Differential Immunogenicity of *H-2* Alloantigens: A Sensitive Immunopharmacologic Test[1] (35815)

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Proliferative responses of mouse lymphoid cells to *H-2* alloantigens complexes are stronger when elicited by K-end than by D-end specificities (1-4). This was demonstrated *in vivo* by graft-versus-host reactions and *in vitro* by mixed lymphocyte interactions. In contrast, survival times of skin grafts are similar in mice incompatible for one or the other end of *H-2* (5, 6). The failure to detect differential immunogenicity of alloantigens with skin grafts might be due to two reasons: (i) the large excess of lymphoid cells available for sensitization to every *H-2* antigen in intact animals; (ii) the fixed size of skin grafts and the limited proliferative activity of cells releasing alloantigens.

In this report, we describe a protocol which overcomes these difficulties and allows detection of differential allograft reactivity *in vivo* to D and K-end *H-2* antigens. The pool of competent host lymphoid cells was reduced by chemical immunodepression; lymphoma cells were used instead of skin tissue for transplantation; and proliferative activity of the graft was controlled by chemotherapy.

Materials and Methods. Male mice of the congenic-resistant lines C57BL/10ScSn or B10 ($H\text{-}2^b$), B10.A ($H\text{-}2^a$), B10.A(2R) ($H\text{-}2^h$), and B10.A(5R) ($H\text{-}2^i$) were used at the age of 2–6 months. Mice of all these lines differ only at a chromosome segment of linkage group IX which includes either the whole *H-2* region (B10 and B10.A), or one of its ends (7). The allele of B10.A(2R) differs from that of B10 at the K end, whereas the allele of B10.A(5R) differs at the D end of *H-2*.

Cells of the transplantable B10 lymphoma S1033 (8) were obtained from grossly enlarged spleens, suspended in medium 199, counted, and injected either by the intravenous (iv) or intraperitoneal (ip) route. Mortality due to generalized lymphoma was verified by autopsy and recorded for 70 days.

Cyclophosphamide (CY) and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) were obtained from the Cancer Chemotherapy National Service Center, National Cancer Institute. The drugs were dissolved in saline immediately before use and injected in a volume of 0.1 ml/10 g of body weight.

The animals were assigned by random numbers to all treatment groups, which included mice of different ages and strains caged together.

Results. Titration experiments with lymphoma S1033 in untreated mice of the strains B10.A and B10.A(2R) were reported elsewhere (9). The dose–response relationships were similar in the 2 strains and complicated by the phenomenon of "dilution escape": tumor takes were more frequent in recipients of 10^3–10^5 cells than in mice injected with 10^6 or 10^7 cells. To avoid this complication, inocula of 10^6 cells were used henceforth. Lymphoma cells were transplanted iv into immunodepressed B10, B10.A, and B10.A(2R)

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mice, and into untreated controls. The drug CY was given ip 24 hr before tumor transplantation at 3 dose levels: 72, 120, and 200 mg/kg. The grafts took in mice of the strain of origin (B10) regardless of immunodepression, and in almost all CY-treated B10.A and B10.A(2R) allogeneic recipients. Each group included 8–9 mice. The median survival times ranged from 11.5 to 15.5 days and were not distinguishable by strains. Most nonimmunodepressed allogeneic recipients rejected the B10 lymphoma cells and survived beyond the 70 days of observation. Under these conditions, no differences were detected in the strength of allograft reactions against cells carrying *H-2* antigens of the K end alone [B10.A(2R) hosts] or of the entire *H-2* locus (B10.A hosts). It was concluded that the tumor challenge of mice immunodepressed with graded doses of CY was not sensitive enough to reveal additive immunogenicity of *H-2* antigens.

If CY-treated mice had residual allograft immunity, inadequate to control allogeneic tumor growth by itself, it should be feasible to improve the effectiveness of this immunity by combined antitumor chemotherapy. Additive or synergistic effects of the antitumor drug BCNU and allograft reactions to weak *H-1* antigens of lymphoma S1033 were described in a preceding study (10). It is the subthreshold level of the residual reactivity of CY-treated mice which would eventually permit detection of quantitative differences in immunogenicity of *H-2* antigens.

