Further Studies on Inhibition and Adaptation of a Parental Tumor in F₁ Hybrid Mice

Richard P. Huemer

Developmental Biology Laboratory, Veterans Administration Hospital, Sepulveda, California 91343

SUMMARY

Growth repression of a parental tumor in F₁ hybrid mice and adaptation of the tumor to such repression were further studied with the 21B lymphoma of C57BL mice. With a tumor line propagated only in C57BL mice, growth repression in F₁ hybrids was found to depend more on heterozygosity of the hybrid at the H-2 locus than on heterozygosity at other loci. Passage of the tumor in F₁ hybrid hosts facilitated its subsequent growth in those specific hosts under certain circumstances. This adaptation was lost after passage of the modified tumor through F₁ hosts other than those to which adaptation had been induced. The adaptation of a tumor to a hybrid containing certain H-2 antigens did not favor its growth in a genetically different hybrid containing the same H-2 antigens. The results are interpreted in terms of a discontinuity between recognition of, and response to, tumor-specific antigens of parental cells.

INTRODUCTION

Numerous reports (5, 8—10, 13—22, 25, 27—35) during the past dozen years have called attention to various examples of F₁ hybrid effect, that is, to cases in which tumors or normal tissues of a particular inbred line do not function or grow well in the F₁ hybrid between that line and another. Burg and Oth (5) have classified such effects into three types: hybrid resistance (8), in which hematopoietic grafts from C57BL mice are rejected by F₁ mice, apparently by a homograft reaction against parental antigens arising from interallelic interaction; allogeneic inhibition (18, 19), in which histocompatibility antigens present in F₁-hybrid cells but lacking in parental-strain cells presumably exert inhibitory effects on the latter; and hybrid hyperreactivity (5, 30), by which F₁ animals are better able to react against tumor-specific antigens carried by parental cells. Unlike the other effects, allogeneic inhibition does not depend on a conventional immunologic reaction, but it does seem to involve cell-surface antigens and their recognition by genetically different cells. The categories largely reflect the approaches and systems used by certain workers; the extent to which the categories overlap or to which common mechanisms may underlie observations made by different investigators has not been determined.

In some cases the deficient growth of parental tumors in F₁ hosts can be ameliorated by passage of the tumor through an F₁ host. This was first demonstrated by Huemer (22), who found that a C57BL/6 lymphoma, which had originally displayed the hybrid effect, failed to do so after it had been passed in a BAF₁ mouse. Subsequently Hellström (16, 18) developed, by passage in F₁ hosts, several tumor lines showing decreased sensitivity to allogeneic inhibition. F₁ passed tumors do not always adapt to better growth in F₁ hosts; Oth and Burg (29) were unable, using methylcholanthrene-induced tumors, to repeat Huemer's results, and they considered that tumors of different origins or histologic types may behave differently with respect to their adaptability. One tumor studied by Oth and Burg (30) acquired more rapid growth in F₁ hybrids without prior F₁ passage; at the same time its antigenicity for the native strain was markedly diminished. Hybrid-induced growth repression is not demonstrable with all tumors (38).

The present studies were designed to explore further the mechanisms responsible for F₁ hybrid effects and to define conditions under which the adaptation effect takes place. Experiments were set up to test the reproducibility, specificity, and persistence of the adaptation effect, and to examine growth-repression of parental tumors in relation to genetic constitution of F₁ hosts. The experiments were done with the 21B lymphoma of C57BL/6 mice; this was the subject of a previous report (22).

MATERIALS AND METHODS

The following inbred mice and F₁ hybrids were used: C57BL/6 (strain of tumor origin); C57BL/10 (a related strain in which the tumor grows well); the F₁ hybrid of C57BL/10 with its congenic strain B10.A; the (C57BL/10 X A/J)F₁ hybrid; F₁ hybrids between C57BL/10 and the two congenic strains A/WySn and A.BY; and F₁ hybrids of C57BL/6 with A/J (B₁D₁F₁), DBA/2 (B₂D₂F₁), CBA, and C3H (B₂C₂H₂F₁). Mice in all experiments, with the exceptions noted in the text, were females obtained from the Jackson Laboratory in Bar Harbor.
Harbor, Maine. In most cases groups of mice did not differ in age from each other by more than six weeks.

