Prevention of Therapeutically Induced Regression of Sarcoma 180 by Immunologic Enhancement

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SUMMARY

The increased incidence of regression of Sarcoma 180 observed in Swiss HaICR mice fed vitamin B6-deficient diet or treated with 6-mercaptopurine or kethoxal-bis(thiosemicarbazone) was greatly reduced by active immunization of the host with frozen-thawed Sarcoma 180 homogenate or by passive transfer of specific hyperimmune serum. Similar results were obtained in C57BL/6 mice implanted with a subline of Sarcoma 180 resistant to vitamin B6 deficiency and treated with the 2 drugs. In vitamin B6-deficient Swiss mice, immunization with frozen-thawed homogenate heated at 57°C for 30 minutes or with homogenates of DBA/2Ha-DD spleen, thymus and liver was also effective in reducing the incidence of regression of Sarcoma 180. Active immunization with homogenates of Walker carcinosarcoma 256 had no effect.

In contrast to the reduction of the incidence of regression mentioned, neither active nor passive immunization altered the growth inhibition of the tumors caused by the therapeutic treatments.

The evidence reported here indicates that active and passive immunologic enhancement of tumor growth can reduce the ultimate therapeutic effects of selective antitumor treatments.

INTRODUCTION

S-180 grows progressively in 80-100% of Swiss HaICR mice. In this host the incidence of regression of the tumor is not increased even after most of the treatments that impair tumor growth effectively (9). Therefore, the increased incidence of complete regressions of S-180 observed in Swiss mice fed vitamin B6-deficient diet (11), or treated with 6-MP (2) or KTS (13) is of particular interest. The fact that the incidence of the therapeutically induced regressions was greatly reduced in neonatally thymectomized mice (5, 6) strongly suggests that the regressions of the therapeutically impaired tumor were actually brought about by immunologic mechanisms. Data obtained in neonatally thymectomized mice, however, did not provide conclusive information on the nature of these mechanisms.

Immunologic enhancement of the growth of S-180 can be induced actively or passively in AKR mice (3, 4). Since immunologic enhancement is a specific phenomenon mediated by humoral antibody (7), it was of interest to see whether the regressions of S-180 elicited in Swiss HaICR mice by therapeutic means could be prevented by active and passive immunization. The observation of such a prevention would be consistent with the hypothesis that the effects of selective therapeutic treatments on S-180 can be influenced by an alteration of the balance between enhancing antibody and cellular immunity (9).

The enhancement of S-180 was studied in Swiss HaICR mice treated with 6-MP or KTS, or fed vitamin B6-deficient diet. The enhancement of S-180/B6 (12) was studied in C57BL/6 mice in which this tumor responds to the effects of 6-MP and KTS better than in Swiss mice (unpublished data). The regressions of S-180 induced by feeding vitamin B6-deficient diet, and those of both S-180 and S-180/B6 induced by 6-MP and KTS, were greatly reduced by active or passive immunization of the host.

MATERIALS AND METHODS

The solid form of S-180 and the subline S-180/B6 (12) are maintained in this laboratory by serial transplantation in female Swiss HaICR mice fed a complete or a vitamin B6-deficient diet respectively. Standard trocar and caliper technics were used for the s.c. transplantation and the measurements of the tumors (11). The female Swiss HaICR and C57BL/6 mice were obtained from the Roswell Park Memorial Institute breeding colony and were used when 4-8 weeks old.

The composition of the purified diets fed was reported previously (13). KTS was obtained from Dr. H. G. Petering, the Upjohn Company (Present address: Kettering Laboratories, Dept. of Environmental Health, University of Cincinnati, Ohio) and was fed mixed in the complete purified diet. Aqueous solutions of 6-MP were prepared immediately before use and were injected i.p. once daily. Drug treatments were started the day after tumor implantation and were continued for 7 con...
J. F. Ferrer and E. Mihich

progressed completely in each of the surviving mice. Tumor measurements were performed on the 8th day and once weekly thereafter until the tumors had regressed completely in each of the surviving mice.

FTH was prepared by homogenizing tumor fragments free of necrotic parts in a blender in saline. The preparation was then diluted with saline to the concentration of 20% wet tumor weight. The final homogenate was frozen and thawed 3 consecutive times. The immunization was carried out by alternative s.c. and i.p. injections of 0.2 ml of FTH, 3-4 times a week during a 2-week period. The tumor was implanted 10-15 days after the end of the immunization. Hyperimmune serum was then diluted with saline to the concentration of 20% wet tumor weight. The preparation was obtained in Swiss HaICR or C57BL/6 mice in which S-180 or S-180/B6 had regressed 3 times, once following therapeutic treatments and twice following consecutive transplantations performed at 2-week intervals. Blood was collected by cutting the carotid artery under nembutal anesthesia 10-15 days after the last tumor implantation. The sera were injected i.p. 4 hours before the s.c. implantation of the test tumors.

