Variation of Virulence of Transplantable Leukemias of Mice on Successive Transfers into Genetically Unrelated Hosts*†

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Owing to differences in histocompatibility genes, most transplanted mouse tumors grow progressively, as a rule, only in animals of their strain of origin. In mice of genetically unrelated strains they usually grow for a variable period of time and subsequently regress. As the number of serial transfers increases, some transplantable tumors, besides killing 100 per cent in their own strain, acquire the ability to grow progressively and kill a certain percentage of animals of other unrelated strains. In other words, they become less specific. Concurrent with this increased virulence, a decrease in histocompatibility gene requirements has been observed. Extreme cases of this kind are the so-called "nonspecific" tumors, such as the Ehrlich carcinoma, which can be made to grow in almost all strains of mice (see revision by Snell [15]).

In agreement with these general statements, Hoecker and Brnčic (9) observed that the A line of leukemia, after more than 100 transfers in its strain of origin, grew and killed 90 per cent or more of mice of the unrelated inbred strain AK. However, contrary to what might have been expected, when a second transfer was made with leukemic material derived from growths of the first transfer (which were destined to kill the AK host), a marked drop in virulence was observed. The intensity of this decrease in virulence depended chiefly on the length of residence of the leukemic cells in the foreign AK hosts. This modification of the virulence was only transient; when the same material was transplanted back to the strain of origin, namely C58, its original virulence was unmodified.

Because of the rapidity with which these changes took place, it seemed improbable that a variation in the histocompatibility genes, either in the host or the tumor, was the cause; an immunological phenomenon seemed at that time the most plausible explanation (2-7, 9). Since there appeared to be an increased specificity in the tumor line because of its passage through the foreign host, it seemed of interest to know more of this particular tumor-host relationship. Thus, more experiments were undertaken with the same A line of leukemia and several inbred strains of mice. Furthermore, three other long transplanted lines of leukemia were tried in an attempt to learn whether the changes observed were of the same kind as those occurring in the A line and whether the changes in virulence could be made permanent. This report is an account of the results.

PLAN OF THE EXPERIMENTS

The leukemic material, coming from a transfer in the strain of origin, was transplanted to the foreign strain. Moribund hosts of the latter strain, with progressively growing tumors, provided the material for a "second transfer" to mice either of the same strain or of other foreign strains. A part of this same leukemic cell population was transferred back to the strain of origin to test for viability of the cells.

The experimental procedure is more easily visualized schematically:
“Third transfers” were made from material which came from “second transfer” donors.

Considering the virulence in the strain of origin as “normal,” a comparison is made between the number of animals dead in the first and second transfers and in the transplants back to the strain of origin. In this way it was expected to obtain a measure of the relative efficiency of the different genotypes tested to resist the tumor graft. The third transfer experiments were intended to show the possibilities of a permanence of the modification in virulence obtained in the second transfer.

MATERIALS AND METHODS

Four lines of transplanted lymphoid leukemias were used. Lines A and F originated in C58 mice and H and G in the AK strain. Each of these lines was invariably transplanted intraperitoneally only in mice of their strain of origin. All four lines originated from spontaneous cases in 1947 in our laboratory, and had been carried through more than 180 routine transfers when the experiments were started. Lines A, F, and H are highly virulent in their own strains and invariably kill all the animals within 4–8 days, with generalized infiltration of most of the organs by leukemic cells, especially the spleen, liver, thymus, and lymph nodes. The G line is less virulent and usually kills the AK hosts in about 12 days. Slight variations which are characteristic of each of the leukemic lines used, such as presence or absence of petechiae, size of the spleen at death, and production of ascitic fluid provide a macroscopic distinction between them. No detailed studies of their morphology have been made. However, the over-all picture, both cellular and histological, is remarkably similar in these four lines and conforms to the descriptions given by Potter and Richter (14) for line I of transmissible lymphatic leukemia.

The tumor suspensions were prepared by finely mincing the enlarged spleens from leukemic donors which were gravely ill or moribund. The mince was suspended in 0.85 per cent saline in the proportion of 1 volume of tissue to 2 volumes of saline and minced further by passing this suspension several times through a 18-gauge needle and then several times through a 25-gauge needle. From this a dilution was made to contain $10^6$ leukemic cells/0.2 cc of suspension for all the experiments, except the one with Rockefeller mice in which a $3 \times 10^6$ suspension was used. This dose is invariably lethal to the mice of the strain of origin of the leukemic material. The cells were considered to “take” only if the injected animal died of leukemia. Takes were further confirmed by macroscopic and sometimes by histological examination.