The stock (unadapted) line of the 21B lymphoma was maintained in C57BL/6J mice prior to the 17th transplant generation and in C57BL/6 or C57BL/10 mice thereafter. Transfer of tumors was accomplished by injection of 0.2 to 0.6 ml of sterile tumor cell suspension in the neck-and-shoulders region of mice. The initial step in preparation of all suspensions was fragmentation of the tumor in balanced salt solution with the aid of a rotating Teflon pestle that fitted loosely in a glass homogenizer tube or test tube. For the earlier experiments (reported in Chart 1a, b, c and the first and third group in Table 1), the fragmented tumors were further broken down into cells and small pieces by passage through a 16- or 18-gauge needle prior to injection. In later experiments, the fragmented tumors were filtered through a layer of coarse bolting silk to yield a more homogeneous suspension suitable for cell counting with a hemocytometer.

Observations for the presence of tumorous and dead mice in the various experimental groups were made at 2- or 3-day intervals. Such observations comprise the experimental results. F₁ mice that failed to develop visible tumors were generally observed for 6 to 10 months after the beginning of an experiment; the exceptions are mice listed in Table 1, line 5 (4.5 months) and line 9 (3 months).

RESULTS
Repeatability, Specificity, and Recidivism of Adaptation Effect

In the original report (22) on the adaptation effect, the 21B lymphoma which had been grown only in C57BL/6 mice killed mice of that strain more rapidly than B₆D₂F₁ mice, whereas a subline of the tumor, which had been carried for 87 days in a B₆D₂F₁ host, killed the two types of host equally rapidly. The mean survival time (MST), not heretofore published, was 12.2 weeks and 29.3 weeks respectively for C57BL/6 and B₆D₂F₁ hosts carrying the stock tumor and 11.5 weeks and 12.8 weeks for those respective hosts carrying the F₁-passed tumor. Subse-
quently the stock tumor was transplanted to nine C57BL/6 and nine B6D2F1 hosts. As shown in Chart 1a, the tumor grew significantly more slowly in F1 than in syngeneic hosts; MST and standard deviations for the first eight animals in each group were 44.8 ± 7.6 days for C57BL/6 mice and 68.1 ± 13.6 days for B6D2F1. The difference in survival time was highly significant (P < 0.01) by Student’s t test. After the tumor had been grown for 93 days in a B6D2F1 host, it was transplanted into C57BL/6, B6D2F1, and B6AF1 hosts. As is evident in Chart 1b and Table 1, the difference between survival of C57BL/6 (MST = 57.8 ± 11.3 days) and B6D2F1 (MST = 59.8 ± 10.7 days) hosts was no longer significant, but B6AF1 hosts survived significantly longer than either (MST = 79.5 ± 15.0 days, P < 0.05). Hence the adaptation effect can be reproduced with this tumor in another type of F1 hybrid, and the adaptation is specific for the hybrid in which the tumor has been passed. Hellstrom (16, 18) also observed a degree of specificity with his adapted tumors.

A subsequent set of experiments was designed to determine whether the growth repression in hybrid hosts was related to the H-2 genotype of the hybrid and whether adaptation of the tumor to hosts of various H-2 constitutions could reveal this. It was thought that by passing the B6-D2F1-adapted tumor (which had been exposed to H-2b antigens) though B6C3H, C57BL/6, B6D2F1, and B6AF1 hosts, adaptation would grow well in B6AF1 hosts, whose H-2b antigens contain no components not also present in H-2d or H-2k. Chart 1c shows that this expectation was not fulfilled; instead the tumor apparently grew less well in B6D2F1 hosts than formerly. This tumor subline was taken from a B6AF1 mouse and transplanted to C57BL/6, B6D2F1, and B6AF1 hosts; now there was no significant difference in survival of the two kinds of F1, and both survived significantly longer than syngeneic mice (Chart 1d and Table 1). The behavior of this F1-passed tumor toward the two kinds of F1 mice did not appear grossly different from the behavior of the stock tumor (Table 1). Evidently the type of adaptation made by the tumor for a specific F1 host was not compatible with persistence of adapted cells in an F1 of different genotype. Adaptation was not a stable, irreversible characteristic of all the tumor cells, and the range of hosts in which the tumor grows well was not broadened by sequential passage in hosts of different H-2 genotypes.

