Inflammation and Tumor Growth

II. Tumor Growth at Sites of Inflammation
Induced by Mitogens in Mice

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Experiments were carried out to determine whether the growth of tumors could be influenced by local inflammatory reactions induced by mitogens; Gram-negative bacterial lipopolysaccharide (LPS), concanavalin A (Con A) and phytohemagglutinin (PHA). Mice received injections, beneath the footpad or subcutaneously in the flank, of cells of syngeneic chemically induced fibrosarcomas with or without varying doses of mitogen. In the footpad (a) LPS caused a dose-dependent increase in the size; (b) Con A caused a decrease in the size of one of the three tumors, the decrease being inversely related to the dose of Con A; (c) PHA caused a dose-dependent decrease in the size of all three tumors; (d) PHA caused much smaller macroscopic inflammatory reactions than LPS or Con A. Subcutaneously injected tumor growth was inhibited by all three agents. Subcutaneous tumors contained a higher proportion of host inflammatory cells when mitogens had been mixed with the tumor inoculum. It is concluded that mitogens that can induce inflammatory reactions in mice can also bring about some suppression of tumor growth but that the depression is site-dependent and not clearly related to the apparent intensity of inflammation. (Am J Pathol 1981, 104:125-131)

THERE IS MUCH EVIDENCE, both direct and circumstantial, that macrophages can suppress the growth of tumor cells both in vitro and in vivo. As with acquired cellular resistance to infection, the antitumor activities of macrophages are largely nonspecific. Macrophages that have been activated in animals injected with any of a wide variety of microbial agents exert, in vitro, a nonspecific cytotoxic effect on tumor cells. Conversely, the carriage of tumors can lead both to the development of concomitant tumor immunity and the stimulation of host macrophages to increase their phagocytic, proliferative, and nonspecific antimicrobial and antitumor capacities. It might therefore be expected that tumor growth would be suppressed at sites where macrophages have congregated as a result of a cell-mediated immune reaction to another (nontumor) antigen. This has, in fact, proved to be the case for some tumors at the sites of delayed-type hypersensitivity (DTH) reactions in mice, chickens, and human patients.

We wished to see whether other agents inducing inflammatory reactions in an immunologically nonspecific way would also depress the growth of tumors at the sites of injection. Phytohemagglutinin (PHA) and concanavalin A (Con A) were chosen, as both have been reported to induce local inflammatory reactions with features resembling those of DTH, conceivably because both cause blastogenesis and the production of lymphokines by thymus-derived lymphocytes (T cells). The lipopolysaccharide (LPS) endotoxins of gram-negative bacteria can also cause local inflammatory reactions and activate macrophages. The effect of LPS on local tumor growth was therefore also examined.

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Materials and Methods

Mice and Tumors

CBA/J male mice, A/J female mice and the methylcholanthrene-induced tumors C-4 and A-2 were used as described in the accompanying paper. Another methylcholanthrene-induced tumor, C-15, of CBA/J mice was also used. All three tumors were immunogenic.

Tumor Challenge

Mice were challenged in the footpad on Day 0 with $5 \times 10^4$ viable cells in a volume of 50 µl, or subcutaneously with $10^7$ viable cells in a volume of 0.2 or 0.3 ml.

Agents Injected With the Tumor

Endotoxin (LPS; Boivin) from Escherichia coli (Difco, Detroit, Mich), concanavalin A (Con A; Calbiochem-Behring, Sydney, Australia), and phytohemagglutinin-P (PHA-P; Difco) were injected admixed with tumor cell suspensions. For footpad challenge, solutions of appropriate concentration in DME were mixed with equal volumes of suspensions of $2 \times 10^4$ viable cells immediately prior to challenge; $5 \times 10^4$ viable cells were rejected under the hind footpad in 50-µl volumes which contained 10, 1, or 0.2 µg of Con A or PHA-P. For subcutaneous challenge, mice received $10^7$ viable tumor cells alone in 0.2 ml, or mixed with a further 0.1 ml of LPS, Con A, or PHA solutions to give the same total quantities of these agents in 0.3 ml as in the footpad challenge.

Measurement of Tumor Growth and Inflammation in the Footpad

The degree of inflammation or tumor growth in the footpad was followed by daily measurement of the increase in footpad thickness by means of a Schnett-taster dial gauge. The un.injected hind foot was used as a control.

Measurement of Subcutaneous Tumor Growth

At various times after subcutaneous injection of tumor cells the animals were sacrificed and the tumors were excised, dissected free of other tissue and weighed. Tumors from each experimental group were pooled and digested with pronase. Smears were made from the washed single cell suspensions and stained with May-Grünwald–Giemsa. Cells were classified according to their microscopic appearance as tumor cells, macrophages, lymphocytes, or polymorphs.

Statistics

The Student $t$ test was used.