All inoculations were made intraperitoneally with a 25-gauge needle.

The strains of mice used were: AK, A', BALB/c, C3H, C58, DBA/1, and Rockefeller (Rk). AK, BALB/c, C3H, C58, and DBA/1 are standard strains inbred for more than 50 generations (17). A' and Rk mice had been inbred in our colony for ten generations at the time they were used. The age of the animals ranged between 70 and 95 days in different experiments, and within each experiment the animals did not differ in age by more than 10 days. In all groups an approximately equal number of males and females was used, though no sex difference in susceptibility had been noticed in previous experiments.

The changes in virulence of the leukemic lines were estimated by both the percentage of takes and the length of survival time of the mice after inoculation. For purposes of comparison virulence is classified as “normal” in the strain of origin and “modified” if a change is observed after transplanting into foreign strains.

RESULTS

Takes were obtained only with the A and H lines (from the C58 and AK strains, respectively). In two experiments, in which the F line from C58 and the G line from AK were cross-transplanted to twelve AK and twelve C58 mice, respectively, there were no deaths in any of the animals.

Results with the A line.—In seven experiments, the A line (C58 strain) was transplanted to hosts of the AK, C3H, BALB/c, Rk, A', and DBA/1 strains. In the first transfer only mice from the AK, Rk, and C3H strains died of leukemia. The numbers of takes were: 179 of 187 (96 per cent) in the AK; 10 of 28 (36 per cent) in the Rk; and 21 of 24 (88 per cent) in the C3H strain. No deaths occurred in twelve DBA/1, eighteen BALB/c, and twelve A' mice. The BALB/c and DBA/1 mice remained healthy, but four of the A' animals became very sick and had enormous spleens which were easily palpable. In all these experiments the leukemic material was fully viable and killed all the animals of strain C58 (Table 1).

Five AK animals had atypical leukemias which, on the basis of histological examinations, were classified as lymphosarcomas. These animals also had a very long survival time. After transplanting one of these lymphosarcomas back to C58 mice, five out of five died within 6–9 days.

The results of the second transfer of the A line in the different foreign hosts, which are summarized in Table 1, show marked differences in several respects, according to the different strains used.

There was a marked drop in the percentage of
"takes" when the A leukemic cells were transplanted a second time into hosts of strains AK and Rk. The intensity of the decrease in takes was related to the days of survival of the donors. "Second transfer" leukemic material killed 48 per cent (20 of 42) and 64 per cent (29 of 45) of strain AK mice, respectively, when the donors had survived 4 and 6 days, respectively, but there were no takes when the A leukemic cells were transplanted a second time into hosts of strains AK and Rk. The intensity of the decrease in takes was related to the days of survival of the donors. "Second transfer" leukemic material killed 48 per cent (20 of 42) and 64 per cent (29 of 45) of strain AK mice, respectively, when the donors had survived 4 and 6 days, respectively, but there were no takes.

| TABLE 1 |
| RESULTS OF FIRST AND SECOND TRANSFER OF THE A LINE OF LEUKEMIA TO SEVERAL FOREIGN STRAINS AND BACK TO THE STRAIN OF ORIGIN, C58 |
| Dose was 10⁶ leukemic cells intraperitoneally |

<table>
<thead>
<tr>
<th>HOST STRAIN</th>
<th>FIRST TRANSFER</th>
<th>SECOND TRANSFER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host strain (No. inoculated)</td>
<td>No. dead</td>
<td>Percents</td>
</tr>
<tr>
<td>AK (179/187)</td>
<td>96</td>
<td>4</td>
</tr>
<tr>
<td>C58 (51/51)</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>Donor C58</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>Donor C58*</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Rk (10/28)</td>
<td>56</td>
<td>4</td>
</tr>
<tr>
<td>C58 (16/16)</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>Donor C58</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>C58 (21/24)</td>
<td>88</td>
<td>4</td>
</tr>
<tr>
<td>C58 (7/7)</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>C58 (16/12)</td>
<td>100</td>
<td>6</td>
</tr>
</tbody>
</table>

* 3 × 10⁶ leukemic cells intraperitoneally.
† Four had clinical signs of leukemia, with large, palpable spleens.

(0 of 38) when the donors had survived 10 days. When this same material was transplanted back from AK donors to C58 mice, all the hosts were killed, irrespective of the days of survival of the AK donors, and the cells immediately recovered their normal virulence.

