Suppression of the Graft-versus-Host Reaction by Passive Immunization of Donor against Recipient Antigens[1] (34537)

JOHN W. SAFFORD, JR.[2] AND SEI TOKUDA[3]
(Introduced by L. C. McLaren)
Department of Microbiology, The University of New Mexico School of Medicine, Albuquerque, New Mexico 87106

Cell-mediated immune responses, such as delayed hypersensitivity, rejection of tumor allografts, skin allografts, and renal allografts, may be specifically suppressed by administration of either antigen or antibody or by a combination of both methods (1-6). Voisin and Kinsky (7) have reported that newborn mice are significantly protected from the cell mediated graft-versus-host reaction (GVHR) induced by injection of allogeneic spleen cells from adult donors if small amounts of antibody against recipient tissue antigens are administered simultaneously. It has also been demonstrated that active immunization of the lymphoid cell donor against recipient tissue antigens may lower the capacity of the lymphoid cells to mount a GVHR against newborn recipients (8) and against adult hybrid recipients (9). The protection afforded the host against the GVHR was believed to operate through the same mechanism(s) by which enhancing antiserum protects a tumor against rejection and was termed immunological enhancement-facilitation (9). This report deals with suppression of the GVHR by passively immunizing the cell donor with antiserum directed against the host's H-2 antigens.

Materials and Methods. Antiserum against Sarcoma I (SaI, a tumor indigenous to the A/J, *H-2*^{a}, mouse strain) was prepared in C57Bl/Ks (*H-2*^{d}) by multiple immunization with the ascites form of the tumor. The resulting antiserum was found capable of provoking passive immunological enhancement of SaI tumor in C57Bl/Ks mice. The hemagglutination titer was determined according to the method of Stimpfling (10) using polyvinylpyrrolidone (Antar Chemical, Inc.) as a developing agent. The hemolysin test was performed essentially as described by Hildemann (11); the complement source was guinea pig serum adsorbed with mouse red blood cells and the target was A/J mouse red blood cells. Titers are expressed as the reciprocal of the highest dilution of antiserum showing a positive reaction. Antiserum against sheep red blood cells (SRBC, Colorado Serum Co.) was prepared by multiple immunization of C57Bl/Ks mice with 0.2 ml 2% (v/v) SRBC. The hemagglutination titer was determined by the use of the microtiter apparatus (Cooke Engineering Co.). Both antisera were subjected to sucrose-density gradient ultracentrifugation on a linear 10–40% (w/w) gradient in a SW 50L rotor at 40,000 rpm and 4° for 16 hr. After centrifugation 12 fractions were collected from the bottom of the tube, and each fraction was assayed for hemagglutinating and hemolytic activities as described above. Analysis of the fractions showed that both sera contained almost all of the detectable antibody activity in the 7S region.

Donor spleen cells were obtained from three groups of C57Bl/Ks male mice: (1) mice which had received no previous treatment; (2) mice which had received 0.1 ml anti-SRBC serum intraperitoneally (ip) 4-8 days before they were killed; and (3) mice which had received 0.1 ml anti-*H-2*^{a} serum ip

[1] This investigation was supported by USPHS Grant 5 RO1 CA 10129 from the National Cancer Institute.

[2] Recipient of USPHS Predoctoral Research Fellowship 5 FO1 GM 35, 862 03.

[3] Recipient of Research Career Development Award USPH Grant 5 KO4 GM 14893.

TABLE I. The Suppression of the Graft-versus-Host Reaction by Passively Immunizing the Donor with Specific Antibody.

Spleen donor treatment	No. dead/no. injected	% Mortality	MST ± SE[a]
None or anti-SRBC serum	30/31	96.8	14.9 ± 0.5
Anti-SaI serum	39/65	60.0	15.3 ± 0.6
		$p < 0.001$[b]	

[a] Mean survival time in days ± one standard error.
[b] Probability as determined by the chi-square test.

4-8 days before they were killed. The anti-*H-2*^{a} serum used to passively immunize spleen donors had a hemagglutination titer of 2^{12} and a hemolysin titer of 2^8. The anti-SRBC serum had a hemagglutination titer of 2^9.

Spleens were removed aseptically from the donor mice and placed in a 35-mm plastic petri dish containing 2 ml of Hanks' balanced salt solution (HBSS). After mincing the spleen with a scalpel the cells were dispersed with a capillary pipet. Single cell suspensions were prepared by successively drawing the slurry through 20- and 27-gauge hypodermic needles. The cells were sedimented by centrifugation for 10 min at 250*g* at 4° and were resuspended in sufficient HBSS to yield a suspension of 140×10^6 nucleated cells per milliliter as determined by counting in a standard hemocytometer. At least 90% of the cells in these suspensions were viable as determined by trypan blue dye exclusion.

