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Anti-Renal-Cell Carcinoma Chimeric Antibody G250 Facilitates Antibody-Dependent Cellular Cytotoxicity with In Vitro and In Vivo Interleukin-2-Activated Effectors

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Summary: Renal cell carcinoma (RCC) is relatively resistant to chemotherapy and radiotherapy, whereas treatment with biologics has achieved limited success. Although monoclonal antibodies able to recognize human RCC have been identified, most induce little complement-dependent cytotoxicity or antibody-dependent cellular cytotoxicity (ADCC), and thus are of limited potential as therapeutic modalities in their natural conformation. We evaluated a human/mouse chimeric derivative of the previously described G250 murine monoclonal antibody (mAb), reactive with RCC, to identify a reagent for potential immunotherapy. This chimeric antibody (ch-G250) is composed of the murine variable region from the G250 mAb, which recognizes a tumor-associated antigen expressed on 95% of primary and 86% of metastatic renal cell carcinomas. The constant region of the ch-G250 is comprised of the human IgG₁ isotype domains. This chimeric antibody does not bind to normal renal tissue or other normal human tissues, with the exception of gastric mucosal cells and large bile-duct epithelium. Clinical radiolocalization studies have demonstrated the relative tumor-targeting potential of this radiolabeled antibody. This ch-G250 antibody facilitated potent ADCC against several RCC lines when using in vitro and in vivo interleukin-2 (IL-2)-activated peripheral blood mononuclear cells obtained from healthy control donors and patients with cancer, respectively. This lymphocyte-mediated ADCC was specific for RCC cells recognized by the ch-G250 antibody. Using flow cytometry, we found that the level of ADCC was directly related to the degree of binding of ch-G250 to the renal cell target. These in vitro data suggest that this antibody may improve efficacy of IL-2 therapy by targeting cytokine-activated effector cells directly to the tumor and facilitating in vivo ADCC. Clinical studies combining this chimeric antibody with IL-2 treatment will be needed to test the antitumor effects of this ADCC effect in vivo. **Key Words:** Immunotherapy—Renal cancer—Monoclonal antibody.

Murine monoclonal antibody mG250 recognizes a determinant expressed preferentially on renal cell carcinoma (RCC). It does not bind to normal kidney tissue (1). An initial clinical study using ¹³¹I-labeled

mG250 demonstrated that this antibody localized preferentially to RCC, expressing the G250 antigen (2) and that the antibody was useful as an imaging agent. The specific localization and accumulation

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rates suggested that the antibody may have therapeutic potential (2). Murine studies demonstrated that mG250 given to nude mice with established RCC xenografts resulted in inhibition of tumor growth (3). In addition, treatment with combined interferon- α and tumor necrosis factor- α (TNF- α) together with mG250 significantly enhanced the antitumor response over that seen with the combined cytokines or antibody alone (3). A phase I/II trial with ^{131}I -tagged murine G250 antibody in patients with RCC demonstrated that a single dose of antibody was well tolerated and minor antitumor activity was noted in 3 of 15 patients (4). These results suggested that repeated infusions of the radiolabeled antibody may be able to obtain better antitumor activity.

Interleukin-2 (IL-2), as a single agent, has had limited success as a treatment for RCC (5). The therapeutic action of this cytokine is not fully understood. Although virtually all patients demonstrate expansion and activation of their natural killer cell population (6,7), only a minority of patients have antitumor responses. A significant proportion of these activated natural killer cells express Fc receptors (8), and combining a RCC-specific antibody able to mediate antibody-dependent cellular cytotoxicity (ADCC) with the cytokine therapy might specifically target the effector cells to the tumor and enhance tumor destruction.

In an effort to expand the utility of mG250 antibody as a therapeutic agent, a human/mouse chimeric antibody was constructed (9). This antibody maintained the murine variable region conferring the specificity of the murine G250 antibody and used the human IgG₁ constant domains. Because some human IgG₁ antibodies can mediate ADCC, we investigated the specificity of ADCC activity by the ch-G250 chimeric antibody.

MATERIALS AND METHODS

Monoclonal Antibodies

The ch-G250 antibody is a human/mouse chimeric antibody (human IgG₁) derived from the parent murine monoclonal G250, which recognizes a tumor-associated antigen homogeneously expressed on RCC (9). The chimerization process was the same as previously described for the 323/A3 chimerization (10). Binding studies and affinity constants show that the ch-G250 antibody has identical binding to the parent murine G250 antibody from which

it was derived (E. Oosterwijk, unpublished data). UMVA-RCC-A6H (A6H) is a mouse monoclonal antibody of the IgG₁ subclass, which reacts with human RCCs. This antibody was provided by Dr. Timothy Moon, University of Wisconsin, Madison, WI (11). ING-1 is a human/mouse chimeric antibody of the human IgG₁ subclass provided by Dr. R. Robinson (Xoma, Santa Monica, CA, U.S.A.) that binds a 40 kDa tumor-associated membrane glycoprotein. This tumor antigen is expressed on colon, lung, and breast carcinomas (12).

