Attempt to Immunize Guinea Pigs Against Leukemia by Skin Scarification with Leukemic Cell Suspensions

(virus in guinea pig leukemia/skin and tumor immunity)

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ABSTRACT An attempt was made to immunize "strain 2" guinea pigs by superficial skin scarification with small doses of L2C leukemic cell suspensions. Among 203 scarified guinea pigs, 32 developed progressively growing leukemic tumors at the site of skin scarification. In 35 guinea pigs small intradermal tumors that appeared at the site of scarification regressed spontaneously; however, 15 guinea pigs in which the intradermal tumor regressed later developed generalized leukemia. In addition, 13 other animals developed generalized leukemia, without an apparent local tumor formation at the site of scarification. A total of 60 out of 203 scarified guinea pigs (30%) died from leukemia.

143 Guinea pigs that survived the scarification were challenged by subcutaneous inoculation of massive doses (0.5 ml each of a 10-fold dilution from a 10% extract) of leukemic cell extracts and only 48 (34%) developed leukemia; 95 guinea pigs (66%) resisted the challenge and remained in good health. In a control experiment, 156 untreated "strain 2" guinea pigs were inoculated subcutaneously (0.5 ml each) with L2C leukemic cell suspensions of 10^-1 or 10^-3 dilution, and all but two (99%) developed generalized leukemia.

For the past several years we have been trying to induce active, specific immunity against leukemia in guinea pigs (1, 2). In our initial studies we inoculated "strain 2" guinea pigs intradermally with very small doses of L2C leukemic cell suspensions. About 40-55% of the induced intradermal tumors persisted only temporarily and later regressed spontaneously. In marked contrast, subcutaneous or intraperitoneal inoculations led uniformly to generalized leukemia (1-3). The majority of those guinea pigs which recovered spontaneously from intradermal tumors were resistant to subcutaneous reinoculation of massive doses of leukemic cell suspensions (1, 2). Immunity was more pronounced in those animals which received about 2-3 weeks after the disappearance of the initial intradermal tumors a second intradermal "booster" inoculation of a leukemic cell suspension. Females were more readily immunized than males (2). The immunity thus induced was lasting; animals tested as long as 2 years after they had recovered from the intradermal tumors were still resistant to a challenging reinoculation of leukemic cells.

In view of the fact that the mortality rate incident to the course of intradermal immunization was relatively high, varying from 40 to 60% even under most favorable conditions (1, 2), an attempt was made to devise an alternative method of immunization which would have a higher safety factor.

Accordingly, in a more recent series of experiments, instead of inoculating the leukemic cell suspensions intradermally, we have attempted to induce immunity by superficial skin scarification with leukemic cell extracts, i.e., by a method in some respects similar to the classical method of smallpox vaccination, except that in our experiments cell suspensions and not a cell-free virus were applied.

In our initial experiments in which the scarification method was first applied the great majority of scarified guinea pigs survived the immunization procedure, and most of them were subsequently resistant to a challenging reinoculation of heavy doses of leukemic cells (4). However, in the course of subsequent experiments described in this study in which large numbers of guinea pigs were employed and observed for prolonged periods of time, it was realized that inoculation of leukemic cell suspensions by superficial skin scarification may quite frequently lead to a delayed development of leukemia; it also became evident that immunity resulting from skin scarification is less predictable than that induced by the previously employed method of intraderal immunization.

MATERIALS AND METHODS

L2C leukemia. The L2C leukemia, employed in this study, originated in 1954 as a spontaneous leukemia in a "strain 2" guinea pig (5) and has been maintained since that time by serial passage, presumably by cell-graft, in animals of "strain 2" inbred line (5, 6). For the past several years we have been transmitting the L2C leukemia by serial subcutaneous cell-graft passage in "strain 2" guinea pigs (1-3). When L2C leukemic cell suspensions are inoculated subcutaneously or intraperitoneally into "strain 2" guinea pigs, the suspensions consistently induce a rapidly progressing and uniformly fatal generalized stem-cell leukemia.

Animals. Our experiments were carried out on "strain 2" guinea pigs bred in our laboratory by brother-to-sister mating. Young adult animals, about 4-6 weeks old, were used for inoculations.

