SERUM-MEDIATED INHIBITION OF GRAFT-VS.-HOST REACTION

By M. N. CAUCHI* AND K. B. DAWSON

(From the Institute of Cancer Research Royal Cancer Hospital, The Royal Marsden Hospital, Sutton, Surrey, England)

When parental type lymphoid cells are injected into F1 hybrid animals there is usually a severe graft-vs.-host reaction (GVHR). It has been shown that a proportion of animals will survive the acute episode, depending on the number of cells injected and the strain combination used, and that these animals are then tolerant to a second challenge by parental type cells (1). We have previously shown that this tolerance could be transferred from GVH-recovered rats to normal rats by cross-circulation or parabiosis (2). X or /0 irradiation of the transfused blood to doses of 1,000 rad did not inhibit the transfer of tolerance, indicating that serum factors were responsible for mediating this phenomenon. Initial attempts to transfer this tolerance by serum alone proved unsuccessful (2, 3), probably because insufficient amounts of serum were transferred.

The following experiments were designed to elucidate the role of serum factors from rats undergoing or recovering from a GVHR using the relatively more sensitive technique of local GVHR (4).

Materials and Methods

Rats. Young adult rats from our own stock of highly inbred Marshall and August strain rats and their F1 hybrids were used.

Cell Suspensions. Spleen and lymph node tissues were diced with scissors and pressed through a steel sieve (Mesh No. 100; Endecotts Test Sieves Ltd.). The cells were washed in tissue culture Medium 199, counted in a hemocytometer, and diluted to the required concentration. Their viability, as judged by trypan blue exclusion was about 90%.

GVH Reaction. Systemic GVHR was produced by intraperitoneal inoculation of 100–150 x 10^6 Marshall spleen cells. The degree of severity of the reaction was assessed on the scale described previously (5). A local GVHR was produced by the technique of Elkins (4). In brief, this consisted of inoculating 50 x 10^6 Marshall spleen or lymph node cells under the kidney capsule of hybrid rats of the same sex. Assessment of the severity of the GVHR was made on the 7th day after transplantation from the relative weights of the kidneys and from the uptake of [125I] iododeoxyuridine ([125I]UdR; The Radiochemical Center, Amersham, England; sp act, 1-6 mCi/mg), when 10 μCi were injected intravenously (i.v.) 2 h before sacrifice. Both kidneys and the spleen were weighed, fixed in formal saline for 24 h and then in several changes of 70% alcohol to remove the acid-soluble 125I (6), and counted in an Autogamma spectrometer (Packard Ltd.). This method has the additional advantage that the tissues can be subsequently histologically examined.

The kidney indices for the weight and [125I]UdR activity were calculated as follows:

\[
\frac{(K_t - K_c)}{K_c} \text{ treated} : \frac{(K_t - K_c)}{K_c} \text{ control}
\]

* Present address: Department of Pathology and Immunology, Monash Medical School, Melbourne, Victoria, Australia 3181.
where the subscripts $i$ and $c$ refer to the weights, or activities of the injected and contralateral (control) kidneys respectively. Similarly the spleen indices were calculated as:

$$\left( \frac{S}{K_i} \right) \text{treated} / \left( \frac{S}{K_c} \right) \text{control}$$

where $S$ is the spleen weight or activity.

**Sera.** Rats were bled under light ether anesthesia, 2-3 ml of blood being taken from the tail vein. The sera were frozen at $-10^\circ$C until required. Absorption of the sera and precipitation with 50% ammonium sulphate were performed by standard techniques.

Lymphoid cells were suspended in Medium 199 at a concentration of $50 \times 10^6$/ml, and serum (25-100 $\mu$l/$10^6$ cells) was added and incubated at $37^\circ$C for 60 min. The cells were then washed and reconstituted to $50 \times 10^6$/ml.

**Results**

When parental spleen cells are injected directly under the kidney capsule the resulting GVHR is predominantly localized to that kidney and there is minimal change in weight or $[^{125}]$UdR activity of the contralateral kidney. There is no evidence of external symptoms at this time and for this dose of cells, but there is some increase in weight and $[^{125}]$UdR activity in the spleen.

When 1 ml of serum from rats with acute systemic GVHR induced 4 wk previously was injected i.v. into hybrid rats 1 h before transplantation of $50 \times 10^6$ Marshall spleen cells under the kidney capsule, there was a moderate depression of $^{125}$I uptake to 60% of controls ($0.1 < P < 0.05$). However, when Marshall spleen cells were preincubated with the serum at a concentration of 20-100 $\mu$l/$10^6$ cells, there was a marked reduction of $^{125}$I uptake to 24% of controls ($P < 0.01$). There were corresponding but less pronounced changes in the splenic indices. Lymph node cells were also inhibited by the serum, indicating a nonspleen-specific factor.