In the Rk mice two out of ten died when the Rk donor had survived for 4 days, and there were no deaths (zero out of six) when the Rk donor had survived for 10 days. This decrease in virulence of the A leukemic cells in the Rk strain was also shown when the leukemic material coming from an Rk donor that had survived for 4 days was inoculated into AK mice: only one-third (33 per cent) as against 96 per cent in the first transfer (Table 2).

The Rk strain appeared to be more effective than the AK in suppressing the virulence of the A leukemic cells, as was shown by the results of transplanting the leukemic material from Rk mice surviving 10 days back to the strain of origin (C58). None of four C58 mice was killed as against all four C58 dead when the leukemic material came from the AK donors that had survived 10 days (Table 1).

An apparent increase in virulence followed the second transfer of the A line in the CSH hosts: 24 of 25 CSH mice (96 per cent) were killed when the CSH donor had survived 4 days and twelve of twelve (100 per cent) when the donors of this same strain survived 6 days. That the leukemic cells had not lost their virulence on passing through CSH hosts was also shown when this same material was transferred to AK mice: fifteen of sixteen (96 per cent) died of leukemia. This figure is practically the same as that obtained in the first transfer of the A line leukemia to this strain.

Results of a third transfer of line A leukemia in AK hosts.—Two attempts, one of which was successful, were made to transfer the A line of leukemia into strain AK mice. In the first experiment (Table 3), the A line took in eleven of eleven AK mice in the first transfer and in two of six animals in the second transfer. The third transfer was made from an animal which was about to die on the 10th day, and six of six AK mice inoculated died of leukemia. Obviously, the line recovered its full virulence.

In the second experiment, only three of five AK mice died in the first transfer and none out of five in the second transfer. In the second experiment, since the donor was critically ill at 10 days, it was killed and the leukemic material transplanted both to AK and C58 mice. In the AK animals none of five inoculated died. In the strain of origin (C58) five mice in the first transfer and in two of six animals in the second transfer. The third transfer was made from an animal which was about to die on the 10th day, and six of six AK mice inoculated died of leukemia. Obviously, the line recovered its full virulence.

In the second experiment, only three of five AK mice died in the first transfer and none out of five in the second transfer. In the second experiment, since the donor was critically ill at 10 days, it was killed and the leukemic material transplanted both to AK and C58 mice. In the AK animals none of five inoculated died. In the strain of origin (C58) five
of five inoculated died of leukemia, but the mean survival time of this group was 15.4 days, which is about 3 times that of A leukemic cells with "normal" virulence.

As was the case with the A line in AK mice (see above), the number of deaths in the H line showed a consistent decrease in the second transfer into AK mice.

**TABLE 3**

RESULTS OF "FIRST," "SECOND," AND "THIRD" TRANSFERS OF THE A LINE LEUKEMIA IN MICE OF THE FOREIGN STRAIN AK AND BACK TO THE STRAIN OF ORIGIN, C58

Dose was $15 \times 10^6$ leukemic cells administered intraperitoneally.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Host Strain</th>
<th>No. of dead/total inoculated</th>
<th>Mean survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>donor</td>
<td>total survival</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dead/total</td>
<td>inoculated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>days</td>
</tr>
<tr>
<td>1</td>
<td>AK</td>
<td>1/12</td>
<td>4/4</td>
</tr>
<tr>
<td>2</td>
<td>C58</td>
<td>3/5</td>
<td>4/6</td>
</tr>
</tbody>
</table>

* The AK donor for this transfer was critically ill, but not moribund.

**TABLE 4**

RESULTS OF "FIRST" AND "SECOND" TRANSFER OF THE H LINE OF LEUKEMIA TO FOREIGN HOSTS (C58) AND BACK TO THE STRAIN OF ORIGIN (AK)

Dose was $10^6$ leukemic cells administered intra-abdominally.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Host Strain</th>
<th>No. of dead/total inoculated</th>
<th>Mean survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>donor</td>
<td>total survival</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>inoculated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>days</td>
</tr>
<tr>
<td>1</td>
<td>C58</td>
<td>11/12</td>
<td>4/12</td>
</tr>
<tr>
<td>2</td>
<td>AK</td>
<td>404/404*</td>
<td>4/7</td>
</tr>
</tbody>
</table>

* Includes all mice inoculated with $10^6$ cells of line H in this and other experiments.

