Potentiation by Interleukin 2 of Burkitt's Lymphoma Therapy with Anti-Pan B (Anti-CD19) Monoclonal Antibodies in a Mouse Xenotransplantation Model


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ABSTRACT

To study the immunotherapeutic potential of monoclonal antibodies (mAbs) directed against the human pan-B-cell antigen CD19, a xenotransplantation model was developed in which the human Burkitt's cell line Daudi is s.c. transplanted into nude mice.

IgG1, IgG2b, and IgG2a isotype variants of the anti-CD19 mAb (CLB-CD19) were tested for their capacity to inhibit the growth of 10^6 Daudi cells injected s.c. into nude mice. When mAb treatment was started 30 min after the injection of tumor cells, only the IgG2a isotype of CLB-CD19 had a marked antitumor effect in vitro. If treatment with IgG2a anti-CD19 mAb alone was delayed until Day 10 after tumor inoculation, no therapeutic effect was observed. However, the combination of this delayed mAb treatment with recombinant interleukin 2 (rIL-2) inhibited the growth of the Daudi cells in the nude mice, while treatment with rIL-2 alone was ineffective.

The results of in vitro experiments showed that peritoneal exudate cells were able to inhibit the proliferation of Daudi cells in the presence of the IgG2a isotype variant of CLB-CD19 but not in the presence of the other CLB-CD19 isotype variants.

Fresh nude mouse spleen cells did not mediate antibody-dependent cellular cytotoxicity against CLB-CD19 mAb-sensitized Daudi cells, irrespective of the isotype used for sensitization. However, preculture of these spleen cells with rIL-2 induced antibody-dependent cellular cytotoxicity against CD19* target cells sensitized with CLB-CD19 mAb of all isotypes.

These results indicate that it is possible to enhance mAb-dependent effector systems in vivo with the lymphokine rIL-2.

INTRODUCTION

The use of mAbs2 for the therapy of malignant disease continues to draw wide attention. Phase I/II clinical trials with mAbs have, e.g., been performed in patients with melanoma (1), colon carcinoma (2, 3), or leukemia/lymphoma (4–9). Although the clinical effects were limited, the results encourage further study with the ultimate goal to improve the efficacy of mAb therapy.

The therapeutic effect of mAbs as such, i.e., not coupled to toxins or radioisotopes, depends on the recruitment of host effector systems, including complement, ADCC, and phagocytosis and/or cytostasis of antibody-coated tumor cells (10–13). Thus, optimal utilization of the therapeutic potential of mAbs requires optimal mobilization of these effector systems.

The importance of the mAb isotype used for immunotherapy has been most elegantly studied with isotype switch variants of mouse mAb (14–16). In these studies, mouse IgG2a was consistently identified as the most effective isotype in depleting antigen-positive cells in vivo and in directing mouse and human effector cells to mediate ADCC activity in vitro. However, although the mouse IgG3 isotype of a series of isotype switch variants was not very effective in mediating ADCC (14), IgG3 mAbs directed against a human melanoma-associated antigen were described as very effective in a clinical trial in melanoma patients (1), in inhibiting the growth of human melanoma in nude mice, and in directing mouse and human effector cells to mediate ADCC activity in vitro (17).

Rat mAbs have been advocated as more active reagents than mouse mAbs for the treatment of malignant disease (18, 19). Of the rat immunoglobulins, IgG2b was shown to be very efficient in directing human and mouse effector cells to mediate ADCC and in activating complement (18), although for complement activation, not only the mAb isotype but also the structure and density of the recognized antigen were shown to be of importance (20).

The limited efficacy of mAbs in clinical trials may in part be explained by a lack of optimally activated host effector systems that are potentially able to clear mAb-coated tumor cells. Biological response modifiers such as INF and IL-2 are known to enhance cellular and humoral immune responses (21, 22) and therefore offer a possibility to overcome this problem. Indeed, it was shown recently that rIL-2 can increase the ADCC activity of mouse (23, 24) and human (25, 26) effector cells. Moreover, a synergistic antitumor effect was observed in vivo with the combined treatment of experimental animal tumors with mAbs and INF-α (27) or rIL-2 (23, 28–30).

