addition, the ALP, frequency, histopathology and topography of the tumors were completely different when the recipients of the virus were normal, cyclic or hormonally deranged, virilized, acyclic female CR mice. Female, normal outbred mice developed a high percentage of carcinomas, mainly located in the mammary region (25 tumors = 62%, Table 2), and a low number of LS or other systemic neoplasms (10 tumors = 25%, Table 2). Acyclic, virilized females, on the contrary, developed a relatively high number of carcinomas (20 tumors = 42%), mainly located in the ovaries and with precocious lung metastasis (10 tumors = 21%), and also a relatively high number of systemic neoplasms, mainly reticulum cell neoplasms type B (11) and LS (7) (total number 19 tumors = 40%). Besides the different organ and tissue topography, the acyclic female mice showed a higher polymorphism of tumor histogenesis when injected with the preparation of leukemogenic virus (Table 2). In all, the topography, distribution, histogenesis and timing of oncogenesis were totally different in the two groups. For example, acyclic females developed 11 RCN type B and 7 LS with an ALP of 20 months, while the normal females developed only a few (4) early LS and a few (3) late RCN type B.

The incidence of spontaneous mammary carcinoma was very low in our normal, untreated, virgin female, outbred CR mice (1% on 200 mice). The masculinized females not injected with the virus developed only a few tumors (Table 2). Thus, while the leukemogenic virus *per se* was a main determinant for the induction of the tumors, the permanent endocrine derangement of cyclicity affected remarkably the incidence, localization and histopathology of the tumors in the virus-injected virilized females.

These findings once more point out the distinguished, specific and prominent role of congenital (SJL/J, AKR mice) or induced endocrine derangements in the genesis of systemic neoplastic processes. The claim that a genetic immune resistance to the selected leukemogenic virus is affected by operational procedures like masculinization cannot be sustained because no impairment of the immune capacity could be found in the adult virilized mice (primary and secondary response to two different antigens and the ability to reject allogeneic skin grafts; W. Pierpaoli, unpublished results).

The observation that orchidectomy does not delay or enhance the onset of LS in C57BL/6 mice raises again the central issue of our theme. As already reiterated [3], it seems that quantitatively deranged hormonal levels do not promote leukaemogenesis, but rather a permanent disturbance of cyclic functions. The 'natural resistance' opposed by mice with a different genetical background to the same carcinogenic stimulus or agent (X-rays, chemical carcinogens, viruses, etc.) seems therefore to be primarily based on the strain-specific (mice) or individual (other species or man) genetic resistance opposed by the exquisitely unique and individual neuroendocrine control mechanisms, which are genetically determined and which also control the immune response [4].

Acknowledgements—We thank Miss Regula Kellerhals for her expert technical help during all the years required for completion of the work.

REFERENCES

1. PIERPAOLI W, HARAN-GHERA N, BIANCHI E, MUELLER J, MESHORER A, BREE M. Endocrine disorders as a contributory factor to neoplasia in SJL/J mice. *J Natl Cancer Inst* 1974, 53, 731–743.
2. PIERPAOLI W, HARAN-GHERA N. Prevention of induced leukaemia in mice by immunological inhibition of adenohypophysis. *Nature (Lond)* 1975, **254**, 334–335.
3. PIERPAOLI W, HARAN-GHERA N, KOPP HG. Role of host endocrine status in murine leukaemogenesis. *Br J Cancer* 1977, **35**, 621–629.
4. PIERPAOLI W, KOPP HG, MUELLER J, KELLER M. Interdependence between neuroendocrine programming and the generation of immune recognition in ontogeny. *Cell Immunology* 1977, **29**, 16–27.
5. HELLMAN A, FOWLER AK. Hormone-activated expression of C-type RNA tumor virus genome. *Nature (New Biol)* 1971, **233**, 142–144.
6. FOWLER AK, REED CO, TODARD GJ, HELLMAN A. Activation of C-type RNA virus markers in mouse uterine tissue. *Proc Natl Acad Sci USA* 1972, **69**, 2254–2257.

7. BARRACLOUGH CA, LEATHEM JH. Infertility induced in mice by a single injection of testosterone propionate. *Proc Soc Exp Biol Med* 1954, **85**, 673–674.
8. BARRACLOUGH CA. Production of anovulatory sterile rats by single injections of testosterone propionate. *Endocrinology* 1961, **68**, 62–67.
9. BARRACLOUGH CA, GORSKI RA. Evidence that the hypothalamus is responsible for androgen-induced sterility in the female rat. *Endocrinology* 1961, **68**, 68–79.
10. HARAN-GHERA N. Leukemogenic activity of centrifugates from irradiated mouse thymus and bone marrow. *Int J Cancer* 1966, **1**, 81–87.