Cell Lines and Culture Conditions

SK-RC-13, SK-RC-30, and SK-RC-52 RCC lines were obtained from the tumor cell bank of the Ludwig Institute for Cancer Research, New York Branch at Memorial Sloan-Kettering Cancer Center. BT-20 is a human breast carcinoma cell line obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). All cell lines were grown as monolayers in RPMI-1640 supplemented with penicillin and streptomycin, 25 mM HEPES buffer, L-glutamine, and 10% fetal bovine serum (FBS) (Hyclone). A solution of 0.05% trypsin-0.53 mM EDTA was used to harvest cells from the flasks before use.

Effector Populations

Normal peripheral blood mononuclear cells (PBMC) from healthy volunteers were isolated from heparinized blood by centrifugation over a Ficoll-Hypaque density gradient. Activated natural killer (NK) cells were obtained by incubating PBMC in RPMI containing 10% heat-inactivated human serum (RPMI-HS) supplemented with 100 U/ml IL-2 (Hoffmann-La Roche, Nutley, NJ, U.S.A.), for 24 h. The cells were washed twice just before use in the ADCC assay. PBMC were also obtained from cancer patients undergoing IL-2 therapy at the University of Wisconsin (UW) Comprehensive Cancer Center at either 1.5 or 3×10^6 U/m²/day. The samples used for the ADCC assays were obtained from patients 24 h after a 4-day continuous infusion of IL-2 (Hoffmann-La Roche) (13). The PBMC were tested immediately after isolation over a Ficoll density gradient or were cryopreserved and then thawed immediately before the assay. All donors signed informed consent forms approved by the UW Human Subjects Review Board.

ADCC Assay

PBMC were resuspended in RPMI-1640 supplemented with 10% human serum, penicillin/streptomycin, L-glutamine, 25 mM HEPES (RPMI-HS), and plated in a 96-well microtiter plate in a 50- μ l volume at three effector-to-target-cell (E:T) ratios. Fifty microliters of RPMI-HS or RPMI-HS supplemented with IL-2 at a concentration of 400 U/ml was added to the wells, and plates were incubated at 37°C in 5% CO₂ for 30–45 min. Monoclonal antibody was added in 50 μ l, resulting in a final concentration of 0.5 μ g/ml for ch-G250 and 1 μ g/ml for ING-1. The target cells were labeled with 250 μ Ci of ⁵¹Cr for 2 h at 37°C in 5% CO₂, washed and resuspended to 1 \times 10⁵ cells/ml in RPMI-HS. Targets were added to quadruplicate wells in a 50- μ l volume. The E:T ratios were 100:1, 33:1, and 11:1. After a 4-h incubation, the supernatant was harvested using the Skatron harvesting system (Skatron, McLean, VA, U.S.A.) and percent cytotoxicity was calculated for each E:T ratio using the following equation:

$$\% \text{ cytotoxicity} = \frac{\text{experimental} - \text{spontaneous}}{\text{maximum} - \text{spontaneous}}$$

Spontaneous is the amount of radioactivity released when targets are incubated in RPMI-HS only; the maximum is the amount of radioactivity released when targets are incubated in cetrimide detergent (Sigma Chemical Co., St. Louis, MO, U.S.A.).

Immunofluorescence

Indirect immunofluorescent staining was used to analyze the expression of the renal carcinoma cell associated antigen detected by the ch-G250 antibody. Tumor cell lines were harvested, washed, and resuspended in Hanks' balanced salt solution with 1% FBS. Fifty microliters containing 2.5 \times 10⁵ cells were added to 50 μ l of the appropriate monoclonal antibody (mAb), which was at 20 μ g/ml. The cells were incubated at 4°C for 30 min and were washed once with phosphate-buffered saline (PBS). The secondary antibody, either goat anti-mouse IgG fluorescein isothiocyanate (FITC) conjugated (Becton Dickinson, San Jose, CA, U.S.A.) or goat anti-human IgG FITC conjugated (Caltag, San Francisco, CA, U.S.A.) at a 1:100 dilution, was

added to the appropriate samples. The cells were incubated at 4°C for 30 min in the dark. The cells were washed in PBS, and 40 μ l of a 4- μ g/ml stock of propidium iodide were added just before analysis on the FACScan (Becton Dickinson) to exclude dead cells.

