Immunological Depression of Tumor Growth in F₁ Hybrid/Parental Strain Systems*

HANS WIGZELL

(Institute for Tumor Biology, Karolinska Institutet Medical School, Stockholm, Sweden)

SUMMARY

Evidence has been presented indicating that the intraperitoneal injection of lymphoid cells of one parental strain into F₁ hybrid mice inhibits the growth of lymphoma cells of the opposite parental strain inoculated subcutaneously. The lymphoid cells were either preimmunized against the other parental strain and inoculated simultaneously with the tumor cells or inoculated, untreated, 5 days before tumor inoculation. In contrast to the lymphoma inhibition, when the tested tumor was a sarcoma or a carcinoma the inhibition was very slight, and in these groups all mice died with tumors. The inhibition with lymphoid cells was obtained when the number of lymphoma cells varied between 10⁵ and 10⁶ cells. The inhibition was specific, because when the lymphoma and the lymphoid cells were derived from the same parental strain, the tumor inhibition was very slight and probably due to the wasted condition of the host animals. In a similar system with skin grafts, as with the carcinomas and the sarcomas but not the lymphomas, the lymphoid cells of the opposite parental strain failed to bring about the rejection of skin grafts of the other parental strain. It is proposed that the inhibition of the lymphomas is due to their unusual sensitivity toward the action of humoral antibodies as compared with the sensitivity of carcinomas and sarcomas.

F₁ hybrids between homozygous strains of mice are universal recipients of grafts from both parental strains. This is due to the fact that the F₁ hybrid contains the genomes of both parental strains in a single dose and that all histocompatibility genes found in the mouse are expressed. Injected parental lymphoid cells can react against the foreign isoantigens of the F₁ hybrid cells derived from the opposite parent and determined by those alleles of the histocompatibility loci which are different in the two strains. If a sufficient number of reactive parental lymphoid cells are injected into an F₁ hybrid, it may succumb due to the graft-versus-host reaction (24). The mortality risk decreases with increasing age of the F₁ hybrid recipient.

Lymph node cells of a homozygous parental strain have been found to be more sensitive than heterozygous F₁ hybrid cells to the in vitro toxic action of an isoantiserum obtained by immunizing animals of the other parental strain (13). In vivo experiments of Simonsen (22) showed a similar gene-dose effect with regard to the severity of the graft-versus-host reaction measured by spleen enlargement.

The experiments described in the present paper were designed to explore the possibility of influencing transplanted isologous tumor cells on the basis of this knowledge. The results obtained may serve as a model of what might possibly be achieved in particularly suitable autologous systems where an antigenic difference between tumor and primary host has actually been demonstrated (16). Young F₁ hybrids were given injections subcutaneously of tumor cells derived from one of the parental strains and intraperitoneally with spleen and lymph node cells of the other parental strain. The age of the F₁ hybrids used varied between 1 and 8 months, and their weight always exceeded 10 gm. in order to diminish the mortality due to the graft-versus-host reaction. The reason that F₁ hybrids survive the graft-versus-host reaction of the paren-

* This work has been supported by grants to Prof. G. Klein from the Swedish Cancer Society and from the National Cancer Institute, U.S. Public Health Service (C-3700).

Received for publication September 19, 1960.
tal lymphoid cells is not clear, but it is known that the injected parental lymphoid cells finally die out (7).

MATERIALS AND METHODS

The mice used belonged to the inbred strains A/Sn, C3H, DBA/2, and one isogenic resistant (IR) subline of A/Sn—namely, A.SW and their F1 hybrids (23). The age of the F1 hybrids used varied between 1 and 3 months. All strains had been maintained by continuous brother-sister mating in single lines. The mice were kept on a standard diet in pellet form with drinking water and pellets available ad libitum.

The term "parental lymphoid cells" refers to a suspension of spleen and lymph node cells derived from mice of one parental strain that had been given inoculations intraperitoneally of spleen cells of the other parental strain 5 days previously. The lymphoid cells and the tumor cells were injected simultaneously. In some experiments parental lymphoid cells from untreated donors were used. The lymphoid cells were then injected into the F1 hybrids 5 days before tumor inoculation in order to permit them to become sensitized. Essentially the same results were obtained with both procedures.

The cell suspensions were obtained by pressing the solid tissues through a 60-mesh stainless steel screen into Ringer's solution containing 100 I.U. of penicillin and 100 μg of streptomycin/ml. The approximate number of viable cells was estimated by the eosin method (21). The skin grafting was done according to Medawar (6).