Antibodies directed against differentiation antigens expressed on lymphocytes are possible candidates for the treatment of leukemia/lymphoma. The results of clinical trials with mAbs directed against the differentiation antigens CD5 or CD20 have been published (7–9). In both systems tumor responses were observed, and side effects that accompanied mAb infusions were mild. Another possible target antigen for immunotherapy of B-cell malignancies is CD19, a B-cell-specific antigen present on most differentiation stages. Recently, a detailed analysis of the CD19 expression on leukemia or lymphoma cells was published (31). This study showed that 88% of B-lineage lymphoma cases and 100% of B-lineage leukemia cases expressed CD19. Importantly, CD19 expression was observed on the putative leukemia stem cells of three B-cell precursor acute lymphocytic leukemia cases.

In order to test the immunotherapeutic potential of the anti-CD19 mAb CLB-CD19 (32), we developed a xenotransplantation model in which human Burkitt's lymphoma cell lines are transplanted in nude mice. We present here the encouraging results of immunotherapy experiments, performed in this model with isotype switch variants of CLB-CD19 mAb and with the combination of IgG2a CLB-CD19 mAb and rIL-2. The results of this study suggest that it is possible to enhance the effect of mAbs in vivo with rIL-2, thus providing a rationale for clinical use.
trials in patients with B-cell malignancies with anti-CD19 mAb and rIL-2.

MATERIALS AND METHODS

Animals. C3H-nu/nu, C57BL/6 Kh-nu/nu BALB/c mice, and R rats were bred and maintained under specific-pathogen-free conditions at the animal department of the Netherlands Cancer Institute. Male and female BALB/c-nu/nu mice were purchased from: TNO, Zeist, The Netherlands; Bomholtgart, Ltd., Ry, Denmark, or Harlan-Olac, Ltd., Blackthorn, England. For in vivo experiments, male or female nude mice were used between 4 and 8 wk of age and maintained in sterile isolators. The mice were used as effector cell sources for in vitro experiments were between 1 and 4 mo of age.

Cell Lines and Cell Culture Conditions. The human Burkitt’s cell lines Namalwa, Ramos, Raji, EB3, Daudi, and Jiyoye were purchased from the American Type Culture Collection, Rockville, MD. The JVM3 cell line was derived from the tumor cells of a B-prolymphocytic leukemia patient after Epstein-Barr virus transformation in vitro (33). The rat myeloma cell line IR983F and the mouse anti-rat e-producing hybridoma MARK-1 (34) were kindly provided by Dr. Bazin (Belgium). The cell lines were cultured in DMEM supplemented with 2 g of glucose/liter, 1 mM sodium pyruvate, 4 mM L-glutamine, nonessential amino acids, 100 IU penicillin/ml, 100 μg of kanamycin/ml, and NBCS (10%, v/v) (Sera Lab., Sussex, England), or FCS (10%, v/v) (PAA Laborgesellschaft, Austria). Murine cells and cell lines were cultured in DMEM/FCS supplemented with 5 × 10^{-3} m mercaptoethanol (DMEM/FCS/2ME).

Monoclonal Antibodies. The production, isolation, and characterization of isotype switch variants of the anti-CD19 mAb (CLB-CD19) were previously described (35). The R24.3 mAb was produced as previously described (35), except that as fusion partner for the rat spleen cells, the rat myeloma cell line IR983F was used. The antigen recognized by R24.3 mAb was identified as HLA Class II by immunoprecipitation followed by SDS/PAGE analysis (36, 37). The R24.3 mAb was determined to be of the IgG2b isotype using the Ouchterlony double-diffusion procedure with rabbit anti-rat immunoglobulin class- and subclass-specific antibodies (Nordic, Tilburg, The Netherlands). The irrelevant control mAbs used in in vivo and in vitro experiments were: K8, S6, NKI-beteb, mouse IgG2a, IgG1, and IgG2b mAbs, respectively; and the rat IgG2b mAb 50B8. The NKI-beteb mAb was obtained from Dr. C. Vennegoor, Amsterdam, and the 50B8 mAb was kindly provided by Dr. A. Sonnenberg, Amsterdam, The Netherlands.

