Relationship Between Tumor Incompatibility and Therapeutic Response.* (32459)

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Recent studies have indicated that the incidence of regression of sarcoma 180 (S-180) observed in Swiss HaICR mice fed vitamin B_6 deficient diets or treated with either 6-mercaptopurine (6MP) or kethoxal-bis-thiosemicarbazone (KTS) is reduced to levels seen in untreated controls in animals which had been neonatally thymectomized(1,2). The present investigation was carried out in order to clarify whether the therapeutically induced regression of so-called non-specific tumors is related to the intensity of the immunological response elicited by the tumor in the host. The occurrence of such a relationship has been suggested by others in the case of S-180(3).

The antigenicity of S-180, a subline capable of growing progressively in vitamin B_6 deficient Swiss mice (S-180/B_6), a subline resistant to 6MP (S-180/MP) and of Ehrlich carcinoma ascites (ECA) was determined by transplantation into various strains of mice. The effects of 6MP, KTS, and dietary vitamin B_6 deficiency on S-180/B_6 and ECA were determined in mice exhibiting different degrees of resistance to these tumors. The results obtained indicate that the effect of these treatments on tumor growth and regression could be correlated with the degree of the host response to the tumor.

Materials and methods. The female mice used in this study were obtained from the breeding colony of the Roswell Park Memorial Institute. Sarcoma 180, S-180/MP,‡ and S-180/B_6(4) were implanted s.c. by standard trocar techniques. ECA was implanted s.c. by the inoculation of 1×10^6 cells per mouse. The growth of the tumors was estimated weekly by a standard caliper technique. Aqueous solutions of 6MP were prepared shortly before use and given i.p. once daily for 7 days. KTS was administered mixed in a purified, complete diet. The composition of the complete and vitamin B_6-deficient diets used were reported previously in detail(5). These diets were fed starting two weeks prior to tumor implantation.

Results. The data in Table I indicate that each of the 4 tumors studied showed a different incidence of regression in 7 strains of mice. The incidence of regression of S-180/MP was similar to that of S-180 in Swiss HaICR, C3Hf/HeHa and AKR mice. The incidence of regression of S-180/B_6 was very low in each strain except for C57Bl/6. No significant rejection of ECA was observed in any of the strains used. To see whether the different regression patterns of S-180/MP and S-180/B_6 was related to a decrease or a loss of S-180 incompatibility properties, these 2 tumors were implanted in mice in which S-180 had previously regressed. Similar tests were performed with the unrelated tumor ECA. These experiments were carried out in Swiss mice and in 3 of the 4 strains which were resistant to S-180.

As shown in Table II, both S-180/B_6 and S-180/MP regressed in a high percentage of cases when implanted in mice of each of the 4 strains used. The incidence of regression of ECA was lower in Swiss HaICR and AKR than in C57Bl/6 and A/St mice.

In order to study the possibility that therapeutic treatments may act synergistically with host defenses, two types of experiments were performed. (A) The therapeutic response of S-180/B_6 to 6MP, KTS or vitamin B_6 deficiency was compared in Swiss HaICR and C57Bl/6 mice, namely in 2 strains of mice showing different responses to S-180 and S-180/B_6 (see Tables I and II). (B) The therapeutic response of ECA to 6MP, KTS or

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TABLE I. Regressions of S-180, S-180/MP, S-180/B_6 and ECA in Different Strains of Mice.

Mouse strain*	S-180 No. Regr./ No. Impl.	S-180 % Regr.	S-180/MP No. Regr./ No. Impl.	S-180/MP % Regr.	S-180/B_6 No. Regr./ No. Impl.	S-180/B_6 % Regr.	ECA No. Regr./ No. Impl.	ECA % Regr.
Swiss HaICR	5/40	13	9/43	21	5/40	13	1/65	2
C 3Hf/HeHa	19/39	49	11/20	55	3/36	8	0/19	0
C 57Bl/6	39/55	70	7/50	14	9/40	24	0/43	0
A/St	17/36	46	3/44	7	0/30	0	0/30	0
DBA/2Ha-DD	0/39	0	0/10	0	0/30	0	0/27	0
DBA/1	0/50	0	0/10	0	0/24	0	0/23	0
AKR	27/30	90	20/23	86	1/30	3	0/46	0

* All mice were obtained from the breeding colony of Roswell Park Memorial Institute. The animals were 8-12 wk old at time of transplantation and were observed until death or complete tumor regression.

TABLE II. Regressions of S-180/MP, S-180/B_6 and ECA in Mice in which S-180 had Regressed Previously.

