Unusually Efficient Tumor Cell Lysis by Human Effectors of Antibody-dependent Cellular Cytotoxicity Mediated by Monoclonal Antibodies

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ABSTRACT

Concentrations ranging between 0.01 and 10 pg per cell of certain monoclonal antibodies (MAbs) are shown to constitute 4-hr 50% lethal doses for tumor cells mixed with human effectors of antibody-dependent cellular cytotoxicity (ADCC). This efficient and rapid tumor cell lysis is achieved at low effector cell levels (effector:target ratios, <25:1) at which the effectors are nonadherent peripheral blood leukocytes (PBL) enriched by density centrifugation. Comparable MAb-mediated ADCC efficiency has not been reported previously, probably because most MAbs (e.g., 10 of 13 tested in this study) are typically inefficient or completely inactive in mediating ADCC, even at 100-fold greater concentrations. By analyzing the ADCC efficiencies of several MAbs specific for murine cell surface alloantigens, it is shown that murine IgG2a and IgG3 MAbs and a rat IgG2b MAb are very efficient mediators of ADCC. However, ADCC efficiency was found not to correlate strictly with subclass, since 4 of 6 murine IgG2a MAbs tested were completely inactive, even though they all bound the target cells readily. It is shown that the relative differences in ADCC efficiencies are not accounted for directly by antibody affinity for antigen; one MAb was very efficient in ADCC but had demonstrably low antigen affinity, while a second MAb showed no ADCC activity in spite of its high affinity for the same target antigen. These results point to other experimentally testable properties of MAbs and of MAb-antigen complexes which may be critical for efficient ADCC reactions. This study underscores an important immunotherapeutic value which certain MAbs potentially have for mediating tumor cell lysis: in low concentrations (and without toxic drug modification), some MAbs efficiently mediate the lysis of tumors by ADCC, which itself is as effective as other immune lytic processes but which requires no prior immunological education of effector cells.

INTRODUCTION

ADCC is an immune mechanism which represents an important link between humoral immunity in the form of specific antigen recognition by antibody and cellular immunity in the form of specific antigen recognition by antibody and cellular immunity in the form of cell-mediated, cytolytic destruction of antibody-coated target cells. In principle, then, any antibody response to a cell surface antigen such as a viral antigen, a tumor-specific antigen, or even a "self" molecule on the membrane surface of normal cells is potentially accompanied by tissue-destructive ADCC reactions (21, 22).

Human PBL contain a subpopulation of cells capable of rapidly lysing nucleated target cells in an antibody-dependent fashion with no prior exposure to antigen required. Effector cells of this nature are thought to be K-cells, which are non-T, non-B, nonphagocytic, and nonadherent lymphocytes that have Fc receptors on their cell surface (3, 11-13, 21, 22, 27). The specificity of target cell recognition resides within the antigen-combining site of the antibody molecule. Effector cells recognize target cell-bound antibody through interaction of an effector cell Fc receptor and the Fc portion of the antibody molecule; target cells are lysed following contact between the effector and target cell (21, 22, 27). The exact molecular details of the mechanism of ADCC and of the process by which antibody bridges effectors with target cells in such a way as to activate the lytic steps are yet to be elucidated.

It would seem that MAbs could shed new light on this process, given their homogeneous nature and the possibility of making many structurally distinct MAbs against the same antigen. However, previous studies of ADCC mediated by monoclonal antibodies (MADCC) with human effectors indicate that individual MAbs are generally ineffective in this process (in comparison to heterologous rabbit antiserum, for example) because lengthy incubation periods, very high MAb concentrations, and high E:T ratios all appear to be necessary in order to achieve even moderate levels of target cell lysis (4, 6, 8, 9, 23). In the course of comparing the effectiveness of tumor cell lysis by ADCC and by CTL, it was observed that certain MAbs facilitated significantly better levels of target cell lysis than those achieved by CTL in the same experiments. This observation has prompted a systematic evaluation of the factors which are critical for effective MADCC. In this study, the relative ADCC efficiencies with human effectors are established for several MAbs against murine cell surface alloantigens in order to begin narrowing the possible reasons which could account for the wide range of ADCC efficiencies commonly observed when monoclonals mediate ADCC reactions. MAbs specific for murine alloantigens are particularly suited for dissecting the recognition requirements of ADCC, because the antigens themselves are well characterized, both structurally and immunogenetically, and because studies in a number of laboratories indicate that it will soon be possible by genetic engineering methods to localize precisely the structural regions within these molecules where individual MAbs bind.