Purification of mAbs. Initially the mAbs MARK-1, K8, and the heavy-chain variants of CLB-CD19 were isolated from ascites on a preparative high-performance liquid chromatography column (Bakerbond AbX; J. T. Baker, Inc., NJ). In later purification procedures, the preparative high-performance liquid chromatography column (Bakerbond AbX (Baker) column matrix was used according to a previously described procedure (38). The purity of mAbs was estimated on the sum of the take-rates of independent experiments with the Fisher exact test. Differences were considered significant if P ≤ 0.05. Therapy experiments with isotype switch variants of CLB-CD19 mAb were performed as follows. On Day 0, BALB/c-nu/nu mice received 3 Gy of whole-body irradiation prior to the s.c. injection of 10 × 10^6 Daudi cells. The mAb was given i.p. (1 mg/injection in 1 ml of PBS, 0.5% (w/v) BSA) on Days 0, 3, and 6. Control animals received 1 ml of PBS/BSA or 1 mg of irrelevant mAb (K8 antiidiotype, IgG2a isotype) in 1 ml of PBS/BSA on Days 0, 3, and 6.

In Vivo Experiments. Human Burkitt’s cell lines used for in vivo experiments were grown in vitro, washed once with medium, and reconstituted at the desired cell density in PBS. These cells were injected s.c. or i.p. (0.2 ml/injection) into nude mice, and the mice were monitored at regular intervals thereafter. Once a week the s.c. developing tumors were measured with precision calipers. These measurements were expressed as the product of two perpendicular diameters (tumor area, mm^2).

In immunotherapy experiments, the take-rate (number of mice with tumor/total number of mice) was recorded on Day 60, and a statistical analysis was performed on the sum of the take-rates of independent experiments with the Fisher exact test. Differences were considered significant if P ≤ 0.05. Fluorescence staining with antibody was analyzed by a FACSCAN cytofluorimeter (Becton and Dickinson, Mountain View, CA) or using a fluorescence microscope, screening at least 200 cells. The intact antibody content of purified CLB-CD19 mAbs and the plasma levels of CLB-CD19 mAbs were determined by indirect immunofluorescence. Mean fluorescence intensity was interpolated on a standard curve of intensities obtained with known concentrations of CLB-CD19 mAb.

In vitro Experiments. Human Burkitt's cell lines were used for in vitro experiments were grown in vitro, washed once with medium, and reconstituted at the desired cell density in PBS. These cells were injected s.c. or i.p. (0.2 ml/injection) into nude mice, and the mice were monitored at regular intervals thereafter. Once a week the s.c. developing tumors were measured with precision calipers. These measurements were expressed as the product of two perpendicular diameters (tumor area, mm^2).

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Combined treatment with IgG2a CLB-CD19 mAb and rIL-2 was started on Day 10; test or control mAb (K8 antiidiotype) was injected i.p. on Days 10, 13, and 17 (1 mg/injection in 1 ml of PBS/BSA); rIL-2 was injected i.p. (5 × 10^5 units in 0.5 ml of PBS/BSA) 3 times daily on Days 10 to 14 and 17, or s.c. (2 × 10^5 units in 0.2 ml of incomplete Freund’s adjuvant, 3% (w/v) BSA (BAO)) on Days 10, 17, and 24. Control treatment consisted of K8 mAb or rIL-2 alone or rIL-2 in combination with K8 mAb. Highly purified human rIL-2 produced in Escherichia coli (41) was a generous gift from Eurocetus, Amsterdam. The growth of the s.c. tumors was monitored once a week as described above.