C58's. Here, too, the intensity of the phenomenon was in relation to the days of survival of the C58 donor in the first transfer. Six out of twelve C58's died when the C58 donor survived 4 days. When the C58 donor survived 7 days, none of seven C58 mice inoculated died of leukemia (Table 4).

**Summary of the results of first and second transfer of leukemic lines to foreign hosts.**—The observed variations in virulence of leukemic cells transplanted a second time to a foreign strain are dependent upon the following main variables: (a) the genetic composition of the leukemic cell popula-

tion; (b) the genotype of the recipient hosts; (c) the survival time of the donors, or, in other words, the length of time which the leukemic cells stay in the foreign host after their first transfer. According to the combinations of these three main factors, the following results were obtained.

* (b) *Virulence maintained or increased.* This happened when transferring the A line (C58 strain leukemia) into the foreign C3H strain mice. In the second transfer through this strain the leukemic material increased in virulence not only in C3H mice but also in mice of the strain of origin (C58) and in mice of the foreign AK strain. The increase in virulence from the first to the second transfer is shown by the shortening of the mean interval, in days, between time of inoculation of the leukemia and death of the host. This time was lowered from 10.7 days in the first transfer to 6 days in the second transfer in animals of the AK strain, and from 7.2 to 5.2 days in C58 mice.

* (c) *Virulence decreased.* This was observed in mice of the AK and Rk strains inoculated with the A line and in C58 mice inoculated with the H (AK strain) line. According to the different combinations of hosts, leukemic cells, and days of residence in the host (survival time), several degrees of diminished virulence were observed.

* (d) *Suppression of the ability to kill in the first transfer with concomitant apparent signs of leukemic disease.* This was observed in some A* animals inoculated with the A line (C58 strain) leukemia and also in C58 mice which received line G leukemic material (AK strain).

* (d) *Suppression both of the ability of the leukemic cells to kill and of clinical signs of leukemia.* This happened in DBA/1 and BALB/c mice inoculated with the A line leukemia.

**DISCUSSION**

The complexity of the results obtained in these experiments calls for several possible interpretations. From the start it is necessary to state that in all the combinations of hosts and transplantable leukemic lines studied there was a clear histocompatibility gene difference between the tumor and the foreign hosts. This has been definitely shown by the strong immunity which resulted when smaller doses of leukemic cells were inoculated in stead of the higher doses used herewith ([6] and unpublished).

Because of these differences it seems safe to assume that the suppression or decrease in virulence observed in the first transfer in some strains, and in the second transfer in others, was due to the immunological processes set up by the antigenic differences between hosts and transplanted cells.
which in turn were due to their differences in histocompatibility genes. This is further stressed by the direct relationship between length of survival of the foreign donors and degree of decreased virulence in the second transfer of tissue taken from such donors. It is interesting to note, however, that the protective efficiency against the graft of a particular tumor line is not the same in different strains. This confirms in a very clear way the assumption that the different histocompatibility gene combinations found in different strains have also a different capacity to elicit a protective response. In this respect the finely graded decrease in takes observed with the A line when transplanted to different unrelated hosts would be a good measure of the relative efficiency of the systems concerned, and a better knowledge of the antigenic differences involved should lead this analysis even further.

The decreased virulence in these experiments was always transient. The inability to “take” in the strain of origin, therefore, has to be interpreted as due to a permanent damage of the leukemic cells by the defenses of the host. Whether such a damage was enough to kill the cells is unanswerable on the basis of actual evidence. It must be noted, however, that the foreign donor in all these cases appeared about to die from the transplanted leukemia, so we can assume that, at that moment at least, the leukemic cells were alive. Furthermore, no gross macroscopic signs of necrosis were noticed in the spleens of the donor animals at the time that the tumor material was taken for transplant. More experiments will be needed to know the fate of this material: whether it is destroyed or, a more interesting possibility, whether it remains alive but unable to carry on its uncontrolled growth.

