Comparative Studies of Subcutaneous and Intradermal Leukemic Tumors in Guinea Pigs

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SUMMARY

Small doses of L2C leukemic cell suspensions inoculated intradermally into strain 2 and F1 hybrid guinea pigs induced leukemic tumors that regressed spontaneously in about 50% of animals, whereas s.c. inoculation induced uniformly generalized leukemia. Because of the behavioral differences between the s.c. and intradermal tumors, it appeared of interest to examine their comparative morphology by light and electron microscopy. Both s.c. and actively growing intradermal tumors are made up of identical nonphagocytic histiocytes and contain virus particles. They differ in the number of proliferating cells, which is larger in the s.c. tumors. Cell necrosis is a conspicuous feature of the regressing intradermal tumors and is manifested by large numbers of macrophages with engulfed lysed cells or cell fragments. The virus particles disappear before the tumors regress.

INTRODUCTION

It was observed in our previous studies (7, 8) that diluted doses of leukemic cell suspensions inoculated intradermally into strain 2 or F1 hybrid guinea pigs induced small intradermal leukemic tumors that regressed spontaneously in about 50% of animals, whereas similar doses inoculated s.c. or i.p. led uniformly to a generalized and fatal leukemia. Those animals that recovered from the intradermal tumors were in most instances immune to reinoculation of massive doses of leukemic cells by any route.

Experiments here reported describe results of light and electron microscopic studies of the intradermal leukemic tumors, as compared with the morphology of the s.c. leukemic infiltrations. We have studied specifically the changes in the morphology of the leukemic cells and their surrounding tissues in different phases of development, temporary growth, and eventual regression of the intradermal leukemic tumors. Virus particles observed in such tumors were also studied in the electron microscope.

MATERIALS AND METHODS

Animals

Strain 2 guinea pigs used in this study were bred by brother-to-sister mating in our laboratory; F1 hybrid guinea pigs, born to Hartley females and strain 2 males, were also used.

L2C Guinea Pig Leukemia. The L2C leukemic strain used in this study originated as a spontaneous leukemia in one of the strain 2 guinea pigs (4) and has been carried by cell graft in animals of this inbred line. This strain of leukemia is uniformly leukemogenic for strain 2 or F1 hybrid guinea pigs; s.c. or i.p. cell graft induces in these animals a rapidly progressing, generalized stem-cell leukemia (9, 10).

Preparation of Specimens for Microscopic Studies

Light Microscopy. Tissues were fixed in Bouin's fluid, followed by 10% buffered formalin, and embedded in paraffin. The sections were stained routinely with hematoxylin and eosin and in some instances with Gomori's silver methenamine, Masson trichrome, and periodic-acid Schiff.

Electron Microscopy. The specimens for electron microscopy were fixed in 4% phosphate-buffered glutaraldehyde, followed by 1% phosphate-buffered osmic acid, and embedded in Epon. The sections were stained with uranyl acetate and lead hydroxide and then examined in an RCA EMU-3G electron microscope.

EXPERIMENTAL STUDIES

Leukemic cell suspensions at 10^-2 to 10^-7 dilutions were inoculated under the skin of the right flank into young adult, 3- to 6-week-old, strain 2 or F1 hybrid guinea pigs. Out of 106 strain 2 guinea pigs inoculated s.c. with leukemic cell suspensions at 10^-2 to 10^-6 dilutions, 102 developed leukemic cell infiltrations, which appeared at the site of inoculation, grew progressively, and led to a generalized stem-cell leukemia (Table 1). In a parallel series, 83 F1 hybrid guinea pigs were inoculated s.c. with leukemic cell suspensions at 10^-2 to 10^-7 dilutions, and 79 of them developed generalized leukemia (Table 2).