### Dosage Effects

Some workers have noted that F1 hybrid effects do not occur when large doses of tumor cells are inoculated (15, 35). However, in the previous experiments with the 21B lymphoma (22), large doses were employed in the early transplant generations (0.2 ml of thick suspension containing about 3 parts by weight of tumor to 1 part of saline. In the experiments referred to above, in which growth repression and adaptation were demonstrated in B6-D2F1 hosts, each mouse received an estimated 3.8 X 10⁸ cells in the sixth generation and 3.3 X 10⁸ in the seventh. (The estimates are based on known volume of tumor injected and correlations between tumor volume and cell content made during later transplantations.) In later transfers, usually fewer cells were needed to achieve similar or shorter mean survival times of syngeneic hosts (e.g., 25 X 10⁶ cells for 40.5 days in the 11th generation, 20.2 X 10⁶ for 32.4 in the 17th, 11.8 X 10⁶ for 31.9 in the 28th). The greatly prolonged mean survival time of 29.3 weeks for B6AF1 hosts was never observed after the third transplant generation, even though smaller tumor doses were used later. Possibly the tumor in its early stages contained cells with various different potentialities for malignancy, and selective pressures for increasing virulence may have excluded cells that would have been most susceptible to growth inhibition in hybrids.

In the 20th transplant generation a comparison was made between the effects of two different doses of tumor cells in C57BL/6 and BDF1 mice (obtained from Simonsen Laboratories, Gilroy, California). The tumor employed was from a 21B mouse and transplanted to nine C57BL/6, B6D2F1, and B6AF1 hosts; now there was no significant difference in survival of the two kinds of F1, and both survived significantly longer than syngeneic mice (Chart 1d and Table 1). The behavior of this F1-passed tumor toward the two kinds of F1 mice did not appear grossly different from the behavior of the stock tumor (Table 1). Evidently the type of adaptation made by the tumor for a specific F1 host was not compatible with persistence of adapted cells in an F1 of different genotype. Adaptation was not a stable, irreversible characteristic of all the tumor cells, and the range of hosts in which the tumor grows well was not broadened by sequential passage in hosts of different H-2 genotypes.

### Table 1

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Hosts</th>
<th>Tumors/total</th>
<th>Mean survival time ± S. D. (days)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passed 93 days through</td>
<td>C57BL/6</td>
<td>6/6</td>
<td>57.8 ± 11.3</td>
<td>Control</td>
</tr>
<tr>
<td>B6-D2F1</td>
<td>B6-D2F1</td>
<td>6/6</td>
<td>59.8 ± 10.7</td>
<td>&gt;0.70</td>
</tr>
<tr>
<td></td>
<td>B6AF1</td>
<td>6/6</td>
<td>79.5 ± 15.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Passed through B6-D2F1 (6th), B6C3H (7th), C57BL/6 (8th) and B6AF1 (9th)</td>
<td>C57BL/6</td>
<td>8/8</td>
<td>59.9 ± 8.03</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>B6-D2F1</td>
<td>7/8</td>
<td>81.4 ± 19.87</td>
<td>&lt;0.05</td>
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<tr>
<td></td>
<td>B6AF1</td>
<td>8/8</td>
<td>78.5 ± 11.11</td>
<td>&lt;0.01</td>
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<tr>
<td>Stock (from 10th generation in C57BL/6)</td>
<td>C57BL/6</td>
<td>10/10</td>
<td>40.5 ± 7.66</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>B6-D2F1</td>
<td>10/10</td>
<td>64.4 ± 14.62</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>B6AF1</td>
<td>9/10</td>
<td>67.2 ± 13.43</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Loss of adaptation after passage of 21B lymphoma through different F1 hybrids.*

### Table 2

<table>
<thead>
<tr>
<th>Cell dose</th>
<th>Hosts</th>
<th>Tumors/total</th>
<th>Mean survival time ± S. D. (days)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.9 x 10⁶</td>
<td>C57BL/6</td>
<td>7/7</td>
<td>36.3 ± 4.50</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>B6-D2F1</td>
<td>7/7</td>
<td>72.4 ± 19.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>0.119 x 10⁶</td>
<td>C57BL/6</td>
<td>7/7</td>
<td>41.7 ± 4.64</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>B6-D2F1</td>
<td>2/7</td>
<td>167.0 ± 29.7</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Effect of cell dose on tumor growth in syngeneic and F1 hosts.*