Tumors.—The following tumors were used: (a) Four lymphomas: DBA ascites lymphoma, lymphoid leukemia LI10, and the lymphomas WL3 and WL4. The DBA ascites lymphoma originated in 1947 and L1210 in 1948 in the DBA/2 strain. They have been maintained in this laboratory for several years by serial intraperitoneal transfers in DBA/2 mice, interrupted by periods of frozen storage. The lymphomas WL3 and WL4 originated spontaneously in two A.SW mice in 1959. They have been maintained by serial subcutaneous transfers. (b) Two spontaneous mammary carcinomas, SDB1 and SDB2, originating in DBA/2 female mice, were used in their first transplant generation. (c) One methylcholanthrene-induced sarcoma, MWG, originated in an A.SW mouse and was in its first transplant generation when used in the experiments.

In the cases where the tumors had originated in male mice, all experiments were carried out in male mice to avoid influences from sex differences, especially the y-antigen.

RESULTS

Experimental system A.—Fourteen A.SW × DBA/2 F1 hybrids were given injections subcutaneously of 10^8 cells of the DBA ascites lymphoma. Half of the group received at the same time 4 × 10^7 A.SW lymphoid cells preimmunized against DBA/2 I.P. (B) Seven A.SW×DBA/2 F1 hybrid mice given inoculations of 10^8 DBA ascites lymphoma cells S.C. (1) Two animals survived without tumors for an observation period of 95 days.

of the other parental strain 5 days previously. The lymphoid cells and the tumor cells were injected simultaneously. In some experiments parental lymphoid cells from untreated donors were used. The lymphoid cells were then injected into the F1 hybrids 5 days before tumor inoculation in order to permit them to become sensitized. Essentially the same results were obtained with both procedures.

The cell suspensions were obtained by pressing the solid tissues through a 60-mesh stainless steel screen into Ringer's solution containing 100 I.U. of penicillin and 100 μg of streptomycin/ml. The approximate number of viable cells was estimated by the eosin method (21). The skin grafting was done according to Medawar (6).

Tumors.—The following tumors were used: (a) Four lymphomas: DBA ascites lymphoma, lymphoid leukemia LI10, and the lymphomas WL3 and WL4. The DBA ascites lymphoma originated in 1947 and L1210 in 1948 in the DBA/2 strain. They have been maintained in this laboratory for several years by serial intraperitoneal transfers in DBA/2 mice, interrupted by periods of frozen storage. The lymphomas WL3 and WL4 originated spontaneously in two A.SW mice in 1959. They have been maintained by serial subcutaneous transfers. (b) Two spontaneous mammary carcinomas, SDB1 and SDB2, originating in DBA/2 female mice, were used in their first transplant generation. (c) One methylcholanthrene-induced sarcoma, MWG, originated in an A.SW mouse and was in its first transplant generation when used in the experiments.

In the cases where the tumors had originated in male mice, all experiments were carried out in male mice to avoid influences from sex differences, especially the y-antigen.
preceding the appearance of palpable tumors was slightly extended in the group given inoculations of parental lymphoid cells and tumor cells as compared with the control animals receiving only carcinoma cells. All animals died with tumors. In Table 1 data from the experiments with MWG and SDB2 are included.

The graft-versus-host reaction is known to give rise to a complex syndrome (4), and all the animals in the carcinoma and sarcoma experiments given injections of parental lymphoid cells showed the signs of the reaction also expressed by a weight loss. This eliminates the possibility that the slightness or absence of the effect on the mammary carcinoma and sarcoma cells might have been due to the use of inactive parental lymphoid cells.

**Experimental system B.**—It may be questioned whether the effect on the lymphoma cells observed in the experimental system A was due to a specific immunological reaction between the sensitized lymphoid cells and the tumor cells or to a nonspecific tumor-inhibiting effect secondary to the wasting syndrome caused by the graft-versus-host reaction. To differentiate between these possibilities eighteen 2- to 3-month-old ASW X C3H F1 hybrids were divided into three groups. All mice received $2 	imes 10^8$ C3H lymphoid cells intraperitoneally derived from donors immunized against ASW. A second group received the same number of ASW lymphoid cells immunized against C3H. The third group received only the lymphoma cells. Here both groups receiving lymphoid cells can be expected to develop the graft-versus-host reaction, but only in one of these are the lymphoid cells immunogenetically competent to react against the neoplastic cells.

The results are shown in Chart 4. Both groups given injections of parental lymphoid cells developed a graft-versus-host reaction of approximately the same severity, as judged by their weight curves. Some of the mice given injections of ASW lymphoid cells that were not competent to react against the WL3 lymphoma cells showed a slight prolongation of survival time as compared with that of the controls. The mice that received the same number of C3H lymphoid cells competent to react against the lymphoma cells showed a clearly increased survival time, and one mouse never developed a tumor. All mice in the other two groups

---

**Table 1.** Data from the experiments with MWG and SDB2 are included.

```
<table>
<thead>
<tr>
<th>Mortality (No of mice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
</tbody>
</table>
```

**Chart 3.** (A) Mean weight. (B) Number of tumor-bearing animals of seven ASW X DBA/2 F1 hybrid mice given inoculations of $10^8$ cells of a DBA/2 mammary carcinoma S.C. and $3 \times 10^7$ ASW lymphoid cells immunized against DBA/2 I.P. (C) Mean weight. (D) Number of tumor-bearing animals of four ASW X DBA/2 F1 hybrid mice given inoculations of $10^8$ cells of a DBA/2 mammary carcinoma, SDB1, S.C.