RESULTS

Effect of Immunization with S-180 Frozen-thawed Homogenates. The data summarized in Chart 1 indicate that the incidence of regression of S-180 in Swiss HaICR mice fed vitamin B6-deficient diet was greatly reduced by the immunization of the host with FTH. The reduction was greater when the total immunizing dose of FTH was 200-240 mg wet tumor weight per mouse than when it was 280-320 mg.

In 2 combined representative experiments, the growth patterns of S-180 were not altered by the immunization in mice fed the complete or the vitamin B6-deficient diet (Chart 2). The initial impairment of growth of S-180 was the same in mice fed vitamin B6-deficient diet regardless of the ultimate fate of the tumor. The differences between average diameters of the regressing tumors were not significant. Only at the end of the second week and later, the tumors growing progressively reached a size significantly larger than that of the regressing tumors.

In other experiments, FTH was heated at 57°C for 30 minutes in order to destroy the activity of virus possibly present in it (14). As shown in Chart 3, heated FTH was still capable of inducing a reduction of the incidence of regression of S-180. No significant reduction of the incidence of regression occurred in mice treated with homogenates of Walker carcinosarcoma 256. In contrast, immunization with homogenates prepared from liver, spleen and thymus of DBA/2Ha-DD (intense brown coat color) mice caused a significant reduction of the incidence of tumor regression. This reduction was not as marked as that caused by FTH, however. It should be noted that both S-180 and DBA/2Ha-DD mice share antigenic components 4 and 13 of the complex specified by the H-2d allele (T. S. Hauschka, personal communication).

As shown in Chart 4, the increase of the incidence of complete regression of S-180 and S-180/B6 caused by KTS and 6-MP was prevented by the immunization of the hosts with FTH. Since FTH prepared from S-180 or S-180/B6 was equally effective in reducing the incidence of regression of S-180/B6 caused by the 2 drugs, the data obtained with the 2 homogenates were grouped together. Data not presented indicated that, similarly to the results shown in Chart 2, the growth pattern of the tumors was not affected by the immunization in mice treated with 6-MP or KTS.

Effects of Hyperimmune Serum. The results summarized in Chart 5 indicate that the i.p. administration of hyperimmune serum prior to the implantation of S-180 reduced the tumor regression elicited by 6-MP, KTS and vitamin B6 deficiency. In each case different amounts of serum were required to cause this effect at a statistically significant level (see legend Chart 5). In mice given 6-MP or fed vitamin B6-deficient diet a direct relationship was apparent between serum dose and effect. Since no difference was noted between mice which were not injected with serum and those which were given normal serum, the corresponding data were pooled for presentation in the chart. It should be mentioned that, in contrast with the highly significant reduction of tumor regression ($P < 0.001$) seen in the 3 experiments performed with vitamin B6-deficient mice which are summarized in Chart 5; in 2 other experiments no passive enhancement could be obtained under comparable experimental conditions. This inconsistency was at variance with the reproducible results obtained in mice treated with 6-MP and KTS.

In 2 combined representative experiments the growth of S-180 in mice treated with KTS was not altered by the passive immunization with specific hyperimmune serum (Chart 6). Thus these data were similar to those shown in Chart 2. Comparable results were obtained in mice treated with 6-MP or fed vitamin B6-deficient diet.

As shown in part A of Chart 7, the incidence of regression of S-180/B6 caused by KTS or 6-MP in C57BL/6 mice was also greatly reduced by the administration of appropriate amounts of hyperimmune serum.

Chart 1. Prevention of the regression of Sarcoma 180 in Swiss HaICR mice fed vitamin B6-deficient diet (---B6) and immunized with frozen-thawed S-180 homogenates (FTH). C, Complete diet. The 95% confidence limits for each value are shown by the vertical lines. The probability value of differences was less than 0.1% between groups C and ---B6, ---B6 and [---B6 + FTH], C and [---B6 + FTH 280-320 mg]. The difference between groups C and [---B6 + FTH 200-240 mg] was not significant at the 5% level.
Chart 2. Growth of Sarcoma 180 in Swiss HaICR mice fed vitamin B<sub>6</sub>-deficient diet (–B<sub>6</sub>) and immunized with frozen-thawed S-180 homogenates (FTH). C, Complete diet. Numbers at each point indicate the number of surviving mice. Within each experimental group the animals were divided into 2 groups depending upon whether their tumor regressed or not.

