enzyme activity in the present case does not seem to be related to its possible effects on nitrogen uptake, as the same is observed both in the absence as well as presence of ammonium nitrate in the nutrient medium.

Increase in enzyme activity during incubation of excised leaf segments in light seems to involve de novo synthesis of the enzyme, as there is a considerable time lag. (Figure 1) and the increase is inhibited by cycloheximide (Table 2). Apparently, the site of enzyme synthesis is cytoplasmic as inhibitors of protein synthesis on 70S ribosomes such as lincomycin, has no effect on the increase. On the other hand, chloramphenicol increases enzyme activity considerably. The mechanism of this increase is not understood at the moment. The enzyme induction by light appears to be dependent on mitochondrial activities related to ATP generation as well, as supply of DNP inhibits the same.

The insensitivity of light-induced increase in NADH-glutamate synthase activity to DCMU, excludes any direct participation of photosynthesis in the process. However, differential effects of carbohydrates glucose and sucrose on dark- and light-induced enzyme activities are observed both during in vivo as well as in vitro supply of carbohydrates (Tables 3 and 4). Further, the degree of activation during storage at 25°C is also different for dark- and light-induced enzyme. We believe therefore, that a new isozyme of glutamate synthase is synthesized during light treatment and that this isozyme is more active than the one synthesized in the dark. Further experiments are in progress to separate the two enzymes.


Drug targeting to a fast growing rat histiocytoma

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AK-5 is a rat macrophage-like tumor line and the effect of daunomycin was tested on this cell line. Daunomycin alone is not highly effective against this tumor, however, when conjugated to cytotoxic anti-AK-5 antibodies, kills the tumor cells thereby regressing tumors in 100% animals. Anti-AK-5 acts as a carrier for daunomycin to reach the target cells.

In effective cancer chemotherapy there is always the risk of toxicity to the normal proliferating cells. This limitation could be improved by the specific targeting of antineoplastic drugs to the tumor cells, using specific carriers as conjugates of the drugs. Polyclonal and monoclonal antibodies against tumor-associated antigens have been used as carriers for various drugs and toxins, and their use in regression of the tumors have been demonstrated.

We illustrate one such example of antibody conjugation to the antitumor drug daunomycin. AK-5 is a rat histiocytic tumor line which possesses several typical characteristics of a macrophage-like cell such as Fc-receptors, la-determinant, non-specific esterase, lysozyme and phagocytosis. AK-5 is highly immunogenic and induces the production of cytotoxic antitumor antibodies in the host when injected subcutaneously. These antibodies are directed against a surface antigen, which has been purified and characterized to be specific to AK-5 cells. When the tumor cells are injected intraperitoneally into rats, they kill all the injected animals. However, the tumor gets completely regressed when a combination of immunomodulators and antineoplastic drug treatment is given.

The drug daunomycin by itself is not completely effective in regressing the AK-5 tumor as ascites. We have raised anti-AK-5 antibodies in rats by injecting $5 \times 10^5$ AK-5 cells subcutaneously. After about 25 days, the animals are injected $5 \times 10^5$ tumor cells s.c. and bled 5 days later to get the antisera. Immunoglobulin fraction was precipitated with ammonium sulphate and passed through a column of Sephadex G-150. The partially purified antitumor antibodies were conjugated to daunomycin using the glutaraldehyde method and the
Table 1. Effect of daunomycin-anti-AK-5 conjugate on the growth of AK-5 tumor.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose/100 g/injection</th>
<th>Single dose</th>
<th>Three doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>10/10(7)</td>
<td>10/10(6)</td>
</tr>
<tr>
<td>anti-AK-5</td>
<td>1 ml (2mg/g)</td>
<td>10/10(7)</td>
<td>10/10(7)</td>
</tr>
<tr>
<td>Daunomycin</td>
<td>5 μg</td>
<td>8/10(15)</td>
<td>6/10(35)</td>
</tr>
<tr>
<td>Daunomycin-anti-AK-5</td>
<td>5 μg</td>
<td>5/10(34)</td>
<td>0/20</td>
</tr>
<tr>
<td>Daunomycin-anti-AK-5</td>
<td>10 μg</td>
<td>2/10(55)</td>
<td>0/20</td>
</tr>
</tbody>
</table>

*Animals in each group were injected with 5 x 10⁶ AK-5 cells intraperitoneally, the tumor develops as ascites by day 3 and the animals die between day 5 and 8. Results are expressed as number of animals that developed the tumor/number of animals in the group. Mean survival time of dead animals in days is given in parentheses. Antibody or daunomycin or the conjugate were injected intraperitoneally on days 0, 2 and 4 of the tumor transplantation. The values given are representative of 3 different experiments and the dose corresponds to the amount of daunomycin in the conjugate.

The conjugate used for the treatment of AK-5 bearing rats, thereby specifically targeting the drug to the tumor cell. The drug-antibody conjugate was tested for its biological potency and specificity by studying the effect on the incorporation of 3H-thymidine in L-929 cells and ability to cause complement-mediated lysis of AK-5 cells.

AK-5 is a fast growing tumor. When injected (5 x 10⁶ cells) intraperitoneally, it kills the animals within 5-10 days. AK-5 is also highly immunogenic as shown by its high spontaneous rejection when injected subcutaneously. The host which rejects the tumor possesses highly specific cytotoxic anti-tumor antibodies, which are capable of lysing the AK-5 cells in the presence of complement. Using these properties of the antibody, we have used it as a carrier for antineoplastic drug, daunomycin. Daunomycin alone is not very effective, since about 60% of the animals still die due to tumor development (Table 1). On the other hand, when the daunomycin-antibody conjugate is injected into tumor-bearing animals, there is total regression of the tumor. The mean survival time is also higher when a single dose of the conjugate is given in comparison to a similar dose of the drug alone. Similar drug antibody conjugates have been used for different tumors possessing a specific marker, such as the expression of Thy-1 antigen, alphafetoprotein or tumor-associated antigen. Since AK-5 is highly immunogenic, it has been possible to direct the drug specifically to the tumor cells with the help of cytotoxic anti-tumor antibodies. It may be possible to direct the conjugates of drug and specific antibodies against a tumor cell marker to either the tumor at the primary site or to the metastatic foci that are left behind after the surgery.


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