**Chart 4.** (A) Six ASW X C3H F1 hybrid mice given inoculations of $10^6$ WL3 lymphoma cells S.C. and $2 \times 10^7$ ASW lymphoid cells immunized against C3H I.P. (B) Six ASW X C3H F1 hybrid mice given inoculations of $10^6$ WL3 lymphoma cells S.C. and $2 \times 10^7$ C3H lymphoid cells immunized against ASW I.P. (C) Six ASW X C3H F1 hybrid mice given inoculations of $10^6$ WL3 lymphoma cells S.C. (1) One animal survived without tumor for an observation period of 105 days.
died with tumors. Additional experiments with the lymphoma WIA confirmed the results obtained with lymphoma WL3 and are included in Table 1.

These results show clearly that the wasting effect of the graft-versus-host reaction cannot explain alone the strong depressing effect of immunologically competent parental lymphoid cells on the lymphoma cells, although it may contribute to it to a minor extent.

Experimental system C.—Skin transplantation is regarded as a sensitive detector of small antigenic differences (1). The skin graft rejection in homokogous systems is probably caused by the cellular component in the homograft reaction, since skin grafts are not rejected in passive immunization experiments (18). In an attempt to find out whether the cellular or the humoral component of the homograft reaction was causing the depressing effect on the lymphoma cells in the experimental system used, skin grafts were used instead of lymphoma cell grafts. Fourteen days after transplantation, when the grafts were all established, all mice received 300 r to increase their sensitivity toward the graft-versus-host reaction (8).

Immediately after irradiation $6 \times 10^7$ lymphoid cells of CSH mice, preimmunized against A cells, were injected intraperitoneally into each mouse. Three weeks after irradiation five out of fourteen mice were dead, owing to the combined effect of irradiation and graft-versus-host reaction; 300 r condition after 160 days. Thus, it would appear that lymphoid cells exert their effect on the lymphoma cells predominantly by releasing humoral antibodies rather than finding their way to the lymphoma cells.

**DISCUSSION**

The possibility of influencing tumor growth by immunological means has been a major objective of cancer research for several decades. Most of the work has been done to ascertain whether cancer cells possess any antigens absent from normal tissues. Whereas the overwhelming part of the findings are negative (12), some critically proved positive results can now be quoted (9, 20).

Although this “qualitative” search for tumor-specific antigens is the basic prerequisite for influ-

**TABLE 1**

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>TUMOR</th>
<th>LYMPHOID CELLS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Designation</td>
<td>Type</td>
</tr>
<tr>
<td>1</td>
<td>L1210</td>
<td>Lymph.</td>
</tr>
<tr>
<td>2</td>
<td>L1210</td>
<td>Lymph.</td>
</tr>
<tr>
<td>3</td>
<td>L1210</td>
<td>Lymph.</td>
</tr>
<tr>
<td>4</td>
<td>WIA</td>
<td>Lymph.</td>
</tr>
<tr>
<td>5</td>
<td>MWG</td>
<td>Sarc.</td>
</tr>
<tr>
<td>6</td>
<td>SDB₂</td>
<td>Care.</td>
</tr>
<tr>
<td></td>
<td>SDB₂</td>
<td>Care.</td>
</tr>
<tr>
<td></td>
<td>MWG</td>
<td>Sarc.</td>
</tr>
<tr>
<td></td>
<td>MWG</td>
<td>Sarc.</td>
</tr>
<tr>
<td></td>
<td>SDB₂</td>
<td>Care.</td>
</tr>
<tr>
<td></td>
<td>SDB₂</td>
<td>Care.</td>
</tr>
<tr>
<td></td>
<td>SDB₂</td>
<td>Care.</td>
</tr>
<tr>
<td></td>
<td>SDB₂</td>
<td>Care.</td>
</tr>
</tbody>
</table>
encuing tumors by immunological means, attention should be paid to the "quantitative" approach. This means that one must have an experimental system in which the tumor cells differ from the normal cells, not in the number of antigens but in the quantities of isoantigens. By using such a system, in which the host animals also are genetically tolerant to immunologically reactive cells which are able to react against these antigens, we have been able to completely suppress the growth of otherwise compatible lymphomas.

