Parental-F_1 Hybrid Bone Marrow Chimeras: High Incidence of Donor-Type Lymphomas[1] (34854)

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Considerations of the relationships between carcinogenesis and immunobiology have received increasing attention and emphasis in recent years (1–5). Apart from the evidence that immunosuppression *per se* may permit the growth and development of autochthonous tumor cells bearing neoantigens (these cells would be held in check by normal immunological responses), it is also possible that lymphoproliferative diseases, such as malignant lymphoma, may be the consequence of chronic, persistent histoincompatibility reactions involving immunocompetent cells in a host animal (cf. 4).

It is known from our previous studies on radiation chimeras (6–8) that syngeneic radiation chimeras (lethally X-irradiated LAF_1 mice receiving a transplant of normal LAF_1 bone marrow or spleen cells) show a vanishingly low lymphoma incidence (approaching zero) for their total life span; whereas by contrast, allogeneic bone marrow chimeras (C_3H strain marrow cells tranfused into lethally irradiated LAF_1 mice) exhibited a 9% incidence of malignant lymphomas, and an additional 9% of the mice showed atypical proliferation in the spleen (7).

Experimentally, the most explicit data relating lymphomagenesis to immunological abnormalities come from the studies of Schwartz and Beldotti (9), and of Walford (10). In the former case, nonirradiated 6-week-old, (C57Bl/6 × DBA/2) F_1 hybrid male mice received four weekly injections of 80×10^6 parental strain (C57Bl/6) spleen cells, resulting in a high percentage of mortality due to the severe graft-versus-host reaction. However, among the survivors, over 40% exhibited nonthymic lymphomas at 8 months or 1 year of age. The tumor-bearing mice were found to be chimeras, but the lymphomas were transplantable only to (C57Bl/6 × DBA/2) F_1 mice, suggesting that the neoplasms were of host rather than donor cell origin. In Walford's experiments (10), definite augmentation of lymphoma incidence was associated with the injection of newborn C_3H mice with spleen cells (2.5×10^6) from an adult congenic C_3HK "partner" strain, differing only at the weak H-1 histocompatibility locus.

In view of these considerations, we have in the present studies compared the occurrence of lymphomas in parental strain-F_1 hybrid bone marrow and lymphoid chimeras with that in appropriate controls. These experiments were designed in the effort to resolve the following questions: (1) are overt graft-versus-host reactions necessary preconditions for increased lymphomagenesis in parental-F_1 hybrid chimeras; (2) are the lymphomas produced of donor or of host origin.

Methods. Adult (10 to 12-week-old) male (C57L X A/He) F_1 hybrid mice (H2a, b), (so-called LAF_1), received a single whole-body sublethal exposure of 500 R of 250 kVP X-rays, followed in a few hours by a single intravenous injection of 15×10^6 parental strain lymph node cells or an ip injection of 30×10^6 cells from adult A/He donors

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TABLE I. Lymphomas in X-Irradiated F_1 Hybrid Mice Surviving Graft-Versus-Host Disease.[a]

Cells injected into irradiated (500 R) LAF_1 mice	Time of sacrifice (mo.)	Lymphoma no./total (%)	Lymphoid hyperplasia no./total
Fresh A-lymph node	25	0/12 (0)	5/12
Incubated A-lymph node	25	10/17 (59)	1/17
A-lymph node incubated with prednisone	25	4/9 (44)	3/9
None (irradiation controls)	20–24	17/132 (13)	—

[a] 15×10^6 cells injected iv, or 30×10^6 injected ip. The cells were incubated at 37° for 2 hr in buffered saline, with or without added prednisone (100 μg/ml) prior to injection.

(H2a). The cell suspensions were prepared in phosphate buffer–saline. In some cases the lymph node cells were incubated *in vitro* at 37° for 2 hr, with or without added prednisone, prior to their injection in order to reduce the severity of graft-vs-host reactions.

One group of 500 R irradiated LAF_1 mice received a single iv injection of 4×10^6 fresh bone marrow cells from normal, adult A-strain donors. An additional group of A-strain donors were first subjected to 900 R of X-radiation, with one limb of each mouse enclosed in an external lead shield during the irradiation. Such marrow-shielding is known to permit the migration of viable bone marrow stem cells into the circulation and into the peripheral tissues and elicits the subsequent functional cellular repopulation of the radiation-depleted hematopoietic and lymphoid systems (cf. 11). Seven days after this procedure, the spleen and liver from these donors were extirpated and injected intraperitoneally as separate cell suspensions in TC-199 medium into LAF_1 mice which had just received 500 R of X-radiation. Each recipient LAF_1 mouse either received the equivalent of 50 mg wet weight of A-liver cells from these donors, or the equivalent of one A-spleen. A control group of 16 sublethally X-irradiated LAF_1 mice received liver cells from whole-body lethally X-irradiated (900 R) A-strain donors.