Litters of 5–8 A/J mice were selected and each mouse was injected ip with 7×10^6 spleen cells (0.05 ml) within 24 hr of birth. Intralitter controls were not used because of the possibility of deleterious competition between stronger and weaker littermates. The mice were observed daily and weighed every other day. The primary criteria used to establish the occurrence of runt disease were failure to gain weight at a normal rate and death which occurred within 2–3 weeks. Additional mice which were not given the injections or had received 0.05 ml HBSS within 24 hr of birth served to establish normal weight gain.

Results. Spleen cell suspensions from either untreated donors or from donors passively immunized against SRBC yielded identical results. These data will, therefore, be considered as a single control group. Newborn A/J mice injected with spleen cells from these donors invariably developed symptoms of the runting syndrome (12). They gained weight at a normal rate for the first 7–8 days and then showed a decreased weight gain as compared to normal mice. Eventually they failed to gain any weight or actually lost weight. During this time the mice appeared emaciated, developed a high-stepping gait, and suffered from diarrhea and alopecia. Of the 31 recipients of spleen cells from this group of donors, only one survived. The mean survival time of the 30 that died was 14.9 days (Table I).

A significant difference in the incidence of runt disease was observed between the control group and recipients of spleen cells from C57Bl/Ks donors passively immunized with anti-*H-2*^{a} serum. The 65 A/J mice injected with spleen cells from donors passively immunized with anti-*H-2*^{a} serum were obtained from 10 litters; 9 spleen cell donors were used. Of the 65 A/J newborns injected with spleen cells from this group of donors, 26 survived. Mice in this group gained weight normally for about 9 days then showed a weight gain increase which was only slightly less than that of untreated controls. After 17-18 days the increase in body weight of the survivors paralleled that of normal mice. Only those mice which eventually died developed the emanciation, diarrhea, and alopecia characteristic of runt disease. The 39 mice that died had a mean survival time of 15.3 days (Table I). Deaths occurred randomly among the litters, and all surviving mice remained healthy for an observation period of 4 months.

Spleen cells from mice injected with anti-*H-2*^{a} serum 8 days previously were just as depressed in their ability to initiate a GVHR as were spleen cells taken 4 days after passive immunization.

It has been reported that extremely small amounts of antiserum are effective in suppressing the GVHR if it is injected into the newborn recipient along with the lymphoid cell inoculum (7). The possibility that the spleen cell inoculum prepared from passively immunized donors may have contained antibody against A/J tissue antigens was investigated. After the spleen cells were sedimented from the 2 ml of HBSS, the supernatant fluid was removed and tested for the presence of A/J red blood cell hemagglutinins. None of the cell washings from the nine spleen donors which had received anti-*H-2*^{a} serum contained detectable antibody. In addition, preliminary experiments have shown that spleen cells from passively immunized C57Bl/Ks donors remain suppressed even after three washings in large volumes of HBSS. Washing the cells three times did not significantly decrease the proportion of cells capable of excluding trypan blue dye.

Discussion. The GVHR may be specifically suppressed either by including small amounts of antibody against recipient tissue antigens in the lymphoid cell inoculum (7) or by actively immunizing the lymphoid cell donor against recipient tissue antigens (8, 9). Our results show that the GVHR may also be suppressed by passively immunizing the cell donor with antiserum directed against the recipient's H-2 antigens and that the reduced capacity of the spleen cells is still manifested up to 8 days after the donor is passively immunized with antibody. It was also observed that spleen cells obtained from passively immunized donors had a reduced capacity to induce a GVHR even after three washings in large volumes of HBSS.

The exact mechanism by which antibody suppresses the development of the immune response has not been satisfactorily explained. Since suppression of the immune response by passively administered antibody is specific for the corresponding antigen, it may be argued that this specificity indicates that the mechanism of suppression involves interaction of antibody with antigen (13). Tumor allografts may be protected from destruction by sensitized lymphoid cells by coating the cells with antibody; this antibody is believed to suppress the immune response at the antigenic level (14). Voisin and Kinsky (7) reported that 0.2–0.4 μl of antiserum mixed with the allogeneic lymphoid cells was sufficient to protect newborn mice from the runting syndrome. In the experiments reported here, it is highly unlikely that enough antibody was contained in the spleen cell inoculum (7 million spleen cells in 0.05 ml) to coat all the antigens of the newborn host. We have calculated (on the basis of antibody half-life, blood volume of the mice, and size of the spleen) that the amount of antibody that could have been transferred with the spleen cell inoculum was about one order of magnitude less than the 0.2–0.4 μl discussed above. The possibility that antibody had adsorbed onto the spleen cells and dissociated after injection into the newborn host has not been ruled out. This appears unlikely, however, due to the observation that the spleen cells remained suppressed even after washing three times in large volumes of HBSS. Furthermore, the supernatant fluid from the first washing contained no detectable antibody. Even if antibody was eluted from the spleen cells *in vivo*, it does not seem possible that enough antibody could be released to saturate all of the antigenic determinants in an intact, newborn mouse.