Preparation of leukemic cell suspensions. A guinea pig with advanced leukemia was sacrificed by ether inhalation. A fragment of the subcutaneous leukemic tumor from the site of inoculation, also a small fragment of spleen, and of the mesenteric tumor, occasionally also a fragment of a metastatic inguinal or axillary lymph node, were removed aseptically, weighed, cut with scissors, and ground in a mortar, sterile physiological saline solution being added to obtain a cell suspension of 10% concentration; the cell extract was then passed through a sterile voile cloth. Serial dilutions of desired
concentration were prepared from the original cell suspension, and used immediately for inoculation.

Scarification of skin. The scarification was performed in a similar manner to that employed in the application of smallpox vaccine to the skin. A few drops of a leukemic cell suspension of desired dilution were placed on the skin of the flank of a guinea pig on an area on which the hair had been closely clipped with an electric shaver. Scarification was performed in most instances with leukemic cell suspensions of $10^{-4}$ dilution. A sharp, sterile, 26-gauge needle was then used to scarify the skin on an area of about one square inch, introducing thereby the leukemic cell extract into the superficial layers of the skin. The scarification was performed gently so as not to pierce the skin too deeply with the needle during this procedure. Each of the scarified animals was then placed on an individual small table for 1–2 hr to allow the scarified skin to dry. No dressing was applied. The animals were then returned to their cages.

RESULTS

Results of Skin Scarification with Leukemic Cell Suspensions in “Strain 2” Guinea Pigs. A total of 203 young adult guinea pigs had their skin scarified with leukemic cell suspensions. 174 Guinea pigs had their skin scarified with cell suspensions of $10^{-4}$ dilution, 21 with a $10^{-3}$ dilution, and only 8 with a $10^{-2}$ dilution. Out of 203 scarified guinea pigs, 67 animals developed small single or multiple intradermal tumors at the site of scarification. In most instances, the intradermal tumors were single, but a few animals developed two or three small intradermal tumors at the site of scarification. In 32 guinea pigs, the intradermal tumors grew progressively and led to generalized leukemia. In the remaining 35 guinea pigs the intradermal tumors regressed spontaneously 6–10 days after they appeared. It is of considerable interest that among those 35 guinea pigs in which the intradermal tumors regressed spontaneously, 15 animals developed generalized leukemia several weeks later. 13 Guinea pigs did not develop skin tumors at the site of scarification, and remained, at first, in good health, but died about 2–3 months later from generalized leukemia. A total of 60 out of 203 scarified guinea pigs (30%) developed, and died from, leukemia within 1–3 months after scarification (Table 1).

Microscopic and Electron Microscopic Studies. Fragments of scarified skin were removed at various time intervals after scarification for preparation of sections for microscopic and electron microscopic studies. Examination of these sections demonstrated the appearance of leukemic cells in strands and clusters, infiltrating diffusely the superficial layers of the dermis between collagen fibers and surrounding also the hair follicles (Figs. 1 and 2). As can be seen in Fig. 3, the cells contained characteristic virus particles previously described (3, 7–9).

In most instances, with the exception of those guinea pigs in which progressively growing intradermal tumors were induced, the leukemic cells present in the superficial layers of the dermis gradually disintegrated and disappeared within 4–5 weeks after scarification. We have recently reported similar observations in our microscopic and electron microscopic studies of the cutaneous tumors induced in guinea pigs by intradermal inoculations of small doses of leukemic cells (10).

The presence of leukemic cells and of virus particles in the dermis could not always be demonstrated; in some of the guinea pigs in which fragments of the scarified skin were excised and studied, neither leukemic cells nor virus particles could be detected at the site of inoculation. However, no serial sections were made of the entire, relatively large (about one square inch) skin surface which was submitted to scarification and it is possible that the few small areas in which the leukemic cells entered into the dermis might have been missed on microscopic examination. It is also possible that some of the guinea pigs in which no microscopic evidence was found of leukemic cell infiltration within the dermis might have had the leukemic cell suspensions rubbed off accidentally from the epi-

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**Table 1. Results of skin scarification with leukemic cell suspensions* in “strain 2” guinea pigs**