Antibody-dependent Cellular Cytotoxicity. Short-term 3^1Cr release experiments and long-term [H]thymidine release experiments were performed essentially as described (42, 43). In brief, mouse effector cells, i.e., spleen cells or PEC, were mixed with 10^6 3^1Cr-labeled target cells (labeling with 3^1Cr: 6.4 mBq/10^6 cells incubated at 37°C for 60 to 120 min; specific activity of 3^1Cr, 13 to 22 GBq/mg of chromium; Amersham, Buckinghamshire, England) or 5 × 10^5 [H]thymidine-labeled target cells (labeling with [H]thymidine: cells incubated for 18 to 24 h with [H]thymidine-labeled target cells) at E/T ratios varying from 10:1 to 20:1 with 3 mBq of [H]thymidine/ml; specific activity of [H]thymidine, 247.9 GBq/mmol; Amersham) at E/T ratios varying from 100:1 to 6.25:1 in the 96-well round-bottomed microtiter plates (Costar). Test or control mAbs were added, at indicated concentrations, to a final volume of 200 μl. Subsequently the plates were centrifuged for 5 min at 1000 rpm and incubated for 4 h (3^1Cr-labeled target cells) or 24 to 48 h ([H]thymidine-labeled target cells) at 37°C in humidified air with 5% CO2. After this incubation period, the plates were centrifuged again (5 min, 1000 rpm), and the 100-μl supernatant was harvested and processed for determining the 3^1Cr or [H]thymidine content in a GAMMA-8000 gamma counter or an LS8000 liquid scintillation counter (Beckman Instruments, Inc., Irvine, CA).

The percentage of specific label release was calculated according to
the following formula

\[
\% \text{ of specific inhibition} = \left( \frac{C - T}{C - S} \right) \times 100
\]

where \( T \) is cpm in test sample, \( M \) is maximal releasable label in 2% (v/v) Triton X-100/0.5% (w/v) SDS/1% (w/v) sodium deoxycholate/10 mM EDTA, and \( S \) is spontaneously released label from target cells alone. The spontaneous label release never exceeded 15% of maximally releasable radioactivity.

Proliferation Inhibition Assay. PEC of BALB/c mice were harvested as described above. The cells were seeded in 96-well flat-bottomed microtiter plates (Costar). After 24-h incubation at 37°C in humidified air with 5% CO₂, nonadhering cells were removed by washing the wells once with medium. Test or control mAbs, at the indicated concentrations, as well as Daudi target cells (5000 target cells/well, \( E/T \) ratios varied from 5:1 to 0:6:1) were added to a final volume of 150 μl. Three days later, 50 μl of \(^{3}H\)thymidine (4.8 kBq/well; specific activity 247.9 GBq/mmol; Amersham) were added for the last 4 h of culture. Subsequently, the cells were harvested with a Titertek cell harvester (Flow Lab, Inc., McLean, VA), and the incorporated \(^{3}H\)thymidine was determined in the LS8000 liquid scintillation counter (Beckman). The percentage of specific inhibition of \(^{3}H\)thymidine incorporation (\% of specific inhibition) was calculated according to the following formula

\[
\% \text{ of specific inhibition} = \left( \frac{C - T}{C - S} \right) \times 100
\]

RESULTS

Development of a Xenotransplantation Model of Human B-Cell Lines Transplanted in Athymic Nude Mice. Because it is well documented that it is very difficult to successfully transplant human primary leukemia/lymphoma cells in nude mice (44–46), established human B-cell lines were used for developing a xenotransplantation model. Nilsson and coworkers (46) described human Burkitt's lymphoma cell lines as the fastest growing human tumors in nude mice. Therefore, Burkitt's lymphoma cell lines BJAB, Daudi, EB3, RAMOS, Jiyoye, and Namalwa were used in the initial xenotransplantation experiments. These cell lines, however, failed to grow when injected i.p. or s.c. (5 × 10⁶ cells) in C3H or C57BL/6 Kh nude mice. In BALB/c nude mice of less than 8 wk, the Daudi cells eventually grew when they were injected s.c. Sublethal whole-body irradiation (3 Gy) of BALB/c nude mice decreased the tumor latency time and reduced the variability of the growth rate of Daudi cells injected s.c. in these nude mice. In subsequent experiments, the growth kinetics of different doses of Daudi cells, injected s.c. in 3-Gy-irradiated BALB/c nude mice, was studied (Fig. 1). From these data, it is clear that a s.c. injection of 5 to 20 × 10⁶ Daudi cells invariably gave rise to the development of s.c. tumor nodules and that the growth rate of the tumor was relatively independent of the number of cells injected. These results guided our decision to inject 10 × 10⁶ Daudi cells s.c. in 3-Gy-irradiated BALB/c nude mice for immunotherapy experiments.

