Tumor-associated Cytotoxicity of Autoantibodies in Sera from Rats with Chemically Induced Hepatomas

P. Lando, F. Blomberg, K. Berzins, and P. Perlmann

ABSTRACT

Complement-dependent cytotoxicity in tumor bearer sera (TBS) from Wistar rats carrying any of five transplantable aminoazo dye-induced hepatomas (D23, D33, D23/Not, D30, or D202) were investigated by a short-term $^{51}$Cr release assay. Extensive tests of various TBS against the hepatomas revealed that the tumors could be divided into two groups. Group 1 hepatomas (D23, D33, and D23/Not) all shared antigens which could induce serum antibodies in syngeneic hosts as shown by complement-dependent cytotoxicity. The Group 2 tumors (D30 and D202) elicited such an immune response occasionally and less efficiently. This immune response was demonstrable only with Group 1 tumors as targets.

It was shown that this reactivity was directed against antigens also present in several homogenates made from normal tissues. Furthermore, absorption experiments with viable tumor cells confirmed that the target antigens for the cytotoxic effect of Group 1 TBS were poorly expressed on the Group 2 tumors. In allogeneic sera raised in Dark Agouti rats against the tumors, a similar immune response was detected when the sera were tested against the tumors and Wistar spleen cells. The alloantisera against the Group 1 tumors reacted strongly with tumor-associated antigens as well as with Wistar alloantigens, but no reactivity against the Group 2 tumors was found. In some of the alloantisera against the Group 2 tumors, reactivities against Group 1 tumor antigens as well as against Wistar alloantigens, but no reactivity against the Group 2 tumors was found. In some of the alloantisera against the Group 2 tumors, reactivities against Group 1 tumor antigens as well as against Wistar alloantigens, but no reactivity against the Group 2 tumors was found. In some of the alloantisera against the Group 2 tumors, reactivities against Group 1 tumor antigens as well as against Wistar alloantigens, but no reactivity against the Group 2 tumors was found. In some of the alloantisera against the Group 2 tumors, reactivities against Group 1 tumor antigens as well as against Wistar alloantigens, but no reactivity against the Group 2 tumors was found. In some of the alloantisera against the Group 2 tumors, reactivities against Group 1 tumor antigens as well as against Wistar alloantigens, but no reactivity against the Group 2 tumors was found. In some of the alloantisera against the Group 2 tumors, reactivities against Group 1 tumor antigens as well as against Wistar alloantigens, but no reactivity against the Group 2 tumors was found. In some of the alloantisera against the Group 2 tumors, reactivities against Group 1 tumor antigens as well as against Wistar alloantigens, but no reactivity against the Group 2 tumors was found.
Paisley, Scotland) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (Flow Co., Irvine, Scotland). The preparation of these cells and the detailed assay procedures have been described previously (15). Wistar spleen cells or hepatoma cells (3 × 10⁴ and 1 × 10⁴ per test tube, respectively) were used. A normal guinea pig serum pool (diluted 1/50) was used as complement source in all experiments.

The percentage of lysis (percentage of ⁵¹Cr release) after incubation for 1 hr at 37°C was calculated according to the formula:

\[
\% \text{ lysis} = \frac{1.5(Y - B)}{Y + X - 2B} \times 100
\]

where Y is cpm in the supernatant, X is cpm in the pellet part, B is cpm counting background, and 1.5 is the dilution factor. Where indicated, the percentage of lysis was corrected by subtracting percentage of ⁶Cr release from cells in preimmune or normal serum from that in immune serum or TBS. Results presented are mean values of duplicates. The mean spontaneous release (medium controls) in the experiments was: D23 cells, 6.9 ± 3.1% (S.D.); D33 cells, 12.1 ± 2.4%; D23/Not cells, 15.3 ± 4.7%; D30 cells, 11.3 ± 1.4%; D202 cells, 10.1 ± 3.9%; and Wistar spleen cells, 9.0 ± 0.9%. The variation between the duplicates never exceeded ±3.2%. The results presented are mean values of duplicates. The mean spontaneous release from experiments in which the different test sera were tested under standardized conditions against one cell type at a time.