Chart 3. Specificity of the prevention of the regression of Sarcoma 180 in Swiss HaICR mice fed vitamin B<sub>6</sub>-deficient diet (–B<sub>6</sub>) by active immunization. C, Complete diet. W256, frozen-thawed homogenate prepared from Walker carcinosarcoma 256. DBA/2Ha-DD, frozen-thawed homogenate prepared from liver, spleen and thymus of DBA/2Ha-DD mice. The confidence limits for each value are shown by the vertical lines. The probability value of differences was less than 0.1% between groups C and –B<sub>6</sub>, –B<sub>6</sub> and [–B<sub>6</sub> + FTH], –B<sub>6</sub> and [–B<sub>6</sub> + heated FTH], C and [–B<sub>6</sub> + W256], –B<sub>6</sub> and [–B<sub>6</sub> + DBA/2Ha-DD]. This value was less than 1% between groups [–B<sub>6</sub> + FTH] and [–B<sub>6</sub> + heated FTH]. The difference between groups [–B<sub>6</sub> + FTH] and [–B<sub>6</sub> + heated FTH], –B<sub>6</sub> and [–B<sub>6</sub> + W256], was not significant at the 5% level.

Chart 4. Prevention of the chemotherapeutically induced regression of S-180 and of S-180/B<sub>6</sub> by immunization with frozen-thawed S-180 homogenates (FTH). Kethoxal-bis(thiosemicarbazone) (KTS) was fed mixed in the diet at the levels indicated. 6-Mercaptopurine (6-MP) was injected i.p. once daily at the doses indicated (mkd, mg/kg/day). Treatments were continued for 7 days starting the day after tumor implantation. The 95% confidence limits for each value are shown by the vertical lines. The probability value of differences was less than 0.1% between groups KTS and [KTS -F FTH], None and 6-MP, and [6-MP - FTH], None and KTS 0.05%. This value was less than 5% between groups None and KTS 0.1%, None and [KTS 0.05% - FTH]. The difference between groups None and [6-MP + FTH], None and [KTS 0.05% + FTH], was not significant at the 5% level.
DISCUSSION

Sarcoma 180 is not a strain-specific tumor and thus results of studies on this tumor cannot contribute directly to basic knowledge in the area of immunity directed against so-called tumor-specific antigens (5, 9). Nevertheless, when S-180 is implanted in Swiss HaICR mice, it grows progressively in 80–100% of the cases, even after most of the therapeutic treat-

ments that impair initial tumor growth effectively (9). Therefore, this tumor-host system provides a model useful in the study of the interrelationships between tumor therapy and immunity under circumstances in which the homograft reaction elicited by the tumor is ineffective (10). Knowledge gained in the study of such interrelationships may be potentially important in cancer chemotherapy.

The data reported indicate that both active immunization with FTH and passive transfer of specific antisera can reduce the incidence of regression of S-180 and of S-180/B6 caused in Swiss HaICR and C57BL/6 mice by selective therapeutic treatments.

The observation that homogenates obtained from Walker carcinosarcoma 256 did not reduce the incidence of regression of S-180 in vitamin B6-deficient Swiss mice suggested that the effects of active immunization with FTH are specific and not due to a general impairment of the immune response. Moreover, the fact that the reduction of tumor regression was caused also by FTH heated at 57°C for 30 minutes suggests that a virus, shown to occur in S-180 (14), is not responsible for the effects of FTH. Indeed, it was demonstrated that this agent is destroyed by heating procedures similar to those applied in this study (14).

The observation that homogenates of normal DBA/2Ha-DD tissues also reduced the incidence of regression of S-180 in vitamin B6-deficient mice is consistent with the fact that both DBA/2Ha-DD mice and S-180 share some antigenic components specified by the H-2a allele. It is known that immunologic enhancement of a tumor can be induced by immunization with normal tissue of the same genotype (8).

Passive transfer of specific antisera caused effects similar to those of active immunization with FTH. Moreover, passive enhancement of S-180/B6 was obtained with serum from mice actively immunized with FTH. Since immunologic tolerance cannot be transferred by humoral antibody, these data further indicate that immunologic enhancement is at the basis of the reductions of the incidence of tumor regression described in this report.

