Selectivity of Inhibition by Anticancer Agents of Mouse Spleen Immune Effector Functions Involved in Responses to Sheep Erythrocytes

M. J. Ehrke, S. A. Cohen, and E. Mihich

ABSTRACT

The selective effects of 24 agents on mouse spleen effector functions involved in the immune responses to sheep erythrocytes have been demonstrated. Five tests were used to assay the effector functions: (1) the complement-dependent cellular cytotoxicity test, which measures complement-mediated lysis in the presence of antibody excreted from B-cell effectors; (2) the complement-independent cellular cytotoxicity test, which measures lysis after non-T-cell, non-B-cell interactions with target cells coated with antibody excreted by B-cells within the same spleen cell populations; (3) the antibody-dependent cellular cytotoxicity test, which measures lysis after non-T-cell, non-B-cell interactions with antiserum-coated target cells; (4) the complement-independent phagocytic test and the antibody-dependent phagocytic test, which measure phagocytosis of target cells coated with endogenous and added antibody, respectively. These five tests were, in general, insensitive to inhibitors of DNA synthesis. Phagocytosis and lysis associated with complement-independent cellular cytotoxicity were selectively sensitive to protein inhibitors, anthracycline antibiotics, bleomycin, actinomycin D, 2-deoxy-D-glucose, and N\(^6\)-benzyladenosine. All functions, except complement-dependent cellular cytotoxicity, were inhibited by agents that affect cellular motility and/or membrane characteristics. The complement-dependent cellular cytotoxicity was only affected by agents that probably acted in a nonspecific cytotoxic manner. Perturbation of immune function was demonstrated in one case, namely, with N\(^6\)-(A\(^2\)-isopentenyl)adenosine. The demonstrated selectivity of action of the agents studied in vitro permitted speculation concerning the cellular effector functions involved in the effenter arm of the mouse spleen cell response to sheep erythrocytes and indicated selectivities on cellular functions that may become relevant in vivo.

INTRODUCTION

The immunosuppressive effects of many anticancer agents have been well documented (13, 21). In most cases these effects have been determined under conditions that measure the action of the agents on a developing immune response; i.e., the agent under study was administered in vivo or in vitro at various times with respect to antigen, and the effect was measured later. It seems reasonable to suppose that the action of an agent on the afferent arm and on the development of an immune response could be different from its action on preformed effector functions. An understanding of the selectivity of drug action on immune effectors may be important in cancer therapeutics particularly in view of the fact that in humans the initial immune responses to the antigenic stimulus of the tumor had probably already occurred at the time of diagnosis (29).

This study was undertaken in an attempt to determine the effects of various agents on effector functions of an immune response that had developed in the absence of drugs. The response of C3Hf/He mice to SRBC\(^{\text{a}}\) was chosen as the model system for study. The assay involves the testing of 3 independent splenic effector functions developed as a result of the immune response of the mouse and 2 effector functions present in the normal mouse spleen. In this assay it is possible to measure not only antibody synthesis and secretion by B-cells but also the function of accessory effector cells, which are known to bind to the free Fc portions of antigen-antibody complexes and then to promote cellular lysis or phagocytosis (see "Materials and Methods"). This is a relatively simple system that includes separate pathways involved in the immune response and may permit the description of drug selectivity in terms of major cellular targets.

The selectivity of inhibition by 24 agents is reported herein. The effects observed are discussed in the light of what is known about the action of these agents and of their possible use in pointing out differences among the immune cellular functions involved at the effector level. A preliminary account of these results has been reported (9).

MATERIALS AND METHODS

Animals

Six- to 12-week-old female C3Hf/He mice were used in all experiments and were obtained from either the Roswell Park Memorial Institute or the Houston, Texas breeding colony.