Mice of the same 3 strains used in the preceding experiment were immunodepressed with CY (200 mg/kg, ip) and grafted iv 1 day later with 10^6 lymphoma cells. Each group included 8 mice. The animals were given a single ip injection of BCNU on the fifth day after transplantation. The doses of BCNU were reduced by a factor of 0.6 starting from 50 mg/kg (Fig. 1). The dose–response curve for BCNU treatment in syngeneic mice was not affected by pretreatment with CY (data not shown in Fig. 1), thus ensuring that CY did not interfere with the antitumor effect of BCNU. All syngeneic and allogeneic animals died with generalized lymphomas except those injected with the

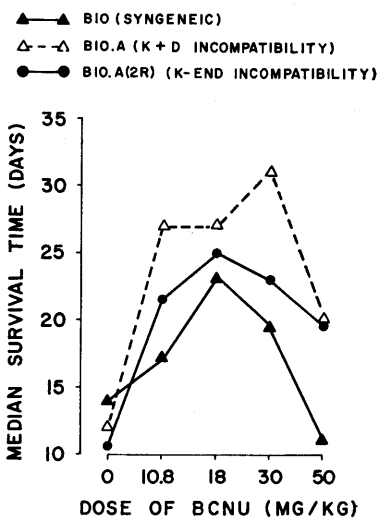

FIG. 1. Median survival time of mice grafted iv with 10^6 S1033 lymphoma cells of B10 origin: Recipients were immunodepressed with a single ip injection of CY (200 mg/kg) 1 day before transplantation, and treated with a single ip injection of BCNU on day. 5.

toxic dose of 50 mg/kg of BCNU, which died before tumor appearance. The B10 and the allogeneic recipients of lymphoma cells differed, however, with respect to the duration of survival. The median survival times were longer in the 6 groups of allogeneic mice given nontoxic doses of BCNU than in similarly treated syngeneic controls (Fig. 1). Moreover, B10.A mice differing from B10 at the entire *H-2* locus, lived longer than B10.A(2R) mice differing from B10 at the K end only. The effect of chemotherapy on the rate of tumor growth was related to the genetic disparity between host and tumor.

As this assay for *H-2* immunogenicity was sensitive enough to distinguish between the entire $H\text{-}2^b$ complex and its K end, a second experiment was done using a similar protocol. Recipient mice (9 animals/group) belonged to the strains B10, B10.A(2R), and B10.A(5R). The *H-2* incompatibility of the latter 2 strains with the B10 lymphoma was restricted to K-end and D-end alloantigens, respectively. The animals were immunodepressed with 3 doses of CY, *i.e.*, 71, 120, and 200 mg/kg ip. Lymphoma cells were grafted ip instead of iv, and BCNU was injected subcutaneously instead of ip to reduce acute

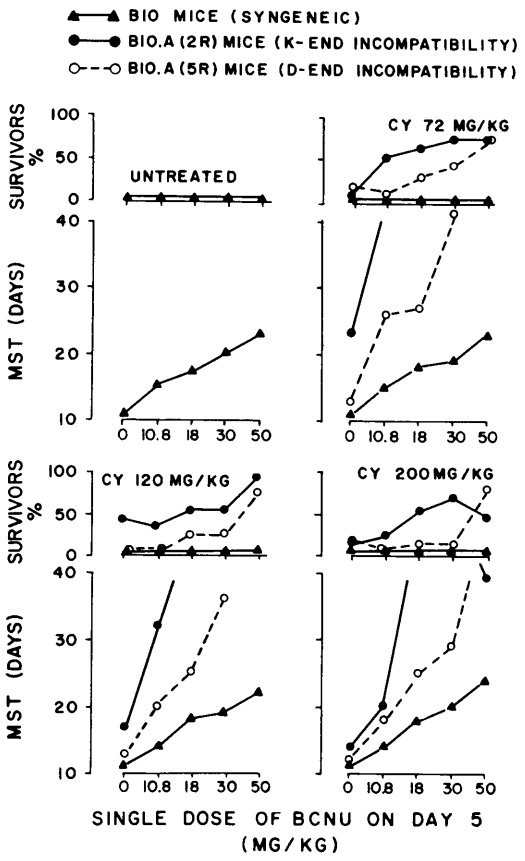

FIG. 2. Percentage of long-term survivors and median survival time (MST) of mice grafted ip with 10^6 S1033 lymphoma cells, Recipients were immunodepressed, as indicated with a single ip injection of CY 1 day before transplantation, and treated with a single subcutaneous injection of BCNU on day 5.