Table I also shows that the globulin fraction obtained from serum by precipitation with 50% ammonium sulphate can inhibit $^{125}$I uptake. Absorption of the serum by either parental strain spleen or kidney homogenates removed all its inhibiting activity.

**Table I**

| Cells                      | Incubation                                | Kidney indices | Spleen indices |
|----------------------------|-------------------------------------------|----------------|----------------|
|                            |                                           | Weight         | $^{125}$I uptake | Weight         | $^{125}$I uptake |
| Spleen                     | GVH-recovered serum (100 $\mu$l/$10^6$ cells) | 121.1 ± 14.9   | 24.1 ± 0.9      | 76.0 ± 3.8     | 44.1 ± 14.1     |
| Spleen                     | GVH-recovered serum (20 $\mu$l/$10^6$ cells) | 119.4 ± 12.6   | 37.7 ± 8.4      | 78.0 ± 1.0     | 68.9 ± 5.1      |
| Spleen                     | Normal serum (100 $\mu$l/$10^6$ cells)    | 102.6 ± 11.9   | 103.1 ± 8.7     | 95.6 ± 2.9     | 89.6 ± 1.1      |
| Spleen                     | Globulin fraction from GVH-recovered serum | 103.8 ± 14.1   | 43.4 ± 11.0     | 82.4 ± 4.2     | 54.9 ± 5.7      |
| Spleen                     | Globulin fraction from normal serum       | 119.4 ± 12.6   | 113.9 ± 5.6     | 58.4 ± 7.3     | 93.5 ± 12.4     |
| Lymph node                 | No preincubation                          | 105.9 ± 10.3   | 96.7 ± 19.1     | 120.5 ± 12.4   | 128.8 ± 17.9    |
| Lymph node                 | GVH-recovered serum (100 $\mu$l/$10^6$ cells) | 37.1 ± 8.7     | 25.3 ± 8.3      | 83.2 ± 1.7     | 120.1 ± 19.5    |
It is to be noted that in the experiments with preincubation of the spleen cells with serum from GVH-recovered animals, there is a discrepancy between the kidney weight and $^{125}$I uptake indices (121 and 24%, respectively), emphasizing that enlargement of the kidney is not due to DNA-synthesizing lymphoid cells. Gross changes could be observed: in the kidneys of rats transplanted with Marshall spleen cells preincubated with GVHR serum there was accumulation of hemorrhage under the kidney capsule. Histological examination of these kidneys showed that whereas in the local GVHR there is massive infiltration of the kidneys with lymphoid cells, in the GVHR induced by spleen cells preincubated with serum from GVH-recovered animals there is accumulation of hemorrhage with minimal mononuclear cell infiltration.

To establish the rate of acquisition of these factors, hybrid rats were given the standard inoculum of Marshall spleen cells to produce a systemic GVHR and serum was taken 1 wk to 8 mo later. Normal Marshall cells were then incubated with this serum and inoculated under the kidney capsule of fresh hybrid rats. Considerable reduction in the $^{125}$I uptake was obtained with serum taken 1 wk after induction of a systemic GVHR ($K_n$, 46.2 ± 8.4). This reduction was still observed with serum taken 8 mo after the initial induction of a systemic GVHR ($K_n$, 45.6 ± 9.5).

When normal Marshall spleen cells were injected under the kidney capsule of rats undergoing a systemic GVHR, there was significant reduction in the kidney and splenic indices only in rats 3 wk after induction of a GVHR, and was absent in rats with GVHR of 6 wk to 8 mo duration, when superficial symptoms were no longer obvious.

**Discussion**

These studies show that during the course of a GVHR, inhibitors appear in the circulation within 1 wk and persist for up to 8 mo after inoculation of parental cells. This phenomenon can be shown to occur by: (a) injection of serum from GVH-recovered animals into F₁ hybrid rats before challenge with parental cells; (b) direct challenge of GVH-recovered F₁ hybrid rats by parental cells systemically or locally under the kidney capsule; and (c) by preincubation of parental cells with GVHR serum in vitro for 1 h before transfer to F₁ recipients. The last technique appears to be the most sensitive index of inhibitory activity in such sera, and indicates that the failure to demonstrate transfer of GVH inhibitory activity by previous workers (2, 3) was due to insensitivity of the technique used, or to the relatively inadequate amount of serum transferred.