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of guinea pigs scarified</th>
<th>No. developing progressively growing i.d.† tumors</th>
<th>No. developing transient i.d. tumors</th>
<th>No. developing generalized leukemia after i.d. tumors regressed</th>
<th>No. developing generalized leukemia with no local skin tumors</th>
<th>Total developing leukemia</th>
<th>Leukemia incidence (%)</th>
<th>Average latency: development of leukemia after scarification (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>124</td>
<td>16</td>
<td>20</td>
<td>8</td>
<td>11</td>
<td>35</td>
<td>28</td>
<td>56</td>
</tr>
<tr>
<td>Males</td>
<td>79</td>
<td>16</td>
<td>15</td>
<td>7</td>
<td>2</td>
<td>25</td>
<td>32</td>
<td>52</td>
</tr>
<tr>
<td>Total</td>
<td>203</td>
<td>32</td>
<td>35</td>
<td>15</td>
<td>13</td>
<td>60</td>
<td>30</td>
<td>54</td>
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* In the great majority of animals cell suspensions of $10^{-4}$ dilution were employed for skin scarification. (See text.)
† Intradermal.

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Fig. 1. Cross section of a fragment of skin and subcutaneous tissue removed from a guinea pig 19 days after superficial skin scarification with leukemic cell suspension. A leukemic cellular infiltrate surrounding hair follicles can be seen in the center of the dermis. There is no involvement of the subcutaneous tissues. Hematoxylin and eosin. Magnification: ×9.
**Table 2.** Resistance of scarified "strain 2" guinea pigs to subcutaneous* challenging reinoculation of leukemic cell suspensions

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of guinea pigs scarified</th>
<th>No. of guinea pigs developing transient i.d. tumors†</th>
<th>No. of guinea pigs challenged by s.c.* inoculation</th>
<th>No. of guinea pigs developing leukemia</th>
<th>Incidence of leukemia developing after s.c. challenge %</th>
<th>Average latency: died from leukemia after s.c. challenge (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>88</td>
<td>12</td>
<td>88</td>
<td>34</td>
<td>39</td>
<td>27</td>
</tr>
<tr>
<td>Males</td>
<td>55</td>
<td>8</td>
<td>55</td>
<td>14</td>
<td>25</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>143</td>
<td>20</td>
<td>143</td>
<td>48</td>
<td>34</td>
<td>28</td>
</tr>
</tbody>
</table>

* 0.5 ml each; s.c., subcutaneous.
† Transient intradermal tumors developed in these animals prior to the subcutaneous challenge, as a result of the initial skin scarification with leukemic cells. (See Table 1.)

dermis promptly after scarification, and for that reason remained negative.

**Resistance of Scarified Guinea Pigs to Subsequent Subcutaneous Challenge with Leukemic Cell Extracts.** 143 Guinea pigs which remained in good health following skin scarification with leukemic cell extracts were challenged by subcutaneous inoculation (0.5 ml each) of leukemic cell suspensions of $10^{-3}$ to $10^{-5}$ dilution. Among the 143 challenged animals, only 48 guinea pigs (34%) developed leukemia; the remaining 95 animals (66%) resisted the challenge and remained in good health (Table 2).

**Control Experiments.** In a control series, untreated young adult "strain 2" guinea pigs were inoculated subcutaneously (0.5 ml each) with leukemic cell suspensions of either $10^{-3}$ or $10^{-5}$ dilution. 60 out of 61 in the first group and 94 out of 95 in the second group (99%) developed generalized leukemia after 3 weeks (Table 3).

In another control experiment, six "strain 2" guinea pigs

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**Fig. 2.** Higher magnification of Fig. 1. Leukemic tumor cells with large nuclei and prominent nucleoli are seen infiltrating and disassociating collagen bundles. There is one mitotic figure (arrow). A small number of lymphocytes is present. Hematoxylin and eosin. Magnification: $\times$625.
had their skin scarified with cell suspensions of 10\% concentration prepared from fragments of normal spleen and thymus removed from healthy “strain 2” guinea pig donors. After 5-7 weeks the six guinea pigs were inoculated subcutaneously (0.5 ml each) with a $10^{-2}$ dilution of a leukemic cell suspension, and all developed generalized leukemia after 3 weeks.

