Treatment of an Established Graft-versus-Host Reaction in AKR Mice by Adoptive Immunotherapy

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SUMMARY

A model system in AKR mice for the induction and cure of a clinically evident graft-versus-host disease is reported. Graft-versus-host disease is initiated by i.p. injections of cyclophosphamide (250 mg/kg body weight) into female AKR mice, on Day 0. This is followed by i.v. injections of 45 x 10^6 normal spleen cells (NSC) from male C57BL/6J mice. Median survival time for these mice is 33.4 ± 4.5 days.

Following the administration of C57BL/6J NSC, AKR mice were rescued from graft-versus-host disease by the following treatment protocol: (a) Day 6, 35 x 10^6 DBA/2 NSC given i.v.; (b) Day 10, cyclophosphamide i.p. (150 mg/kg body weight); (c) Days 11 and 16, 35 x 10^6 AKR NSC given i.v.

These experiments demonstrate that graft-versus-host reaction can be eliminated by coupling a graft-versus-host reaction with a graft-versus-graft reaction and restoring the host by immunocompetent syngeneic cells.

INTRODUCTION

Adoptive immunotherapy, in which cells from a specifically immunized donor are transferred into a tumor host, is potentially useful in the treatment of leukemia or solid tumors. Indeed, this method has been shown to have significant antitumor effect in a number of animal systems (6, 12). The major difficulty with this general therapy in human disease is the induction of graft-versus-host disease by immunologically competent cells from an allogeneic donor. Immunosuppressed recipients of allogeneic immunocompetent cells undergo severe wasting, clinically manifested by loss of weight, diarrhea, and a high incidence of mortality usually between the 3rd and 7th week (4, 9, 19). Data from Boranic and Tonkovic (7), however, show that some degree of GVHR is necessary for antitumor effect. Thus, the problem is to induce a GVHR which, after being allowed to become established, can be brought under control.

Many approaches have been utilized to minimize GVHR in both clinical transplantation and experimental models. These include tissue matching of donor and recipient, modifying the immunological capacity of the donor prior to transplantation (5, 22), fractionating the donor cells in vitro prior to transplantation (1, 21), and using immunosuppressive drugs after transplantation (3, 4, 14). None of these methods has been totally satisfactory.

This report describes a new method for the prevention of GVHR in mice. It utilizes histoincompatible, immunocompetent donor cells to react against the initial GVHR-causing donor cells. This was followed by immunosuppression of both donor cells and reconstitution with syngeneic cells.

MATERIALS AND METHODS

Animals. Inbred female AKR (H-2^k) and DBA/2 (H-2^d) and male C57BL/6J (H-2^b) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. All mice were 8 to 12 weeks old, maintained in plastic cages (10/cage), and fed Purina chow ad libitum. The chow was finely ground and placed in an open container on the floor of the cage from the 10th to the 70th day so that animals weakened by treatment would be able to obtain adequate food without recourse to feeder boxes. The drinking bottles were autoclaved and contained distilled water with streptomycin (40 μg/ml) and penicillin (40 units/ml). Before initiation of an experiment, the animals were randomized to prevent small differences in age or weight from being factors in the results. The animals were weighed and carefully observed daily. Each animal, with the exception of the long-term survivors, was autopsied and the spleen and thymus were obtained for histological examination.

Drugs. Solutions of Cyclo, obtained from Mead Johnson & Co., Evansville, Ind., were prepared in 0.9% NaCl solution and injected within 15 min.

Antisera. Anti-AKR (D-3b), anti-C57BL/6J (D-33), and anti-DBA/2 (D-4) sera were generously provided by Dr. John G. Ray, Jr., of the NIH.

Cell Suspensions. Spleen cells were prepared according to the method of Boranic and Tonkovic (7). Viable nucleated cells were counted in a hemocytometer and the final volume was adjusted to give a cell count of 1 x 10^6/ml. Cell viability was determined by trypan blue dye exclusion (8).

Procedure for Induction and Treatment of GVHR. The
protocol for our experiment is illustrated in Chart 1. GVHR was induced in AKR mice by the method of Owens and Santos (16). Control female AKR mice were injected i.p. on Day 0 with Cyclo (250 mg/kg body weight); within 24 hr 45 x 10^6 NSC from male C57BL/6J mice were injected i.v. into the tail vein. Groups 2 to 8 received, in addition to the above, the treatments as listed in Table 1. Spleen cells from a 2nd donor were injected i.v. on Day 6. By this time clinical evidence of GVHR, manifested by wasting and ruffled fur, was observed.