It can also be seen in these studies that while there was a good correlation between the weight and $^{125}$I uptake in kidneys injected with normal parental cells, in animals which were given cells preincubated with inhibitor serum there was gross enlargement of the kidney and poor $^{125}$I uptake. Histological examination confirmed that this enlargement was largely due to accumulation of hemorrhage with a relative reduction in the number of lymphoid cells.

The presence of antihost antibody activity in the sera of animals undergoing a GVHR has been well documented. Kano et al. (7) found antibodies to C57BL/6 cells in the serum of (C57BL/6 × DBA)F₁ mice after multiple injections of DBA/2.
spleen cells, and similarly, when C57BL/6 cells were injected in the hybrid mice, an autoimmune hemolytic anemia developed where the antibody eluted from the erythrocytes was shown to react with DBA/2 erythrocytes. Similarly, Gleichmann et al. (8) showed that immunoglobulin deposits in renal lesions had allotypic markers of the donor, indicating that in chronic GVHR antibodies are produced by the donor and directed against the host cells.

The above findings indicate that during the course of a GVHR, factors appear in the serum of the host which can react with parental-type cells. It has been shown that during a GVHR the host cells are activated and capable of lysing parental cells nonspecifically and that parental tumor cells exposed in vitro to spleen cells from F hybrid mice undergoing a GVHR had a markedly decreased ability to grow in syngeneic recipients (9). Our findings would indicate that similar reactivity against parental cells can be demonstrated in the serum of animals recovering from GVH disease. In this respect these factors are different from anti-idiotype antibodies which have recently been described by McKearn and colleagues (10, 11) in hybrid rats undergoing a GVHR.

Summary

Rats recovering from a systemic graft-vs.-host reaction (GVHR) possess factors in the serum which can inhibit the production of a local GVHR. After incubation in vitro for 1 h at 37°C these factors reduce the GVH-producing potential of parental spleen or lymph node cells to 24% of control cells treated with normal serum. These factors appear within 1 wk after initiation of a systemic GVHR and some residual activity persists for up to 8 mo. The serum activity was present in the globulin fraction and was completely removed by absorption with spleen, lymph node, or kidney homogenates from either parental strain rats.

These studies indicate that during the course of a systemic GVHR, serum factors directed against the host appear in the circulation and tend to inhibit the production of further GVHR by a second challenge of either parental strain cells.

Received for publication 30 December 1974.

References

1. Field, E. O., and J. E. Gibbs. 1966. Reduced sensitivity of F hybrid rats to re-challenge with parental strain spleen cells. Clin. Exp. Immunol. 1:195.
2. Field, E. O., M. N. Cauchi, and J. E. Gibbs. 1967. The transfer of refractoriness to GVH disease in F hybrid rats. Transplantation (Baltimore). 5:241.
3. Fox, M., and J. G. Howard. 1963. An acquired type of refractoriness to graft-versus-host reaction in adult F hybrid mice. Transplantation (Baltimore). 1:2.
4. Elkins, W. L. 1970. Specific and nonspecific lymphoid cell proliferation in the pathogenesis of graft-versus-host reactions. Transplantation (Baltimore). 9:273.
5. Field, E. O., and J. E. Gibbs. 1965. Effects of thymectomy and irradiation on graft-versus-host disease. Transplantation (Baltimore). 3:634.
6. Bryant, J. B., and L. J. Cole. 1967. Evidence for pluripotentiality of marrow stem cells. Modification of tissue distribution of in vivo 125I-UdR labelled transplanted marrow. In The Lymphocyte in Immunology and Haemopoiesis. J. M. Yoffey, editor. Arnold Publishers Ltd., London, England. 170.
7. Kano, K., L. Beldotti, F. Milgrom, and R. S. Schwarz. 1974. Glomerulonephritis in
the graft-vs-host reaction: serologic demonstration of anti-host antibodies. *J. Immunol.* 112:410.

8. Gleichmann, H., E. Gleichmann, J. Andre-Schwartz, and R. S. Schwartz. 1972. Chronic allogeneic disease. III. Genetic requirements for the induction of glomerulonephritis. *J. Exp. Med.* 135:516.

9. Singh, J. N., E. Sabbadini, and A. F. Sehon. 1972. Cytotoxicity in graft-versus-host reaction, I. Role of donor and host spleen cells. *J. Exp. Med.* 136:39.

10. McKearn, T. J. 1974. Anti-receptor antiserum causes specific inhibition of reactivity to rat histocompatibility antigens. *Science (Wash. D. C.).* 183:94.

11. McKearn, T. J., Y. Hamada, and F. W. Stuart. 1974. Anti receptor antibody and resistance to graft-versus-host disease. *Nature (Lond.).* 251:648.