Mouse Strain*	S-180/MP No. Regr./ No. Impl.	S-180/MP %	S-180/B_6 No. Regr./ No. Impl.	S-180/B_6 %	ECA No. Regr. No. Impl.	ECA %
Swiss HaICR	22/22	100	9/11	82	10/29	34
C 57 Bl/6	21/22	96	17/22	77	16/16	100
A/St	14/14	100	14/14	100	13/17	76
AKR	—	—	12/15	80	8/14	57

* Mice in which S-180 had regressed 2 to 4 weeks previously. In Swiss HaICR mice, S-180 had regressed once after therapeutic treatments and a second time after rechallenge. In the other 3 strains S-180 had regressed once in the absence of treatment.

vitamin B_6 deficiency was compared in Swiss HaICR mice immunized and non-immunized with S-180. For comparative purposes the effects of the 3 treatments were also evaluated on S-180 in Swiss HaICR mice. This tumor regressed in 60 to 90% of vitamin B_6 deficient Swiss mice, in 30 to 50% of mice treated with 6MP at the dose of 25 mg/kg/day and in 40 to 80% of those treated with this drug at the dose of 50 mg/kg/day, and in 30 to 50% of mice treated with KTS at the dietary level of 0.1%. These results were not significantly different from those reported previously(1,2, and 5).

As shown in Chart 1, after treatment with 6MP at the dose of 25 mg/kg/day, the incidence of regression of S-180/B_6 was 14% in Swiss HaICR mice and 51% in C57Bl/6 mice. At the dose of 50 mg/kg/day the drug caused tumor regression in 28% of Swiss mice and was toxic in C57Bl/6 (not shown). Also when KTS was used to impair the growth of the tumor, the incidence of drug-induced regression was higher in C57Bl/6 than in Swiss mice. Data not shown indicated that the incidence of regression of S-180/B_6 was not significantly increased in Swiss HaICR, C57Bl/6, A/St and AKR mice which were fed the vitamin B_6 deficient diet starting 2 weeks prior to implantation.

The data summarized in Chart 2 indicate that ECA does not regress in Swiss HaICR mice treated with 6MP which had not been exposed to S-180. In animals in which S-180 had previously regressed once or twice, the incidence of regression of ECA was significantly increased after 6MP treatments. Similarly, a significant increase of the incidence of tumor regression was seen in vitamin B_6-deficient mice in which S-180 had previously regressed once. In contrast to the results obtained in mice treated with 6MP or fed a vitamin B_6 deficient diet, no increase of incidence of regression was seen after administration of KTS.

Discussion. The histocompatibility characteristics of S-180, S-180/MP, S-180/B_6 and ECA were studied by the transplantation technique in 7 different strains of mice. The data suggested that in general S-180/MP and S-180/B_6 were more compatible than the parent S-180. The compatibility of ECA was

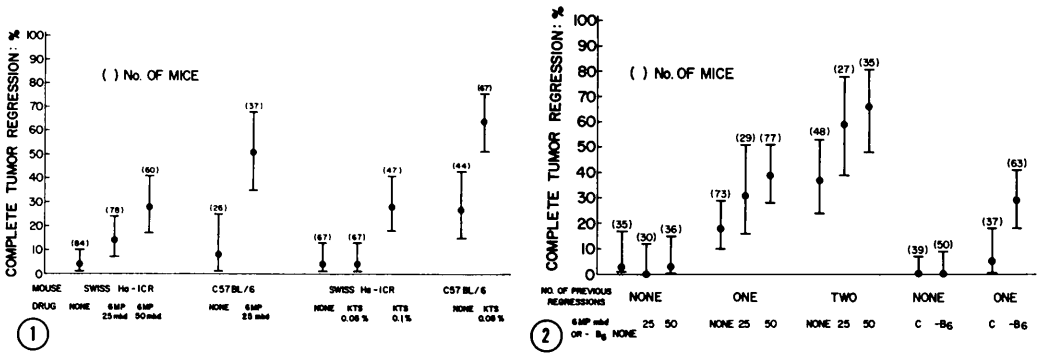

Chart 1. Incidence of regression of S-180/B_6 after therapeutic treatments in Swiss HaICR and in C57 Bl/6 mice. The 95% confidence limits are shown by vertical lines. Differences between groups Control and 6MP, Control and KTS 0.05 in C57Bl/6, Control and KTS 0.1 in Swiss HaICR were significant at the 1-0.1% level.

Chart 2. Incidence of regression of ECA induced by 6MP and vitamin B_6 deficiency in Swiss HaICR mice immunized with S-180. The 95% confidence limits are shown by the vertical lines. Differences between groups 1 × R and [1 × R 6MP 50], 2 × R and [2 × R 6MP 50], [1 × R 6MP 50] and [2 × R 6MP 50], 1 × R and [1 × R-B_6] were significant at the 5-1% level.

clearly less than that of S-180. Transplantation experiments performed in mice, in which S-180 had previously regressed, suggested that both S-180/MP and S-180/B_6 as well as ECA have antigenic properties in common or cross-reacting with S-180. The question was asked whether the effectiveness of therapeutic treatments can be influenced by the extent of the host defenses to the tumor. The possibility was investigated that S-180/B_6 may be more sensitive to 6MP, KTS, or dietary depletion of vitamin B_6, in a mouse strain, other than the Swiss HaICR, in which this tumor might elicit a stronger host response. Similarly, the possibility was tested that the incidence of regression of ECA might be increased by the three treatments under study in Swiss mice in which S-180 had regressed once, or twice, namely in animals exhibiting a stronger response to the tumor than those not previously exposed to S-180.