Statistical analysis was performed by means of a paired t test.

**TBS.** At the time for harvesting the hepatomas, blood was drawn by cardiac puncture while the rats were under ether anesthesia. Sera were prepared and kept frozen (−20°C) until used.

**Rat Anti-Tumor and Anti-Spleen Antisera.** Attempts were made to raise antisera by injection of tumor or spleen cells, all of Wistar origin, into inbred allogeneic DA rats. In no case did the tumors grow in the allogeneic DA rats. After collection of preimmune sera from all animals, DA anti-D23, anti-D30, anti-D202, and anti-Wistar spleen cell antisera were prepared by s.c. injections of 0.5 to 1 × 10⁶ cells every second week. The rats were bled 1 week after the second booster or the last injection as indicated in Table 5.

Inactivation of endogenous lytic complement in sera to be used for the cytotoxicity experiments was accomplished by incubation for 1 hr at 56°C. The treated sera were cleared by centrifugation for 1 hr at 100,000 × g.

Absorption experiments were carried out with viable cells from hepatomas or Wistar spleen, respectively. Absorptions with trypsinized tumor cells (D23, D23/Not, D202, or D30) were performed in 3 steps using 7 × 10⁷ cells in total per ml test serum. Absorptions with spleen cells were carried out twice with 2 × 10⁷ cells in total per ml. Moreover, absorption experiments with tissue homogenates from various organs were performed as previously described (15). All absorptions were done at 4°C, and all test sera were cleared by centrifugation for 1 hr at 100,000 × g prior to use.

Fractionation of serum on Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) and processing of the eluted fractions was performed as described previously (15).

**RESULTS**

A time study was performed to investigate the development of complement-dependent cytotoxic antibodies in the sera of D23 tumor bearers. For this purpose, sera were collected from 6 rats at Day 0, and each animal was thereafter given a s.c. injection of 10⁶ freshly trypsinized and ≥95% viable tumor cells. During the outgrowth of the tumors, the rats were bled on Days 7, 9, 11, and 14. Heat-inactivated TBS were then tested for their complement-dependent cytolytic activity against chromium-labeled D23 cells (Chart 1). No cytotoxic activity was found in TBS from any of the 6 rats until Day 11, or sometimes at Day 14, although readily visible tumors had developed in all animals by Day 7.

Because the D23 hepatomas grew very quickly in the syngeneic rats, despite the presence in their sera of highly cytotoxic tumor-induced antibodies, we found it important to investigate whether tumor growth reflected an impaired lytic function of the complement system. Therefore, 4 different, freshly drawn D23 TBS (15 days after tumor inoculation) were tested for their endogenous lytic complement activity (Chart 2). It was found, however, that fresh D23 TBS, without addition of exogenous complement, lysed D23 cells just as efficiently as did the heat-inactivated sera in the presence of added guinea pig serum.

In addition to the 2 hepatomas D23 and D33, kept in our laboratory for about 125 passages, we extended our tumor test panel to also include 3 other chemically induced transplanted hepatomas. These tumors (D23/Not, D30, and D202) were transplanted only 33, 39, and 2 times, respectively, before we received them. Prior to testing in our laboratory, they had not been propagated for more than 5 further passages. It is noteworthy that these newly included hepatomas grew much more slowly than did D23 and D33. Thus, D23/Not grew 20 to 30 days before it reached a size comparable to that of D23 or D33 at 14 to 20 days of tumor growth (15). D202 and D30 always needed more than 30 days to reach a comparable size. Moreover, although all transplanted animals fi-
nally developed tumors, some irregularities in tumor growth were observed especially for the slowly growing tumors D30 and D202. Thus, the time for development of visible tumors were observed especially for the slowly growing tumors D30 and D202. Thus, the time for development of visible tumors differed in our test, inasmuch as it could be expected that our fast-growing D23 had undergone some immunomodulation versus D30 or D202 tumor inoculum at the same day and under identical conditions.

It should be noted that D23/Not is of the same origin as our D23 but has been transplanted fewer times (33 times as compared to >125 times for D23) and has thus been kept frozen for a long time. The particular reason for including D23/Not was to see whether this tumor would behave immunologically differently in our test, inasmuch as it could be expected that our fast-growing D23 had undergone some immunomodulation or immunoselection.

