The growth of tumours in T-cell deprived mice and their response to treatment with *Corynebacterium parvum*

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Severe depletion of thymus-derived lymphocytes does not reduce, and may significantly increase, the resistance of mice to syngeneic tumours, although it grossly impairs their ability to reject allografts.

Injection of killed *C. parvum* causes further inhibition of tumour growth in T-cell deprived mice to an extent comparable to that resulting from the same dose of *C. parvum* in normal mice. This appears to lend support to the hypothesis that the antitumour effect of *C. parvum* depends primarily on macrophage stimulation.

It has been reported from this laboratory (Woodruff & Boak 1966; Woodruff & Dunbar 1973; Woodruff & Inchley 1971; Woodruff, Inchley & Dunbar 1972) that the growth of primary isogeneic transplants of mouse fibrosarcomas and mammary carcinomas may be inhibited by a single i.v. or i.p. injection of a killed vaccine of certain strains of *Corynebacterium parvum*.

In attempting to elucidate the mechanism underlying this phenomenon we have recently studied the growth of similar tumours in T-cell deprived mice ('B' mice), and their response to *C. parvum*. For comparison we have also studied the behaviour of allografts of an A-strain sarcoma in T-cell deprived CBA mice.

**Material and methods**

The strains of mice and the preparation and inoculation of tumour cell suspensions were as described previously (Woodruff et al. 1972). *C. parvum* preparation WEZ176 has been used throughout.

Details of the tumours and cell-dosage are shown in table 1.

<table>
<thead>
<tr>
<th>Tumour and strain of origin</th>
<th>Transplant generation in present expt.</th>
<th>Viable cells for immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBA fibrosarcoma</td>
<td>17</td>
<td>$10^4$ (isotransplantation)</td>
</tr>
<tr>
<td>A mammary carcinoma</td>
<td>2</td>
<td>$10^6$ (isotransplantation)</td>
</tr>
<tr>
<td>A fibrosarcoma</td>
<td>22</td>
<td>$10^6$ (allotransplantation to CBA)</td>
</tr>
</tbody>
</table>

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Preparation of 'B' mice

CBA and A-strain mice were thymectomized at the age of 4 to 6 weeks, and given whole body X-irradiation (850 rad for CBA and 750 rad for A mice) and an i.v. injection of untreated isogeneic bone marrow (at least $5 \times 10^6$ viable nucleated cells) 10 days later.

These mice, which will be designated ‘B’ (CBA) and ‘B’ (A) mice respectively, were tested for their ability to mount immune responses known to be dependent on the participation of thymus derived cells and it was found that (a) they failed to produce any significant amount of antibody to sheep erythrocytes and (b) they have failed to reject H2-incompatible (A-strain) skin allografts (period of observation so far 3 months). It seems clear, therefore, that they had been made severely deficient in respect of their T-cell population. In this connexion it is interesting to note the findings of Dr A. J. S. Davies (personal communication) showing a 90% depletion of phytohaemagglutinin responding cells in the peripheral blood of mice prepared in this way.

Statistical procedure

The statistical procedure has been modified on the advice of Dr Frank Yates in that the measure of response in each mouse has been taken as the sum of the tumour diameters measured every 2 days up to a time chosen so that most of the animals survived and none of the tumours had begun to ulcerate (26 days for the fibrosarcoma and 32 days for the mammary carcinoma), instead of the diameter on one particular day. This is essentially equivalent to basing comparisons on linear regression lines fitted to each treatment group.

Results

Isotransplants of CBA-strain sarcoma

The behaviour of sarcoma isotransplants in various categories of CBA mice is summarized in figure 1 and table 2. The main conclusions which emerge are as follows:

1. The transplants grow more slowly in untreated ‘B’ mice than in untreated normal mice.

2. A single i.p. injection of C. parvum 3 days after tumour inoculation inhibits tumour growth not only in normal mice but also in ‘B’ mice.

3. Immunization of normal mice with $10^6$ irradiated tumour cells completely prevents tumour growth following a subsequent inoculation of $10^4$ viable cells in normal mice, but has no inhibitory effect on subsequent tumour growth in ‘B’ mice.

Isotransplants of A-strain mammary carcinoma

Groups corresponding to the first four groups in the experiment with the CBA sarcoma (see above) were set up in A/HeJ mice using the mammary carcinoma. The results are illustrated in figure 2. Owing to the early death of three mice in group 2 the evidence of slower growth of the tumour in untreated ‘B’ mice as
Antitumour effect of C. parvum in T-cell deprived mice

**Figure 1.** Growth of isogeneic fibrosarcoma in CBA female mice after subcutaneous injection of $10^4$ viable cells. o, normal mice, untreated; □, 'B' mice, untreated; ●, normal mice, C. parvum i.p. day +3; ■, 'B' mice, C. parvum i.p. day +3; ▲, 'B' mice immunized with $10^6$ irradiated cells. No tumour grew in pre-immunized normal mice.

