Treatment of a Moloney Lymphoma with Cyclophosphamide and H-2-incompatible Spleen Cells

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SUMMARY

This study was performed to determine whether, as an adjunct to cyclophosphamide (CY), a number of H-2-incompatible lymphoid cells too small to induce clinically evident graft-versus-host disease would exert a significant antitumor effect and whether clinically significant antitumor specificity might be imparted to a subclinical graft-versus-host reaction by preimmunization of the spleen cell donors. Adult BALB/c mice bearing a disseminated transplantable BALB/c Moloney virus-induced lymphoma (LSTRA) with tumor-associated antigens were treated with sublethal CY plus spleen cells from C57BL/6 mice that either were nonimmune or had been immunized with normal BALB/c tissue, a BALB/c chemical sarcoma, an antigenically related murine sarcoma virus (Moloney), or LSTRA cells. The results, represented by long-term survivors, showed that, in the absence of detectable graft-versus-host disease and in conjunction with CY, nonimmune spleen cells exerted a significantly greater antitumor effect than was observed with CY alone; that spleen cells immune to host isoantigens were no more effective than nonimmune cells; but that preimmunization of the spleen cell donors with murine sarcoma virus (Moloney), or LSTRA rendered their cells significantly more immunotherapeutically effective in the CY-treated tumor-bearing hosts.

INTRODUCTION

Clinically detectable tumors, are, with rare exceptions (1, 10), not susceptible to eradication by immune lymphocytes alone. However, in several tumor models, lymphoid cells were quite effective in inhibiting or eradicating disseminated antigenic tumors if used as an adjunct to sublethal noncurative chemotherapy, provided that the drug had a potent antitumor effect and that the lymphoid cells were viable and immune to TAA3 (6-8, 12, 17, 18, 23, 27).

The most extensively studied model involved the treatment of a BALB/c Moloney lymphoma with sublethal CY plus spleen cells from syngeneic (11), hemisyngeneic F1 hybrid (17), or allogeneic but H-2-compatible mice (11, 18) preimmunized against TAA by exposure either to a Moloney lymphoma of C57BL/6 origin or to an antigenically-related MSV. All of the studies highlighted the critical importance of lymphoid cells specifically immune to TAA.

Application of this adoptive chemoimmunotherapy approach to H-2-incompatible donor-host combinations can be potentially complicated by GVH reactions which, when severe enough to be fatal, have an antitumor effect detectable by bioassay or autopsy (2-5, 21, 25, 26). The aim of this study was to determine whether, as an adjunct to CY, H-2-incompatible lymphoid cells in numbers that would not induce clinically evident GVH disease would exert a significant antitumor effect and whether clinically significant antitumor specificity might be imparted to any subclinical GVH reaction by the preimmunization of spleen cell donors against normal host tissue antigens or TAA.

MATERIALS AND METHODS

Mice. Adult BALB/c (H-2d) and C57BL/6 (H-2b) mice were obtained from the production colonies of Texas Inbred Mice, Houston, Texas.

Virus. A pool of MSV, designated RP 114, was kindly provided by Dr. John B. Moloney (National Cancer Institute, Bethesda, Md.). Tumors were induced with this pool in adult BALB/c mice and were removed on Day 10 when they contained abundant MSV (14). Virus was extracted by differential ultracentrifugation (1 ml of extract per g tumor) (24). The resultant pool, designated RP-114-S-1, was stored at −70° in 0.3-ml aliquots in heat-sealed vials and was used for all experiments.

Immunization with MSV. Adult mice were inoculated with 0.05 ml of a 10−1 dilution of MSV i.m. into the right hind limb. As previously reported with the same MSV-M pool (9), tumors developed within 1 week and regressed within 2 to 3 weeks. One to 3 weeks after regression, the mice were reinoculated i.m. with the same amount of MSV and did not develop tumors. Most donors were immunized with a 3rd dose of MSV 2 to 4 weeks after the 2nd one, and some received a 4th dose. Spleens were obtained and used 2 to 3 weeks after the last MSV inoculation. The immunization scheme induces resistance to histocompatible Moloney lymphoma cells (13) and renders donor lymphoid cells effective for chemoimmunotherapy (11).