Changes of this kind in the first transfer of a tumor to foreign strains have been observed and analyzed in particular by Mitchison (18) in both immunized and nonimmunized animals. He observed a decrease in viability of the tumor cells after being transplanted to foreign hosts, and found this to be a function of their length of residence in the foreign strain and more marked and starting earlier in animals passively immunized against the tumor. The critical period in his experiments is about 10 days in untreated animals. In the experiments here presented the critical period of residence in a foreign host, as determined by the ability of the cells to “take” on retransplanting, is lowered to 7 days in some combinations (H line in C58 hosts). What is more significant, other factors being held constant, our data show that the degree and timing of the critical period are a function of the strain, i.e., owing to genetic differences. Hauschka (7) has obtained comparable results in some of his experiments on transplantation of tumors into F1 hybrids. His experiments, which include several transfers both to F1’s and the parent strains, showed in some cases a slow and lasting shift of the tumor lines toward an increased specificity and lower virulence with complete loss of virulence eventually even in the strain of origin of the tumor. MacDowell et al. have reported one instance of a similar modification of virulence after implanting a leukemic line in animals which had been immunized against it. The cells in the first transfer grew locally and slowly as a lymphosarcoma. This pattern of growth was maintained for five transfers. In all the other cases the lymphosarcomas obtained in the first transfer reverted to the original generalized virulent form of leukemia (19) in the second transfer.

Increase in virulence.—The increase in virulence of the A leukemic cells obtained in C5H mice when these animals receive a highly lethal dose is clear indication that the histocompatibility gene differences are not enough to explain all these results and that other mechanisms must exist to account for them. An analogy can be drawn here with results of other authors in which an increase in tumor takes in unrelated strains has been observed as a result of passing the transplanted tumors into F1 hybrids (Barrett et al. [1]). It is doubtful whether the analogy can be broadened to include all cases in which a treatment of the host has also led to an increase in takes in animals of other strains, such as in the foster-nursing experiments of Law (11), the parabiosis experiments of Cloudman (4), and the enhancing effect of cancerous or normal tissues or their extracts (Casey [3], Snell et al. [16], Kaliss [10], Day et al. [5]). The acute and rapid development of the leukemic disease and the marked uniformity of the lesions in nearly all the animals which die on the same day would lead us to rule out selection of more virulent cells as a major factor in the observed increase in virulence. One is therefore led to postulate the existence of “facilitating substances” in the host which in some obscure way become a part of the tumor cells and reproduce along with them, thereby conferring upon them the increased virulence. The analogy with Barrett’s findings is further stressed by the specificity of this particular enhancement of tumor growth; only one out of seven strains tested showed this effect. In the other six there was a depression of virulence.

Recovery of virulence.—The recovery of virulence of the A line in its third transfer in AK hosts can be explained on the same grounds as above,
but because of the number of intervening cell generations after the first transferred to the foreign strain, other interpretations are also possible. Selection, which cannot be ruled out in these experiments, seems to be the most probable of the explanations. This situation would parallel the cyclic variations of trypanosomic populations on the same host observed by Ehrlich (quoted from Woglon [18]).

It is tempting to imagine that, under the prolonged action of the antibodies from the foreign host, a change in antigenicity takes place which affects the majority of the cell population (though no direct serological proof of an antigenic change has appeared in the literature), the antigenic change being postulated on the basis of the histocompatibility gene requirements in transplantation (Hauschka [7]). That this is not the only possibility has been suggested by Hoecker (8), based on the lack of actual antigenic loss observed in serological experiments with long-transplanted tumors in mice.

**SUMMARY**

The reciprocal transplantability of four long-transplanted lines of leukemia, A and F from strain C58 mice and G and H from strain AK mice, was studied. Of these, lines F and G were strain-specific and did not kill mice from unrelated inbred strains. A and H, however, grew and killed more than 90 per cent of the foreign hosts in the first transfer. In the second transfer a marked drop in the number of takes was observed, the degree of reduction being related to the length of residence of the leukemic cells in the foreign host used as donor for this transfer.

In a more detailed analysis, A leukemia was transplanted into mice of seven different inbred strains. Of these, 96 per cent of the AK, 86 per cent of the C3H, and 38 per cent of the Rk mice died in the first transfer. None of the A7, BALB/c, or DBA/1 mice that were inoculated died. In the second transfer a decrease in the number of takes was observed in mice of strains AK and Rk, which was more marked in Rk mice. In C3H, on the contrary, an increase in virulence was observed, as judged from the high mortality and the shortening of the interval before death when leukemic material from these hosts was inoculated in a second transfer into C3H hosts, and into AK and C58 strain mice.

Of two experiments involving a third transfer of line A leukemia into AK hosts, one was successful, and the A line recovered the virulence observed in the first transfer to these hosts.

It is postulated that the results measure the "protective efficiency" of the different genotypes against the particular leukemias involved. Several hypotheses to explain these complex results are discussed.

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