Influence of presensitization with allogeneic lymphoma cells on the growth and response to therapy of radiation-induced lymphomas in mice[1]

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Summary. Congenic mice were sensitized with viable *H-2*-incompatible radiation-induced lymphomas (RIL), challenged with syngeneic RIL and treated with bis-chloroethyl-nitrosourea. Either enhancement or inhibition of RIL was found in presensitized mice, depending on the tumor-host system used.

Lymphomas induced by total-body irradiation (radiation-induced lymphomas, RIL) of C57B1 mice, are slightly or not at all immunogenic for the strain of origin[3]. It has been hypothesized that mice are tolerant to tumor-associated transplantation antigens (TATA) specified by radiation leukemia virus (RadLV), as a consequence of neonatal exposure to the virus[4,5]. However, it has been shown that transplantation resistance against RIL can be induced in adult mice by intrathymic inoculation of RadLV[6,7].

Studies conducted in our laboratory with RIL of B10 and B10.129 (5M) origin, showed that transplantation resistance could not be induced by pretreatment of the host with inactivated (i.e. irradiated) syngeneic tumor cells given i.p.[3]. However B10.129 (5M) mice have been sensitized successfully against lymphoma cells incompatible for the *H-1* locus[3]. Host's graft responses were abrogated by treatment with cyclophosphamide prior to tumor transplantation. On the other hand, the therapeutic effectiveness of antineoplastic chemotherapy performed with bis-chloroethyl-nitrosourea (BCNU) given after tumor challenge, was markedly improved by the antilymphoma allograft reaction of the host[3].

In the present study, further investigations were performed to explore the possibility of sensitizing the mice against syngeneic RIL, using viable allogeneic lymphomas of the same origin, incompatible for the *H-2* complex. In this case, the cells used for immunization presumably share the same RadLV-mediated TATA with syngeneic RIL and do not require inactivation in vitro, being rejected by *H-2*-incompatible recipients.

The experiments were conducted with 2-4-month-old mice of B10. 129 (5M), *(H-2^b, H-1^b)* and congenic C57B1/10 ScSn (abbreviated as B10, *H-2^b, H-1^c)* strains, obtained from the Mammalian Genetics and Animal Production Section of the National Cancer Institute (NIH, Bethesda, Maryland, USA). The RIL used in the present studies were L5MF-22 of B10.129 (5M) origin, S-1033 of B10 origin and LAF-17 originated in B10.A mice *(H-2^a, kkkkkdddd)*, congenic with B10 mice, but incompatible for the entire *H-2* complex. These lymphomas were kindly supplied by Dr G. Cudkowicz (Dept. of Pathology, State Univ. of New York at Buffalo, Buffalo, N.Y., USA).

Male and female B10.129 (5M) mice were sensitized with viable allogeneic LAF-17 cells. Control mice were either nonsensitized or immunized with unrelated L1210 leukemia, a chemically-induced tumor of DBA/2 *(H-2^d)* origin[8], which presumably does not express TATA related to RadLV. The animals were challenged with 10^3 or 10^7 cells of syngeneic L5MF-22 lymphoma. Some of the mice, inoculated with 10^7 cells, were subjected to further treatment with graded doses of BCNU (supplied by the Drug Synthesis and Chemistry Branch, NCI, NIH, Bethesda, Maryland, USA). In this case, drug efficiency would have been amplified if the presensitization had generated lymphoma graft resistance in the host[3].