An alternative to inhibition at the antigenic level is that antibody acts directly on the lymphoid cell to suppress the development of the immune response. Rowley and Fitch (15) reported that exposure of normal spleen cells to antibody, either *in vivo* or *in vitro*, suppressed their response to the corresponding antigen after transfer to X-irradiated recipients. Supernatant fluid from their cell suspensions contained no detectable antibody. In addition, it has been reported that amounts of antibody 100 times too small to saturate all antigenic sites in a dose of SRBC will suppress the immune response to SRBC (16).

Rowley and Fitch (15) suggested that ei-

ther free antibody or the free antigen-binding sites on antibody molecules complexed with antigen may combine on or in the potential antibody-forming cell which carries configurations similar to the antigen. This combination of antibody (or the free site on an antibody-antigen complex) with the potential antibody-forming cell would then limit the immune response through a "homeostatic or feedback mechanism." In our studies we can not rule out the possibility that antibody against H-2 antigens was adsorbed to potential antibody-forming cells and prevented the induction of the cellular immune response necessary to provoke the GVHR.

Voisin *et al.* (9) noted the similarity between immunological enhancement of tumor allografts and the suppression of the GVHR in adult F_1 hybrid mice injected with spleen cells obtained from parental strain mice immunized against the F_1 antigens. In our current studies, the same lot and dosage of anti-*H-2*^{a} serum was also used to provoke passive immunological enhancement of the SaI tumor in C57Bl/Ks mice and to suppress the development of the GVHR in A/J mice. It was observed that immunological enhancement of the SaI tumor could be provoked in C57Bl/Ks mice up to 7 days after passive immunization. If the SaI inoculation is delayed until 14 days after passive immunization, the tumor is rejected normally (unpublished observations). Spleen cells obtained from C57Bl/Ks mice 8 days after the passive immunization with anti-*H-2*^{a} serum failed to provoke a virogous GVHR in newborn A/J mice. Thus, our data are consistant with the concept that the suppressed GVHR activity and the enhancement of tumor growth by passive immunization represent two manifestations of the basic phenomenon of the suppression of the cell-mediated immune response by humoral antibody.

Summary. A graft-versus-host reaction was produced by injecting newborn A/J mice with C57Bl/Ks spleen cells which resulted in 96.8% mortality. Passive immunization of the spleen donors with antibody against the A/J tissue antigens reduced mortality to 60.0%. Spleen cells taken from donors passively immunized up to at least 8 days previously were depressed in their ability to mount a graft-versus-host reaction.

1. Asherson, G. L. and Stone, S. H., Immunology **9**, 205 (1965).

2. Crowle, A. J. and Hu, C. C., J. Allergy **43**, 209 (1969).

3. Axelrad, M. A., Immunology **15**, 159 (1968).

4. Kaliss, N., Ann. N. Y. Acad. Sci. **129**, 155 (1966).

5. Chutna, J., Nature **217**, 175 (1968).

6. Stuart, F. P., Saitch, T., and Fitch, F. W., Science **160**, 1463 (1968).

7. Voisin, G. A. and Kinsky, R., *in* "Ciba Foundation Symposium on Transplantation" (G. E. W. Wolstenholme and M. P. Cameron, eds.), p. 286. Little, Brown, Boston, Massachusetts (1962).

8. Schlesinger, M. and Goitein, R. J., J. Immunol. **95**, 323 (1965).

9. Voisin, G. A., Kinsky, R., and Maillar, J., Transplantation **6**, 187 (1968).

10. Stimpfling, J. H., Transplant. Bull. **27**, 109 (1961).

11. Hildemann, W. H., Transplant. Bull. **4**, 148 (1957).

12. Simonsen, M., Progr. Allergy **6**, 349 (1962).

13. Uhr, J. W. and Möller, G., Advan. Immunol. **8**, 81 (1968).

14. Möller, G., Transplantation **2**, 405 (1964).

15. Rowley, D. A. and Fitch, F. W., *in* "Regulation of the Antibody Response" (B. Cinader, ed.), p. 127. Thomas, Springfield, Illinois (1968).

16. Haughton, G. and Nash, D. R., Transplant Proc. **1**, 616 (1969).

Received Oct. 9, 1969. P.S.E.B.M., 1970, Vol. 133.