Agents

Maytansine (NSC 153858), busulfan (NSC 750), procarbazine (NSC 77213), hydroxyurea (NSC 32065), 5-(3,3-di-
methyl-1-triazeno)imidazole-4-carboxamide (NSC 45388),
streptozotocin (NSC 85986), bleomycin (NSC 125666), BCNU
(NSC 409962), MTX (NSC 740), 1-β-d-arabinofuranosylcyto-
sine (NSC 63878), and VCR (NSC 67574) were kindly sup-
plied by Dr. David Houchens, National Cancer Institute,
Washington, D. C. DR and Adriamycin were the gift of Dr.
L. Lenaz, Adria Laboratories Inc., Wilmington, Del. Levam-
isole was from Janssen Pharmaceutica, N.V. Beerse, Bel-
gium. CB was purchased from Aldrich Chemical Co. Inc.,
Milwaukee, Wis. 2-Deoxy-d-glucose, colchicine, cytohexi-
mide, puromycin, and actinomycin D were purchased from
Sigma Chemical Co., St. Louis, Mo. N6-Benzyladenosine
and IPAR were synthesized in this department and were a
gift from Dr. Fleysher and Dr. Hakala. Prednisolone was
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Effector Functions Assayed

Since Govaerts (14) first described immunologically spec-
cific cell damage mediated by lymphocytes, mechanisms
involving thymus-processed killer T-cells, which specifically
interact with and destroy the target cell, have been exten-
sively studied. This mechanism, however, is not involved in
SRBC lysis by cellular effectors (19, 43). A brief account of
what is known about the much-less-studied cellular effector
functions measurable in the SRBC immunological assay
system seems required to define the methods used in this
investigation and to interpret the results.

There are at least 2 specific immunological mechanisms by
which SRBC's can be lysed; both involve the humoral
response. The first is the complement-dependent mecha-
nism that can be measured by the direct plaque assay, as
described by Jerne and Nordin (22), or by the CDCC test
for nucleated cell targets, as described by Mawas et al.
(26), and later applied to SRBC (2, 5, 28). This response
peaks early (Day 4 or 5) (28), is anti-Ig insensitive, and can
be blocked by anti-IgM (3, 26). In fact, if the effector cells
are pretreated with antisera in the absence of complement
and are then washed, anti-IgM but not anti-IgG completely
blocks the subsequent CDCC assay (3, 26), suggesting that
the IgM antibody required in this assay is on the outer
surface of the membrane at the time that the effectors are
added to the incubation mixture.

The second mechanism is a complement-independent
process (19, 25, 32, 42, 43) in which the cytotoxic effector
cells, which bear receptors for the Fc portion of the anti-
body (11, 12, 30, 34), have no affinity for target cell antigens
such and are triggered into inducing specific lytic activity
on interaction with antibody complexed to the target cell
antigens. This process has been described with immune
cell populations (2, 5, 19, 25, 28, 32, 39, 43), in
which clear evidence has been obtained that at least 2
cell types are involved, namely, the AFC and the killer cell,
both of which are present in the same immune spleen cell
populations (31, 38, 43). This mechanism has also been
described with target cells coated with specific exogenous
antisera and normal spleen cell populations, in which case
the test has been called ADCC (4, 12, 25, 33). It has been
suggested that the killer cell in the immune spleen cell
population is different from the killer cell in the nonim-
une spleen cell populations (25, 38, 43). Further data,
which are essentially confirmatory, establishing the similar-
ities of the lytic effector in the 2 systems have been obtained
in this laboratory (unpublished data).

In the normal spleen, 2 populations of effector killer cells
have been defined: the first population, which is glass
adherent and carbonyl iron removable, is involved in initial
rapid lysis; the second population is responsible for the
induction of a slower but continuous lysis (4, 12, 15, 40).
It is likely that under appropriate conditions 1 subpopulation
functionally predominates over the other (7, 10, 12, 16, 25).

The AFC involved in this complement-independent, anti-
body-dependent mechanism is not a direct plaque-forming
cell (3, 43). The peak of the response is on Days 10 to 12 (28), which is consistent with the kinetics of appearance of indirect plaques (8). The antibody involved has been shown to be IgG (12, 25, 33, 39). A recent report (24) has indicated that 19S and 7S antibodies can induce ADCC to a viral-induced tumor target cell, but IgM does not appear to be involved in the complement-independent response to SRBC. In fact the lysis occurring in the presence of immune spleen cells can be inhibited by adding aggregated immunoglobulins (17, 25) or immune complexes (3, 25, 38, 42).

Specific cytotoxic activity by normal spleen cell populations can be induced with cell-free supernatants from immune cells cultured in vitro as well as by serum from immune animals (39, 43); in both instances the factor that induces antigen-specific cytotoxic activity could be absorbed on anti-mouse immunoglobulin columns (39) or inactivated by rabbit anti-mouse y-globulin (43). These results have also been confirmed in this laboratory (3).