Crosswise testing of TBS induced by D23, D33, and D23/Not, respectively, against the 5 hepatomas revealed that the strong cytolytic activity was not restricted to the homologous tumors, but marked cross-reactions were also encountered within the group of D23, D33, and D23/Not hepatomas. Representative results are presented in Table 1 and Chart 3.

When the cytotoxic effects (percentage of $\text{Cr}^{51}$ release) of D23 TBS against D23 cells and against the other two tumors [D33 and D23/Not (Chart 3)] were compared by a paired $t$ test, a significantly stronger reactivity was demonstrated in the homologous combinations (Table 2). Thus, when 11 D23 TBS were tested against D23 cells or D33 cells, the sera gave a mean percentage of $\text{Cr}^{51}$ release (corrected for the percentage of $\text{Cr}^{51}$ release obtained with normal Wistar serum) of 34.0 ± 8.1% (S.D.) against D23 cells and 23.8 ± 6.9% against D33 cells. The difference between these means ($d = 10.2 ± 7.3\%$) was statistically significant ($p < 0.001$) as calculated by a paired $t$ test. The mean difference in reactivity of D23 TBS against D23 cells as compared with D23/Not cells was even larger ($d = 24.9 ± 5.6\%$) than that with D33 cells. These data could indicate a certain amount of tumor-specific reactivity of D23 TBS. When, however, the cytotoxic reactions of D33 and D23/Not TBS against the 3 hepatomas were analyzed in the same manner by paired $t$ test, no elevated reactivities against the homologous tumors were obtained, except for the reactivity of D33 TBS against D33 cells as compared with D23/Not cells ($d = 9.3 ± 6.7\%$). Thus, for example only, D23/Not TBS reacted significantly more weakly with D23/Not cells than with D23 or D33 cells giving negative $d$ values (−19.5 ± 7.0% and −10.3 ± 3.2%) in Table 2.

For comparison, we also tested 4 D23/Not TBS received from Dr. Baldwin’s laboratory against our 5 different tumors (Table 1, Sera 4, 5, 6, and 7). The results corroborated those obtained with the D23/Not TBS from our laboratory (Table 1). D23, D33, and D23/Not TBS did not lyse D30 and D202 cells (Table 1). However, TBS from rats carrying hepatomas D30 or D202 had a somewhat similar cytotoxicity pattern (Table 3). Thus, no cytotoxicity against D30 or D202 cells was detectable in these sera either, but some D30 and D202 TBS were cytotoxic for D23, D33, and D23/Not cells. In comparison with the reactions of D23, D33, and D23/Not TBS against the corresponding tumors, the occasional reactivity of D30 and D202 TBS against D23, D33, and D23/Not hepatomas was generally lower.

It was of importance to establish if the differences in reactivity between the tumors were due to differences in antigen expression on the surface of the nonresponsive tumors or due to differences in lytic susceptibility of the tumor cells. For this purpose, D23 TBS (Pool 3) was absorbed with either D23, D23/Not, D30, or D202 cells and was then tested against D23 target cells (Chart 4). No or only minor reductions of cytotoxicity were detected when D30 or D202 cells were used for absorption. The same amount (7 × 10$^7$ cells/ml test serum) of D23 or D23/Not cells completely abrogated the cytolytic effect of D23 TBS. Similar results were obtained when a D202 TBS,
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Chart 3. Complement-dependent cytotoxicity of D23 TBS, D33 TBS, and D23/Not TBS against D23, D33, and D23/Not hepatoma cells. The lines connect test values obtained with the same TBS against different target cells. All sera were diluted $3 \times 10^{-1}$ in the tests. Percentage of lysis was calculated by subtracting the mean percentage of $^{51}$Cr release obtained with TBS from the mean percentage of $^{51}$Cr release obtained with Wistar normal serum (see legend to Table 1).