Immunotherapy with Isotype Variants of CLB-CD19 mAb. Immunotherapy experiments were performed in the xenotransplantation model described above. On Day 0, the mice received 3 Gy of whole-body irradiation prior to the s.c. injection of 10 × 10⁶ Daudi tumor cells. mAbs were injected i.p. on Days 0, 3, and 6 [1 mg/injection, in 1 ml of 0.5% PBS (w/v)/BSA]. Control animals received 1 ml of PBS (Experiment 1) or 1 mg of irrelevant mAb (K8, IgG2a isotype in 1 ml of PBS/BSA; Experiments 2 and 3) injected i.p. on Days 0, 3, and 6.

The results of three such experiments are shown in Table 1. In Fig. 2, the result of Experiment 1 is shown in terms of the growth rate of the tumor nodules, and the take-rate is indicated at the end of each curve. The results show that treatment of the mice with CLB-CD19 IgG2a mAb resulted in a reduction of the tumor take-rate (\( P < 0.02; \) Table 1) and an extension of the tumor latency time (Fig. 2), while treatment with the two other isotype variants of CLB-CD19 mAb had no significant effect on these parameters.

Possible Mechanism of the IgG2a CLB-CD19 mAb-mediated In Vivo Antitumor Effect. Because differences in plasma half-life (\( t_{\text{½}} \)) of the isotype variants of CLB-CD19 mAb could explain their different antitumor effect in vivo, the \( t_{\text{½}} \) of the isotype variants of CLB-CD19 mAb was determined. BALB/c mice were injected i.p. with 1 mg of mAb, and at various time intervals, the plasma CLB-CD19 mAb level was determined by
indirect immunofluorescence, in which mean fluorescence intensity obtained with the plasma samples was interpolated on a standard curve of intensities obtained with CLB-CD19 mAb solutions of known concentrations. The \( t_0 \) (days ± SD, \( n = 6 \)) of the IgG1, IgG2b, and IgG2a was 7.4 ± 2.5, 6.8 ± 1.9, and 6.8 ± 1.4, respectively. This result indicates that it is not likely that the different therapeutic activities of the isotype variants are caused by differences in distribution and/or breakdown in vivo.

To identify the involved effector mechanisms, in vitro ADCC experiments were performed. In these ADCC experiments, using mouse spleen cells or PEC as effector cells, none of the CLB-CD19 isotype variants was able to induce CD19+ target cell lysis, while target cells sensitized with the anti-HLA Class II mAb R24.3 were readily lysed by PEC (data not shown) and, although to a limited extent, also by freshly isolated BALB/c nude mouse spleen cells as shown in Fig. 4.

CLB-CD19 mAb-mediated effects were obtained with a modification of a cytostasis assay (11). In this assay, Daudi target cells are incubated for 3 to 4 days with mouse PEC in the presence of specific mAbs or irrelevant isotype-matched control mAbs. At the end of this incubation period, the target cell survival is quantitated by measuring [\(^{3}H\)]thymidine incorporation. The results of such a representative experiment are shown in Fig. 3. The R24.3 mAb, rat IgG2b directed against human HLA Class II antigens, was found to be very potent in this assay. Of the IgG1 and IgG2a isotype variants of CLB-CD19 included in this experiment, only the latter was able to inhibit significantly the [\(^{3}H\)]thymidine incorporation of the target cells. In an identical experiment, the IgG2b isotype variant was equally ineffective as was the IgG1 isotype variant shown in Fig. 3 (data not shown). The results obtained with these experiments correlate with the isotype-dependent antitumor activity of CLB-CD19 mAb observed in vivo and therefore, suggest that the effector cells in PEC that are responsible for the antitumor effect of the treatment with mAb alone.