In another series of experiments, small doses (0.1 ml) of leukemic cell suspensions, varying in dilution from 10^-3 to 10^-7, were inoculated intradermally into young, adult 3- to 6-week-old strain 2 guinea pigs. Among 120 guinea pigs inoculated intradermally, 94 developed small intradermal tumors at the site of inoculation after a latency varying from 11 to 20 days; in 48 animals (51%) these tumors regressed spontaneously after 4 to 7 days (Table 3). In a parallel series, out of 46 F1 hybrid guinea pigs inoculated intradermally, 39 developed intradermal tumors; 12 of these tumors (31%) regressed spontaneously (Table 4).
Table 1

Results of s.c. inoculation of leukemic cell suspensions into young adult strain 2 guinea pigs

<table>
<thead>
<tr>
<th>Leukemic cell dilution</th>
<th>No. of guinea pigs inoculated</th>
<th>No. that developed s.c. tumors</th>
<th>Av. time s.c. tumors developed (days)</th>
<th>No. that developed generalized leukemia</th>
<th>Leukemia incidence (%)</th>
<th>Av. time of death (days)</th>
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<td>$10^{-2}$</td>
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<td>53</td>
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*With 0.3 to 0.5 ml each, s.c.

Table 2

Results of s.c. inoculation of leukemic cell suspensions into young adult F1 hybrid guinea pigs

<table>
<thead>
<tr>
<th>Leukemic cell dilution</th>
<th>No. of guinea pigs inoculated</th>
<th>No. that developed s.c. tumors</th>
<th>Av. time s.c. tumors developed (days)</th>
<th>No. that developed generalized leukemia</th>
<th>Leukemia incidence (%)</th>
<th>Av. time of death (days)</th>
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<td></td>
<td>95</td>
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*With 0.3 to 0.5 ml each, s.c.

Table 3

Results of intradermal inoculations of small doses of leukemic cell suspensions into strain 2 guinea pigs

<table>
<thead>
<tr>
<th>Leukemic cell dilution</th>
<th>No. of guinea pigs inoculated</th>
<th>No. that developed i.d. tumors</th>
<th>Av. time i.d. tumors developed (days)</th>
<th>No. of i.d. tumors that regressed</th>
<th>Av. time i.d. tumors regressed (days)</th>
<th>Incidence of i.d. tumors that regressed (%)</th>
<th>No. that developed generalized leukemia</th>
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<td>11</td>
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<td>Total</td>
<td>120</td>
<td>94</td>
<td>48</td>
<td>51</td>
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*a* With 0.1 ml each, intradermally.

**Light Microscopy**

**Morphology of the s.c. Tumors.** There was widespread and massive leukemic cell infiltration extending through the s.c. fat into striated muscle (Fig. 1). The muscle fibers were widely infiltrated, separated, and surrounded by tumor cells. In some of the s.c. tumors muscle fibers were replaced by sheets of closely packed leukemic cells. The tumor infiltrated around small arteries and arterioles, occasionally invading the walls of the vessels. However, there were no intrinsic vascular changes. The infiltrating tumor was made up of a single cell type, resembling a histiocyte with scanty cytoplasm. The large vesicular nucleus was occasionally indented, with finely dispersed chromatin and 1 or 2 slightly enlarged nucleoli (Fig. 2). There were many mitotic figures ranging from 2 to 10 per high-power field. Silver reticulin stains in some of the tumors showed numerous thick fibers with occasional areas of finely branching reticulin fibers surrounding individual cells. There were a few small areas of necrosis in the periphery of the tumors, usually associated with hemorrhage and demarcated...
from the rest of the tumor. The necrotic foci consisted of clusters of degenerated cells with pycnolic nuclei and eosinophilic cytoplasm. Intermingled among the tumor cells were variable small numbers of polymorphonuclear blood cells and occasionally lymphocytes.

**Morphology of the Growing Intradermal Tumors.** The leukemic cells infiltrated the dermis in small clusters, separating collagen fibers without destroying them. In a few instances, small sheets and clusters of tumor cells infiltrated the entire thickness of the dermis, often extending into the fat and occasionally into the superficial portion of the underlying striated muscle. The number of cells present was always relatively small when compared with the s.c. tumors. The cell type was similar to that of the s.c. tumors and mitoses were frequently encountered. There were occasional cells with pycnolic nuclei (Figs. 3 and 4).

**Morphology of the Regressing Intradermal Tumors.** In the dermis there was extensive necrosis and lysis of the tumor cells. There were numerous fragmented cells with pycnolic nuclei and nuclear debris, and many cells stained poorly. There was an intermingling of macrophages containing cell debris, lymphocytes, polymorphonuclear cells and, occasionally, eosinophils (Fig. 5). Small blood vessels or arterioles, particularly in the deeper part of the dermis, showed endothelial hyperplasia and alteration of the muscle layers.