Results

Inflammatory Agents and Tumor Growth in the Footpad

LPS

Figure 1 shows the degree of swelling due to inflammation and subsequent tumor growth in the footpads of CBA/J mice given injections of the syngeneic tumor C-4 alone or with LPS in doses of 0.2, 2, or 20 µg. After the initial dose-dependent inflammatory response, tumor growth appeared to be potentiated by LPS. The results were essentially similar to the other tumors, although the degree of apparent potentiation was less.

Con A

Mice received injections into the foot of the syngeneic tumor C-4, C-15, or A-2, alone or with Con A in doses of 5, 10, and 20 µg. In each case Con A induced inflammatory reactions, which were most intense at Day 1, and most intense with the highest dose of Con A. Only in the case of C-4, however, was there any eventual difference between tumor growth in feet given injections of Con A and the tumor inoculum and tumor growth in feet given injections of tumor cells alone (Figure 2). The degree of inhibition of C-4 growth was inversely proportional to the dose of Con A. Inhibition appeared to be due to a delay in the onset of rapid tumor growth rather than to a slower final growth rate.

PHA

Figure 3 shows the degree of swelling in the footpads of A/J mice given A-2 tumor, alone or with PHA in doses of 5, 10, and 20 µg. Only very slight inflammatory reactions were apparent at Day 1, and then only with the highest dose of PHA. Thereafter tumor growth was depressed when PHA had also been injected, all doses of PHA causing about the same degree of depression. Depression appeared to involve both a delay in onset and a slowing of tumor growth. There was a similar marked depression of the growth of C-15. The growth of C-4 was at first retarded, in a dose-dependent fashion, but later proceeded at a normal rate.
Inflammatory Agents and Subcutaneous Tumor Growth

Table 1 shows the weight of A-2 tumors from a subcutaneous site in A/J mice 11 days after inoculation of 10^7 cells with or without the addition of varying concentrations of LPS, Con A, or PHA. The differential cell count of a pooled tumor digest from each experimental group is also shown, together with the calculated weight of tumor cells in the tissue, based on the mean total tumor weight and percentage of tumor cells.

The mean weight of tumor tissue from animals receiving LPS was decreased only in those receiving 20 μg. However, the proportion of tumor cells was reduced, in a dose-related manner, the highest dose of LPS giving rise to a tumor only 31% of which consisted, morphologically, of tumor cells. The other cells were mostly polymorphs.

The presence of Con A in the tumor inoculum at doses of 20 and 10 μg resulted in tumors that were significantly smaller than control tumors, but 5 μg of Con A had no effect. The proportion of tumor cells in the Con A groups was much less than in the con-
control groups, and the polymorph content was higher. The calculated weight of tumor cells was much less in all Con A groups than in the control. The effect of PHA was similar.

A similar experiment was carried out with C-4 in CBA/J mice. All three agents caused reductions in tumor weight, LPS having the strongest effect. The reductions were dose-dependent for LPS and PHA but not for Con A. The polymorph content of the control tumor was low (1%) and was not affected by any of the agents. Each agent caused an increase in the macrophage content, but these increases were not clearly dose-related.

**Discussion**

These experiments were begun with the hypothesis that mitogenic agents that cause inflammatory reactions containing macrophages would also, when injected, mixed with tumor cells, into mice, cause a reduction in tumor size. The hypothesis is not tenable in this simple form, as there was no consistent pattern in the effects of the agents. On the one hand, different agents had different effects on the same tumor in the footpad. The growth of A-2, for example, was apparently potentiated by LPS, inhibited by PHA (Figure 3) but unaffected by Con A. On the
other hand, the same agent could have different effects on different tumors in the footpad: Con A inhibited the growth of C-4 (Figure 2) but had no effect on the growth of C-15 or A-2. Nor was there any relationship between the intensity of the initial inflammatory reaction in the footpad and the subsequent growth of the tumor. The reactions to Con A injected with C-4, C-15, and A-2 were similar, but only C-4 was inhibited, and even then the degree of inhibition was inversely proportional to the dose of Con A and

Figure 3—Effect of PHA on the growth of A-2 tumor in the footpad. O—O, control, tumor alone; •—•, tumor with 20 μg PHA; △—△, tumor with 10 μg PHA; ▲—▲, tumor with 5 μg PHA.