Lymphomas of one inbred mouse strain inoculated subcutaneously into F1 hybrid hosts, produced by outcrossing this strain with another strain, were retarded in their growth or failed to grow altogether if the host received an intraperitoneal injection of lymphoid cells derived from the opposite parental strain at the time of tumor inoculation. The effect was much weaker when a sarcoma or a carcinoma was used for the test. The following considerations may be relevant for the explanation of this difference.

One fairly regular difference between the lymphomas on the one hand and sarcomas and carcinomas on the other lies in the greater sensitivity of the former group to humoral antibodies (11). Whether the lymphoma cells also differ in their sensitivity to the cellular component of the homograft reaction is not known. Results have also been obtained (13), suggesting a gene-dose effect in lymphoma cells with regard to their sensitivity to cytotoxic antibodies, homozygous cells being more vulnerable than heterozygous. This may have contributed to their sensitivity in this test where homozygous lymphomas were grown in heterozygous hosts, although control tests were F1 lymphomas will be needed to confirm this assumption.

To investigate whether the wasting syndrome induced by the reaction of the graft of lymphoid cells against the host was responsible for the tumor-depressing effect in a secondary way or a more specific immunological reaction took place between the immunogenetically competent lymphoid cells and the foreign isoantigens of the tumor cells, control experiments were carried out in which the F1 hybrid mice were given inoculations of lymphoma cells and lymphoid cells of the same parental strain. In this case the parental lymphoid cells were competent to react only against the host animal but not against the lymphoma cells. The results showed that lymphoma growth was slightly retarded as compared with the lymphoma growth in the animals receiving only lymphoma cells. The degree of this inhibition was much smaller than that in the experiments in which the lymphoid cells were competent to react against the lymphoma cells as well. It can be concluded that the major part of the lymphoma-depressing effect must be attributed to a specific immunological reaction. In addition, there is a slight nonspecific effect contributed at least in part by the wasting syndrome. Wasting alone has been shown to cause slight retardation of tumor growth in other systems (2, 25).

Another factor that may conceivably contribute to the nonspecific part of the lymphoma inhibition can be postulated if it is assumed that the inoculation of lymphoid cells may depress the homeostatic mechanism controlling the production of lymphoid cells in the host (19). If the lymphoma cells are still sensitive to this mechanism, such an increase in the number of normal lymphoid cells may retard their growth to some extent. This possibility is now being studied by inoculating lymphoid cells isologous with the F1 hybrid host. The preliminary results show a slight retardation of lymphoma growth in this system, but more facts are needed.

Thus, the experiments indicate that the major part of the lymphoma-depressing effect is due to an immunological mechanism, although several factors may play additional small parts. Gorer (9) has shown that it is possible to transfer immunity against lymphoma cells passively by antisem. Animals of one strain were immunized against a lymphoma of another strain, and the antisem was then injected into animals isologous with the lymphoma cells. A protective effect could be demonstrated when these animals were given inoculations of the lymphoma cells. This effect was due to the humoral antibodies to which lymphoma cells are usually eminently sensitive.

To investigate whether humoral antibodies produced by the lymphoid cells were operating also in our system or whether a cellular reaction was primarily responsible, skin grafts were used as targets instead of lymphoma cells. F1 hybrid mice given inoculations of lymphoid cells of one parental strain received skin grafts from both parental strains. Skin is very sensitive to the homograft reaction (1, 17), but here humoral antibodies seem to play a very small part and may even have an enhancing effect (5). The skin grafts were not rejected despite a severe graft-versus-host reaction indicated by the weight curves of the animals. This is somewhat surprising, since the scruffy condition of the fur is one of the usual symptoms of a graft-versus-host reaction (4), accompanied by a histological damage of the skin (14). The failure to get skin graft rejection despite the presence of actively reacting cells, which are needed to produce and maintain the symptoms of the graft-versus-host reaction (3), might be due to the fact that the
whole host is antigenic for the parental lymphoid cells, and they might be prevented from reaching the graft through a cellular reaction or what Gorer and Boyse (10) call "allergic" death.

Although the quantitative difference in isoantigens between the homozygous tumor cells and the heterozygous F1 hybrid hosts may have contributed to the retarding effect upon the growth of the former, this cannot be determined with certainty from the present experiments. Direct proof for a former, this cannot be determined with certainty and Boyse (10) call "allergic" death. the graft through a cellular reaction or what Gorer cells, and they might be prevented from reaching the

ACKNOWLEDGMENTS

The author wishes to thank Prof. G. Klein and Dr. E. Klein for valuable help and criticisms.

REFERENCES

Immunological Depression of Tumor Growth in F₁ Hybrid/Parental Strain Systems

Hans Wigzell


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/21/3/365

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.