Leukemia Induced by Sarcoma 180 and the Development of Reciprocal Immunity

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SUMMARY

Rockland mice which reject a S-180 challenge remain nearly immune to further transplants. Thirty-five per cent of these S-180-immune mice eventually died of Leukemia 180 (L-180). One of these leukemias (L-180/s) has proved transplantable within the strain, with an incidence of 69 per cent; the remaining 31 per cent are L-180/s-immune mice.

S-180-immune mice proved completely resistant to L-180/s challenge, whereas, vice versa, L-180/s-immune mice were also resistant to S-180 but to a lesser degree.

In 27 S-180-immune mice unsuccessfully challenged with L-180/s eventual death from leukemia (L-180) was observed in only one case (4 per cent) as compared with 35 per cent in the control group.

This reciprocal immunity is not easily attributable either to a selection of those animals more apt to develop nonspecific immunity or to histocompatibility genes. Tentatively, a subcellular agent common to both tumors might be held responsible for this phenomenon.

In a previous paper it was reported that, when Sarcoma 180 is transplanted into Rockland mice, some animals survive, rejecting the tumor and becoming immune to it (5). Of these S-180-immune mice, 33 per cent eventually die of a leukemia which we have termed Leukemia 180 (L-180) (6).

L-180 proved transplantable within the original strain; one of the transplants (L-180/s) is now in its 35th serial passage, with an incidence of “takes” of 69 per cent. It then became possible to attempt a comparison of the immunity conferred by S-180 with that obtained with L-180.

MATERIALS AND METHODS

Rockland mice are being inbred in this laboratory and are now in their 13th sib-mating generation. Most of the experiments here reported

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have been performed with animals coming from the third through the thirteenth generation of inbreeding—i.e., with coefficients of inbreeding ranging from 0.50 to 0.94. They belong to five sublines of the same strain which have arisen in the course of inbreeding and are maintained at present.

Immunity to Sarcoma 180 is induced in Rockland mice as described in previous publications (5, 6): out of the total of Rockland mice which receive a subcutaneous transplant of the tumor, from 13 to 18 per cent which survive by rejecting the tumor become almost immune to further challenges and are known as S-180-immune mice.

L-180/s originated in a S-180-immune Rockland mouse belonging to the third generation of inbreeding. This animal was a full brother of the pair from which subline 5 is descended. L-180/s is being serially transplanted in the cellular form, with a homogenate of solid tumor administered subcutaneously or pure ascites intraperitoneally. The subcutaneous tumor usually remains localized
but terminally may show either a diffuse invasion of subcutaneous tissue and/or generalized leukemia; the animals usually die within 30–40 days. Intraperitoneal transplantation leads to the development of abundant ascites within 10 days, the survival time ranging from 15 to 30 days. Microscopically, both forms were classified as either prolymphocytic or lymphoblastic leukemia.

The animals which reject the leukemia after two or more challenges are termed Leukemia 180/s-immune mice.

RESULTS

Out of a total of 139 S-180-immune mice, 49 (35 per cent) have died of leukemia when 14–27 months of age. Out of 168 stock Rockland mice dying when more than 12 months old (range: 12–27 months old), leukemia was observed in three of them (1.7 per cent), all three dying when 15 months old.

Upon histological observation, these leukemias proved to be mainly of the lymphocytic type, similar to the one observed in leukemic strains of mice, such as C58; but four were reticulum-cell neoplasm, type B (1).

Cellular transplantations of these leukemias were attempted in 29 cases, with positive results in four of them; the four reticulum-cell sarcomas were also transplanted in cellular form, two of them taking, but only at the end of 8 months—proving that biologically they are different from S-180. These transplants were all lost in their second passage except the above-mentioned leukemia (L-180/s).

