Behavior of L1210 Leukemia after Heterologous Transplantation in Rats*

R. R. ERDMANN, C. T. ASHWORTH, AND V. BUTTRAM

(Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas)

The survival and growth of normal and neoplastic cells inoculated into a heterologous species are subjects of increasing interest. In this phenomenon, the reaction of tissues against neoplastic cells is accentuated and more clearly delineated than in homotransplants. Although the mechanisms of resistance against heterologous tumor transplants may be different in degree and nature from that which occurs in the animal's natural environment, such exaggerated reactions may have a bearing upon the general aspects of the progressive and unrestrained growth which characterizes malignant neoplastic cells. Furthermore, studies of the successful restraint and subsequent destruction of heterotransplanted cells may shed some light on a means of neutralizing the growth of malignant cells in their original and natural locus.

The present study is an endeavor to determine some of the quantitative and qualitative aspects of the successful transplants and of the regressive phenomena which occur later, after inoculation of L1210 mouse leukemia cells into rats. An evaluation is also made of the influence of prior conditioning of the recipient animals, with whole-body x-radiation and cortisone administration upon the course of the heterotransplants.

MATERIALS AND METHODS

In total, 81 Sprague-Dawley rats were used in this study. L1210 cells were obtained from BDF-1 mice, in which they were being maintained as isotransplants. The leukemic cells were injected intraperitoneally, with the object of studying the ascites form of growth. Injections of $5 \times 10^6$ to $7 \times 10^7$ of the leukemic cells were made into the lower abdominal cavity. Heterotransplants were attempted in the following groups: (a) 26 newborn rats from 1 to 4 days of age; (b) 13 newborn rats which were also given 0.05 cc. of cortisone subcutaneously; (c) 13 untreated young rats from 1 to 3 months of age and weighing 75–170 gm.; (d) 14 young rats weighing 75–170 gm. which had received total-body irradiation (400 r) 4 days prior to the inoculation of leukemic cells, and which were given 0.1 cc. of cortisone subcutaneously 2 and 4 days after irradiation; and (e) 15 untreated old, adult rats weighing 180–320 gm.

The animals were sacrificed 2–27 days after intraperitoneal injection. The volume of peritoneal fluid was estimated by aspiration to dryness and then lavaging the peritoneal cavity with a known volume of 0.85 per cent NaCl. Cell counts were performed upon the recovered peritoneal fluid, and the total number of recovered tumor cells was approximated. Differential cell counts were made on Giemsa-Wright-stained smears of the peritoneal fluid. Estimations were made of the number of viable as compared with degenerated cells, and of cells in mitosis.

Histologic study was carried out on the following tissues: heart, lungs, mediastinum, liver, spleen, pancreas and retroperitoneal tissue, intestine, kidney, abdominal wall at the general site of inoculation, and brain. Sections were prepared with hematoxylin-eosin, toluidine blue for mast cells, and Feulgen stains.

RESULTS

1. Incidence of successful heterotransplants.—All successful transplants of the L1210 leukemia were believed to be of the regression type. Death, however, occurred in a few animals at the height of the growth of the transplant, before regression had occurred.

Of the 81 animals studied, 29 successful heterotransplants were demonstrated histologically. All these were found in animals sacrificed less than 10 days after the inoculation. Viability of leukemic cells was confirmed in 21 of the 29 successful heterotransplants by the production of progressive ascites tumor following the re-inoculation of the peritoneal fluid in BDF mice. Histological evidence was also obtained in fourteen of 37 animals surviving past 10 days, which suggested that...
growth of neoplastic cells had occurred earlier. At the time of sacrifice, however, these tumor cells had regressed completely.

The incidence of positive heterotransplants in the individual groups of animals is shown in Table 1. The highest incidence of successful transfers was in the group of young adult rats which had been conditioned with cortisone and total-body x-radiation prior to inoculation.

2. Rate of growth of injected L1210 cells.—Estimates of the total number of leukemic cells in recovered peritoneal fluid fail to indicate accurately the number of all viable cells present, since many tumor cells have already invaded the tissues. The total number of leukemic cells recovered from the peritoneal cavity 2 days after inoculation increased, on the average, by sixfold, while at 4 days and later the number averaged less than that injected, and nearly no viable cells were found after 7 days. There was no apparent difference in numerical increases of leukemic cells in different age groups, nor in conditioned as compared with unconditioned animals. In the first 2 days, most of the cells in smears from the peritoneal fluid were intact and appeared viable (Fig. 1). After this time dead or degenerating cells increased rapidly in number, comprising the majority after 5 days, when the total number of cells was also markedly reduced (Fig. 2). As long as viable cells remained, the frequency of mitoses and of the different phases of mitosis remained unaltered.