MATERIALS AND METHODS

Human Effector Cells. PBL samples were obtained from 7 normal, healthy adult donors. Leukocytes were separated by centrifugation on a Ficoll-Hypaque (10) gradient (ρ = 1.08 g/ml). Cells were washed 2 times

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with Ca²⁺- and Mg²⁺-free 0.15 M phosphate-buffered saline (pH 7.2) and then once with CM [RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, penicillin, streptomycin, 4-(2-hydroxyethyl)-1-piperazinoethanesulfonic acid, and glutathione]. Adherent cells were removed by incubation in CM in plastic flasks twice for 1 hr each at 37°C, at an initial cell density of 5 x 10⁶ cells/ml (8). Nonadherent cells were used as effectors. Primary human CTL were educated against murine cells by 7-day stimulation in an in vitro mixed-lymphocyte culture with 9 x 10⁶ human PBL plus 18 x 10⁶ irradiated C57BL/6 splenocytes.

Murine Lymphoma Target Cells. The following murine lymphoma cells used as targets were maintained in CM: EL4 (5), H-2b; RADA1 (18), H-2b; RI.1 (7), H-2b; and R1E (7), derived from an H-2D, TL, β₂M-negative variant of R1.1 cells.

MAbs and Antiserum. The MAbs and hyperimmune antiserum used in the ADCC assays are listed in Table 1, along with their specificities. The M1/42 and 11-4.1 cell lines were obtained from the Cell Distribution Center, Salk Institute, La Jolla, CA, and maintained in tissue culture as described above. MAbs from the following hybridomas in parentheses were generously provided by: Drs. L. Sherman, Scripps Clinic and Research Foundation (5F1.2); K. Ozo, NIH (30.5.7, 28.14.8, 34.5.8, and 34.2.12); U. Hammerling, Memorial Sloan-Kettering Institute (S19.8); and R. Hyman, Salk Institute (30-H12). MAbs from the following hybridomas in parentheses were purchased from: Accurate Scientific and Chemical Co., New York, NY (5a-8); Biotest, W. Germany (H141-30, H142-23); and New England Nuclear, Boston, MA (NEI-026). RamlgG and RatlgG were purchased from Miles Laboratories, Inc., Elkhart, IN. Rabbit anti-R1E antiserum was generated in this laboratory by repeated immunization with 3 x 10⁶ R1E cells.

Determination of MAAb Concentration. Immunoglobulin concentrations of ascites fluid were determined by competition radioimmunoassay. Mouse ascites fluid (50 µl) was serially diluted and incubated for 15 min at room temperature with 10 µl of 5% SA (Cowan 1 strain). As a positive control, protein-A purified BBM.1 (mouse anti-human β₂M, IgG2b; Ref. 1) of known immunoglobulin concentration was treated in the same manner. Ten µl of 14C-labeled MPC-11 (lgG2b myeloma) culture supernatant, diluted 1:40, was then added to all tubes. After an additional 15 min at room temperature, the SA was pelleted, washed, dissolved in scintillation cocktail, and then counted by liquid scintillation. Dilutions of ascites yielding 50% inhibition of SA-precipitable MPC-11 counts were considered to contain the same amounts of antibody as in the dilution of BBM.1, which also resulted in 50% inhibition. Samples of M1/42 and 30-H12 culture supernatants were affinity purified over a RatlgG Sepharose-4B column, and concentrations of eluted immunoglobulin were determined spectrophotometrically. The concentrations of all MAbs listed in Table 1 represent the averages (±10%) of 2 determinations (except M1/42 and 30-H12).