The recipient mice received no further treatment, and were maintained until sacrifice at 24 or 25 months after injection. The mice were housed five per cage with access to tap water and Purina Laboratory Chow *ad libitum*.

Results. A definite increase in lymphoma incidence occurred in the recipient mice injected with incubated parental strain lymph node cells, as compared with radiation controls (Table I): a total of 14 among the 26 animals (combined) which received the incubated lymph node cells exhibited lymphomas (54%), compared to a 13% incidence among radiation controls ($p < .01$). Histologically, the lymphomas did not differ significantly from those previously observed in either irradiated or nonirradiated LAF_1 mice. The majority were lymphosarcomas composed of sheets of medium and large lymphocytes, usually extrathymic in origin and involving the spleen, various lymph nodes, kidney and liver. The most common variant consisted of more undifferentiated and pleomorphic cells shading into a reticulum-cell pattern. Tumors resembling follicular lymphoma, Hodgkin's disease, and plasmacytoma were observed once or twice each. The lesions listed as "marked lymphoid hyperplasia, possibly neoplastic," nearly all involved only the spleen. Despite marked splenomegaly and frequently massive enlargement of lymphoid follicles in these particular cases, neither the cellular morphology, follicular growth pattern, or general splenic architecture was sufficiently abnormal to permit a definite diagnosis of "lymphoma." The distinct possibility exists, however, that these do represent early neoplastic processes. The spectrum of lymphoid proliferation, both in the spleen and also in such organs as kidney, is a continuous one, not infrequently requiring somewhat arbitrary decisions as to what is a true neoplasm and what is not. Lymphoid proliferation was

TABLE II. Lymphomas in X-Irradiated F_1 Hybrid Mice (500 R) Receiving Parental Strain Marrow Cells.

Irradiated (500 R) LAF_1 mice	Time of sacrifice (mo.)	Lymphoma (no./total)	Lymphoma (%)
4×10^6 A-marrow	23½	13/17	76
Liver from 900 R limb-shielded A-mice[a]	23	8/10	80
Spleen from 900 R limb-shielded A-mice[a]	23	6/8	75
Liver from 900 R whole-body irradiated A-mice[a]	25	0/16	0
None (irradiation controls)	20–24	17/132	13

[a] Tissue removed 7 days postirradiation.

considered to be definitely neoplastic only if there were aggregates of immature lymphoid cells replacing areas of parenchyma in various organs, such as the liver and kidney; or, in those cases in which only the spleen was involved, the splenic architecture was largely obliterated by sheets of such immature cells.

Of perhaps greater interest in the present context are the results of a set of our experiments in which parental strain *bone marrow cells,* instead of lymph node cells, were transplanted. Marrow cells from adult A/He strain mice were injected intravenously into sublethally X-irradiated LAF_1 hybrid mice in numbers (4×10^6 cells) that did not elicit overt graft-versus-host mortality or morbidity. When these mice were sacrificed 23 months later, 76% of the group of 17 exhibited malignant lymphomas (Table II). Similarly, when liver or spleen cells from 900 R X-irradiated A-strain mice (with one limb lead-shielded during irradiation) were injected into sublethally X-irradiated (500 R) LAF_1 mice, about 80% of a group of 18 showed lymphomas 23 months later; the donor liver and spleens were extirpated 7 days after the irradiation and shielding. As a control, liver cells (equivalent to 50 mg liver) from 900 R whole-body irradiated A-strain mice were injected into LAF_1 mice; under these conditions, no lymphomas were seen at 25 months among 16 mice so treated. Thus, the transplantation of parental strain bone marrow cells directly, or of cells in the spleen and liver recently migrated from the shielded femoral bone marrow (11), leads to a significant augmentation of lymphoma frequence ($p<.01$), since the incidence of lymphomas in 132 control mice of this strain was only 13%.

In a limited number of cases, the lymphomas arising in the marrow cell-injected mice were transplanted intraperitoneally as a cell suspension into nonirradiated adult A-strain and LAF_1 hybrid recipient mice, respectively, in order to determine whether these cells would exhibit malignant growth and show specificity as regards their donor or host origin. Among lymphomas from six mice thus examined, all grew and infiltrated in the A-strain recipients sacrificed 9 weeks after injection of the lymphoma cells; in all these cases the spleens, as well as the mesenteric lymph nodes were greatly enlarged and lymphomatous; thymic and liver involvement was grossly evident in two cases. At the same time, no evidence for lymphoma takes was seen in the LAF_1 recipients. The lymphomas from three of these A-strain recipient mice were each retransplanted in turn into five nonirradiated adult LAF_1 mice and five A-strain mice. In all cases, the tumors again grew in the A-strain recipients but not in the LAF_1 mice. We conclude from these observations that many, if not all, of the lymphomas arising in parental strain F_1 hybrid bone marrow chimeras are of donor, *i.e.,* parental strain, origin.

