Effect of Immunosuppressive Drugs on a Localized Graft-Versus-Host Reaction in the Rat (37234)

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The response of the popliteal lymph nodes of F_1 hybrid rats or mice to the subplantar injection of parental lymphocytes has been described (1–3). This localized graft-versus-host reaction (GVHR) has been used primarily to determine the relative activities of various parental lymphoid cell populations to initiate such a reaction (4, 5). Camiener and Tree (6) demonstrated the inhibitory effect of cyclophosphamide, and Levy *et al.* (7) demonstrated the effects of cyclophosphamide, cycloleucine, azathioprine, and methotrexate on the reaction.

The present communication describes our experience with the assay and the inhibitory effects of a variety of anti-tumor/immunosuppressive agents. The assay is recommended as a convenient and reliable method for the routine assessment of the immunosuppressive activity of drugs.

Methods. Splenic cell suspensions were prepared from adult female Fischer rats[1] weighing 150–170 g. These rats were killed by a blow to the head, the spleens rapidly removed and placed into a beaker containing cold Hank's balanced salt solution (BSS). The spleens were then macerated by gentle scraping on a 200-mesh stainless steel wire cloth (Small Parts, Inc., Miami, FL) contained within a Petri dish filled with cold BSS to which a few drops of serum from the donor rats were added. The splenic cell preparations were centrifuged at approximately 2000 rpm in a bench top clinical centrifuge for about 10 sec to remove debris and cell aggregates. The supernatant was then centrifuged at 3000 rpm at 10° for 15 min in a Servall centrifuge (SS-1 rotor). The cell pellet obtained was washed once with cold BSS and suspended in BSS to give an approximate cell concentration of 30×10^6 cells/0.15 ml.

Recipient rats were young F_1 hybrid males (Wistar × Fischer)[1] weighing 75–95 g. Injection of 0.15 ml of the final splenic cell suspension (*ca.* 30×10^6 cells) was made into the plantar tissues of the left hind paw. Occasional checks of cell viability by the dye exclusion technique showed the donor cells to be greater than 90% viable.

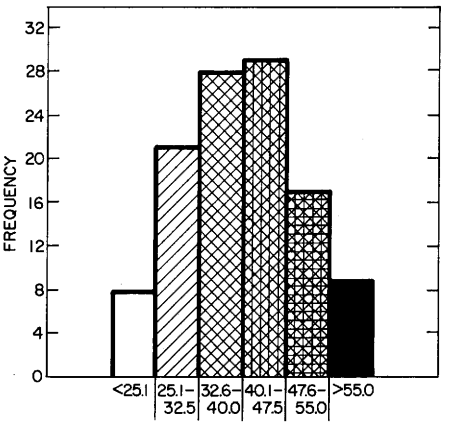

FIG. 1. Frequency distribution of the wet weights of the ipsilateral lymph nodes of F_1 hybrid rats four days after the subplantar injection of 30×10^6 parental splenic lymphocytes into one hind paw. The mean weight of the node based on values obtained from 112 rats is 39.5 mg. The distribution is normal, with a coefficient of kurtosis of −0.003 and a coefficient of skewness of 2.661.

[1] Female Fischer (HLA-F344) and male Fischer/Wistar F_1 hybrid (HLA-F344/W) rats were purchased from Hilltop Laboratories, Scottdale, PA. All Riker research animals are housed and cared for in accordance with the recommendations of the Institute of Laboratory Animal Resources as issued in the latest published *Guide for Laboratory Animal Facilities and Care,* and the facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

TABLE I. Effect of Certain Immunosuppressive Drugs on Body Weight and Wet Weights of the Popliteal Lymph Nodes and Thymus of Rats in Which a Localized GVHR was Elicited.[a]