ADCC Assay. Aliquots of 1 x 10⁶ target cells, prelabeled with ⁵¹Cr, were sensitized with log dilutions of MAAb or rabbit heteroantisera for 10 min at room temperature in microtiter wells containing 100 µl of CM. One hundred µl of effectors in CM were then added to each microtiter well, and the plate was incubated for 3.5 to 4 hr at 37°C, in 5% CO₂. At the conclusion of the assay, 100-µl aliquots of supernatant were withdrawn and counted by liquid scintillation.

For assays using RamlgG or RatlgG as a second antibody, target cells (3 x 10⁶/ml) were incubated with MAAbs at a final dilution of 1:500 (M1/42 and 30-H12) or 1:1000 (all other MAAb), for 10 or 30 min at room temperature, washed once, and resuspended at 2 x 10⁶/ml in CM. Dilutions of the second antibody in 50 µl were added to 50 µl of treated targets and incubated for an additional 10 min. Effectors in 100 µl were then added, and the assay was performed as described above.

All data represent averages of duplicate wells in a single experiment. Specific lysis was determined as follows:

\[
\frac{ER - SRC}{FRC - SRC} \times 100
\]

where ER is the average effector-mediated release of ⁵¹Cr from the labeled target cells from duplicate wells, SRC is the average "spontaneous" ⁵¹Cr release control for target cells in the absence of effectors for duplicate wells, and FRC is the average "full" ⁵¹Cr release control for targets with 1 N HCl or 1% Nonidet P-40 detergent added for duplicate wells. In all experiments, the SRC was less than 20% of the FRC. Error bars are included in the figures when a symbol did not encompass both data points. Each experiment shown is representative of at least 2 different experiments with each monoclonal and effector cell donor listed in the figure legends.

Affinity of Target Cell-bound MAAs. EL4 target cells were incubated with a high concentration of MAAb, washed twice, and then resuspended at 1 x 10⁶/ml in CM at room temperature. At various time points up to 6 hr, aliquots of cells were removed, washed, and resuspended at a final concentration of 5 x 10⁷/ml in microtiter wells and assayed for either direct MADCC or ADCC using RamlgG in order to detect all target cell-bound MAAbs.

RESULTS

A Comparison Between ADCC and CTL Cytotoxicity. In the course of comparing tumor cell lysis by human ADCC and CTL, it was discovered that certain MAAbS considerably enhanced the lysis of murine tumor targets by human PBL taken from mixed lymphocyte cultures after being stimulated with mouse splenocytes. A representative experiment is shown in Table 2, in which human PBL stimulated with C57BL/6 splenocytes for 7 days in vitro were tested for lysis of EL4 tumor cell targets derived from the same mouse strain. Whereas the level of lysis was low at

Table 1

<table>
<thead>
<tr>
<th>MAbs and heteroantisera tested for human anti-mouse ADCC activity</th>
<th>IgG</th>
<th>IgG expression</th>
<th>mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse-derived monoclonals</td>
<td>NEI-026</td>
<td>2a</td>
<td>1b</td>
</tr>
<tr>
<td>11-4.1</td>
<td>2a</td>
<td>1a</td>
<td>H-2k*</td>
</tr>
<tr>
<td>30.5.7</td>
<td>2a</td>
<td>1a</td>
<td>H-2d*</td>
</tr>
<tr>
<td>28.14.8</td>
<td>2a</td>
<td>1a</td>
<td>H-2d*</td>
</tr>
<tr>
<td>34.2.12</td>
<td>2a</td>
<td>1a</td>
<td>H-2d*</td>
</tr>
<tr>
<td>5a-8</td>
<td>2b</td>
<td>3a</td>
<td>Thy-1.2</td>
</tr>
<tr>
<td>51F1.2</td>
<td>2b</td>
<td>3a</td>
<td>H-2k*</td>
</tr>
<tr>
<td>H142-23</td>
<td>2b</td>
<td>3a</td>
<td>H-2k*, D*</td>
</tr>
<tr>
<td>S19.8</td>
<td>2b</td>
<td>3a</td>
<td>A/M</td>
</tr>
<tr>
<td>H141-30</td>
<td>3</td>
<td>H-2d*</td>
<td>4.7</td>
</tr>
</tbody>
</table>