It has been suggested by several workers (25, 38) that, in the test with AFC and killer cells from immune spleen cell populations, the antibody is synthesized during the incubation of the assay mixture. The data from this laboratory, shown in Table 1, further supports this contention. The reduced effectiveness in the complement-dependent assay of supernatants from cultures of immune spleen cells harvested on Day 11 is consistent with the fact that the CDCC assay peaks on Days 4 or 5 after immunization and the response declines thereafter. In contrast, the increased effectiveness in the ADCC of supernatants from cultures of immune spleen cells harvested on Day 11 is consistent with the fact that the complement-independent response peaks on Days 10 to 12, with little or no response as early as Day 4 (28, 43). The salient point of this data, however, is that, with the complement-dependent lysis assay, there was no increase in any case in the activity elicited by the 4-h supernatant over that elicited by the 90-min supernatant, whereas in the complement-independent assay the 4-h supernatants from the spleen cells taken from animals 11 days after immunization induced a higher cytotoxic response than that induced by the 90-min supernatant at every dilution studied. Thus, in transferring the information gained with supernatants to the actual conditions of the CDCC, CICC, and ADCC tests, it is apparent that the IgM that predominates in the CDCC appears to be preformed at the start of the incubation (28), whereas the IgG involved in the CICC appears to increase with time. This could indicate synthesis or, simply, secretion of preformed antibody, but in either case additional parameters are measurable in the CICC with respect to the ADCC in which antiserum (preformed IgG) is added to the target cells before the start of the assay incubation.

Hersy (20) has shown that, under conditions similar to those for ADCC, it is possible to measure the phagocytic function of a nonimmunized spleen cell population. As shown by Cohen et al. (4), lytic functions and phagocytic functions are clearly independent. Bennett et al. (2) used the same technique to measure phagocytic function in immune spleen populations.

Based on the above considerations, the functions that can be assayed by the tests used in this study can be summarized as follows: Function 1, a CDCC that depends predominantly on IgM marking of the target cell for complement-mediated lysis. The IgM appears to be preformed and on the outer membrane of certain cells in the immune spleen cell populations at the start of the assay. With this test it is possible to evaluate the effects of drugs on IgM-dependent, complement-mediated lytic function. Function 2, a CICC that depends on the presence of the AFC in immune spleen cell populations, synthesizing and/or secreting IgG, which marks the target cell for lysis after interaction with the Fc receptor-bearing effector cell. Function 3, an ADCC that involves the interaction of Fc receptor-bearing effector cells from normal spleens with target cells coated with specific antisera (IgG) added to the incubation mixture. Since the basic lytic effector functions are assumed to be the same in CICC and ADCC, by comparing drug effects on ADCC with those on CICC, it should be possible to determine whether the effect is only on effector:target cell lytic interactions (ADCC) or whether it is also on the AFC function of IgG synthesis and/or secretion (CICC). Functions 4 and 5, by the hypotonic shock method of Hersy (20), it is possible to study drug effects on phagocytic function only (ADCC phagocytosis) or on the combined functions of antibody synthesis and/or secretion and phagocytosis (CICC phagocytosis).

### Assay Conditions

The 5 separate tests outlined here were used to assay the effects of the agents on the immunological functions of sensitized and nonsensitized spleen cells as defined in the preceding account.

**CDCC.** This test involves the use of immune spleen effector cells. Forty-five min after the initiation of the incubation, 25\(\lambda\) of a 1:12 dilution of guinea pig complement...
ADCC. In this test nonimmunized spleen cells and target cells precoated with mouse anti-SRBC antibody are used. The incubation is for 4 hr and is stopped by the addition of 2 ml cold media (4).

ADCC Phagocytosis. This assay follows the procedure described by Hersey (20), which is based on the fact that a macrophage-engulfed SRBC is protected from lysis during osmotic shock. The incubation is the same as that for ADCC and is stopped after 4 hr by the addition of 1 ml cold H2O, followed in 15 sec by 1 ml cold twice-concentrated medium (4).

CICC Phagocytosis. This assay is performed exactly as that used with the ADCC phagocytosis assay, except that sensitized spleen cells and target cells that have not been precoated with antibody are used (2).