Discussion. The present results stand in contrast to those of Schwartz and Beldotti (9), who reported that the lymphomas in mice surviving graft-versus-host disease were of host origin. As a matter of fact, the experimental conditions of Schwartz and Beldotti are quite different from ours, and it is likely that a critical consideration of these differ-

ences, as proposed here, permit further insights into questions of lymphomagenesis—particularly as they related to the role of immunosuppression and to histoincompatibility reactions involving immunocompetent cells. In view of the extensive lymphoid tissue atrophy and aplasia observed in nonirradiated adult F_1 hybrid mice after injection of parental strain immunocompetent lymphoid cells (12) as well as the resultant immunologic functional loss (13), it is evident that mice such as those studied by Schwartz and Beldotti are severely compromised immunologically. The lymphomas observed by these workers may, therefore, represent the end result of the immunosuppressive effects of severe graft-versus-host reactions, analogous to the known tumor-inducing effects of chemical immunosuppressive compounds and of heterologous antilymphocyte sera (14).

However, the results of Walford, and also the present data suggest that overt graft-versus-host reactions are not a *sine qua non* for lymphomagensis in these chimeras. The key point here may in fact be not so much a matter of immunosuppression by graft-versus-host reactions, but that of persistent histoincompatibility associated with the parental strain-F_1 hybrid reticuloendothelial cell chimerism. This condition would, according to Tyler (4), lead to isoantigenic stimulation of the parental strain lymphocytic cells, resulting in their blastogenesis, new DNA synthesis, and proliferation. We suggest that such repeated and prolonged proliferative activity would increase the probability of the occurrence of a carcinogenic alteration in the stimulated parental strain lymphocytic cells. The occurrence of such carcinogenic changes would depend in part on the genotype of the cells. This follows from recent observations by Huebner *et al.* (15) which reveal marked differences among various inbred mouse strains in their susceptibility to be activated or "turned on" with respect to vertically transmitted oncogenic virus. These workers (16) also postulate, from cell culture evidence and sero-epidemiological studies, that cells of vertebrates contain information in the genome (the virogene), for producing C-type RNA viruses. They propose that the virogene, including that portion (the oncogene) responsible for transforming a normal cell into a tumor cell, is transmitted vertically cell-to-cell progeny, and parent to offspring, in a covert form. According to this hypothesis, chemical carcinogens (17), radiation, and the normal aging process all favor the partial or complete activation of these genes.

In the present context, we propose that chronic histoincompatiblity reactions, involving as they do, new DNA synthesis, constitute an additional mode of activation of endogenous oncogenic viruses. Consistent with this hypothesis is the well-documented observation [cf. Dulbecco, (18)] that cellular DNA synthesis is required for the transformation of cells by oncogenic virus—such a requirement for DNA synthesis being presumably related to the activation of a portion of the genome.

Although the parental strain-F_1 hybrid bone marrow is obviously a laboratory artifact, it may, nevertheless, provide a model system for considering the consequences of "natural" chimera-like states that may give rise to chronic cellular histoincompatibility. We suggest that NZB mice may be such natural chimeras, *i.e.*, that cells bearing slight histocompatibility differences exist side by side within the NZB host organism. The abnormal lymphocytic proliferations, autoimmune phenomena, and lymphoma production may then be consequences of the persistent chimeric-like state. Peripheral blood lymphocytes from such animals would also be expected—on this basis—to yield anomalously high values for incorporation of ^{3}H-thymidine into DNA, on *in vitro* culture.

Our general thesis on carcinogenesis (19) is predicated on the concept of its multievent nature—whether elicited by ionizing radiations, chemicals, or "latent" endogenous oncogenic viruses. We consider that the carcinogenic initiating events induce the formation of relatively small numbers of specifically "transformed" or altered cells. Once formed, these transformed cells can remain dormant for long periods; or they can be triggered into proliferation by a variety of influences, both

specific and nonspecific, physiologic and pathologic, giving rise to clones of such cells. Our present studies are directed toward elucidating the factors that may influence the expression of lymphomagenesis in parental strain-F_1 hybrid chimeras; for example, the role of mouse genotype and the effect of fetal parental strain hematopoietic cells.

Summary. Adult LAF_1 hybrid mice received a whole-body exposure to 500 R of X-rays, followed by a single intravenous injection of 4×10^6 normal, parental strain (A/He) bone marrow cells, which produced no overt graft-versus-host morbidity or mortality. On sacrifice, 2 years later, a striking increase in the incidence of malignant lymphoma (76%) over that of appropriate controls, was observed. Transplantation tests with cell suspensions prepared from these lymphomas revealed that they are of donor strain—rather than host—origin. The key point for lymphomagenesis here may be the persistent, tolerated histoincompatibility associated with the parental strain-F_1 hybrid bone marrow cell chimerism, rather than immunosuppression by graft-versus-host reaction.

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