L-180/s has now been passed in 500 Rockland mice with an over-all incidence of takes of 69 per cent (Table 1). Autopsy reveals enlarged spleen, liver, thymus, and lymph nodes along with extensive mesenteric infiltration and ascites; leukocytosis ranges from 16,000 to 80,000 WBC.

L-180/s is specific for the Rockland strain; cellular passage was attempted in 185 BALB mice, with negative results, as compared with 80 per cent of takes in 60 simultaneous Rockland controls; in the DBA strain, out of seventeen animals, two developed leukemia—i.e., 11 per cent as compared with 100 per cent in fifteen Rockland controls.

Of the animals in which L-180/s did not take, even after two to four challenges, 126 were transplanted with S-180. As can be noted in Table 1, 41 per cent of these L-180/s-immune mice were also immune to S-180. Simultaneous controls of the same age, challenged with S-180 alone, gave 14 per cent immunity (P < 0.001).

Doing the reciprocal of the experiment (Table 1), S-180-immune mice were challenged with L-180; in none of the 27 mice were there any signs of leukemia. Moreover, when these 27 mice eventually died, months later, only one of them presented leukemia, as compared with 35 per cent of the 139 S-180-immune controls (Table 2) (P < 0.01).

In order to elucidate this problem further, the above mentioned results were also investigated, taking into account the differences among the five sublines of the Rockland strain (Table 3). It can be seen that the percentage of takes both of S-180 and L-180/s differed significantly among these sublines, whereas no correlation existed between the percentage of takes of sarcoma and leukemia (r = -0.43; P > 0.05). The highest value of S-180 was found in subline 2, where L-180/s had its lowest percentage of takes and, conversely, in subline 5 (where L-180/s arose) the highest value of L-180/s met one of the lowest percentage of S-180. However, reciprocal immunity became apparent in all five sublines when pretreatment with L-180/s was instituted, thus decreasing the percentage of takes of S-180 in vary-

### Table 1

#### PERCENTAGE OF “TAKES” IN ROCKLAND MICE RECEIVING S-180 AND L-180/s CHALLENGES SEPARATELY AND CONSECUTIVELY

<table>
<thead>
<tr>
<th>Challenge</th>
<th>No. mice</th>
<th>Percent dying</th>
<th>$x^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-180</td>
<td>100</td>
<td>86</td>
<td>20.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>L-180/s-immune+S-180</td>
<td>126</td>
<td>59</td>
<td>0.01</td>
<td>---</td>
</tr>
<tr>
<td>L-180/s</td>
<td>500</td>
<td>69</td>
<td>58.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S-180-immune+L-180/s</td>
<td>27</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* $x^2$ comparing influence of previous immunization.

### Table 2

#### INCIDENCE OF LEUKEMIA AT DEATH (L-180)

<table>
<thead>
<tr>
<th>Challenge</th>
<th>No. mice</th>
<th>Percent dying</th>
<th>$x^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-180-immune</td>
<td>139</td>
<td>35</td>
<td>8.94</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>S-180-immune+L-180/s</td>
<td>27</td>
<td>4</td>
<td>---</td>
<td></td>
</tr>
</tbody>
</table>

Note that one or more passages of L-180/s (negative ones) almost inhibit the appearance of L-180.
ing proportions, up to one-third of its previous value in subline 2.

DISCUSSION

In a previous publication the appearance of leukemia in S-180-immune mice was attributed to a subcellular agent (6). At the time there was no direct proof for this hypothesis, but since then it has been possible to demonstrate that acellular extracts of S-180 are capable of inducing leukemia in a number of mice. Thus, immunity to S-180 would be a necessary step for the appearance of leukemia in that it makes possible the survival of the animals after challenge with the tumor.

It is interesting to note that S-180-immune mice are resistant to L-180/s, since none of the sublines in which the percentage of takes of S-180 is highest (i.e., subline 2, in which naturally occurring immunity is lowest) (Table 3).