Statistical Analyses

Data from multiple experiments were used to determine least-squares means. The means were adjusted for individuals where all possible combinations of antibodies, cytokines, and targets were not included in each assay. Paired *t* tests were used to determine significance.

RESULTS

Expression of G250 Surface Antigen Detected by ch-G250

To determine the density of the G250 cell surface antigen expressed on several RCC lines, we analyzed them by indirect immunofluorescence. Figure 1 shows that ch-G250 detected the antigen on all three RCC lines tested. The expression was greatest on SK-RC-13 cells, intermediate on SK-RC-30 cells, and SK-RC-52 cells had the lowest expression. All three of these lines stained positively with the murine anti-RCC antibody, A6H. The BT-20 breast carcinoma line did not bind either A6H or ch-G250, but was positive for ING-1, which recognizes a tumor-associated 40-kDa glycoprotein. SK-RC-30 cells also expressed the antigen recognized by ING-1.

Antibody-Dependent Cellular Cytotoxicity Is Associated with the Expression of G250 Antigen on the Target Cell

The ability of activated NK cells to lyse the tumor cell targets was related to the degree of binding of the monoclonal antibody shown in Fig. 1. Figure 2 summarizes the results from six separate experiments performed with PBMC obtained from 10 normal donors. The mean percent cytotoxicity values are presented. G250 expression was greatest on the SK-RC-13 line, and this target demonstrates the specificity of ADCC mediated by ch-G250 (*p* = 0.0002) comparing cytotoxicity in media versus cy-