- Monoclonal antibodies
- Mouse-derived monoclonals
- NEI-026
- 11-4.1
- 30.5.7
- 28.14.8
- 34.2.12
- 5a-8
- 51F1.2
- H142-23
- S19.8
- H141-30

- Rat-derived monoclonals
- M1/42
- 30-H12

- Hyperimmune heteroantisera
- Rabbit anti-R1E
- Rabbit anti-Rat lgG
- Mouse anti-Rat IgG

Table 2

<table>
<thead>
<tr>
<th>MAAb enhancement of the cytotoxicity of tumor cells by human PBL effectors</th>
<th>E:T ratioa</th>
<th>NEI-026</th>
<th>% of specific lysisb</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>–</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>24</td>
<td>–</td>
<td>–</td>
<td>9</td>
</tr>
<tr>
<td>50</td>
<td>–</td>
<td>–</td>
<td>13</td>
</tr>
<tr>
<td>50</td>
<td>–</td>
<td>–</td>
<td>66</td>
</tr>
</tbody>
</table>

- Human PBL effectors, recovered from a 7d primary in vitro MLC with irradiated C57BL/6 splenocytes, were tested for lysis of 1 x 10⁶ ⁵¹Cr-labeled EL4 target cells in 4 hr.
- MAAb (4.75 µg) was added at the start of the E:T incubation.
- Percentage of specific lysis was calculated according to the equation given in "Materials and Methods" with FRC = 12500 and SRC = 2100. Duplicate sample values were within 5% of each other.

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E:T = 50:1 (or less) without antibody, the addition of the NEI-026 MAb against \( \beta_2 \)M on the surface of these target cells dramatically enhanced target cell lysis by at least 5-fold. While much more effective CTL could be generated by multiple additions of stimulating splenocytes with longer stimulation periods in vitro, this MAb always significantly enhanced the level of killing over that achieved without antibody (data not shown). However, unlike the CTL killing in these experiments, the ADCC effectors required no prior education, since this MAb also mediated just as efficient ADCC with freshly prepared nonadherent PBL. Hence, unstimulated nonadherent PBL enriched by density centrifugation were used as effectors for the remaining experiments described in this study.

ADCC Activity of Different MAbs. In order to determine whether or not the NEI-026 MAb was unusual in its ability to mediate such efficient ADCC reactions with human effector cells, a variety of MAbs, all listed in Table 1, were tested for their comparative ADCC activities over 3 log dilutions in a 4-hr assay at E:T = 25:1. Four different murine T-lymphomas were used as targets in order to accommodate the various specificities of these antibodies. Chart 1 shows representative results of several such assays. Three MAbs from the 13 tested were particularly effective in directing target cell lysis. At concentrations of less than 50 ng/well (200 \( \mu \)l) or 5 pg/target cell, NEI-026 and H141-30 facilitated greater than 40% lysis of EL4 target cells (Chart 1A). Most striking are the data obtained with 30-H12, however, where 50 pg/well or 5 fg/target cell (about 20,000 molecules/cell) resulted in 40% lysis of all 4 target cells tested (Chart 1A). A fourth MAb, 11-4-1, was capable of mediating up to 40% specific lysis (Chart 1B), but only at considerably higher antibody concentrations (1.75 \( \mu \)g/well), with no lysis observed at less than 50 ng/well. The 9 remaining MAbs mediated less than 15% or no specific lysis at all concentrations tested. The NEI-026 MAb recognizes murine \( \beta_2 \)M of the \( b \) allele, and the H141-30 MAb recognizes the H-2D\( ^b \) antigen, both of which are present only on the EL4 target cells. The 30-H12 MAb recognizes the Thy 1.2 antigen, which is present on all of the targets used here. Finally, the 11-4-1 MAb recognizes the H-2K\( ^a \) antigen on R1.1 targets (and RADA1 cells which were not tested with this MAb). These MAbs do not cross-react with human cells, as judged by complement assay plates or MADCC (data not shown). As one specificity control in Chart 1B, the NEI-026 MAb was also tested on R1.1 target cells, and no lysis by MADCC was detected, proving that the MADCC reaction with this MAb is antigen specific. Another finding here is illustrated in Chart 1C, where it is shown that Class I antigens need not be on the target cells for ADCC (as is the case for CTL), since R1E cells were efficiently lysed but expressed no H-2 antigens (7).