Calculations. After the addition of cold medium in each test, the cell suspensions are centrifuged at 500 × g for 5 min, and the supernatants are decanted into 12- × 75-mm disposable glass tubes. The radioactivity in both the pellet and supernatant is determined as cpm in a Packard Auto-Gamma counter. The percentage of 51Cr released from the target cells is calculated in the following way:

\[
\% \text{ 51Cr release} = \frac{\text{pellet cpm + supernatant cpm}}{\text{supernatant cpm}} \times 100
\]

The percentage of specific release in a test is the percentage of release in the experimental tube minus the percentage of release in the base-line tube. The base-line tube contains non-antibody-coated target cells plus spleen cells from nonimmunized mice (4). For lytic reactions the base-line tube gives a 5 to 10% 51Cr release, which represents spontaneous lysis. The percentage of specific protection from osmotic shock, namely, the indication of phagocytosis, is the percentage of release in the base-line tube minus the percentage of release in the experimental tube; in this case the base-line tubes give 90 to 95% release because no phagocytosis has occurred. The control value of any test is the percentage of specific release in the absence of added test agent.

For comparison of the data from the 5 different tests, the control value in each case was normalized to 100, and the value obtained at each concentration of test agent was compared to this normalized control. For previously documented (28) kinetic reasons, it was decided to assess the function of spleen cells from mice challenged with SRBC either 4 or 11 days earlier. It was thought that perhaps the test agents would have qualitatively or quantitatively different effects on cells at the peak of the immune response than on cells early in the response before the peak is reached (Day 4 for CICC) or late in the response after the peak is past (Day 11 for CDCC). No statistically significant differences, however, could be found in this respect with any of the agents studied (results not shown). In computing the arithmetic mean of the data reported herein, therefore, the data obtained on Days 4 and 11 were pooled.

RESULTS

The average percentage of specific release or protection ± S.E. obtained for the controls in each test at the highest effector:target ratio used for that test are given in Table 2. It is apparent that the maximal obtainable control values varied depending on the test. As explained in “Materials and Methods,” each test had its appropriate controls without the drug, and the values of percentage of specific release or protection for these controls were normalized to 100%. Additional controls with target cells plus normal spleen cells and drug excluded the possibility that the drug directly affected the lysis of the target cells.

The 24 agents tested are divided into 7 groups (Table 3), with respect to their selectivity of action on the 5 effector functions assayed. The function that was sensitive to more agents was CICC phagocytosis, and the least sensitive function was CDCC. ADCC lysis was also quite insensitive to inhibition.

Graphic examples of the selectivity of action of only 1 representative agent from each group are presented in detail. The results obtained with streptozotocin on the 5 tests are shown in Chart 1 as representative of a group of 6 agents. These agents represent a control group in that, at the range of molarity tested, they are completely without effect in all 5 tests.

The results obtained with maytansine at the molarity range of 10^-6 to 10^-11 are shown in Chart 2. It is apparent that this agent has a significant inhibitory effect on CICC phagocytosis but has little or no effect on the other 4 tests.

The effects of DR are shown in Chart 3. DR clearly affects both phagocytic functions and, to a lesser extent, CICC lysis.

The effects of puromycin are shown on Chart 4. Puromycin inhibits both phagocytic functions and CICC lysis.

CB (Chart 5) inhibits CICC lysis and phagocytosis and ADCC lysis and phagocytosis but has no effect on CDCC.

Table 2

Maximal control values obtained in the 5 tests with 51Cr-labeled SRBC as targets

The C3Hf/He effector cells for the CDCC and CICC tests were sensitized to SRBC in vivo. In the CDCC test the effector and target cells were incubated for 45 min, guinea pig complement was added, and the incubation was continued for another 45 min. In both CICC tests the incubations were for 4 hr. In the ADCC tests nonsensitized C3Hf/He spleen cells were used as effector cells, and the target cells were precoated with specific antisera prior to combining effector and target cells. The incubation time for the ADCC tests was also 4 hr.