**Electron Microscopy**

**Electron Microscopic Studies of the s.c. Tumors.** The s.c. leukemic infiltrations consisted of large numbers of tumor cells (Fig. 6, T). These cells resembled nonphagocytic histiocytes; they were large, with an irregular, sometimes elongated shape and contained cytoplasmic processes. The nuclei were large and often indented.

Occasionally, collagen fibers and striated muscle fibers (Fig. 6, S) were found between the tumor cells. A few areas of cellular necrosis were observed. Several phagocytic histiocytes or macrophages containing engulfed remnants of destroyed cells were also present.

Virus particles similar to those previously described (5, 11, 12) were found budding from the endoplasmic reticulum, or free within the cisternae of the endoplasmic reticulum, of tumor cells (Fig. 7, arrows). Mature virus particles were found in the intercellular spaces.

**Electron Microscopic Studies of Actively Growing Intradermal Tumors.** In the initial phase of their active growth, all intradermal tumors contained considerable numbers of tumor cells. These cells could be identified as histiocytes; they were large, with cytoplasmic processes and large indented nuclei (Fig. 8). Most of the histiocytes were nonphagocytic; some, however, contained ingested material made up of fragments of destroyed cells. Many of the tumor cells showed no signs of degenerative changes, while others appeared to be necrotic. Besides the tumor cells, the intradermal tumors contained collagen, fibroblasts, granulocytes, lymphoid cells, and erythrocytes.

Virus particles were observed in both nonphagocytic and phagocytic histiocytes. Immature particles, without electron-dense nucleoids, appeared in few to moderate numbers within the cisternae of the endoplasmic reticulum (Figs. 9 and 10, arrows). A few mature particles were present in the intercellular spaces.

**Electron Microscopic Study of the Regressing Intradermal Tumors.** Those intradermal tumors that were regressing contained fewer nonphagocytic histiocytes. Large numbers of phagocytic histiocytes or macrophages were present (Figs. 11 and 12, M), containing engulfed destroyed cells or parts of cells and lysosomes. Initially, there was intense cellular destruction followed by extensive phagocytosis of cell debris. Besides the histiocytes, collagen (Fig. 11, C), fibroblasts (Fig. 11, F), granulocytes, lymphoid cells (Fig. 12, L), and erythrocytes were also observed. In spite of a careful search, virus particles were not found in the regressing intradermal tumors.

**Comparison of the s.c. and Intradermal Tumors.** Intradermal and s.c. tumors were both made up of large nonphagocytic, histiocytic tumor cells with many mitotic figures. However, these tumors differed in the relative number of cells and in the type of cells observed. The s.c. tumors contained mostly tumor cells; collagen, striated muscle fibers, and a few macrophages were also present. Fewer tumor cells were found in intradermal tumors; these tumors contained much collagen; macrophages and several fibroblasts, granulocytes, lymphoid cells, and erythrocytes were also observed.

There was less cellular destruction in s.c. tumors than in the actively growing intradermal tumors. The regressing
intradermal tumors showed many areas of cell necrosis. The tumor cells in s.c. tumors were usually compact and densely packed, whereas tumor cells in the intradermal tumors were more loosely arranged with wider intercellular spaces.

Virus particles were found within, or proximal to, the tumor cells in both the s.c. and intradermal tumors. Since tumor cells were more numerous in s.c. tumors, virus particles appeared more frequently in the s.c. than in the intradermal tumors. Mature virus particles, in particular, were observed more often in the s.c. tumors.

**DISCUSSION**

It has been known since the early studies on experimental cancer that those animals in which implantation of tumors led to an initial "take" and a temporary growth of the tumor, followed by a subsequent spontaneous regression, were usually immune to reinoculation of the same tumor by any route. Clowes and Baeslack (3) were probably the first to describe this interesting and important phenomenon.