*Compared with C57BL/6 that received 0.119 x 10⁶ cells.*
was associated with a 5.4-day increase in MST of syngeneic hosts. To make up for a hundred-fold deficiency in the number of tumor cells, each cell would have had to divide 6.64 times; the corresponding mean cell cycle time is 20 hours, which is not unreasonable. If all of the injected cells are assumed to be dividing tumor cells, it can be calculated that a single injected cell would multiply at the stated rate to kill the average host in no more than 56 days. This interval is considerably shorter than the MST of BDF₁ hosts; hence the survival time of F₁ hosts cannot be accounted for solely by very small numbers of selected cells multiplying at the average “normal” rate, but rather other factors, such as a lengthened cell cycle and the destruction (or nondivision, Ref. 25) of some daughter cells, must be considered. In BDF₁ hosts, destruction of injected cells or their failure to grow was more evident at the lower dose level. Although all seven mice in each of the other groups developed tumors, in the low-dose group only two of seven F₁ mice had visible, progressively growing tumors, which is a significantly smaller number than in any other group (P = 0.010* by Fisher’s exact test, one-tailed). Presumably the failure of visible tumors to appear represents an extreme case of growth repression.

Relation of Growth Repression and Adaptation to H-2 and Non-H-2 Genes

In the eighth transplant generation a subline of the tumor was propagated in (CBA × C57BL/6)F₁ female hosts (produced by crossing strains in our laboratory). When tested for adaptation by retransplantation in C57BL/6 and B₆C3H/ F₁ hosts, this subline killed the former hosts more rapidly (MST, 34 days) than the latter (MST, 54 days). Tumor cells from one of the C57BL/6 mice carrying this subline were then transplanted to nine B₆C3H/F₁ and nine (CBA × C57BL/6)F₁ male hosts. The former, all of which developed tumors, survived significantly longer than the MST of BDF₁ hosts; and least slowly in C57BL/6 and C57BL/10 mice. Passage of the tumor through the (C57BL/10 × A.BY)/ F₁, which is similar to the previous hybrid except for homozygosity for H-2b; and least slowly in C57BL/6 and C57BL/10 mice. Passage of the tumor through the (C57BL/10 × A.BY)/ F₁, did not enhance its subsequent ability to grow in (C57BL/10 × A.WySn)/ F₁. Both before and after the F₁-passage, the hybrids heterozygous at H-2 survived significantly longer than the pure-strain mice, whereas the H-2-homozygous hybrids did not. A subsequent experiment (Table 4) was done with male and female F₁ hybrids produced in our laboratory by crossing C57BL/10 with B10.A (congenic with C57BL/10, carries H-2b) and A/J (carries H-2α and also differs from C57BL/10 at many other loci). After being grafted with the stock tumor, both types of hybrid survived significantly longer than C57BL/10, and there was no significant difference between survival of hybrids heterozygous at H-2 and those heterozygous at H-2 and many other loci. These two experiments, which involved stock or nonadapted tumor lines, showed that the host’s H-2 genotype is the major influence on tumor-growth repression in F₁ mice, as others (20, 35) have previously indicated.

However, when a tumor that had been propagated for 117 days in a (B10.A × C57BL/10)/ F₁ mouse was transplanted into C57BL/10, (B10.A × C57BL/10)/F₁, and (C57BL/10 × A/ J)/F₁ hosts, the growth repression subsequently observed did not seem to depend on the H-2 genes, which were similar in both types of hybrid. The “adapted” tumor grew significantly more rapidly in the adaptation-inducing (B10.A × C57BL/10)/F₁ hosts than it did previously, but it did not grow more rapidly in the (C57BL/10 × A/J)/F₁ hosts (Table 4). In the latter hybrids there were significantly fewer successful tumor takes than in C57BL/10 mice (P = 0.014 by Fisher’s exact test, one-tailed) or (B10.A × C57BL/10)/F₁ mice (P = 0.033). Thus F₁ passage and adaptation of the tumor revealed a formerly apparent difference in the resistance of the two classes of F₁ hosts against the tumor.