Table 1—Weights and Cell Contents of Tumors Growing Subcutaneously in A/J Mice After Injection of Tumor Cell Suspensions Mixed With LPS, Con A, or PHA

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Tumor weight (mg) on Day 11 (mean ± SE)</th>
<th>P*</th>
<th>Percentage of cells in tumor digest</th>
<th>Calculated weight of tumor cells (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tumor</td>
<td>Macrophage</td>
</tr>
<tr>
<td>A-2 (10⁵)</td>
<td>210 ± 15</td>
<td></td>
<td>71</td>
<td>13</td>
</tr>
<tr>
<td>A-2 (10⁵) +</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPS, 0.2 μg</td>
<td>187 ± 15</td>
<td>&lt; 0.0005</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>LPS, 2 μg</td>
<td>180 ± 26</td>
<td>&lt; 0.025</td>
<td>46</td>
<td>18</td>
</tr>
<tr>
<td>LPS, 20 μg</td>
<td>168 ± 15</td>
<td>NS</td>
<td>31</td>
<td>16</td>
</tr>
<tr>
<td>A-2 (10⁵) +</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con A, 5 μg</td>
<td>178 ± 12</td>
<td>NS</td>
<td>54</td>
<td>19</td>
</tr>
<tr>
<td>Con A, 10 μg</td>
<td>148 ± 18</td>
<td>NS</td>
<td>39</td>
<td>17</td>
</tr>
<tr>
<td>Con A, 20 μg</td>
<td>120 ± 11</td>
<td>&lt; 0.0005</td>
<td>27</td>
<td>21</td>
</tr>
<tr>
<td>A-2 (10⁷) +</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHA, 5 μg</td>
<td>175 ± 8</td>
<td>&lt; 0.05</td>
<td>42</td>
<td>11</td>
</tr>
<tr>
<td>PHA, 10 μg</td>
<td>172 ± 13</td>
<td>&lt; 0.05</td>
<td>37</td>
<td>25</td>
</tr>
<tr>
<td>PHA, 20 μg</td>
<td>130 ± 4</td>
<td>&lt; 0.0005</td>
<td>44</td>
<td>27</td>
</tr>
</tbody>
</table>

* Statistical significance of difference between weight of tumor injected alone and tumor injected with LPS, Con A, or PHA (Student t test). NS = not significant.
the degree of early footpad thickening (Figure 2). PHA, which induced the smallest initial reactions in the footpads, caused the greatest consistent inhibition of growth (Figure 3). The apparent stimulation by LPS of tumor growth in the foot is puzzling, especially in view of the inhibition of subcutaneous tumor growth by LPS. It is at present unexplained, but conceivably some of the increase in tumor size is due to host polymorphs (see Table 1).

These observations can be compared with other experiments on the effect of local DTH reactions on tumor growth. Such reactions have been found to suppress tumor growth in mice,7,8 chickens,9 and man.10 It is noteworthy, however, that effective suppression has often been associated with the use of particulate antigens. In mice, mycobacteria7 or sheep erythrocytes8 were effective in appropriately immunized animals, whereas a soluble antigen (tuberculin) was not,7 and in some experiments could even potentiate tumor growth.20 There is also some species variation, as a soluble antigen (human gammaglobulin) was effective in chickens8 and contact sensitizing agents were effective in man19 but not in guinea pigs.21 It may well be that there are different forms or components of DTH reactions,7,22,23 only those with a pronounced granulomatous component being associated with tumor suppression.21,22

In our experiments there appeared also to be differences between the effects of the same agent at different sites (foot and flank). All three agents inhibited both tumors (C-4 and A-2) that were grown subcutaneously in the flank. When account was taken of the relative proportions of tumor cells and host inflammatory cells, as judged from stained smears of tumor digests, the degree of inhibition of tumor cells was generally pronounced and dose-related. For the growth of the tumors subcutaneously in the flank, therefore, the predictions of the hypothesis were fulfilled.

The reasons for the differences between the two sites are quite obscure. In mice the foot of the foot contains more mast cells than does trunk skin, and it has been suggested that this is the reason DTH reactions are much more easily elicited in the foot than in the trunk.23 On this basis, however, it might be expected that inflammatory reactions akin to DTH would also be more readily elicited in the foot and would exert a more tumor-suppressive effect.

The possible modes of action of the inflammatory agents in reducing tumor growth deserve comment. Our original hypothesis was that all might in one way or another induce the local accumulation of macrophages, with a selective cytotoxic effect on tumor cells. LPS is well known to induce local tumor suppression, even in unprimed animals,24,25 and to induce the formation of cytotoxic macrophages in vivo26 and in vitro.15,16 Its antitumor effect appears, however, to be independent of its capacity to induce a granulomatous reaction,26 and A/J mouse macrophages are resistant to the induction of cytotoxicity by LPS.28 Con A and PHA could induce lymphocyte-in formation29 by T cells. Finally, all the agents could induce large changes in vascular permeability,24,30,31 allowing the local accumulation of other naturally cytotoxic effector cells.32

In essence, these experiments show that mitogens which are capable of inducing inflammatory reactions in mice can also bring about some suppression of tumor growth but that the suppression is site-dependent and not clearly related to the apparent intensity of inflammation. They provide further reasons for the exercise of caution in attempts at local immunotherapy of cancer in man.

References
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