Combination Therapy with IgG2a CLB-CD19 mAb and rIL-2. When IgG2a CLB-CD19 mAb therapy was delayed until Day 10, instead of started immediately after tumor cell injection (1 mg of mAb-i.p. on Days 10, 13, and 17), the antitumor effect was not significantly different from control-treated animals (Table 2). However, when this delayed mAb treatment was combined with rIL-2, the antitumor effect was again significant (Table 2). Treatment with rIL-2 alone had no effect on the growth of Daudi tumor cells. This result indicates that rIL-2 is able to potentiate the therapeutic efficacy of IgG2a CLB-CD19 mAb in vivo.

ADCC Activity of rIL-2-activated BALB/c Nude Mouse Spleen Cells. As already mentioned before, freshly isolated, nonactivated BALB/c nude mouse spleen cells were poor ADCC effector cells; only with the R24.3 mAb could some specific \(^{51}Cr\) release be obtained. However, activation of these spleen cells in rIL-2 resulted in a dramatic increase in ADCC activity with all tested mAbs as shown in Fig. 4. This result suggests that rIL-2-induced ADCC activity of lymphoid effector cells may contribute to the observed potentiation by rIL-2 of IgG2a CLB-CD19 mAb-mediated antitumor activity in vivo.

DISCUSSION

In this paper we present evidence that mAbs directed against the B-cell-specific antigen CD19 powerfully inhibit the growth of human Burkitt's Daudi lymphoma cell line transplanted into nude mice. Of IgG1, IgG2b, and IgG2a isotype switch variants of CLB-CD19 mAb, only the IgG2a isotype exhibited this antitumor activity. Furthermore, we show for the first time in a xenotransplantation model that rIL-2 dramatically potentiates the therapeutic effect of tumor-specific mAbs.

The observed superior antitumor activity of the IgG2a isotype variant is in agreement with previously published studies by other investigators (14, 15). However, in these studies, IgG2b and, to an even lesser extent, also IgG1 showed some antitumor activity. In our study these isotypes were completely inactive.

Most investigators ascribe the mechanism of in vivo antitumor activity, mediated by tumor-specific antibodies, to ADCC reactions (13–15, 42). Therefore, we performed extensive in vitro ADCC assays with mouse spleen cells or mouse PEC as effector cells. However, none of the CLB-CD19 isotype variants was able to mediate ADCC activity with these effector cells. A modification of a cytostasis assay (11), called the proliferation inhibition assay, eventually gave positive results; PEC inhibited the proliferation of Daudi target cells in vitro only in the presence of the IgG2a isotype variant of CLB-CD19 mAb and...
POTENTIATION BY IL-2 OF THE THERAPEUTIC EFFICACY OF mAbs

Table 2  Combination therapy with IgG2a CLB-CD19 mAb and rIL-2

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
<th>Total</th>
<th>P</th>
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<td>Control mAb</td>
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<td>4/4</td>
<td>8/9</td>
<td>15/18</td>
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</tr>
<tr>
<td>Control mAb + rIL-2(^a)</td>
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<td>ND</td>
<td>6/8</td>
<td>6/8</td>
<td>NS</td>
</tr>
<tr>
<td>rIL-2(^b)</td>
<td>5/5</td>
<td>3/4</td>
<td>ND</td>
<td>8/9</td>
<td>NS</td>
</tr>
<tr>
<td>CLB-CD19 IgG2a</td>
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<td>4/5</td>
<td>6/9</td>
<td>12/19</td>
<td>NS</td>
</tr>
<tr>
<td>CLB-CD19 IgG2a + rIL-2(^b)</td>
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<td>1/3</td>
<td>3/6</td>
<td>4/13</td>
<td>≤0.01</td>
</tr>
<tr>
<td>CLB-CD19 IgG2a + rIL-2(^b)</td>
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<td>ND</td>
<td>2/8</td>
<td>2/8</td>
<td>≤0.02</td>
</tr>
</tbody>
</table>

* mAbs were given i.p. (1 mg in 1 ml of PBS/BSA) on Days 10, 13, and 17.
\(^a\) rIL-2 was given s.c. (2 × 10^6 units in 0.3 ml of Freund's incomplete adjuvant, 3% BSA) on Days 10, 17, and 24.
\(^b\) ND, not done; NS, not significant.
\(^c\) rIL-2 was given i.p. (5 × 10^6 units in 0.5 ml of PBS/BSA) 3 times daily on Days 10, 11, 12, 13, 14, and 17.