**Table 2. Growth of Isotransplants of Fibrosarcoma in CBA Mice**

<table>
<thead>
<tr>
<th>group no.</th>
<th>treatment</th>
<th>no. of mice</th>
<th>sum of tumour diameters (mm) in each mouse measured every 2 days up to day 26</th>
<th>t-test comparison of group means</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>normal mice, no treatment</td>
<td>5</td>
<td>95, 80, 112, 92, 108</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>'B' mice, no treatment</td>
<td>4</td>
<td>73, 62, 77, 72</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>normal mice, C. parvum</td>
<td>5</td>
<td>44, 62, 73, 40, 47</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>'B' mice, C. parvum</td>
<td>5</td>
<td>57, 44, 29, 32, 42</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>pre-immunized normal mice</td>
<td>5</td>
<td>0, 0, 0, 0, 0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>pre-immunized 'B' mice</td>
<td>5</td>
<td>88, 67, 85, 67, 77</td>
<td></td>
</tr>
</tbody>
</table>
compared with normal mice falls just short of statistical significance at the $P = 0.05$ level, but the tumour-inhibitory effect of *C. parvum* in 'B' mice has been confirmed ($P < 0.01$).

**Figure 2.** Growth of isogeneic mammary carcinoma in A/HeJ female mice after subcutaneous injection of $10^6$ viable cells. $\bigcirc$, normal mice, untreated; $\square$, 'B' mice, untreated; $\bullet$, normal mice, *C. parvum* i.p. day +3; $\blacksquare$, 'B' mice, *C. parvum* i.p. day +3.

**Figure 3.** Growth of A/HeJ fibrosarcoma in A/HeJ and CBA mice. $\bullet$, normal A/HeJ; $\bigcirc$, normal CBA; $\ast$, 'B' (CBA); $\square$, CBA which had been irradiated and reconstituted with isogeneic marrow but not thymectomized.
Antitumour effect of C. parvum in T-cell deprived mice

Allotransplants of A-strain fibrosarcoma in CBA mice

The results are summarized in figure 3. In normal CBA mice nodules appeared at the site of tumour inoculation but thereafter disappeared. Subsequently, however, nodules reappeared, grew slowly but progressively, and were shown to consist of tumour tissue. This tumour can therefore grow across an H-2 barrier but takes a long time to become established. In ‘B’ (CBA) mice, on the other hand, the tumour grew at virtually the same rate as in normal A-strain mice.

Discussion

The results indicate that severe depletion of thymus-derived cells does not reduce, and may significantly increase, the resistance of mice to syngeneic tumours although it grossly impairs their ability to reject allotransplants of tumours and also of skin. This strongly suggests that the rejection of isogeneic tumours is mediated by mechanisms which differ from those operative in the rejection of allografts.

The reason for the relatively slow growth of tumour isotransplants in ‘B’ mice has not yet been elucidated. One hypothesis which is being investigated is that, while the direct effect of T-cell deficiency is to weaken cell-mediated immunological responses, lack of T-cell helper function diminishes production of blocking antibody (or antibody capable of combining with tumour antigen to form blocking antigen–antibody complexes), and thus indirectly enhances the cell-mediated response. Slower tumour growth would result if the indirect effect predominated.

One must also take into consideration, however, the fact that non-thymic cells may mediate tumour cell destruction. In the case of macrophages, this has been demonstrated both in vitro (Den Otter, Evans & Alexander 1972) and in vivo (Sezzi, Bellelli & Nista 1972). The role of non-thymic lymphocytes is less well defined, but it has been shown by Lamon, Skurzak, Klein & Wigzell (1972) that lymphoid cells from T-cell deficient mice may cause complement-independent destruction of antibody coated target cells in vitro. This has recently been confirmed by Greenberg and his colleagues (Greenberg, Hudson, Shen & Roitt 1973), who have shown also that the effector cells were non-phagocytic (Greenberg, Shen & Roitt, 1973).

The quite strong antitumour effect of C. parvum in ‘B’ mice is consistent with the view that it may depend primarily on macrophage stimulation (Keller & Hess 1972; Woodruff & Dunbar 1973; Adlam & Scott 1973).

We are deeply grateful to Dr F. Yates, F.R.S., for advice on the statistical analysis of the data, and to Dr A. H. Griffith of the Wellcome Foundation for providing the C. parvum suspension. Figure 1 has been redrawn (with additions) from a figure in Immunopotentiation (Ciba Foundation Symposium, no. 18, new series), by kind permission of Associated Scientific Publishers, Amsterdam.
REFERENCES

Woodruff, M. F. A. & Dunbar, Noreen 1973 In: *Ciba Foundation Symposium on 'Immunopotentiation'.* (In the Press.)