Table 2
Comparison by paired t test of the cytotoxicity of D23, D33, and D23/Not TBS against cells of D23, D33, and D23/Not hepatomas

<table>
<thead>
<tr>
<th>Test serum</th>
<th>Target cells compared</th>
<th>No. of TBS assays</th>
<th>Difference$^c$</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>D23 TBS</td>
<td>D23, D33</td>
<td>11</td>
<td>10.2 ± 7.3$^c$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>D23, D23/Not</td>
<td>8</td>
<td>24.9 ± 5.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D33 TBS</td>
<td>D33</td>
<td>7</td>
<td>-22.2 ± 4.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>D33, D23/Not</td>
<td>7</td>
<td>9.3 ± 6.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D23/Not TBS</td>
<td>D23/Not, D23</td>
<td>8</td>
<td>-19.5 ± 7.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>D23/Not, D33</td>
<td>7</td>
<td>-10.3 ± 3.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^a$ All sera were diluted $3 \times 10^{-1}$ in the experiments.

$^b$ Difference between the means of cytotoxicity (percentage of $^{51}$Cr release corrected by subtracting the percentage of $^{51}$Cr release for normal Wistar sera).

$^c$ Mean ± S.D.

Cytotoxic for D23 cells, was absorbed with D23 or D30 cells ($7 \times 10^7$ cells/ml test serum) and tested against D23 cells. When the serum was absorbed with D23 cells, the percentage of $^{51}$Cr release of the serum against D23 cells was completely abrogated, from 30.8 ± 2.1% to -2.9 ± 0.4%. Absorptions of the serum with D30 cells reduced the percentage of $^{51}$Cr release to only 20.2 ± 1.2%, indicating that the D30 cells also contain the target antigens for the cytotoxic activity, although to a much lesser degree than does D23. Furthermore, it was also found that all 5 hepatomas were equally susceptible to complement-dependent cytotoxicity when tested with rabbit antisera prepared against isolated plasma membranes from the tumors.$^5$ The results suggest that the target antigens for the cytolytic attack of these tumor-induced antibodies are present on the surface of D30 and D202 cells but only in low concentrations.

From the cytotoxicity results, the hepatomas could be divided into 2 groups. Group 1 tumor cells (D23, D33, and D23/Not) elicited formation of antibodies which cross-reacted within the group but did not react with the Group 2 tumors (D30 and D202). The Group 2 tumors (D30 and D202) also evoke a humoral immune response although less efficiently than the Group 1 tumors did. However, these responses were detected when Group 1 tumors but not Group 2 tumors were the target cells.

Our previous results suggested that the complement-dependent cytotoxicity of D23 TBS against the homologous tumor cells was of autoimmune nature (15). We found that absorption with plasma membranes from adult liver and especially with tissue homogenates of kidney and small intestine from normal adult rats readily abrogated the cytotoxic activity. The abrogation of cytotoxicity was not due to an anticomplementary effect of the homogenates which did not affect lysis by rabbit anti-chicken erythrocyte antisera of chromium-labeled...
Antibody Cytotoxicity against Hepatomas

Chart 4. Complement-dependent cytotoxicity against D23 hepatoma cells of D202 TBS before and after absorption with hepatoma cells (7 x 10^7 cells/ml test serum). (a), percentage of lysis obtained with D23 TBS before absorption; (b), percentage of lysis of D202 TBS after absorption with D23/Not, D30, or D202 cells, respectively. The sera were finally diluted 3 x 10^{-1} in the test. Values are corrected for percentage of lysis with normal Wistar rat serum diluted 3 x 10^{-1} (10.6 ± 0.9). The height of each column represents the mean of test duplicates; bars, ranges.

Table 4

<table>
<thead>
<tr>
<th></th>
<th>% of lysis a after absorption b of D202 TBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>37.0</td>
</tr>
<tr>
<td>Testis</td>
<td>27.1</td>
</tr>
<tr>
<td>Heart</td>
<td>8.6</td>
</tr>
<tr>
<td>Muscle</td>
<td>24.2</td>
</tr>
<tr>
<td>Kidney</td>
<td>7.1</td>
</tr>
<tr>
<td>Lung</td>
<td>23.1</td>
</tr>
<tr>
<td>Small intestine</td>
<td>13.8</td>
</tr>
<tr>
<td>Liver</td>
<td>3.7</td>
</tr>
</tbody>
</table>

a Percentage of 51Cr release in test serum (diluted 3 x 10^{-1}) corrected by subtracting the percentage of 51Cr release in normal Wistar serum (10.5 ± 1.5%) at the same dilution.