<table>
<thead>
<tr>
<th>Test</th>
<th>Maximum effector:target ratio used</th>
<th>Maximum % of specific effect obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDCC</td>
<td>20:1</td>
<td>87 ± 4$^a$</td>
</tr>
<tr>
<td>CICC lysis</td>
<td>50:1</td>
<td>44 ± 6$^a$</td>
</tr>
<tr>
<td>ADCC lysis</td>
<td>30:1</td>
<td>48 ± 5$^a$</td>
</tr>
<tr>
<td>CICC phagocytosis</td>
<td>50:1</td>
<td>32 ± 5$^b$</td>
</tr>
<tr>
<td>ADCC phagocytosis</td>
<td>30:1</td>
<td>26 ± 5$^b$</td>
</tr>
</tbody>
</table>

$^a$ Percentage of specific release ± S.E.
$^b$ Percentage of specific protection ± S.E.
Table 3

Selectivity of inhibition of normal and immune C3Hf/He spleen effector functions by various agents in vitro

Assay conditions are the same as those given in Table 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Drugs*</th>
<th>CICC</th>
<th>ADCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5-FdUrd, DTIC, ara-C, hydroxyurea, procarbazine, streptozotocin</td>
<td>- c</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Maytansine, MTX</td>
<td>± c</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Bleomycin, 2-Deoxy-D-glucose, Actinomycin D</td>
<td>+ c</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Adriamycin, DR, N4-benzyladenosine</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>5</td>
<td>Cycloheximide, puromycin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>CB, colchicine, VCR, Levamisole</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Prednisolone, BCNU, acridine derivative</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Range of molarity tested: 5-FdUrd, DTIC, ara-C, hydroxyurea, procarbazine, and streptozotocin, 5 x 10^-4 to 5 x 10^-6; maytansine, 10^-4 to 10^-6; MTX, 10^-3 to 5 x 10^-6; bleomycin, 10^-3 to 5 x 10^-6; 2-deoxyglucose, 5 x 10^-3 to 10^-4; actinomycin D and Adriamycin, 10^-4 to 10^-6; DR, 5 x 10^-3 to 10^-6; N4-benzyladenosine, 10^-5 to 10^-4; cycloheximide, 10^-4 to 10^-6; puromycin, 10^-4 to 10^-7; CB, 5 x 10^-3 to 10^-7; colchicine, 10^-3 to 10^-4; VCR, 10^-4 to 5 x 10^-7; levamisole, 10^-3 to 10^-4; prednisolone, 5 x 10^-4 to 10^-6; BCNU, 5 x 10^-4 to 10^-6; acridine derivative, 10^-4 to 10^-6; IPAR, 10^-4 to 5 x 10^-8.

a 5-FdUrd, 5-fluorodeoxyuridine; DTIC, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide; ara-C, 1-β-D-arabinofuranosylcytosine.

The effects of BCNU are shown on Chart 6. BCNU is 1 of the 3 drugs that inhibited all 5 tests.

The only example, among the agents that were tested, of a drug causing an immunostimulatory effect on the preformed effector functions is shown on Chart 7. IPAR, at the molarity range of 10^-4 to 5 x 10^-6, consistently affected both ADCC and CICC phagocytosis in such a way that it caused values for these functions that were significantly above the 100%-normalized control levels. At high molarity concentrations, however, this agent caused inhibition of all tests except CDCC.

The effects of 5 of the agents on the lysis or phagocytosis of 51Cr-labeled SRBC or antibody-coated SRBC by Day 11 immune spleen cell populations are shown on Table 4. In general, the final molarity was the highest tested for each chart.

These data are in confirmation of a previous preliminary report (27).

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* Range of molarity tested: 5-FdUrd, DTIC, ara-C, hydroxyurea, procarbazine, and streptozotocin, 5 x 10^-4 to 5 x 10^-6; maytansine, 10^-4 to 10^-6; MTX, 10^-3 to 5 x 10^-6; bleomycin, 10^-3 to 5 x 10^-6; 2-deoxyglucose, 5 x 10^-3 to 10^-4; actinomycin D and Adriamycin, 10^-4 to 10^-6; DR, 5 x 10^-3 to 10^-6; N4-benzyladenosine, 10^-5 to 10^-4; cycloheximide, 10^-4 to 10^-6; puromycin, 10^-4 to 10^-7; CB, 5 x 10^-3 to 10^-7; colchicine, 10^-3 to 10^-4; VCR, 10^-4 to 5 x 10^-7; levamisole, 10^-3 to 10^-4; prednisolone, 5 x 10^-4 to 10^-6; BCNU, 5 x 10^-4 to 10^-6; acridine derivative, 10^-4 to 10^-6; IPAR, 10^-4 to 5 x 10^-8.

a 5-FdUrd, 5-fluorodeoxyuridine; DTIC, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide; ara-C, 1-β-D-arabinofuranosylcytosine.