The data obtained indicate that the effects of 6MP and KTS on S-180/B_6 were greater in C57Bl/6 than in Swiss mice. Similarly the effects of 6MP and vitamin B_6 deficiency on ECA were significantly greater in hosts exhibiting a stronger response to the tumor. In the case of S-180/B_6, it should be noted, however, that possible metabolic differences between the two mouse strains may be as important as differences in histocompatibility or RES function in determining differences of effect of therapeutic treatments. This may be particularly so in the case of KTS, a drug known to be dependent in its action upon metabolic factors such as dietary levels of trace metals(6). This reservation is not valid, however, for the experiments with ECA. In fact, in this case the same mouse strain was studied and the only variable was the degree of reactivity of the host. The data indicated clearly that the effects of 6MP and vitamin B_6 deficiency on ECA became evident only in hosts that had rejected S-180.

The data presented in this report indicate that resistance to therapeutic treatments may be modified under conditions in which the seemingly resistant transplantable tumor is subjected to both the therapeutic treatment and a more effective response of the defenses of the host. In this sense, the results of this study also indicate that synergism can occur between antitumor chemotherapy and host defenses directed against the tumor, in further support of data reported previously(1,2,7).

Summary. A comparison of the histocompatibility characteristics of S-180, S-180/MP, S-180/B_6 and ECA was carried out by transplantation techniques in 7 strains of mice. S-180/MP and S-180/B_6 appeared to be less immunogenic than S-180; ECA was less immunogenic than the two S-180 sublines. S-180/MP, S-180/B_6 and ECA appeared to have antigenic properties in common with S-

180. The therapeutic effects of 6MP and KTS against S-180/B_6 were greater in C57Bl/6 than in Swiss HaICR mice. The effects of 6MP and vitamin B_6 deficiency against ECA were evident only in Swiss HaICR sensitized to S-180. Thus, in this case, the effectiveness of therapeutic treatments was influenced by the degree of the host response to the tumor.

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Improvement of Human Growth Hormone Immunoassay Using ^{125}I.* (32460)

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Immunoassay of circulating human growth hormone (HGH) utilizing HGH-^{131}I as tracer has already been reported(1-5). Morgan(6) has also reported a radio-immunoassay for HGH using ^{125}I but the sensitivity appeared to be insufficient. The present report describes a radio-immunoassay technique utilizing a double antibody procedure and HGH-^{125}I which provides a high degree of sensitivity and permits determinations of HGH in unextracted small plasma samples.

Materials and methods. Antiserum was produced in rabbits by repeated administration of Raben's preparation of HGH(7) emulsified in complete Freund's adjuvant. Anti-rabbit gamma globulin serum was obtained from goat and was prepared commercially.‡ A highly purified preparation of HGH (Wilhelmi HS 612 A) served as standard and for the iodination procedure. Phosphate buffer (0.05 M, pH 7.4) containing 2‰ of bovine serum albumin was used as diluent both for the HGH-^{125}I and the antisera. Dilutions of the standards were made in bovine serum.

Preparation of HGH-^{125}I. HGH was labelled with ^{125}I§ by the method of Greenwood *et al*(8) (1.5-2.5 mC ^{125}I/20-30 μl; 50 μl phosphate buffer (0.5 M, pH 7.4); 2-4 μg HGH/20-40 μl; 100 μg Chloramine-T/25 μl; 250 μg $Na_2S_2O_5$/100 μl; 50 μl normal human serum; 1 mg KI/100 μl). HGH, Chloramine-T, $Na_2S_2O_5$ and KI were diluted in phosphate buffer (0.05 M, pH 7.4). After removal of unreacted ^{125}I on a Sephadex G-50 column (10 × 1 cm), the HGH-^{125}I was purified by chromatography on a column of Sephadex G-200 (50 × 2 cm). The first peak of radioactivity corresponded to damaged products and the second peak to "pure" labelled hormone (Fig. 1.). The effect of reaction times of Chloramine-T, ranging from 5 to 60 seconds, on the specific activity and the purity of HGH-^{125}I has been analyzed (Table I). In general, the reaction was stopped after 30-40 seconds because longer times appeared to increase the amount of radioactive impurities. For 15 iodination procedures at 8 different times (Table I), the estimated mean

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