3. Early reaction to intraperitoneally inoculated leukemic cells.—Shortly after inoculation, there was an accumulation of small numbers of polymorphonuclear leukocytes, mast cells, and fibrin within the peritoneal cavity. This reaction reached its peak in about 3 days, following which interval the inflammatory constituents decreased and finally almost completely disappeared after the 5th day. In differential counts of this fluid, mesothelial cells also were noted. They were quite numerous in the first 2 days, but were overshadowed by the leukemic cells after this time.

At approximately 2 days, fibrin deposits containing viable L1210 cells were noted over the peritoneal surfaces. Later, the leukemic cells were found invading the tissues of the peritoneum and retroperitoneal areas (Fig. 3), especially around the pancreas, and solid masses of the tumor could be seen growing in the omentum. Within the invaded tissues, edema and slight infiltration of lymphocytes, eosinophils, a few polymorphonuclears, and plasma cells were observed. Growths of tumor cells in the anterior abdominal wall near the site of inoculation were also present.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>No. examined before 10 days/number showing viable leukemic cells</th>
<th>No. examined after 10 days/number showing histological evidence of regressed heterotransplants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn, unconditioned</td>
<td>9/4</td>
<td>17/5</td>
</tr>
<tr>
<td>Newborn, cortisone-conditioned</td>
<td>13/10</td>
<td>0</td>
</tr>
<tr>
<td>Young adult, unconditioned</td>
<td>12/12</td>
<td>8/4</td>
</tr>
<tr>
<td>Young adult, cortisone- and x-ray-conditioned</td>
<td>5/1</td>
<td>10/5</td>
</tr>
<tr>
<td>Old adult, unconditioned</td>
<td>81/44</td>
<td>37/14</td>
</tr>
<tr>
<td>Total</td>
<td>54/29</td>
<td>26/14</td>
</tr>
</tbody>
</table>

The lesions of these heterotransplants were similar to isotransplants in BDF mice, except that in the former the growths were much less numerous and extensive.

4. Disseminated lesions in heterotransplanted L1210 leukemia.—Small nests of the leukemic cells were found in the sinusoids of the liver, pulmonary capillaries, and in the splenic pulp in animals...
studied at 5–6 days, but were not found in the heart and kidney. No large, nodular metastases were found, and no gross nor histological evidence of involvement of the brain was seen. The metastatic lesions were more extensive and more numerous in the animals previously treated with x-radiation and cortisone.

5. Regressive stage of heterotransplants.—The gradual disappearance of viable leukemic cells and increase in degenerated cells in the peritoneal fluids were considered to indicate the beginning of the stage of regression. The volume of peritoneal fluid became markedly reduced, and at 7–9 days and later regressive changes had occurred in the infiltrative lesions of the abdominal wall, omentum, peripancreatic tissue, and in the spleen and liver (Figs. 5 and 6). Large focal areas of the leukemic cells were found to have undergone nuclear pyknosis, karyorrhexis, and ultimately complete loss of stainable nuclear material. These changes were frequently accompanied by recent interstitial hemorrhage. The necrotic areas were nodular in form, but tended to become confluent and diffuse. At this stage, considerable infiltration of polymorphonuclears, lymphocytes, plasma cells, and macrophages was observed in and around the necrotic areas. Later, the necrotic cells were partially or completely autolyzed, leaving a collapsed, vascular tissue, and in some instances an early encapsulating fibroblastic layer was found (Figs. 7 and 8). These regressive changes were found in animals conditioned with x-ray and cortisone, as well as in unconditioned animals. They were more marked, however, in the latter group.

In the spleen, reticuloendothelial hyperplasia was often present in the unconditioned animals (Fig. 9). In those that had received x-radiation and cortisone, a marked reduction of lymphocytes in the splenic pulp was observed (Fig. 10).

DISCUSSION

The failure of heterotransplants of L1210 cells to survive for longer periods may be due either to a passive inability of inoculated cells to multiply in the new environment, or it could be the result of some active resistance by the host tissue producing injury and death of the transplanted cells. Since during the first 2 days the leukemic cells increase markedly in number, it is believed that the host tissues, at this stage, do not constitute a hostile environment. Further, the inflammatory reaction which occurs at this time is not marked, either in the peritoneal fluid or in the tissues with the advent of invasion by the leukemic cells. These findings are similar to those of von Haam and Horava (5), who transplanted Ehrlich mouse ascites tumor in rats. In severity, this local reaction does not appear to exceed that which is noted in isografts of the leukemic cells in BDF mice, which survive to the death of the animal.