b Test serum was absorbed with 2 x 1.6 mg protein per ml of various Wistar tissue homogenates.

chicken erythrocytes (15). To investigate if the cytotoxic Group 2 TBS also reacted with nontumorous tissues, the following absorption experiments were performed. One D202 TBS, cytotoxic against D23 cells, was absorbed with washed tissue homogenates from Wistar rat testis, heart, muscle, kidney, lung, small intestine, and liver and tested for complement-dependent cytotoxicity against D23 cells. As shown in Table 4, heart, kidney, small intestine, and liver in particular abrogated the cytotoxic activity. Thus, the cytotoxic antibodies in this Group 2 TBS exhibited a similar autoimmune type of specificity as those in TBS from Tumor Group 1.

As the Group 2 tumors appeared to be weakly immunogenic in the syngeneic Wistar hosts, we also investigated the possibility of inducing a tumor-associated immune response in allogeneic DA rats. Since the rejection of the Wistar hepatomas in DA rats might involve an immune response against Wistar transplantation antigens, it was of importance to establish if the tumors expressed these antigens. Therefore, antisera were prepared against Wistar spleen cells, known to express histocompatibility antigens (12). These antisera showed only a weak cytotoxic reactivity against Wistar spleen cells, while their reactivity against D23 cells was considerably higher (Table 5). No reactivity against the Group 2 tumors was detected in these sera. Thus, D23 hepatoma cells expressed surface antigens, also present (but less well expressed) on Wistar spleen cells.

Antisera made in DA rats against D23 cells showed a cytotoxicity pattern similar to that of the DA anti-Wistar spleen cell antisera (Table 5). The lysis of D23 cells was, however, considerably higher than that obtained with the anti-spleen cell antisera. Moreover, the low lysis of Wistar spleen cells was strongly abrogated by absorption of these sera with spleen cells. For instance, absorption of DA a-D23 Antiserum 4 (Table 5) with 2 x 10^7 Wistar spleen cells per ml serum reduced the cytotoxic activity against Wistar spleen cells by 71.9%. In contrast, the cytotoxicity of this serum against D23 cells was only slightly influenced by such absorptions (12.5% reduction after absorption with 2 x 10^7 Wistar spleen cells per ml serum). This would seem to indicate that the major antibody response in these sera was against tumor-associated antigens rather than against Wistar alloantigens.

The different DA anti-D30 antisera were inconsistent in their reactivity against the various hepatomas, one being cytotoxic only for D23 cells (Table 5, Serum 2) and another only for D30 cells (Table 5, Serum 4). In contrast, the D202 alloantisera showed no cytotoxicity for any of the target cells tested. The interpretation of these results is complicated since some of the DA preimmune sera also had an elevated cytotoxicity to D23 as compared to D30, D202, or spleen cells. For the DA a-D30 Sera 3 and 4 in Table 5 which appear to be negative when tested with D23, this selective cytotoxicity of the preimmune sera was exceptionally high (Table 5). On the average, the mean cytotoxicities (percentage of 51Cr release in serum minus percentage of 51Cr release in medium control) of 15 DA preimmune sera for D23, D30, D202, and Wistar spleen cells were 8.9 ± 8.0, 0.6 ± 0.5, 0.9 ± 1.3, and 1.5 ± 0.7%, respectively. These differences may, however, be more apparent than real since the spontaneous 51Cr release (medium control) from D23 was generally lower than that from the other target cells (Table 5). Similar but less pronounced differences between the target cells were also seen with the syngeneic preimmune Wistar sera. In general, this does not affect the interpretation of the TBS results given in Tables 1 to 4 and Chart 3.

We previously reported that the complement-dependent cytotoxic components in syngeneic D23 TBS were restricted to a high-molecular-weight fraction of the serum, thereby suggesting that the antibodies were of the IgM class (15). When a DA a-D23 alloantiserum was similarly fractionated, the major cytotoxic activity also chromatographed in the IgM region. In contrast to what has been found in syngeneic TBS (15), a lower but significant reactivity in this alloantiserum was also seen in the IgG region. Whether or not the cytotoxic activity in the high-molecular-weight region was directed to the same antigens as that in the low-molecular-weight region has not as yet been established.