**Criteria of GVHR.** Clinical criteria for GVHR on Day 6 were ruffled fur, loss of weight, and hunched posture (10). Dead mice were autopsied. The spleen histologically showed features characteristic of GVHR, including a pronounced decrease of lymphoid tissue, a severe depopulation of cells in the Malpighian follicles, extensive "fibrinoid" necrosis, and foci of recent hemorrhage (19). Similarly, the thymus showed severe lymphoid depletion (19).

**RESULTS**

Table 1 summarizes the results of our experiments. Cyclo at the given doses exhibited no obvious toxicity. All control mice (Cyclo on Day 0 followed by the injection of C57BL/6J NSC within 24 hr) developed clinically evident GVHR by Day 6 and were dead within 50 days. The addition of Cyclo on the 10th day increased the MST by about 23 days. No further significant change in the MST was observed when syngeneic cells were given on the 6th day, either with or without cyclo on the 10th day or with Cyclo (Day 10) plus syngeneic cells on the 11th and 16th days. Substituting DBA/2 NSC for AKR cells on the 6th day, with or without Cyclo on the 10th day, resulted in decreased MST. However, when 70 x 10^6 syngeneic cells were given after Cyclo (Day 10) and DBA/2 cells (Day 6), the animals were alive and healthy on Day 200 with no clinical signs of GVHR disease.

**DISCUSSION**

In this study, GVHR was induced in adult Cyclo-immunosuppressed AKR mice by allogeneic immunocompetent C57BL/6J NSC. Similarly to what has been reported by Russell (17), survival time was extended by the administration of syngeneic cells on the 5th day after GVHR to within 80 days (Table 1, Group 3). Possibly, as the work of Kaliss (11) suggests, the allogeneic C57BL/6J spleen cells, residing in a suppressed host for 5 days, become sensitized to the host antigens and are capable of mounting a strong attack against the administered syngeneic cells. This led us to believe that substitution of the syngeneic cells with histoincompatible cells, i.e., DBA/2 spleen cells, might eliminate this adverse reaction. Essentially, we wanted to establish a graft-versus-graft reaction with the host only
passively involved. However, when we substituted a 2nd strain of allogeneic spleen cells for the syngeneic cells on the 6th day, survival time was further decreased, probably as a result of additional GVHR induced by the histoincompatible DBA/2 cells. It was therefore necessary to eliminate the DBA/2 cells, as well as any residual C57BL/6J cells. This was achieved by administering Cyclo followed by syngeneic cells.

As seen in Table 1, essentially 100% of the mice so treated were alive and healthy, without clinical evidence of GVHR on Day 200. One possible factor contributing to the success of this therapy may be the heightened immune reactivity of the host that has been observed in the early stages of GVHR (13, 15). However, it would appear that the allogeneic, GVHR-inducing cells have been destroyed or inactivated by our treatment. Thus, the substitution of DBA/2 cells (Table 1, Group 8) for the AKR cells (Table 1, Group 5) was necessary for attainment of the cure. The GVHR-producing C57BL/6J cells are already sensitized to AKR, so no counter-GVHR could be set up in Group 5. In addition, we have shown that C57BL/6J spleen cells in immunosuppressed AKR hosts are detected by specific cytotoxic antisera 45 days after inoculation (unpublished results). However, if the AKR mice grafted with C57BL/6J spleen cells were given DBA/2 spleen cells, followed by Cyclo and syngeneic spleen cells, neither strain of allogeneic cell was detectable with specific antisera by cytotoxicity testing at 47 days. These results suggested that these cells have been removed. Furthermore, Santos and Owens (18) in rats and Uphoff et al. (20) in mice have shown that when 2 or more strains of pooled marrow are injected into an irradiated animal, long-term chimerism is established with the donor most closely related to the host. This suggests a reaction between the strains of injected pooled marrow whereby one lymphocyte population eliminates the other.

The results of these experiments demonstrate that GVHR can be controlled and eliminated by coupling a GVHR with a graft-versus-graft reaction in a host ultimately restored by immunocompetent syngeneic cells. We are currently using this model to explore the parameters necessary for successful treatment of murine leukemia and osteogenic sarcoma.

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REFERENCES

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