Chart 2 shows an effector titration against EL4 targets sensitized with NEI-026, H141-30, 30-H12, or rabbit anti-R1E. These MAbs were nearly as efficient in mediating ADCC as was the hyperimmune rabbit antiserum control, and each gave greater than 50% lysis of EL4 target cells at E:T ratios as low as 12:1. One important control shown in this experiment is that the 30-H12 MAb did not itself lyse targets in the absence of effector cells, proving that the culture supernatants containing this MAb did not have an autolytic factor which lysed target cells nonspecifically. A second important control in Chart 2 shows that effector cells did not lyse targets without MAbs, proving the antibody dependence of this lytic process.

Human effector cells which mediated the lysis of MAb-coated target cells were found in the peripheral blood of all 7 healthy adult volunteers tested. Effector cells from each individual generated similar patterns of MADCC; i.e., for each person, all (or in some cases, most) of the MAbs listed in Table 1 were tested in an identical MADCC assay, and 30-H12, NEI-026, and H141-30 consistently generated the highest levels of lysis within 4 hr compared to the other MAbs with E:T ratios similar to those above (data not shown for all donors).

One possible explanation for the inability of some MAbs to mediate MADCC in these experiments is that they failed to bind effectively to the target cells. Since rabbit antibodies mediate ADCC very efficiently (cf. Chart 2), RamlgG or RarlgG was used to detect the binding of MAbs to target cells by rabbit antibody-mediated ADCC. Chart 3 as compared to Chart 1 demonstrates that the second antibody dramatically enhanced MAb-precocated
target cell lysis by ADCC, thus proving that all MAbs tested in Chart 1 readily bound the targets (data for 11-4.1 and H142-23 not shown), with one possible exception being the 5F1.2 MAb (Chart 3A); the latter MAb produced only a slightly enhanced level of ADCC lysis with RamlgG, suggesting that it bound the target cells only weakly. As indicated by the prozone-like appearance of the curves in Chart 3, optimal lysis was critically dependent on the final dilution of RamlgG or RarigG used in the assay. In general, very high as well as very low dilutions of the second antibody resulted in relatively low levels of lysis of the MAb-precoated target cells, whereas intermediate dilutions produced optimal lysis. The high dilution effect is probably explained by a lowering of the concentration of the second antibody below some minimal amount necessary for an efficient ADCC reaction; the low dilution effect, where the concentration of RamlgG or RarigG was relatively high, is not presently understood. The fact that identical dilutions of the second antibody gave optimal lysis for almost every different MAb-precoated target cell tested in Chart 3 suggests that comparable amounts, or densities, of each MAb were bound to the targets under the conditions of this experiment.