The results of the experiment, illustrated in table 1, showed that the presensitization with LAF-17 cells did not protect mice challenged with syngeneic lymphoma. On the contrary, shorter survival times of female mice challenged with 10^3 cells were found in animals sensitized with allogeneic RIL compared with those of mice nonsensitized or immunized with the unrelated L1210 leukemia (groups 1-3). Similar results were obtained in female mice treated with relatively low doses of BCNU (10.8 or 18.0 mg/kg; groups 7-12). The efficacy of drug treatment was superior in mice nonsensitized or immunized with L1210 cells than in ani-

Table 1. Mortality of B10.129 (5M) mice, nonsensitized or preimmunized with LAF-17 or L1210 lymphomas, challenged with syngeneic L5MF-22 tumor and treated with graded doses of BCNU

Group	Sex of host mice	Challenge No. of L5MF-22 cells (day 0, i.p.)	Treatment BCNU mg/kg (day+5, s.c.)	Mortality data Nonsensitized MST	D/T[d]	P_1[e]	Sensitized with LAF-17[a] MST	D/T	P_1	P_2[f]	Sensitized with L1210[b] MST	D/T	P_1	P_2
1-3	Female	10^3	-	31.0	6/8	-	19.5	8/8	-	A	27.5	6/8	-	C
4-6	Female	10^7	-	17.0	8/8	-	14.0	8/8	-	C	18.0	8/8	-	C
7-9	Female	10^7	10.8	22.0	8/8	A	16.0	8/8	C	A	24.0	8/8	A	C
10-12	Female	10^7	18.0	29.0	8/8	A	20.5	8/8	B	A	33.0	5/8	A	C
13-15	Female	10^7	30.0	>60.0	1/8	A	>60.0	2/8	A	C	>6.0.	1/8	A	C
16-18	Female	10^7	50.0	>60.0	2/8	A	>60.0	3/8	A	C	>60.0	0/8	A	C
19-21	Male	10^3	-	27.0	8/8	-	24.5	8/8	-	C	27.5	8/8	-	C
22-24	Male	10^7	-	19.5	8/8	-	18.0	8/8	-	C	20.0	8/8	-	C
25-27	Male	10^7	10.8	26.0	8/8	A	22.5	8/8	C	C	25.0	8/8	A	C
28-30	Male	10^7	18.0	28.5	8/8	A	31.5	6/8	A	C	28.0	6/8	A	C
31-33	Male	10^7	30.0	>60.0	4/8	A	>60.0	4/8	A	C	36.5	5/8	A	C
34-36	Male	10^7	50.0	>60.0	4/8	A	>60.0	0/8	A	C	>60.0	3/8	A	C

[a]Viable LAF-17 cells were given i.p. as follows: 10^6 on day -26; 10^7 on day -18; 5×10^7 on day -11 before tumor challenge. No deaths occurred in mice not challenged with syngeneic L5MF-22 cells (data not shown). [b]Viable L1210 cells given i.p. as for LAF-17 cells (see footnote a). [c]MST, median survival times. [d]D/T, dead mice over total animals tested. [e]P_1, probability values calculated according to the Mann-Whitney 'U'-test, comparing control mice (nonsensitized or immunized) not treated with BCNU, with hosts subjected to BCNU chemotherapy; A, $p<0.01$; B, $p<0.05$; C, $p>0.05$ (not significant). [f]P_2, probability values calculated as for P_1, comparing nonsensitized mice with animals preimmunized with lymphoma cells, subjected to the same challenge and treatment.

Table 2. Mortality of B10 female mice, nonsensitized or preimmunized with LAF-17 or L1210 lymphomas, challenged with syngeneic S-1033 tumor and treated with graded doses of BCNU

Group	Challenge No. of S-1033 cells (day 0, i.p.)	Treatment BCNU mg/kg (day+5, s.c.)	Mortality data Nonsensitized MST[c]	D/T[d]	P_1[e]	Sensitized with LAF-17[a] MST	D/T	P_1	P_2[f]	Sensitized with L1210[b] MST	D/T	P_1	P_2
1-3	10^3	-	19.0	8/8	-	22.0	6/8	-	B	20.0	7/8	-	C
4-6	10^6	-	14.0	8/8	-	14.0	8/8	-	C	14.0	8/8	-	C
7-9	10^6	10.8	17.5	8/8	A	18.0	8/8	A	C	20.0	8/8	A	C
10-12	10^6	18.0	23.0	8/8	A	20.0	8/8	A	C	25.0	8/8	A	C
13-15	10^6	30.0	26.5	8/8	A	>60.0	4/8	A	B	26.0	6/8	A	C
16-18	10^6	50.0	>60.0	4/8	A	>60.0	1/8	A	B	26.5	6/8	A	C