Two additional facts deserve attention: (a) Immunity deriving from S-180 pretreatment is more efficient against L-180/s than the other way around; (b) the animals immunized to S-180 in which L-180/s did not take revealed (Table 1) a significant refractoriness to L-180 when they became older, whereas 35 per cent of their controls developed L-180 (Table 2). It is known that leukemias are considerably more sensitive to destruction by isoantibody than are sarcomas. Thus, if S-180 and L-180/s possess a common antigen, it would be reasonable to expect that immunity to this antigen would be more marked against L-180/s whereas S-180 would tend to overgrow the immune response; and yet it must be emphasized that L-180/s is able to complete the immunity of S-180 against the appearance of leukemia at death, and this fact points to the existence of some antigenic difference. In other words, as mentioned before (6), the agent inducing leukemia in S-180-immune animals is in some way escaping immunity, and this immunity in turn may be reinforced and/or perfected by one or more passages (negative ones) of L-180/s. Might it not follow from the previous considerations that this agent might be the common cause of both tumors? Had it been a mere passenger of S-180, this reciprocal immunity would remain unexplained.

TABLE 3

PERCENTAGE OF "TAKES" IN FIVE SUBLINES OF ROCKLAND MICE

<table>
<thead>
<tr>
<th>Sublines</th>
<th>S-180</th>
<th>L-180/s</th>
<th>L-180/s-immune+S-180</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. mice</td>
<td>Per cent dying</td>
<td>No. mice</td>
</tr>
<tr>
<td>1</td>
<td>42</td>
<td>78</td>
<td>42</td>
</tr>
<tr>
<td>2</td>
<td>94</td>
<td>97</td>
<td>94</td>
</tr>
<tr>
<td>3</td>
<td>162</td>
<td>90</td>
<td>61</td>
</tr>
<tr>
<td>4</td>
<td>99</td>
<td>87</td>
<td>37</td>
</tr>
<tr>
<td>5</td>
<td>148</td>
<td>51</td>
<td>114</td>
</tr>
<tr>
<td>( x^2 )</td>
<td></td>
<td>&lt; 0.001</td>
<td>35.8</td>
</tr>
<tr>
<td>( P )</td>
<td></td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

* \( x^2 \) comparing the five sublines.
† \( x^2 \) comparing S-180 with L-180/s-immune+S-180.

The correlation coefficient between the percentages of L-180/s and S-180 in the different sublines is —0.43; \( P > 0.05 \).

27 animals responded. On the other hand, in L-180/s-immune mice, a challenge with S-180 gave a significant decrease in the percentage of takes when compared with controls.

Thus it can be concluded that S-180-immune mice are immune to L-180/s whereas, vice versa, L-180/s-immune mice are also immune to S-180, but to a lesser degree.

Now, since both S-180 and L-180/s-immune mice reject these tumors spontaneously, it might be argued that this procedure leads to the selection of those animals more apt to develop immunity of either type unspecifically or that the crossed immunity is due to a common histocompatibility gene shared by both S-180 and L-180/s, in spite of the fact that L-180/s has arisen de novo in a Rockland mouse, thus possessing a number of antigens from its Rockland mouse of origin.

Table 3 shows that neither of these explanations is valid, because the histocompatibilities of L-180/s and S-180 are different (e.g., subline 2, Table 3) while, on the other hand, it is possible to induce immunity by pretreatment with L-180/s even in the subline in which the percentage of takes of S-180 is highest (i.e., subline 2, in which naturally occurring immunity is lowest) (Table 3).

1 Unpublished results.
It would be interesting to correlate the present results with those of Moloney (4), who describes a passenger leukemogenic virus in S-37, but no immunological study of this virus in connection with S-37 has been reported so far. The underlying phenomenon might be similar to that described by Habel (3) and Sjögren et al. (7)—that is, the rejection of tissue-compatible, polyoma virus-induced tumors by animals previously immunized against this virus. Friend has also described a similar type of immunity (2).

REFERENCES

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