DISCUSSION

We first investigated when cytotoxic antibodies appeared in serum during D23 tumor growth. It was found that in most sera tested the lytic reactivity was detectable after Day 11 although growing tumors were readily seen at Day 7. This is in agreement...
Table 5
Complement-dependent cytotoxicity of alloantisera against Wistar hepatoma or spleen cells

<table>
<thead>
<tr>
<th>Test serum</th>
<th>D23</th>
<th>D30</th>
<th>D202</th>
<th>Wistar Spleen</th>
</tr>
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<tr>
<td></td>
<td>Preimmune serum</td>
<td>Immune serum</td>
<td>Preimmune serum</td>
<td>Immune serum</td>
</tr>
<tr>
<td>DA a-spleen</td>
<td>1</td>
<td>15.9</td>
<td>24.0</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>13.9</td>
<td>30.6</td>
<td>ND</td>
</tr>
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<td></td>
<td>3</td>
<td>14.3</td>
<td>34.4</td>
<td>11.6</td>
</tr>
<tr>
<td>DA a-D23</td>
<td>1</td>
<td>10.9</td>
<td>46.6</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
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<td>4</td>
<td>11.1</td>
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<td>13.8</td>
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<td></td>
<td>4</td>
<td>29.2</td>
<td>27.5</td>
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<tr>
<td>DA a-D202</td>
<td>1</td>
<td>19.4</td>
<td>16.5</td>
<td>11.5</td>
</tr>
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<td></td>
<td>2</td>
<td>14.2</td>
<td>7.7</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>11.7</td>
<td>10.0</td>
<td>12.4</td>
</tr>
</tbody>
</table>

[a] Individual sera (diluted $3 \times 10^{-3}$) were tested.
b Immune serum taken after 4 injections of cells.
c ND, not determined.
d Immune serum taken after 6 injections of cells.

with results reported by Price et al. (20) who also found cytotoxic antibodies in D23 TBS at about the same time after hepatoma transplantation. The late appearance of the cytotoxic activity during tumor growth might be one explanation why this immune response is ineffective in causing a regression or elimination of the tumors. However, several other mechanisms have been proposed to explain the successful in vivo escape of tumors from complement-dependent cytotoxicity (1, 22). Boyle et al. (8) found that syngeneic sera from tumor-immune guinea pigs did not contain sufficient amounts of functionally intact lytic complement to kill enzyme-treated tumor cells. In our system, however, this was probably not the explanation since fresh TBS was found to contain enough endogenous complement to lyse the labeled hepatoma cells in vitro. It has been shown that the lytic susceptibility of hepatoma cells increases after pretreatment of the cells with proteolytic enzymes (8). Thus, it is possible that the cytotoxic activity with syngeneic complement measured by us on freshly trypsinized cells is an in vitro phenomenon taking place with much lower efficiency in vivo.

In order to more thoroughly investigate the specificity of the cytotoxic activities found in TBS, our hepatoma panel was enlarged to include 3 other chemically induced hepatomas (D23/Not, D30, and D202) in addition to hepatomas D23 and D33 used previously. Price et al. (20) have reported on the presence of complement-dependent lytic antibodies directed against private, tumor-specific antigens in TBS from D23 hepatoma. In our experiments, TBS from animals carrying Group 1 (D23, D33, and D23/Not) hepatomas contained cytotoxic activities that cross-reacted between the Group 1 tumors. A quantitative difference in lysis was, however, detected between D23 cells and D23/Not cells, although the 2 hepatomas were of the same origin. Modulation of antigen expression due to immunoselection during in vivo transplantation of the tumors might be an explanation of this phenomenon since our D23 has been transplanted for at least 125 passages while D23/Not had been transplanted only 33 times.