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not in the presence of the other two isotype variants (Fig. 3). Because this proliferation inhibition assay measures the net result of antibody-dependent cellular effector functions including ADCC, phagocytosis, and/or cytostasis of antibody-coated tumor cells, the exact mechanism and the identity of the effector cell in the PEC population remain to be elucidated. The effector cells most likely are macrophages because the assay is performed with adherent PECs, which consist of over 90% macrophages. However, we cannot exclude the possibility that, in the adherent PEC population, a considerable fraction of the cells are Thy-1-positive T-cells.

In contrast to the results of the experiments shown in Table 1, the antitumor activity of mAb alone treatment was lost when this treatment was delayed until Day 10 after tumor cell inoculation (Table 2). An obvious explanation for this observation is that, by Day 10, the tumor burden is too great to be eradicated by antibody-dependent effector mechanisms. While treatment with rIL-2 alone did not affect the growth of the Daudi cells in the nude mice, the combination of IgG2a CLB-CD19 mAb with rIL-2, started on Day 10, resulted in a strong antitumor effect (Table 2). From these results we conclude that rIL-2 increased the therapeutic efficacy of IgG2a CLB-CD19 mAbs in the nude mice. This result is in agreement with previously published studies in syngeneic animal models (23, 28–30).

As already mentioned, fresh nude mouse spleen cells did not mediate ADCC activity irrespective of the isotype of the CLB-CD19 mAb used to sensitize the CD19+ target cells. However, culture of these spleen cells in vitro with rIL-2 induced ADCC activity with all isotypes of CLB-CD19 mAb. In view of this result we are currently testing whether rIL-2 addition to the treatment with IgG1 or IgG2b CLB-CD19 mAb would result in antitumor activity in our xenotransplantation model. Our results clearly show that IL-2 can enhance the ADCC activity of BALB/c nude mouse spleen cells. This is in contrast with the results published by Bernstein et al. (23). In their study the culture of spleen cells in 2500 units of rIL-2/ml for 3 days did not induce ADCC activity. A possible explanation for this discrepancy could be that we used spleen cells from nude mice, which are enriched for NK cells (47), while Bernstein et al. used spleen cells from immunocompetent mice. The results of our ADCC experiments suggest that rIL-2-induced ADCC activity of lymphoid effector cells may contribute to the in vivo observed increased antitumor activity of mAb and rIL-2 combination therapy. Since it is known that IL-2 activation increases the expression of various surface molecules, such as IL-2 receptor (CD25), HLA-DR, transferrin receptor, and leu 23, on cultured human NK cells (48–52), a possible mechanism of the enhancement of ADCC by IL-2 could be an increased expression and/or function of FcR on ADCC effector cells. Moreover, it was shown recently that FcR (CD16)-ligand interaction on human NK cells increased the expression of CD25 and the production of the lymphokines tumor necrosis factor and interferon-γ (53), suggesting a linked regulation of the activation of FcR and IL-2 receptor expression.

The rat mAb R24.3, which is directed against HLA Class II antigens, proved to be very potent in the proliferation inhibition assay (Fig. 3) and in ADCC assays with rIL-2-stimulated nude mouse spleen cells (Fig. 4). R24.3 mAb was identified as rat IgG2b isotype and, indeed, this rat isotype is known to be very efficient in activating complement and in mediating ADCC reaction (Fig. 3) and in ADCC assays with rIL-2-stimulated nude mouse spleen cells (Fig. 4). R24.3 mAb was identified as rat IgG2b isotype and, indeed, this rat isotype is known to be very efficient in activating complement and in mediating ADCC reactions with human and mouse effector cells (18). However, the results obtained in these in vitro assays with R24.3 mAb cannot directly be compared with the results obtained with CLB-CD19 mAb, because it is possible that HLA Class II and CD19 antigen densities and the affinity of the mAbs for their antigens are very different. We therefore are in the process to produce rat mAbs, preferentially of the IgG2b isotype, directed against CD19 in order to be able to compare the antitumor activity of such a reagent in vivo and in vitro with the mouse CLB-CD19 mAb.

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