### Table 3

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Cell dose</th>
<th>Hosts</th>
<th>Tumors/total</th>
<th>Mean survival time ± S. D. (days)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock (from 16th generation in C57BL/6)</td>
<td>20.2 × 10⁶</td>
<td>C57BL/6, C57BL/10</td>
<td>7/8</td>
<td>32.4 ± 3.80 Control</td>
<td></td>
</tr>
<tr>
<td>Passed through (C57BL/10 × A.BY)/F₁ for 42 days in 17th generation</td>
<td>21.0 × 10⁶</td>
<td>(C57BL/10 × A.BY)/F₁, (C57BL/10 × A/WySn)/F₁</td>
<td>9/9</td>
<td>40.3 ± 9.80 Control</td>
<td></td>
</tr>
</tbody>
</table>

Effect on tumor growth in hybrids of homozygosity and heterozygosity at the H-2 locus.

*Two exceptional mice that survived 82 and 92 days are excluded.
Richard P. Huemer

Table 4

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Cell dose</th>
<th>Sex</th>
<th>Hosts</th>
<th>Type</th>
<th>Mean survival time ± S. D. (days)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock (from 18th</td>
<td>11.9 x 10^6</td>
<td>5 d, 10 y</td>
<td>C57BL/10</td>
<td>15/15</td>
<td>66.7 ± 19.8</td>
<td>Control</td>
</tr>
<tr>
<td>generation C57BL/10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passed in</td>
<td>11.8 x 10^6</td>
<td>4 d, 10 y</td>
<td>C57BL/10</td>
<td>14/14</td>
<td>57.4 ± 10.5</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>(B10.A x C57BL/10)F1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Similar survival times of hybrids heterozygous at H-2 only, and those heterozygous at H-2 and other loci; adaptation of the tumor to the former hosts but not the latter.

*Compared with mean survival time in the same kind of host prior to passage in the F1.

DISCUSSION

In his initial investigation of the F1 hybrid effect, Snell (34) found that the native inbred strain is a slightly more favorable host for tumor transplantation than is the F1 hybrid and that immunization does not increase the hybrid's resistance to the parental tumor. Subsequently Snell and Stevens (35) reported that the effect is strongly dose susceptible and increases in proportion to the histocompatibility disparity of the parent strains; immunization did not occur, as the proportion of deaths among regrafted F1 survivors of tumor grafts was the same as among animals first grafted. Hellström (14, 15) described an F1 hybrid effect, which was not sensitive to X-irradiation, in F1 hosts grafted with parent-compatible isoantigenic variant sublines of an F1 lymphoma. Oth et al. (32) reported an additional example of hybrid effect in F1 hosts bearing a parental tumor, and Cudkowicz (8) and Cudkowicz and Stimpfling (9, 10) explained the resistance of F1 hybrid mice to C57BL parental-marrow grafts. The latter phenomenon, termed hybrid resistance, depends on nonexpression in the hybrid of certain H-2d antigens that are expressed in the homozygote (8). Its mechanism differs from that of allogeneic inhibition (18, 19) in which parental tumor cells are thought, by analogy to in vitro effects (18—21, 26), to be damaged or destroyed in vivo by intimate contact with foreign isoantigens carried by F1 host cells. Allogeneic inhibition is proposed as a general surveillance mechanism (19) by which the body recognizes and disposes of aberrant cells whose surfaces are different from, but not necessarily antigenically incompatible with, those of normal cells. If tumor cells are immunologically competent, as Tyler (37) has argued cogently, allogeneic inhibition might be viewed as a manifestation of the "allergic death" described by Gorer and Boyse (11).

Certain results of Oth and Burg have led these investigators to conclude that not all forms of hybrid resistance to tumors are alike. These include their findings that X-irradiation of F1 hosts abolished their resistance (27), that the C3H milk factor influenced the behavior of the hybrids (30), and that F1 hosts in which the tumor failed to grow were more resistant to regrafting (28, 31). These workers consider that they are dealing with a distinct phenomenon, which they term hybrid hyperreactivity (5, 30), that involves the enhanced ability (due to heterosis) of F1 animals to react immunologically against tumor-specific antigens. Probably a similar mechanism was operative in the system of Sanford (33), who reported radiosensitivity of a hybrid effect and reinforcement of the effect by specific immunization. Immunization of F1 hosts also was suggested by the experiments of Huemer (22), who found that regrafted F1 survivors of a parental tumor inoculation were more resistant to the tumor than were F1 mice which had never been grafted; it was not possible to distinguish operationally between immunization of F1 mice and selection of resistant animals, although the high degree of genetic uniformity among F1 hybrids of highly inbred strains would make the latter possibility less likely.