**DISCUSSION**

In our previous studies we have succeeded in producing immunity against L2C leukemia in “strain 2” guinea pigs by inoculating intradermally small doses of diluted leukemic cell suspensions. The great majority of those guinea pigs in which the intradermal tumors regressed spontaneously were immune to a challenging reinoculation of leukemic cells (1, 2). However, only about 42-54\% of the intradermal tumors regressed spontaneously. Because of the high mortality rate incident to that method of immunization we have attempted to induce immunity by inoculating diluted leukemic cell suspensions by superficial skin scarification.

The majority of guinea pigs did not show any significant injurious local effect of skin scarification beyond a short-lasting and promptly-healing superficial skin abrasion. Some animals in this group, even though they did not develop leukemic tumors at the site of scarification, developed, nevertheless, after a delay of several weeks, generalized leukemia. Among the remaining animals, some acquired immunity against a challenging reinoculation of leukemic cells; other guinea pigs in this group, however, remained unaffected by the scarification, and did not acquire immunity; it is possible that in such animals the leukemic cell extracts, applied super-

![Fig. 3. Electron micrograph of a fragment of skin removed 24 days after superficial skin scarification with leukemic cells. Areas of intradermal tumor showing immature guinea pig leukemia virus particles in cisternae of endoplasmic reticulum (a, arrows) and demonstrating the dense inner membrane and rough outer coat (b, arrows) of the virus particles. Magnification: a, $\times 60,000$ and b, $\times 80,000$.](image-url)
In conclusion, it appears that it is possible to immunize "strain 2" guinea pigs against L2C leukemia by superficial skin scarification with diluted leukemic cell suspensions. However, it is also apparent that, under experimental conditions employed in this study, skin scarification with leukemic cells was not a harmless procedure, since it induced in some animals progressively growing tumors at the site of inoculation, or a delayed development of generalized leukemia, without a preceding appearance of a leukemic tumor at the site of inoculation.

I am grateful to Miss Yolande Dreyfuss for excellent and conscientious assistance and Dr. Theodore Ehrenreich for reviewing the microscopic slides and providing the photomicrographs. I also thank Dr. Dorothy G. Feldman of our staff for the preparation and interpretation of the electron micrographs. This study was supported, in part, by grants from the Damon Runyon Memorial Fund (DRG 102-N), the American Cancer Society (VC-87M), and the Chemotherapy Foundation of New York.


TABLE 3. Subcutaneous inoculation* of leukemic cell suspensions into young adult "strain 2" guinea pigs†

<table>
<thead>
<tr>
<th>Leukemia</th>
<th>No. of pigs</th>
<th>No. of inoculations</th>
<th>Leukemia incidence (%)</th>
<th>Average latency (days)</th>
<th>Died (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell dilution</td>
<td>10⁻²</td>
<td>61</td>
<td>60</td>
<td>98</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>10⁻³</td>
<td>95</td>
<td>94</td>
<td>99</td>
<td>12</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>156</td>
<td>154</td>
<td>99</td>
<td>13</td>
<td>21</td>
</tr>
</tbody>
</table>

* 0.5 ml each.
† Normal, healthy animals, serving as controls to those reported in Table 2.

officially to their skin, might have been rubbed off accidentally, without having had an opportunity to penetrate the epidermis and enter the susceptible layers of the skin.

In the second, relatively smaller, group, the animals developed intradermal tumors at the site of scarification. Some of these tumors grew progressively, leading to generalized leukemia. In other animals, the small intradermal tumors regressed spontaneously. The great majority of those guinea pigs in which the intradermal tumors regressed remained in good health, and proved to be immune to reinoculation of leukemic cells. A few animals in this group, however, in spite of the fact that they had not been challenged, unexpectedly developed generalized leukemia after a delay of about 7-11 weeks. However, the overall mortality rate following skin scarification (30%) compared favorably with that observed in animals immunized by the intradermal method (about 50%).

Among 143 guinea pigs which survived scarification, 66% resisted a challenging inoculation of leukemic cell suspensions. In comparison, up to 90% of those guinea pigs, particularly females, which had been immunized by the previously described intradermal method resisted a challenging inoculation of heavy doses of leukemic cells (1, 2, also unpublished data).