When no second antibody was added to the MAb-precoated and washed target cells (NA, no second antibody, in Chart 3), only the NEI-026 and 30-H12 MAbs could be detected by direct MADCC. However, target cells precoated by these 2 MAbs appeared to be lysed considerably less efficiently than did targets that were assayed directly in the presence of these 2 MAbs, as shown in Charts 1 and 2. This difference was found to depend primarily on the length of time in which the MAbs were preincubated with the targets before washing, as illustrated in Chart 4A. In this experiment, MAb-coated targets were prepared by incubating them for 30 min (rather than for 10 min, as in Chart 3) with the MAbs before washing and assaying for direct MADCC. The lytic levels for the NEI-026 and 30-H12 MAbs in Chart 4 returned to those levels shown in Charts 1 and 2. In a control test, no significant lysis of EL4 targets was found when they were precoated with the 28.14.8 MAb which binds the H-2D<sup>e</sup> antigen of this cell type; this result was anticipated from Chart 1D, where it was observed that RADA1 targets are not lysed in the presence of this MAb, which also binds the H-2L<sup>d</sup> antigen of this cell type. An unanticipated result from the experiment shown in Chart 4A was the observation that H141-30-precoated targets were not susceptible to lysis by direct MADCC, even though this MAb behaved comparable to the NEI-026 MAb in the experiments shown in Charts 1 and 2. For reasons which are examined in the next section, the amount of H141-30 MAb remaining on the surface of precoated target cells following antibody incubation and washing was insufficient for direct MADCC, even though this MAb could be effectively detected by RamlgG (Chart 3B).

ADCC Efficiency and Target Antigen Affinity. In order to determine whether H141-30-precoated targets were resistant to direct MADCC because insufficient amounts of this MAb were used or because insufficient times were allotted to precoat the targets, targets were prepared with a 50-fold greater amount (1:20 versus 1:1000 dilution) of H141-30 and incubated for 30 min (versus 10 min). However, as shown in Chart 4B, even with this dose of MAb, which is probably supersaturating (about 1 billion molecules/cell), and with a 3-fold lengthening of the incubation time, direct MADCC was still not detected after the cells were washed. This result shows that the cell surface-bound H141-30 MAb, as detected by MADCC, is rapidly lost during the washing procedure at room temperature, even from cells exposed to supersaturating amounts of MAb. However, as also shown in Chart 4B, MADCC could be restored if additional H141-30 MAb were added back to the precoated targets. Equally efficient levels of target cell lysis by direct MADCC were observed when H141-30 MAb was added back either immediately following the wash-
ing procedures (0 hr) or 4 hr later after the targets had been incubated at room temperature (in the absence of effectors). These latter results show that this MAb does not directly induce, even after 4 hr, a general loss in the target antigen which can be detected by MADCC. Therefore, it is unlikely that this MAb induces a loss in the accessible antigen:antibody complexes in 10 min from the cell surface through some type of modulation, endocytosis, and/or shedding. The most likely explanation for the behavior of this MAb is that it readily dissociates from the target cells by virtue of a low antigen affinity.

In order to test this possibility further, target cells were examined for the temporal loss of their susceptibility to ADCC. In these experiments, the targets were precoated with supersaturating doses (very low dilutions) of either the H141-30 or the 28.14.8 MAb, both of which directly bind H-2D<sup>α</sup>. After the unbound antibody was washed away, the precoated targets were resuspended at a low concentration, 1 x 10<sup>4</sup> cells/ml, and were incubated at room temperature in the absence of effectors or added antibody; at this concentration, antibody dissociation is favored, since the association rate is proportional to the concentration of free antibody, which is negligible under these conditions. At various intervals, the precoated targets were washed once more and then analyzed for lysis by RamlgG-mediated ADCC. As shown in Chart 5A, H141-30-precoated targets lose their susceptibility to lysis with increased incubation times, there being a 60% loss of RamlgG-mediated ADCC after 6 hr as compared to the target susceptibility initially (0 hr after precoating). In comparison, the susceptibility of 28.14.8-precoated targets as a function of incubation time showed no significant decrease in the same time period. Since both of these MAbs bind the same target cell antigen and since it was shown above that H141-30 does not induce a generalized decrease in this antigen from the cell surface (Chart 4B), it appears that the most likely explanation for the different behavior of these 2 MAbs is that one, 28.14.8, has a high affinity for antigen, whereas the other, H141-30, has a relatively low affinity, causing it to gradually dissociate from precoated target cells with the kinetics shown in Chart 5A.

