Requirement of Live Friend Leukemia Virus for Enhanced Expression of *Hybrid Histocompatibility* Genes[1] (35988)

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We had previously reported that the relatively weak antiparent reactions of F_1 mice against transplants of bone marrow and spleen cells from parental DBA/2 donors are considerably increased when the cells come from mice or tissue culture lines infected with Friend leukemia virus (FLV) (1, 2). These enhanced reactions, reflected by the decrease in growth potential of the infected DBA/2 cells in (BALB/c × DBA/2)F_1 hybrids, were attributed to the influence of the virus on one or more *Hybrid histocompatibility* (*Hh*) genes controlling "hybrid resistance." The products of these genes are regarded as parental and tissue-specific transplantation antigens (3–5).

Although there was no doubt that the virus played a role in enhancing the expression of the *Hh* gene(s) of the virus-infected cells, it was not known whether this effect was independent of virus replication, as is the cell fusion-inducing function of the inactivated Sendai virus (6, 7). A suggestion that virus proliferation was a prerequisite to the enhanced expression of *Hh* gene(s) came from the negative results obtained from experiments in which a virus preparation, inactivated by having been thawed in transit from one laboratory to another, had been used. Experiments described below were, therefore, set up to compare the effects of infectious, as well as deliberately inactivated, virus on the expression of *Hh* gene(s) of DBA/2 cells.

Experimental Design and Methods. DBA/2J mice from Jackson Memorial Laboratory, Bar Harbor, Maine were used. Some were injected intraperitoneally with 0.3 ml of infectious FLV filtrates ($LD_{50} = 10^{-2.5}$) prepared from leukemic spleens, as previously described (8), and others with a sample of the same virus stock inactivated by treatment with *beta*-propiolactone (BPL) in a final concentration of 0.04%, as used by Neff and Enders (7). Bone marrow from the inoculated mice, as well as from control untreated animals, was harvested at short intervals; and washed cells were transplanted into syngeneic and F_1 hybrid mice of two strains [(BALB/cCr♀ × DBA/2Cr♂)F_1 and (C57BL/6Cr♀ × DBA/2Cr♂)F] hereafter designated as CDF_1 and BDF_1, respectively. In one set of experiments, the recipients were exposed to 750–800 R of whole-body X-rays a few hours before transplantation to create a hemopoietic graft bed, without abrogating hybrid resistance (5, 9). The grafted cells (3×10^5/mouse) were allowed to multiply for 5 days. At this time, the size of the donor-derived hemopoietic population was estimated by injecting each mouse with 10^{-7} *M* of nonradioactive 5-fluoro-2′-deoxyuridine and 1 hr later with 0.2 μCi of radioactive 5-iodo-2′-deoxyuridine-^{125}I (IUdR). The former drug was given to favor incorporation of IUdR into the DNA of dividing cells. Details of these procedures were previously described (10). The IUdR retained in individual recipient spleens was measured by crystal scintillation counting and expressed as percentage of injected radioactivity. In a

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TABLE I. Expression of *Ih* Genes in Mouse Bone Marrow Cells upon *in Vivo* Infection with FLV, as detected by Transplantation into Syngeneic and F_1 Hybrid Recipients.[a]

Treatment of bone marrow donors	Interval between infection and transplantation (hr)	No. of transplanted cells ($\times 10^6$)	Mean uptake of ^{125}IUdR (% ± SE) in recipient spleens of the following mouse strains[b]		
			DBA/2	CDF_1	BDF_1
			X-irradiated recipients		
None	—	3	0.42 ± 0.06 (6)[c]	0.44 ± 0.05 (4)	0.25 ± 0.04 (4)
FLV	2–3	3	0.32 ± 0.04 (13)	0.32 ± 0.09 (8)	0.23 ± 0.02 (13)
FLV	4–24	3	0.44 ± 0.06 (26)	0.22 ± 0.04 (20)[d]	0.11 ± 0.02 (26)[d]
FLV	24	3	0.57 ± 0.08 (9)	0.18 ± 0.06 (13)[d]	0.09 ± 0.04 (13)[d]
BPL-FLV	24	3	0.46 ± 0.08 (3)	0.35 ± 0.07 (7)	0.20 ± 0.09 (6)
			Nonirradiated recipients		
FLV	24	10	1.13 ± 0.08 (10)	0.53 ± 0.15 (5)	No uptake (5)
FLV	24	50	2.05 ± 0.18 (6)	1.00 ± 0.13 (7)	No uptake (7)
BPL-FLV	24	50	No uptake (4)	No uptake (8)	No uptake (8)

[a] Abbreviations used: *Ih*, hybrid histocompatability; FLV, Friend leukemia virus; BPL-FLV, Friend leukemia virus inactivated by *beta*-propiolactone.