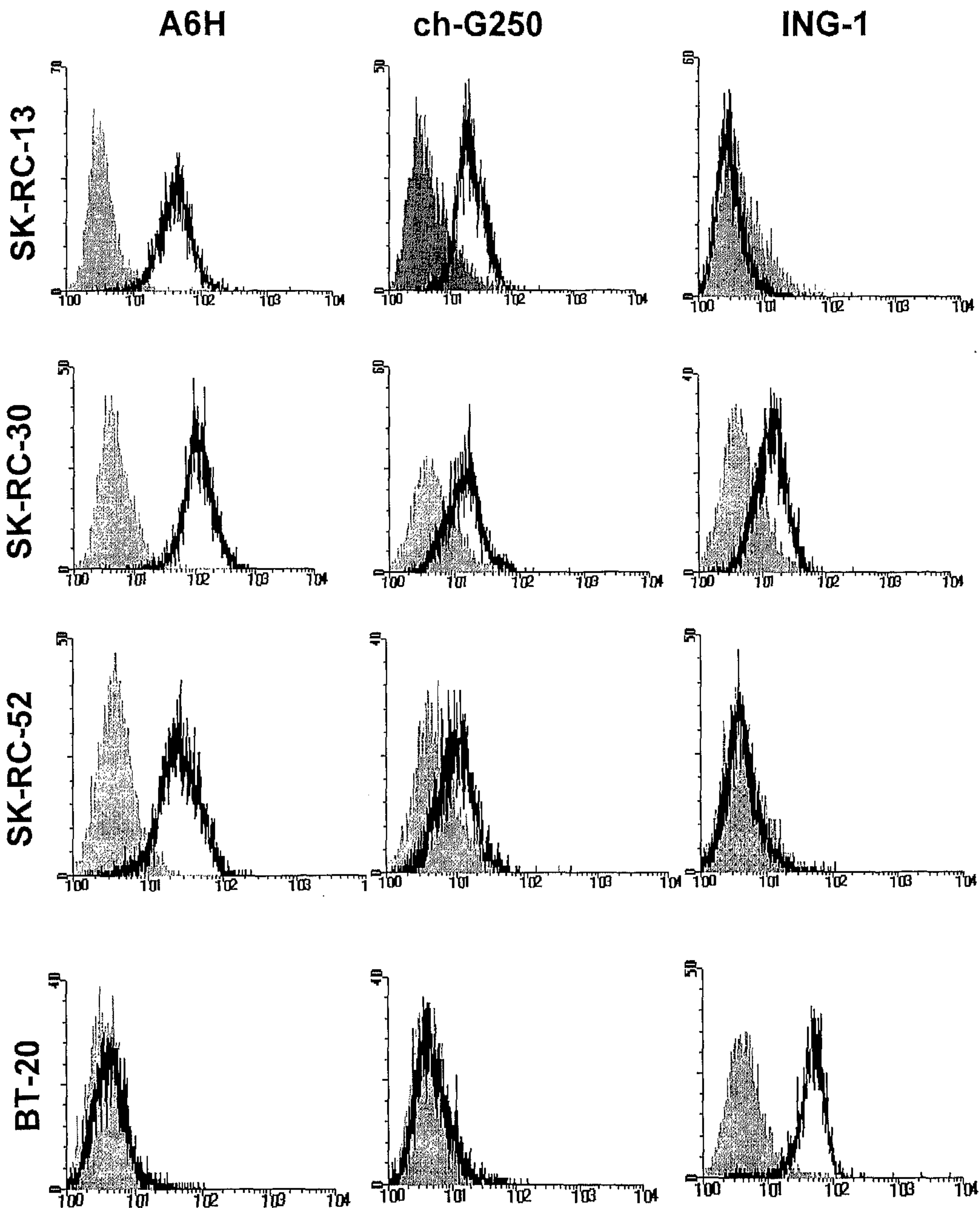


FIG. 1. Chimeric antibody G250 binds renal carcinoma tumor lines. Three renal carcinoma lines (SK-Rc-13, SK-Rc-30, and SK-Rc-52) and a breast carcinoma line (BT-20) were tested in indirect immunofluorescence by flow cytometry. Fluorescence intensity on a \log_{10} scale is plotted on each x axis. Primary antibodies included ch-G250, A6H, which is a murine monoclonal antibody reactive with RCC, and ING-1, a human/murine chimeric antibody that recognizes a 40-kDa membrane glycoprotein expressed on the majority of breast, colon, and lung carcinomas.

totoxicity with the addition of ch-G250. The SK-Rc-30 RCC cells demonstrated a low yet reproducible level of ADCC with ch-G250 ($p = 0.067$) for the comparison of cytotoxicity in media versus ch-G250. The BT-20 targets did not bind the ch-G250 antibody, and no significant ADCC was seen with

the ch-G250 antibody, whereas the ING-1 antibody did bind specifically to this target and facilitated significant ADCC ($p = 0.020$) for cytotoxicity in media versus ING-1. Neither the ch-G250 nor ING-1 antibodies facilitated lysis of the SK-Rc-52 (data not shown).