- , no effect; ±, significant inhibition, but the highest drug tested did not reduce the value obtained to lower than 50% of control; +, inhibition to values lower than 50% of controls; S, stimulation of response.
agent and the molarity at which significant inhibition of the action of the immune spleen population on the $^{51}$Cr-labeled SRBC resulted. As can be seen, however, the effects on lysis of all agents except VCR were prevented when antisera was added to the incubation mixture to coat the targets with antibody during the assay.

DISCUSSION

The use of the assay involving the 5 described tests for measuring in vitro drug effects on performed immunological functions of mouse spleen cells has permitted the demonstration of selectivity of action by the 24 agents studied. This drug selectivity should be interpreted in the light of the phenomena involved in the 5 tests used, which have been described in "Materials and Methods."

During the short time (4 hr maximum) involved in this immunoassay of preformed effector functions, the 24 agents could affect any or all of the following phenomena: (a) synthesis, secretion, and/or release of antibodies (CDCC and CICC tests); (b) binding of antibody to target cells (CDCC and CICC tests); (c) effector cell-dependent lytic pathways (ADCC and CICC tests); (d) phagocytosis (ADCC and CICC phagocytic tests). Thus, the CICC lysis involves a combination of phenomena under a, b, and c whereas CICC phagocytosis involves a combination of phenomena under a, b, and c.

The 6 drugs in the first group in Table 3 are antitumor agents; all have been reported to be immunosuppressive (for review see Refs. 13 and 21). All of these agents are known to affect overall DNA synthesis although at different steps. Under the conditions of this study, as would be expected, it was impossible to show any immunosuppressive effect by these agents when preformed effector functions were assayed. Therefore, DNA synthesis is not a limiting factor during the time involved in this immunoassay.

The 2 drugs (maytansine and MTX) that make up the next group in Table 3 may also affect DNA synthesis. The effects of MTX are well documented, whereas the potential alkylating function of maytansine has only been suggested (23); notwithstanding their different proximal mode of antiproliferative action, both of these drugs have a clear inhibitory effect on CICC phagocytosis (see Chart 2 and Table 3). Notably, in the presence of target cells precoated with exogenous antibody (as in the ADCC test), these agents did not affect phagocytosis. It would seem likely therefore that in this case a site of action involving antibody produc-
tion, secretion, or binding to target cells may be sensitive to these drugs. Differences between macrophage function in nonsensitized and sensitized spleen may also be responsible for the unique sensitivity of CICC phagocytosis to maytansine and MTX.

In Table 3 there are 4 antibiotics and 2 antimetabolites in Group 3. All 4 antibiotics have been reported to bind to DNA, prevent DNA replication, and under proper conditions also affect RNA and, at least in some cases, protein synthesis. The antimetabolite N⁶-benzyladenosine interferes with precursor incorporation into RNA and DNA. Also reported is the observation of a rapid reduction in the ATP pool in liver after drug administration. The metabolic poison 2-deoxy-D-glucose could have similar effects on the energy pools of sensitive cells. All the agents in this group inhibited both ADCC and CICC phagocytosis and to a lesser degree, in most cases, CICC lysis, but they did not inhibit ADCC lysis. Moreover, the addition of heat-inactivated, Day 11 anti-SRBC antisera to the CICC test completely prevented the inhibitory effect of these agents on lysis (Table 4). Both the lack of effect on ADCC lysis and the prevention of the inhibition of CICC lysis by the addition of Day 11 antisera would suggest that, in the case of CICC lysis, the action of these agents is on the antibody production or secretion by the AFC and not on the binding of the antibody to the target cells. The effect of these agents on phagocytic functions, however, must involve an action on phagocytic cells, in addition to that on antibody production and secretion, because ADCC phagocytosis is also affected. CICC phagocytosis was somewhat more sensitive than was ADCC phagocytosis. This also suggests the possibility of 2 sites of action for these agents; in the case of CICC phagocytosis, these 2 effects would be additive.