A similar comparison was made between the NEI-026 and S19.8 MAbs which also bind the H-2D<sup>α</sup> antigen, but which do so indirectly through its noncovalently associated β<sub>2</sub>M subunit (which is also a subunit of the H-2K<sup>α</sup> antigen, the other Class I antigen of EL4 cells). As shown in Chart 5B, targets precoated with supersaturating doses of either of these MAbs show only an 8% loss of RamlgG-mediated ADCC in 3.5 hr. (A similar result was obtained after 6 hr: data not shown.) Furthermore, the small change in the RamlgG-mediated ADCC susceptibilities shown in Chart 5B exactly parallels the change (again, 8% in 3.5 hr) in the susceptibility of NEI-026-precoated targets to direct MADCC (also shown in Chart 5B). In conclusion, 3 of 4 MAbs which detect H-2D<sup>α</sup> on EL4 cells appear to bind this antigen tightly, with little change in antibody:antigen complex being detected in 6 hr by ADCC. Only the H141-30 MAb behaves differently in this respect, since direct MADCC does not occur with precoated targets in the absence of additional MAb, and indirect ADCC becomes less efficient with time. Because 3 of 4 MAbs do not appear to induce any significant modulation of H-2D<sup>α</sup> antigen:antibody complexes, the most reasonable explanation for the properties of the H141-30 MAb is, again, that it has an intrinsically low affinity for H-2D<sup>α</sup> which causes it to gradually dissociate from the target cells.

**DISCUSSION**

The results presented here lead to several important conclusions concerning ADCC. The first major conclusion is that MAbs themselves can be extremely efficient mediators of ADCC, or MADCC (as abbreviated here), by human nonadherent PBL. Three of the MAbs tested, 30-H12, NEI-026, and H141-30, were particularly notable in this respect, because they mediated efficient MADCC reactions at concentrations ranging between 0.01 and 10 pg/target cell (or 40,000 to 40 million MAbs/cell) (Charts 1 and 2). The unusual efficiency of these MAbs in mediating MADCC reactions was gauged by the fact that, within 4 hr at these concentrations, 50% target cell lysis was observed, with low E:T ratios comparable to ratios used for CTL killing with unfractionated PBL effector cells (Table 2). Unlike CTL-mediated killing, however, there was no prior education requirement for ADCC effectors, and there was no CTL-like requirement (29) for the presence of major histocompatibility complex-associated gene products on the cell surface, since R1E cells, which entirely lack cell surface H-2 antigens, are fully susceptible to lysis by MADCC mediated by the 30-H12 (anti-Thy 1.2) MAb (Chart 1C).

The second major conclusion is that considerable variation exists between different MAbs in their capacity to mediate MADCC. Only 9 of 13 MAbs tested showed appreciable levels of MADCC activity. Of these, 30-H12 was the most efficient in that only 50 pg (about 20,000 molecules/cell) were required for 40% lysis in 4 hr of 1 x 10<sup>4</sup> tumor cell targets with 2.5 x 10<sup>5</sup> effectors (Chart 2). At least 35-fold greater concentrations of NEI-026 (anti-murine β<sub>2</sub>M) and H141-30 (anti-H-2D<sup>α</sup>) were required for a similar level of lysis (Chart 2A), and a 35,000-fold greater amount of 11.4.1 (anti-H-2K<sup>α</sup>) could facilitate the same
level of lysis (Chart 2B). For the remaining MAbs tested, amounts in excess of 10,000-fold or greater, as compared to the 30-H12 MAb, gave no appreciable target cell lysis (Chart 2). The inability of these MAbs to mediate efficient MADCC is not the result of poor binding to their respective target cells (Chart 3).