toxicity. The results are shown in Fig. 2. The mortality was 100% in syngeneic B10 mice regardless of chemotherapy. However, there was a prolongation of median survival time directly related with the dose of BCNU. The antitumor activity of BCNU was not influenced by any of the pretreatments with CY, as judged from the dose–response curves of Fig. 2. Mortality was greatly reduced by chemotherapy in the allogeneic immunodepressed mice, as indicated by the percentage of long-term survivors. In most treatment groups, more K-end incompatible B10.A(2R) than D-end incompatible B10.A(5R) mice survived tumor challenge. Such a relationship was also reflected by consistent and substantial prolongation of the median survival times, which were longer in B10.A(2R) than in B10.A(5R) mice. This occurred in animals immunodepressed by each of the 3 doses of CY and subsequently treated with 4 different doses of BCNU. The allograft reactions induced by K-end alloantigens [B10 lymphoma in B10.A(2R) mice] were unequivocally stronger than those induced by D-end antigens [same lymphoma in B10.A(5R) mice].

Discussion. The greater immunogenicity of *H-2* alloantigens associated with the K end has now been observed in three systems: lymphoma cells used in this study to challenge immunodepressed mice; normal lymphoid cells used in one-way mixed lymphocyte interactions (3); and normal target cells of graft-versus-host reactions (1, 2, 4). All three systems tested allograft reactivity. The similarity of results obtained with normal and neoplastic cells make it unlikely that the differential immunogenicity described here merely resulted from altered expression of *H-2* antigens in the serially transplanted lymphoma. Instead, it is conceivable that mice sharing the C57BL/10 background are better "genetic responders" to K-end than to D-end alloantigens of the $H\text{-}2^b$ allele. Indeed, C57BL-derived mice are "genetic nonresponders" for humoral antibodies to the D-end specificity H-2.2 (11). This could imply that differential immunogenicity is not an inherent property of the alloantigens under test, but rather the expression of cellular competence for recognition of, or reactivity to given antigens. Genetic control of transplantation immunity has recently been described for reactions to the murine male antigen H-Y of skin tissue (12-14) and to *H-2* antigens of bone marrow cells (15, 16).

It should be noted that the immunopharmacologic approach was essential for detecting the additive immunogenicity of *H-2* alloantigens which eluded other test systems *in vivo* (1, 2, 5, 6). Although it requires proper administration of cytotoxic drugs, this assay proved to be manageable and a particularly sensitive tool for immunogenetic analysis.

Summary. Allograft reactions directed

against antigens of the entire $H\text{-}2^b$ allele or of its K and D ends were compared in 3 congenic-resistant strains of mice sharing the C57BL/10 background. Lymphoma cells of B10 origin were transplanted into partially immunodepressed mice; residual host antigraft immunity was potentiated by a single administration of the antitumor agent BCNU. The immunogenicity of $H\text{-}2^b$ components was distinct and additive, with strength of elicited allograft reactions decreasing in the following order: D + K> K>D.

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1. Eichwald, E. J., Hart, E. A., and Eichwald, B., Folia Biol. (Prague) **15**, 254 (1969).

2. Lengerová, A., and Matousek, V., Folia Biol. (Prague) **15**, 263 (1969).

3. Rychlíchová, M., Démant, P., and Iványi, P., Folia Biol. (Prague) **16**, 218 (1970).

4. Démant, O., Folia Biol. (Prague) **16**, 273 (1970).

5. Klein, J., Folia Biol. (Prague) **12**, 168 (1966).

6. Eichwald, E. J., Wright, H., Eichwald, B., and Rud, B., Transplantation **6**, 797 (1968).

7. Snell, G. D. and Stimpfling, J. H., *in* "The Biology of the Laboratory Mouse" (E. L. Green, ed.), p. 457. McGraw-Hill, New York (1966).

8. Snell, G. D., and Bunker, H. P., Transplantation **3**, 235 (1965).

9. Bonmassar, E., Goldin, A., and Cudkowicz, G., Transplantation, in press.

10. Bonmassar, E., Cudkowicz, G., Vadlamudi, S., and Goldin, A., Cancer Res. **30**, 2538 (1970).

11. Lilly, F., Jacoby, J. S., and Coley, R. C., *in* "Immunogenetics of the H-2 System" (M. Vojtísková and A. Lengerová, eds.). Karger, Basel, in press.

12. Gasser, D. L., and Silvers, W. K., J. Immunol. **106**, 875 (1971).

13. Bailey, D. W., and Hoste, J., Transplantation **11**, 404 (1971).

14. Bailey, D. W., Transplantation **11**, 426 (1971).

15. Cudkowicz, G., and Bennett, M., J. Exp. Med. **134**, 83 (1971).

16. Cudkowicz, G., J. Exp. Med. **134**, 281 (1971).

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