[b] Irradiated recipients were injected with radioactive IUdR 5 days after transplantation of marrow cells, and nonirradiated recipients, 7 days. For each experiment, a group of control mice received identical treatment except that the transplanted cells had been killed beforehand by sonication.

[c] Number of mice tested in parentheses. Net geometric means ± standard errors; values of isotope retention in control spleens were subtracted from the values of experimental spleens.

[d] The differences with the means of corresponding DBA/2 recipients were significant ($p < .01$).

second set of experiments, the recipients were not irradiated and the grafted cells ($1–5 \times 10^7$/mouse) were allowed to multiply for 7 days. Negative control animals were grafted with comparable numbers of infected and washed DBA/2 cells but killed by sonication. The values of splenic uptake of IUdR of these controls were subtracted from those of experimental mice, taking into account the variance of mean values in both groups of animals.

Results. Cells from noninfected control mice grew without impairment in the spleens of irradiated DBA/2 and CDF_1 hosts, and slightly less in those of BDF_1 hybrids (Table I, upper section). Marrow cells of FLV-infected donors, harvested 2 and 3 hr after infection, grew at a similar rate. Thereafter, infected hemopoietic cells became histoincompatible to irradiated F_1 hybrids. At 4 hr and at 5, 6, and 24 hr after infection (pooled data in Table I), the donor cells were partially resisted by CDF_1 hybrids and to a greater extent by BDF_1. That this change could be induced only by proliferating virus is shown by the results obtained with the cells from mice which had been inoculated with BPL-inactivated FLV. The growth of bone marrow cells harvested from the donors injected 24 hr earlier with BPL-inactivated FLV was no different from that of cells from untreated donors.

We had previously reported that spleen cells shortly after infection acquire the ability to grow autonomously upon transplantation into *nonirradiated* mice (1). This was taken as evidence of early transformation. In the present study, it was found that bone marrow cells were similarly transformed, as judged by the growth of $1–5 \times 10^7$ infected cells in the absence of a radiation induced graft bed (Table I, lower section). Although noninfected cells (11) and cells harvested 1–2 hr after infection (our unpublished data) failed to grow, bone marrow taken from donors 24 hr after infection promoted the uptake of IUdR in the spleens of nonirradiated DBA/2 recipient mice. This mitotic activity upon transplantation indicated that the cells had acquired the potential for autonomous growth. In the spleens of F_1 hybrid recipients the uptake of IUdR was lower than in those of DBA/2 mice, an expression of hybrid resistance to the transformed cells. In con-

trast, the marrow cells of donor mice injected with BPL-inactivated FLV did not promote splenic uptake of IUdR in nonirradiated recipients.

Discussion. No titrations were done to determine the extent of viral proliferation since we have rarely been able to detect virus as early as 1 day after infection, the time at which the cells were taken from the donors. It would appear, however, that FLV replication is required for the enhanced expression of *Hh* gene(s) in DBA/2 mice since inactivation of the virus abolished this viral effect. It is assumed that BPL does not disrupt the virions in inactivating viruses, since Sendai virus treated with this compound retains its fusion and hemagglutinating functions (7).

It is known that infection with other oncogenic viruses results in uncovering components of the normal cell surface. Mild proteolytic treatment of normal and polyoma-transformed cells exposes antigenic sites which cross react with those of SV40-transformed cell surfaces (12). Both polyoma and SV40 unmask carbohydrate-containing receptor sites for various plant glycoproteins in cells they transform (13–16). Although cells infected by RNA tumor viruses do not necessarily react to these glycoproteins (17), it is very likely that live FLV is capable of uncovering certain specific surface structures such as the products of *Hh* gene(s) and that this event, which occurs within a few hours after infection, may be critical in the evolution of leukemia. It is noteworthy that the FLV effect is detected in target cells of splenic and bone marrow origin, even though the normal hemopoietic progenitor cells in the two tissues differ with respect to potential for hemopoietic proliferation and differentiation (18).

Summary. Bone marrow cells of DBA/2 mice became histoincompatible to irradiated (BALB/c × DBA/2)F_1 and (C57BL/6 × DBA/2)F_1 hybrids a few hours after infection with Friend leukemia virus (FLV). This alteration was attributed to enhanced expression of *Hybrid histocompatibility* (*Hh*) gene(s), *i.e.*, to more effective immunogenicity of the parental-specific alloantigens specified by *Hh* gene(s). The viral effect required live virions since it was abolished by inactivation of FLV with *beta*-propiolactone.

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