The skin proved to be an organ with an immunological potential capable, in certain instances at least, of limiting the growth of otherwise progressively growing implanted tumors (1, 2). In previous studies carried out on mice (1, 6) and chickens (2), tumors implanted in small doses intradermally produced in very few instances local and transient growths, persisting for a few days and eventually disappearing spontaneously; whereas the same tumors implanted under the skin grew progressively and led to a generalized and fatal dissemination. In these early experiments, spontaneous regression of the implanted intradermal tumors was very rare and always unforeseen. In our present experiments on guinea pigs, one-half of the intradermal tumors, induced with small doses of leukemic cells, regressed spontaneously; whereas, in contrast, all leukemic tumors induced by s.c. inoculation led to a generalized and fatal disease.

As already discussed (7, 8), the majority of guinea pigs that recovered spontaneously from the intradermal tumors proved to be immune to reinoculation of heavy doses of leukemic cells by any route.

Both the s.c. and the growing intradermal tumors contain virus particles. At a certain point, however, about 6 to 9 days following their appearance, about 50% of the intradermal leukemic tumors begin to regress. The virus particles disappear before the complete regression of tumor cells.

Those intradermal tumors in which regression is very apparent contain large numbers of macrophages with engulfind lysed cells or parts of cells. In such tumors only a few nonphagocytic histiocytes are present; virus particles have already disappeared. The origin of the phagocytic macrophages characteristic of the regressing phase of the intradermal tumors is not apparent, and one may speculate that they are either tissue or circulating macrophages or, perhaps, derived from altered leukemic histiocytes.

These observations are interesting but they do not explain why the intradermal tumors often regress, whereas the s.c. tumors grow progressively in all instances. Furthermore, the question remains to be answered concerning the mechanism and nature of the general and specific immunity developing in those animals in which the intradermal tumors regressed spontaneously. Immunity could not be transferred by a serum, collected from such animals, to other guinea pigs (8).

**REFERENCES**

Fig. 1. A s.c. tumor. Closely packed leukemic cells invading muscle with very little stroma. H & E, × 55.

Fig. 2. A s.c. tumor. High power of Fig. 1, illustrating the monotonous appearance of the cells, large nuclei, scanty cytoplasm, and mitotic figures. H & E, × 575.

Fig. 3. Intradermal tumor. Clusters of leukemic cells in upper dermis infiltrating around hair follicles and separating collagen bundles. H & E, × 55.

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Fig. 4. Intradermal tumor. Detail of Fig. 3. The cells are similar to the s.c. tumor cells and infiltrate around collagen bundles. A mitotic figure and small nuclear fragments are in the center. Small number of lymphocytes in the periphery. H & E, × 575.

Fig. 5. Intradermal tumor, regressing phase. There are many lymphocytes and polymorphonuclear leukocytes. About one-half of the cells are leukemic and many are fragmented and pycnotic. H & E, × 575.
Fig. 6. Electron micrograph of section from s.c. tumor from a leukemic F₁ hybrid guinea pig, 18 days after s.c. inoculation of a leukemic cell suspension. Tumors cells (T) with guinea pig leukemia virus particles (arrow). Also present are striated muscle fibers (S). X 30,000.

Fig. 7. Part of a cell from a s.c. tumor from a leukemic F₁ hybrid guinea pig, 30 days after s.c. inoculation of a leukemic cell suspension. Guinea pig leukemia virus particles (arrows) are present within the perinuclear cisternae and in the cisternae of the endoplasmic reticulum. X 56,000.

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Fig. 8. Electron micrograph of a tumor cell from an actively growing intradermal tumor from a F, hybrid guinea pig, 15 days after intradermal inoculation of a leukemic cell suspension at $10^{-6}$ concentration. Guinea pig leukemia virus particles (arrows) are present. $\times$ 24,000.

Figs. 9 and 10. Electron micrographs of guinea pig leukemia virus particles (arrows) in an actively growing intradermal tumor from a F, hybrid guinea pig, 20 days after intradermal inoculation of a leukemic cell suspension at $10^{-5}$ concentration. $\times$ 42,000.
Figs. 11 and 12. Sections of a regressing intradermal tumor from a strain 2 guinea pig, 14 days after intradermal inoculation of a leukemic cell suspension at $10^{-4}$ concentration. Collagen (C), fibroblast (F), macrophages (M), and a lymphoid cell (L) are shown. Fig. 11, $\times$ 16,000; Fig. 12, $\times$ 20,000.
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