[a]Viable LAF-17 cells given i.p., uding the same treatment schedule described in footnote a) of table 1. [b]Viable L1210 cells given i.p. as for LAF-17. [c]MST, medium survival times. [d]D/T, dead mice over total animals tested. [e]P_1, probability values calculated according to the Mann-Whitney 'U'-test, comparing control mice (nonsensitized or immunized) not treated with BCNU, with hosts subjected to BCNU chemotherapy; A, $p<0.01$; B, $p<0.05$; C, $p>0.05$ (not significant). [f] P_2, probability values calculated as for P_1, comparing nonsensitized mice with animals preimmunized with lymphoma cells, subjected to the same challenge and treatment.

mals presensitized with LAF-17 lymphoma. The data obtained in male mice showed that enhancement might also have occurred in animals presensitized with allogeneic RIL and challenged with 10^3 lymphoma cells (groups 19-21), or inoculated with 10^7 neoplastic cells and treated with 10.8 mg/kg of BCNU (groups 25-27). However, the influence of presensitization on the host's survival was marginal and the differences in MST's did not reach statistically significant levels. When high doses of BCNU were used, (i.e. 30 and 50 mg/kg) no influence of presensitization was found in either male or female recipients.

Similar studies were carried out in female B10 mice presensitized with LAF-17 or L1210 lymphoma cells and challenged with syngeneic S-1033 cells. The results illustrated in table 2 show that no enhancement produced by presensitization could have been detected. On the contrary, marginal graft resistance seems to have been produced by presensitization with allogeneic RIL. This was detectable in mice challenged with 10^3 cells (groups 1 and 2) or with 10^6 cells and treated with 30 or 50 mg/kg of BCNU (groups 13-18). In conclusion, the results of the studies described in the present report evidenced that presensitization with allogeneic lymphomas can produce enhancement of syngeneic tumors sharing, presumably, the same TATA. On the other hand, protective effects can also be obtained, although they appear to be less pronounced and detectable mainly following appropriate chemotherapy. No data is available at present to explain the mechanism underlying the observed enhancement of lymphoma growth. It can be hypothesized that this effect may be the results of activation of suppressor cells[9-11] induced in mice by presensitization with allogeneic RIL. In any case, the present findings should make us aware of the potential risk of indiscriminate use of sensitization with allogeneic tumor cells for clinical tumor immunotherapy[12,13].

1 This work was supported by Progetto Finalizzato Virus, CNR (Rome, Italy), Contract No. 770025284/1152505.
2 Acknowledgments. I wish to thank Dr J. Mayo and Mr C.R. Reeder of the Mammalian Genetics and Animal Production Section, Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda (Maryland, 20014, USA) for providing the mice used in this study. I also thank Dr H.B. Wood of the Drug Synthesis and Chemistry Branch, DCT, NCI, NIH, for furnishing the BCNU used.
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SEM surface morphology of the contractile cells in the rat seminiferous tubules

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Summary. The SEM observation of the basal surface of the contractile cells in the boundary tissue of the seminiferous tubule of the rat has revealed that the contractile cells are extremely flat, vary in shape from rectangular to hexagonal, and are arranged close to each other, in the fashion of a tiled floor, around the seminiferous epithelium.

Recently, the ultrastructural organization of the boundary tissue surrounding the seminiferous tubules has been the subject of considerable interest for many investigators, in relation to such functions as mechanical supporting of the tubules and the passage governing of metabolic materials between tubule and interstitial matrix. Especially since the first description by Clermont[2] in the rat testis, the presence of a contractile cell which is characteristic of a smooth muscle cell and contains bundles of fine filaments in the boundary tissue, has been confirmed in nearly all specimens studied[3-13], and it is now generally accepted that the contractile cell may play an important role in the discharge of spermatozoa from the seminiferous epithelium and in their transport to the rat testis. However, to our knowlege