The fact that a mechanism as specific as immunologic enhancement can counteract the therapeutic effects of vitamin B6 deficiency, 6-MP, and KTS is consistent with the observation that these therapeutic effects are greatly reduced in neonatally thymectomized mice (5, 6), and indicate that specific immunologic mechanisms are responsible for the regression of S-180 observed.

It is of interest that both in mice actively or passively immunized (Charts 2, 6) and in neonatally thymectomized animals (5, 6) the initial growth of the tumors was not altered in relation to the immunologic status of the host, and that the initial tumor growth inhibition was dependent solely upon the therapeutic treatment, irrespective of the ultimate fate of the tumor. Two weeks after implantation or later, however, the rate of S-180 growth was increased as a result of neonatal thymectomy (5, 6), whereas it was not increased as a result of immunologic enhancement. This difference may be related to the fact that in the intact mice in which immunologic enhancement was elicited, but presumably not in the immunologically incompetent neonatally thymectomized mice, the growth rate
Chart 6. Growth of Sarcoma 180 in Swiss HaICR mice treated with kethoxal-bis(thiosemicarbazone) (KTS) and injected with 0.3 ml of normal serum (NS) or hyperimmune serum (HS) 4 hours prior to tumor implantation. Numbers at each point indicate the number of surviving mice. Within each experimental group the animals were divided into 2 groups depending upon whether their tumor regressed or not.

...of the tumor was still under some influence of the cellular defenses of the host. A similar explanation may also apply to the observation that the growth rate of tumors in untreated mice was unaltered by immunologic enhancement (Charts 2, 6), whereas it was increased in neonatally thymectomized mice (5, 6). Thus the growth rate of established S-180 may be influenced by changes in the balance between cellular defenses and immunologic enhancement.

Of the 3 treatments studied, vitamin B6 deficiency was the least sensitive to passive enhancement. Moreover, this phenomenon could not be elicited consistently in mice fed this diet. Since it is known that this vitamin depletion markedly inhibits humoral antibodies (1), it seems possible that, under the experimental conditions of this study, in contrast with 6-MP and KTS, vitamin B6 deficiency affects the production of humoral antibodies which possibly cause active enhancement also in mice given hyperimmune serum. Indeed it was shown that in AKR mice active as well as passive enhancement was reduced after splenectomy, namely under conditions of reduced humoral antibody response (3).

The data presented in this report further support the conclusion that two phenomena are involved in the overall therapeutic effects caused by the treatments studied: the primary selective inhibition of the growth of the tumor by the treatments themselves, which is evident during the 1st week after implantation, and the secondary regression of the impaired tumor, brought about by the specific immunologic response of the host which becomes apparent 2 weeks after implantation or later (9). Moreover, these observations are consistent with the concept that synergism can occur between selective therapeutic treatments and immunity directed against transplantable tumors. They are also consistent with the hypothesis that immunologic enhancement may play a role in determining the progressive growth of certain transplantable tumors and may counteract the effects of some antitumor treatments. Conversely, as was suggested previously in the case of vitamin B6 deficiency (9), certain therapeutic treatments may reduce the immunologic enhancement of the tumor. Thus the effectiveness of an antitumor agent may depend not only upon its selectivity of action on tumor cells, but also upon its selective effects on the complex balances between opposite immunologic actions influencing tumor growth.

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REFERENCES

Chart 7. Prevention of chemotherapeutically induced regression of S-180/B6 in C57BL/6 mice by the administration of hyperimmune serum (HS) or immune serum. 6-Mercaptopurine (6-MP) was injected i.p. once daily at the doses indicated (mkd, mg/kg/day). Kethoxal-bis(thiosemicarbazone) (KTS) was fed mixed in the diet at the levels indicated. Drug treatments were given for 7 days starting the day after tumor implantation. Serum was injected 4 hours prior to tumor implantation. The 95% confidence limits for each value are shown by the vertical lines. In part A the probability value of differences was less than 0.1% between groups None and KTS, KTS and [KTS + HS], None and 6-MP. This value was less than 2% between groups 6-MP and [6-MP + HS]. The difference between groups None and [KTS + HS], None and [6-MP + HS], was not significant at the 5% level. In part B the probability value was less than 0.1% between groups None and KTS, KTS and [KTS + serum from donors immunized with 240 mg FTH and implanted with S-180/B6]. This value was less than 1% between groups KTS and [KTS + serum from donors only implanted with S-180/B6].

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