Treatment [b]	Oral Dose (mg/kg/day)	Change in Body Weight (g)	Wet Weight, (mg/100 g ± S.E.) "Stimulated" Lymph Node [c]	Contralateral Lymph Node	Thymus
Control	—	+25	34.0 ± 2.3	3.6 ± 0.2	363.4 ± 11.2
Methotrexate	5.1	- 1	5.6 ± 0.3 [d]	2.5 ± 0.3 [d]	241.2 ± 12.0 [d]
	1.7	+10	6.9 ± 0.5 [d]	2.6 ± 0.5	330.2 ± 32.3
5-Fluorouracil	90	-10	17.1 ± 2.5 [d]	2.4 ± 0.3 [d]	129.5 ± 8.4 [d]
	30	+15	29.2 ± 4.6	2.4 ± 0.3 [d]	305.2 ± 27.2
Hydroxyurea	450	+17	18.8 ± 1.9 [d]	3.0 ± 0.2	117.6 ± 5.7 [d]
	150	+22	29.7 ± 1.5	3.0 ± 0.3	253.3 ± 17.0 [d]
Control	—	+26	38.5 ± 2.6	3.6 ± 0.3	341.9 ± 14.3
Dexamethasone	0.3	+ 7	11.7 ± 1.5 [d]	1.4 ± 0.1 [d]	58.8 ± 1.8 [d]
	0.1	+10	19.4 ± 2.4 [d]	1.7 ± 0.3 [d]	105.8 ± 3.6 [d]
Chlorambucil	9	- 1	9.3 ± 0.7 [d]	1.5 ± 0.2 [d]	85.6 ± 3.0 [d]
	3	+18	19.0 ± 1.5 [d]	1.8 ± 0.2 [d]	176.1 ± 8.4 [d]
Busulfan	27	- 9	21.1 ± 2.7 [d]	2.7 ± 0.3	110.7 ± 3.1 [d]
	9	+10	29.2 ± 1.7 [d]	3.2 ± 0.2	176.7 ± 6.9 [d]
Control	—	+25	37.7 ± 4.7	4.4 ± 0.4	389.1 ± 14.8
Azathioprine	90	+14	18.0 ± 3.2 [d]	3.3 ± 0.2 [d]	225.4 ± 9.3 [d]
	30	+20	42.8 ± 2.6	3.3 ± 0.4	293.8 ± 9.9 [d]
6-Mercaptopurine	90	+13	28.0 ± 2.4	3.0 ± 0.3 [d]	215.2 ± 10.4 [d]
	30	+17	39.4 ± 2.3	3.6 ± 0.2	321.8 ± 7.0 [d]
Control	—	+33	20.3 ± 1.4	3.3 ± 0.3	339.9 ± 7.2
Hydrocortisone	90	+23	12.8 ± 1.2 [d]	2.6 ± 0.3	117.7 ± 8.3 [d]
	30	+25	17.7 ± 1.2	2.8 ± 0.2	180.3 ± 10.7 [d]
Melphalan	9	+ 4	7.8 ± 0.7 [d]	2.6 ± 0.3	88.3 ± 3.3 [d]
	3	+ 8	20.4 ± 0.9	3.3 ± 0.3	153.0 ± 4.6 [d]
Control	—	+23	34.4 ± 3.3	4.4 ± 0.5	385.9 ± 13.8
Cyclophosphamide	30	+11	6.1 ± 0.3 [d]	3.0 ± 0.3 [d]	92.5 ± 2.0 [d]
	10	+20	11.0 ± 0.7 [d]	3.5 ± 0.4	192.0 ± 14.5 [d]
Colchicine	6	- 3	13.4 ± 2.5 [d]	3.4 ± 0.3	230.9 ± 23.7 [d]
	2	+19	27.7 ± 2.0	3.8 ± 0.3	349.0 ± 9.0 [d]
Thioguanine	30	- 3	9.6 ± 0.5 [d]	3.0 ± 0.2 [d]	152.3 ± 3.2 [d]
	10	+ 6	16.5 ± 3.1 [d]	3.7 ± 0.3	192.0 ± 10.5 [d]

[a] The GVHR was elicited in F_1 hybrid male rats by the injection of 30 x 10^6 parental lymphocytes into the plantar tissues of one hind paw.

[b] Drugs were administered once a day for four consecutive days. The first dose was administered on the day of transfer of parental lymphocytes to F_1 hybrid rats. The rats were killed one day following the last dose of drug.

[c] The lymph node ipsilateral to the injection site.

[d] Indicates a value significantly ($P < .05$) different from the control (vehicle-treated) group mean value. Each mean value is based on 6 or 7 rats.

Drugs, suspended in 4% aqueous acacia, were administered intragastrically for four consecutive days. The first dose was given within 1 hr after splenic cell transfer. Control groups of rats were administered an equivalent volume of the drug vehicle (0.5 ml/100 g body weight). The rats were killed by CO_2 asphyxiation 4 days after splenic cell transfer, and the wet weights of the "stimulated" (ipsilateral to injection site) and contralateral popliteal lymph nodes and of the thymus determined.

Results. The increase in weight of the stimulated popliteal lymph node of F_1 hybrid rats 4 days after the subplantar injection of parental lymphocytes is normally distributed (Fig. 1), and parametric statistics may be applied to the data. The effects of representative chemotherapeutic steroids, alkylating agents, antimetabolites, and alkaloids on the localized GVHR are summarized in Table I. At the doses used, only 6-mercaptopurine failed to produce statistically significant inhibition of the GVHR at either dose (90 mg/kg; $0.1 > p > 0.05$). Dose-related reductions in rate of body weight gain and weights of the thymus and usually the contralateral popliteal lymph node occurred in these rats (Table I). All drugs produced significant reductions in the wet weight of the thymus, and all except methotrexate and 5-fluorouracil did so at both dose levels used. In seven instances, significant reduction in the weight of the thymus without concomitant inhibition of the GVHR occurred. In only one case (methotrexate, 1.7 mg/kg) was the GVHR inhibited in the absence of a significant reduction in weight of the thymus.

Discussion. It has been shown that the localized GVHR produced in F_1 hybrid rats by the subplantar injection of viable parental lymphocytes is sensitive to the effects of a variety of classes of cytotoxic or antiproliferative drugs. Levy *et al.* (7) suggested that such a reaction could find use as a routine assay for rapidly evaluating potential immunosuppressive drugs, and the data obtained from the present studies tend to support such a conclusion. The advantages of inducing a local rather than systemic GVHR were discussed by Ford *et al.* (2). We would suggest the inclusion of an additional parameter when using the method as a screen for potential immunosuppressive drugs; *viz,* the wet weight of the thymus of the rats in which the GVHR is induced. This tissue appears particularly sensitive to the effects of cytotoxic/antiproliferative drugs in adrenalectomized as well as intact rats (8) and may indicate the nonselective immunosuppressive nature of such agents. An agent producing selective inhibition of the GVHR without affecting this sensitive lymphoid population might be presumed *a priori* to possess a mechanism of action different from the available classes of immunosuppressive agents.

Summary. The effects of 13 immunosuppressive agents, which included representative steroids, alkylating agents, antimetabolites, and alkaloids, on a localized graft-versus-host reaction in the rat were examined. The results suggest that the method could find use as a routine screen for detecting agents with immunosuppressive activity. Decrease in the wet weight of the thymus of the recipient rats produced by these agents may reflect the nonselective nature of currently available immunosuppressive drugs.

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