The 2 well-known protein inhibitors cycloheximide and puromycin are the only drugs in Table 3, Group 4. The same 3 tests that were inhibited by the agents in Group 3
are inhibited by these 2 drugs, and it might be argued that they do not constitute a separate group. However, the inhibition of CICC lysis was clearly greater in this case than in the case of the agents in Table 3, Group 3 (see for example Charts 3 and 4). When the same argument as developed with the agents of Group 3 is invoked for these agents, it certainly seems to imply that, at least in the case of these inhibitors of protein synthesis, it is antibody synthesis that is affected primarily. The lack of effect on CDCC supports the contention that the antibody involved in this test is preformed at the time of effector cell addition to the assay tubes (26).

The agents in Table 3, Group 5, inhibit all tests except CDCC lysis. Group 5 includes the fungal extract, CB, and 2 plant alkaloids, colchicine and VCR, both of which are mitotic inhibitors. A demonstrated effect of these agents is their interactions with the microtubules or microfilaments of treated cells. As a probable consequence of this interaction, secondary effects such as changes in intracellular and cellular motility and in cytological appearance of the treated cells are observed (6). The most probable site of the activity of these agents as measured herein would seem to be on cell motility and/or cellular membrane changes. The fourth drug in this group is levamisole, a broad spectrum anthelmintic that has been reported to produce either immunopotentiation or immunosuppression under appropriate conditions (37). Levamisole has been reported to affect cellular levels of cyclic 3':5'-AMP and cyclic 3':5'-GMP and cell motility. It has been suggested that microtubular assembly may be the cellular site involved (1). This would be consistent with its grouping with these other agents. The recently reported critical dose depend-
Table 4  
Effects of agents on the lysis and phagocytosis of ¹¹¹Cr-labeled SRBC or antibody-coated ¹¹¹Cr-labeled SRBC by Day 11 immune spleen cell populations

<table>
<thead>
<tr>
<th>Agent</th>
<th>Final mobility</th>
<th>Phagocytosis</th>
<th>Lysis</th>
<th>Antibody-coated ¹¹¹Cr-labeled SRBC</th>
<th>Phagocytosis*</th>
<th>Lysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Deoxy-D-glucose</td>
<td>10⁻²</td>
<td>46</td>
<td>54</td>
<td>80</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>N₆-Benzyladenosine</td>
<td>10⁻²</td>
<td>49</td>
<td>60</td>
<td>97</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>10⁻²</td>
<td>19</td>
<td>78</td>
<td>30</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>VCR</td>
<td>10⁻⁴</td>
<td>14</td>
<td>61</td>
<td>43</td>
<td>105</td>
<td></td>
</tr>
</tbody>
</table>

* With the exception of Footnote a, all conditions are the same as those indicated for CICC in Table 2.

Anticancer Drug Selectivity on Immune Effectors

shown to accumulate at the plasma membrane and thereby to affect cytosine and uridine transport (18). It is conceivable that the accumulation of IPAR in the cell membrane may be related to its unique effect on preformed effector functions.

As shown in this report, selective action on preformed mouse effector functions can be exerted by agents with different biochemical modes of action. There seems to be no direct correlation between the apparent proximal site of biochemical action of the agents, as known to date, and the selective effect induced on immune effector functions. Moreover, as expected, there was no correlation between antiproliferative action and effect at the level of preformed effectors. In general, the functions studied appeared to be insensitive to inhibitors of DNA synthesis, whereas phagocytosis and CICC lysis were sensitive to inhibitors of protein synthesis. Cell-mediated lysis, as measured by ADCC, appears to be sensitive only to agents that affect cell motility and/or membrane parameters in addition to agents that may have a nonspecific cytotoxic action. CDCC is only sensitive to this latter group.

The differences in sensitivity between ADCC lytic and phagocytic tests further indicate that these are separate functions. The similar sensitivity of the ADCC and CICC lytic tests, when antibody was not limiting, is in support of the suggestion that the same killer cell is involved in both and that the salient difference between the 2 tests is the source of antibody. The striking differences in the sensitivity of the CICC and CDCC lytic tests further support the suggestion that a different product is involved in the marking of the target for complement-dependent (IgM) and for cell-mediated (Fc-positive IgG) lysis.

REFERENCES

10. Evans, R., and Alexander, P. Rendering Macrophage Specifically Cytotoxic by a Factor Released from Immune Lymphoid Cells. Transplanta-


Selectivity of Inhibition by Anticancer Agents of Mouse Spleen Immune Effector Functions Involved in Responses to Sheep Erythrocytes

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