The regressive changes occur only several days after the transplantation. Some of these changes may be due to local circulatory disturbances, such as thrombosis occurring after the foci of tumor cells have grown to a relatively bulky size. However, the rapid and widespread development of the regressive lesions, and the complete destruction and disappearance of the tumor cells from the recipient animal, would favor the development of a cytotoxic agent or antibody which would cause rapid injury and death of the neoplastic cells. The virtually complete disappearance of the tumor cells a few days after the regressive changes have begun might indicate that cytolytic enzymes have been produced or are available for rapid autolysis of the dead cells. Toolan (12) and others (2, 3) have recently demonstrated cytotoxic antibodies to heterotransplanted neoplastic cells in animals, and this may represent the mechanism that leads to regression of heterotransplants.

The transplantation of neoplastic cells into foreign animal species might be expected, especially after several transfers, to result in some modification of behavior of the cells. Skiff et al. (8), in a study of heterologous transplantation of several human neoplasms into hamsters, observed some tendency for the tumor cells to undergo maturation. In the transfers to BDF mice from ascitic growths of L1210 cells in rats for viability studies, we have observed no modification of the growth characteristics nor of the morphologic appearance of the leukemic cells.

Young adult rats appear to be more susceptible to heterotransplantation than either newborn or old rats. This contrasts with the greater facility with which cell-free filtrate transmission of experimental leukemia (4) can be accomplished in newborn animals.

The enhanced growth of the heterotransplanted L1210 cells in animals conditioned with x-radiation and cortisone substantiates the observations of others (1, 7, 11). This effect is probably due to a lessening of the activity or production of cytotoxic antibodies, attributable to the influence of suppression of reticuloendothelial cells or lymphocytes by x-radiation and cortisone. It is apparent, however, since the heterotransplants in treated animals did shortly begin to undergo regression as in other animals, that there is no sustained or complete suppression of antibody by the amount of conditioning given in this experiment.
In conditioned and unconditioned rats, the reticuloendothelial cells of the spleen and lymph nodes became hyperplastic and swollen. Murphy (7) also observed hyperplastic changes in reticuloendothelial tissues of mice bearing growing tumors. These changes are probably related to an antigenic stimulation by products of the growing tumor cells, leading to antibody formation.

**SUMMARY**

In studies on the heterotransplantation of L1210 mouse leukemia cells into the peritoneal cavity of rats, successful takes were obtained in 35 per cent. Previous conditioning of young adult rats by whole-body x-radiation and cortisone administration produced the highest percentage of successful heterotransplants. The leukemic cells also grew more abundantly in prepared rats, and the lesions were more numerous and larger than in unprepared animals.

Regression in heterotransplants first occurred after 3–4 days and was characterized by decrease in the number of cells in the peritoneal cavity, decrease in volume of peritoneal cavity, decrease in volume of peritoneal fluid, and by the development of focal areas of necrosis and cytolysis of the leukemic cells, followed by fibroblastic proliferation. It is believed that the development of cytotoxic antibodies is responsible for the regressive changes which were observed.

**REFERENCES**


**FIGS.**

**Fig. 1.**—Viable L1210 leukemia cells in smear of peritoneal fluid 9 days after inoculation. Note characteristic lipid vacuolization. One cell in mitosis is demonstrated. Wright-Giemsa stain. Mag. X880.

**Fig. 2.**—L1210 leukemia cells in peritoneal fluid after 5 days in heterologous host. Note clusters of degenerating tumor cells. Wright-Giemsa stain. X880.

**Fig. 3.**—Early stage of heterotransplantation of L1210 cells showing interstitial invasion. Two days after inoculation in unconditioned animal. Hematoxylin and eosin (H & E) stain. X370.

**Fig. 4.**—Early stage of omental invasion by leukemic cells in x-ray- and cortisone-conditioned animal. Note abundance of cells. Two days after inoculation. H & E stain. X370.

**Fig. 5.**—Regressive changes of leukemic cell infiltration in omentum in unconditioned animal, consisting of nuclear pyknosis, karyorrhexis, and final complete disintegration of the cells. H & E stain. X370.

**Fig. 6.**—Early regressive changes: Nuclear pyknosis and degenerating cells, associated with recent interstitial hemorrhage. H & E stain. X370.
FIG. 7.—Regressive changes in leukemic cells and early fibrous encapsulation of heterotransplant involving omentum. Nine days after intraperitoneal inoculation. H & E stain. ×370.

FIG. 8.—Late regression of omental tumor mass of L1210 leukemia cells. Note presence of fibroblasts, plasma cells, lymphocytes, and macrophages. Residues of autolyzed leukemic cells are phagocytized by the macrophages. Twelve days after inoculation. H & E stain. ×370.

FIG. 9.—Spleen of unconditioned animal 6 days after intraperitoneal inoculation of L1210 leukemia cells. Note hyperplasia of the reticuloendothelial cells and follicular proliferation of young lymphocytes. H & E stain. ×370.

FIG. 10.—Spleen of x-radiated animal 3 days after intraperitoneal inoculation of leukemic cells. Note sparsity of lymphocytes. H & E stain. ×370.
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