The Group 2 tumors (D30 and D202) were never lysed by any TBS tested. In quite a few Group 2 TBS, however, lytic activity was detected against the Group 1 tumors. Moreover, absorption studies of a Group 2 TBS (D202) revealed that this cytolytic activity could be abrogated with normal adult tissue homogenates. Thus, the cytotoxic activity of the Group 2 TBS seemed to be of a similar autoimmune type as previously reported for D23 TBS (15). The target cell antigens were, however, poorly expressed on the surface of Group 2 tumors inasmuch as the cytolytic antibodies were absorbed out to only a small extent with any of the tumors from this group. In more recent experiments, we have, however, shown that cytotoxicity could be abrogated more efficiently by Group 2 tumors as well when KCl extracts of the tumors were used for absorption. Thus, although the antigenic activity is poorly expressed on the surface of the cells of Group 2 hepatomas, it may be present within the cells.

From these results, we conclude that the complement-dependent cytotoxic immune responses detected by us in syngeneic TBS are not directed against “private” tumor-specific transplantation antigens. Rather they are directed against antigen(s) common for all tumors tested but differing in both quantitative expression and subcellular distribution. This is in contrast to the findings reported by Price et al. (20). These authors reported that the complement-dependent cytotoxic activity found by them in serum from rats carrying DMAB-induced hepatomas was directed against tumor-specific antigens expressed by these tumors. However, the fact that the cytotoxic antibodies in TBS can be absorbed out by homoge-
Antibodies of the IgM class reacting with several syngeneic and allogeneic tumor cell lines have also been reported to occur in mouse serum (16). The target antigens for this cytotoxic activity were, to some extent, also present in normal tissue homogenates. It was proposed that these antibodies might contribute to the elimination of circulating tumor cells in vivo. Whether the cytotoxic antibodies described herein take part in similar reactions remains, however, to be established. An increased incidence of autoantibodies, directed against erythrocytes, in mice with growing, chemically induced tumors has been reported by Morton et al. (18). Whether or not these autoantibodies reacted with tumor cells was not determined, and their significance for tumor surveillance remains unclear.

It has been reported (14, 23) that normal tissues may contain antigens of type-C viruses, and it cannot be excluded that the immune response detected in TBS may, at least in part, be directed against such antigens. The presence of type C viral antigens in chemically induced in vivo transplanted rat hepatomas has recently been reported by Becker and Sherr (6). These authors found that fast-growing poorly differentiated tumors produce high amounts of a virus antigen with a molecular weight of 30,000, while slowly growing and more differentiated hepatomas did not.

In order to further investigate the nature of antigen expression on the hepatoma cells, allogeneic antisera against Wistar tumors and spleen cells were prepared. When DA animals are immunized with Wistar lymphoid tissue, antibodies of low titer but specific for Wistar histocompatibility (Ag-B\(^2\)) antigens are formed (19). Our results with alloantisera to Wistar spleen cells suggested that some antigens expressed by D23 hepatomas were also present on Wistar spleen cells. Whether or not these shared antigens are products of the major histocompatibility locus of the rat is not known. In general, the Group 2 tumors showed a poor expression of such common antigens, although an apparent cross-reactivity between the tumor D30 and Wistar spleen was seen occasionally (Table 5). In addition, the DA-D23 antisera reacted strongly with D23 cells, and this activity could not be abrogated by absorption with Wistar spleen cells. Preliminary experiments indicate a similar pattern of reactivity for D33 hepatoma cells.\(^5\) Taken together, these results suggest (but do not prove) that both alloantisera and syngeneic TBS react with similar antigens expressed on Group 1 tumors but only poorly or not at all on Group 2 tumors.

As already indicated, the significance of the autoantibodies described by us for surveillance against hepatoma growth in vivo is presently unknown. Although in our test system these antibodies are the predominating humoral component directed against the hepatomas during the entire period of tumor growth, they do not seem to affect tumor growth. It is, however, known (5, 13) that tumor-associated antibodies, in free or immune complexed form, can inhibit or block cellular antitumor responses and thus promote rather than inhibit tumor growth.

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REFERENCES

22. Ran, M., Klein, G., and Witz, I. P. Tumor-bound immunoglobulins. Evidence for the in vivo coating of tumor cells by potentially cytotoxic anti-tumor


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