Tumor. The target for chemoimmunotherapy was an ascitic lymphoma, designated LSTRA, which was induced in a newborn BALB/c mouse with MLV and which has been serially transplanted in adult BALB/c mice for >300 genera-

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3The abbreviations used are: TAA, tumor-associated antigens; CY, cyclophosphamide; MSV, murine sarcoma virus (Moloney); GVH, graft-versus-host; MLV, murine leukemia virus.

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642 mice thus treated or left totally untreated were dead by Day 13. CY alone prolonged the median survival time to Day 25, with 1 mouse surviving inexplicably beyond Day 100.

The results of the 4 experiments performed were reproducible and are pooled and summarized in Chart 1. Treatment with immune spleen cells but no CY had no effect, so that all 48 mice thus treated or left totally untreated were dead by Day 13. CY alone prolonged the median survival time to Day 25, with 1 mouse surviving inexplicably beyond Day 100.

DISCUSSION

The application of adoptive chemoimmunotherapy to grossly histoincompatible donor-host combinations would be fraught with and complicated by the problem of a GVH reaction, which, in turn, may have a role in tumor therapy. Unfortunately, the GVH reaction as an antitumor reaction usually has been studied under conditions of fatal GVH disease, so that its antitumor effect has been demonstrated by bioassay or by postmortem examination rather than by long-term tumor-free survivors (2–5, 21, 25, 26). Such studies have shown that fatal GVH disease induced by nonimmune H-2-incompatible lymphoid cells did, in fact, inhibit or eradicate lymphoid tumors (2–5, 21, 25) but exerted little effect on primary Moloney sarcomas (9) or on mammary...
compatible spleen cells were used, but significant GVH disease was avoided by the use of H-2-compatible DBA/2 donors. The results showed that treatment with CY plus spleen cells from DBA/2 mice that were nonimmune, or immune only to host isoantigens, was no more effective than was treatment with CY alone, whereas treatment with CY plus DBA spleen cells immune to MSV was far more effective. In the present study, H-2-incompatible spleen cells were used, but significant GVH disease was avoided by the use of fewer cells than would be required to cause fatal GVH disease under comparable conditions (15).

The results of these studies, in contrast to those obtained with H-2-compatible cells, showed that administration of nonimmune H-2-incompatible spleen cells in conjunction with CY was indeed associated with a greater antitumor effect than that observed with CY alone, as reflected by significantly more long-term survivors. With CY, spleen cells from donors preimmunized against host isoantigens were also effective, but no more so than nonimmune cells. However, as in all previous studies, adoptive chemoimmunotherapy with cells preimmunized to TAA was most effective.

Although none of the pools of the C57BL/6 spleen cells induced grossly detectable GVH disease in tumor-bearing or non-tumor-bearing CY-treated BALB/c controls, it is likely that a subclinical GVH reaction did occur and was by itself immunotherapeutically effective even when induced by nonimmune cells or cells immune to normal host isoantigens. However, it is probable that the postulated GVH reaction was immunotherapeutically even more effective against the tumor cells when the GVH reaction was induced by cells specifically immune to TAA.

The specificity of immunization must be qualified by the fact that immunization with LSTRA cells would of course immunize against host isoantigens, Moloney antigens, and any non-Moloney antigens that the tumor might have acquired in the course of its long transplantation history, and that, although the critical immunogen in the MSV is presumed to be the cellular and/or virion tumor-specific antigens present on the primary tumors induced by MSV in the C57BL/6 mice, the MSV used may certainly have carried with it some normal BALB/c isoantigens. However, the therapeutic efficacy of the cells preimmunized with MSV cannot be attributed totally to possible immunization with BALB/c isoantigens because of the relatively lesser efficacy of cells immune only to host isoantigens present on normal BALB/c cells or on an unrelated BALB/c tumor.

The results of the γ-globulin allotype studies presented suggest that a permanent chimerism was not necessary, since no correlation was observed between the titer or duration of donor allotype in host serum and the fate of the mouse with regard to the tumor. The possibility remains, however, as suggested in other studies (9), that the donor cells must persist for some indeterminate period of time, perhaps 2 weeks or more, in order to be immunotherapeutically effective against the tumors.

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REFERENCES

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