There arises the question of whether the present results represent hybrid resistance, allogeneic inhibition, or hybrid hyperreactivity. Allogeneic inhibition is considered unlikely for the following reason: Although the behavior of the stock tumor accords with the expectations for allogeneic inhibition in displaying an association between growth repression and parental F1 antigenic disparity, the behavior of the adapted sublines does not always do so. In one case a tumor that had adapted to a host containing H-2d antigens (factors 3, 4, 6, 8, 10, 13, 14, 27, 28, 29, and 31) not only failed to adapt to hosts containing other antigens, but lost its presumed adaptation to H-2d after exposure to the other antigens (H-2 factors 1, 3, 5, 8, 11, 25, and 32). As the stock tumor had previously been shown capable of adapting to a host carrying the H-2d complex (factors 1, 3, 4, 5, 6, 8, 10, 11, 13, 14, 25, 27, 28, and 29), there seemed to be no reason why a tumor already adapted to most of those factors should not adapt to the rest of them on a subsequent F1 passage. In another case, the adaptation of the tumor to a hybrid possessing only an H-2 difference from the tumor-maintenance strain did not ensure its good growth in an F1 possessing the H-2 plus other differences. The experiments generally indicated that heterozygosity at H-2 was necessary but not sufficient for the manifestation of hybrid growth repression. This contrasts sharply with findings in certain in vitro systems (20, 23) in which H-2 disparity alone is sufficient for F1 antiparent reactions.

For reasons to be discussed below, the data are believed to be consistent with an immunologic reaction directed against parental tumor cells, and thus could be classified as either hybrid resistance or hybrid hyperreactivity. The two would be rather difficult to distinguish under the present circumstances, as hybrid resistance is said to occur against C57BL tumors (cf. Cudkowicz in discussion following Ref. 19) as well as against...
hematopoietic cells. However, adaptation of parental cells to hybrid resistance has not been achieved (8), and Cudkowicz (8) differentiates between hybrid resistance and other examples of F1 effect partially on the basis that adaptation does not occur in his system. If that criterion be accepted, the present results would be classified by exclusion as hybrid hyperreactivity.

The present data are believed to be explicable in terms of an immune response by F1 hosts against antigens carried by parental tumor cells and subsequent adaptation of the tumor cells by modification of their antigenic characteristics. However, this interpretation is made cautiously in view of the recent finding of Steinmuller (36) that, in normal tissue transplantation, a significant degree of host immunization may be due to contained leukocytes. A demonstration of persistent adaptation following syngeneic retransplantation would exclude the possibility that the observed specific adaptations might have been related to acquisition by the tumor of host blood and stromal elements. In one of the presently reported experiments, done with (CBA X C57BL/6)F1 and B6.C3HF1 mice, adaptation to the former F1 may have persisted through one passage in C57BL/6, but interpretation is hindered by the lack of a parallel experiment with the unadapted tumor line. Hellström refers to some unpublished experiments in which decreased syngeneic preference after F1 passage and specificity of the decrease were maintained following one passage in homozygous (syngeneic) mice (16). Loss of adaptation to a former F1 host would, in any case, be accompanied by adaptation to a new F1 host, if adaptation involved merely the acquisition of new stromal and blood elements; but such secondary adaptation was not observed (Chart 1d).

The antigenic-modification interpretation is consistent with earlier findings of others that passage of parental tumors in F1 hosts leads to an apparent alteration in their histocompatibility requirements, such that F1-passed tumors will grow in a greater (3, 12) or smaller (12) proportion of resistant backcross animals than will the stock tumors. It is also consistent with variations in expression of TL and H-2 antigens of murine leukemias, occurring in response to anti-TL antibody (4). It was observed in the present experiments that a strong antigenic disparity between F1 hybrids of similar H-2 genotypes but of otherwise different genetic constitutions, and adapted cells may not enjoy a neutral selective advantage during subsequent passages in diverse F1 hosts.

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