The third major conclusion reached in this study is that antibodies of the mouse IgG2a and IgG3 of the rat IgG2b (30-H12) subclasses can be efficient mediators of ADCC but that MAb subclass itself is not predictive of the ADCC potential of an antibody. Only 1 (NEI-026) of 6 IgG2a antibodies tested mediated efficient MADCC. Of the 4 mouse IgG2b MAbs tested, none showed lytic kinetics comparable to the 3 MAbs just discussed. However, in recent studies, we have found IgG2b MAbs which are moderately effective in mediating MADCC. No IgG1 MAbs were tested here, and we have yet to find an efficient MAb of this subclass. Our recent studies also indicate that IgG allotype is probably not a factor which itself determines the MADCC effectiveness of an antibody. The discovery that MAbs, even of the same subclass, show widely disparate MADCC efficiencies contrasts with the results of Ralph and Nakoinz (23), who concluded that no apparent IgG subclass restrictions exist among the MAbs for MADCC reactions.

The fourth major conclusion reached in this study is that the antigen affinity of a particular MAb does not correlate with its ability to mediate the rapid lysis of target cells. Whereas the 28.14.8, NEI-026, and S19.8 MAbs all appear to have high antigen affinity (Chart 5), only NEI-026 mediates direct MADCC. Conversely, the H141-30 MAb has a low affinity (Charts 4B and 5A), and yet it was one of the most potent mediators of MADCC when added directly to the assay mixtures (Chart 1). These results and conclusions contrast with those of Larsson et al. (12), who found a statistical correlation between antigen affinity and ADCC activity when heterogeneous populations of antibodies were compared. As one possible explanation for this discrepancy, the behavior of MAbs in ADCC may not be reflected in the behavior of populations of heterogeneous antibodies when examined as a whole. One final point is drawn from the experiments shown in Charts 4A and 5. Although a minimum density of target cell antibody-antigen complexes is probably required to initiate ADCC (16), sufficient antibody density on the target cell will not, in and of itself, necessarily lead to a triggering of target cell lysis by ADCC. The lack of a correlation between antibody density and efficient MADCC is indicated by the sharply contrasting efficiencies of MAbs which bind the same target cell antigens (Chart 5).

More recent studies in this laboratory confirm the exceptional activity of MAbs NEI-026 and 30-H12 in that they are also found to mediate the lysis of normal mouse splenocytes with precisely the same efficiency and kinetics as shown for the tumor targets in the present study (data not shown). Also, our findings here that only some MAbs are effective in mediating target cell lysis by human PBL effectors have been confirmed using a panel of MAbs against human β2M and using human tumor cells as targets. We still find that, of all the murine MAbs which are efficient mediators of MADCC, those of the IgG2a subclass are usually the best. Experiments are currently underway to examine further the reasons for such wide variation in the ability of MAbs to mediate MADCC. Antibody orientation on the target cell surface may be critical in this process in order that antibody Fc regions interact effectively with the effector cell Fc receptors. Currently in progress is the characterization of the human cell type responsible for the ADCC activity in these experiments. We find that the ADCC effectors in our studies cause the rapid fragmentation of target cell nuclear DNA in a manner analogous to CTL-induced fragmentation (2, 24). In our attempts to use the mouse as a model for studying ADCC, we have been unable to identify comparable ADCC activity with normal mouse splenocytes or with Concanavalin A-stimulated murine splenocytes as effectors used together with most of the MAbs listed in Table 1 or with the rabbit anti-R1E antiserum under identical assay conditions (data not shown). This agrees with the conclusions reached by Tada et al. (27), who were also unable to identify a murine cell type functionally equivalent to a human K-cell in mouse spleen, peripheral blood, or bone marrow. Thus, the mouse is probably not a useful animal model for studying efficient ADCC processes like those described here. ADCC is potentially a powerful immunological defense mechanism against transformed or virally infected cells and is possibly an important factor in autoimmune diseases (21, 22). The relative importance of ADCC in the human immune system has yet to be established, but the lytic power of human PBL against MAbs-sensitized targets, as demonstrated in this study, warrants the further investigation of this immune mechanism.

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REFERENCES

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