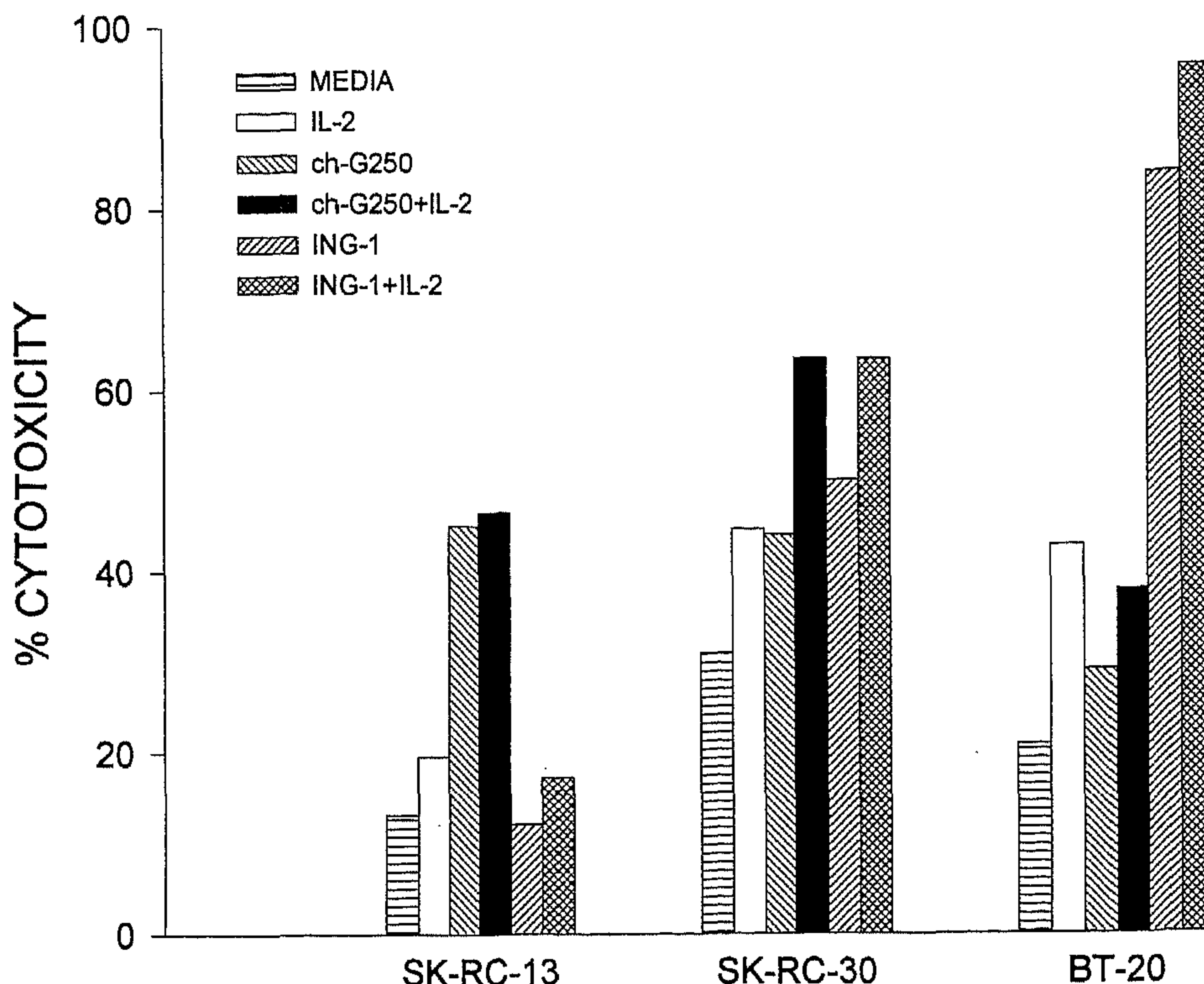


FIG. 2. Specificity of chimeric antibody G250 in antibody-dependent cellular cytotoxicity. Renal carcinoma lines SK-RC-13 and SK-RC-30, and breast carcinoma line BT-20 were targets in a 4-h ^{51}Cr release assay. Effectors were peripheral blood mononuclear cells (PBMC) obtained from healthy control donors cultured in interleukin-2 (IL-2) for 24 h before the assay. Assay conditions included standard tissue culture media, media supplemented with IL-2 at 100 U/ml, ch-G250 at 0.5 $\mu\text{g}/\text{ml}$, ING-1 at 1 $\mu\text{g}/\text{ml}$, and these antibody concentrations plus IL-2 at 100 U/ml. Results shown are the means of percent cytotoxicity values for effector-to-target ratios of 100:1 obtained in six separate assays, involving PBMC from 10 healthy donors.

Effect of In Vivo IL-2 on ADCC with ch-G250 Chimeric Antibody

To determine whether ch-G250 would facilitate the augmented ADCC mediated by freshly obtained PBMC activated in vivo by IL-2 (14), patient PBMC were obtained 24 h after completion of a 96-h continuous infusion of IL-2 (Fig. 3). These effectors mediated lymphokine activated killer (LAK) activity against SK-RC-13, which was best detected in the presence of IL-2 during the 4-h assay. ADCC activity was also noted with the ch-G250 antibody. The combination of IL-2 and antibody induced almost complete lysis of this target. The ING-1 chimeric antibody, which did not bind to this target, had no effect on cytotoxicity. In two subsequent experiments, cryopreserved PBMC, which were obtained from three separate patients after a 96-h continuous infusion of IL-2, were thawed and assayed. These in vivo activated effectors demonstrated a pattern of ADCC on SK-RC-13 target cells similar to that shown by the fresh patient PBMC

(Fig. 3); however, the degree of cytotoxicity was less with the freshly thawed PBMC (data not shown). Freshly obtained effectors from the normal control donor also mediated ADCC against the SK-RC-13 target; however, this cytotoxicity was not augmented by the addition of IL-2 during the 4-h assay.

Figure 4 presents data obtained using freshly thawed PBMC from a second patient, obtained and cryopreserved before and 24 h after a 96-h infusion of IL-2, to demonstrate the effect of in vivo IL-2 treatment on ch-G250-mediated ADCC. Minimal LAK activity and ADCC on the SK-RC-13 target were seen with PBMC from the patient obtained before IL-2 treatment. A slight increase was seen in the ADCC activity when the ch-G250 antibody and IL-2 were combined in the assay. PBMC obtained 24 h after IL-2 therapy mediated low-level LAK activity in media alone. This lytic activity was augmented when IL-2 was included in the assay, as noted previously (7). When the ch-G250 was added to the in vivo primed PBMC, cytotoxicity was dou-

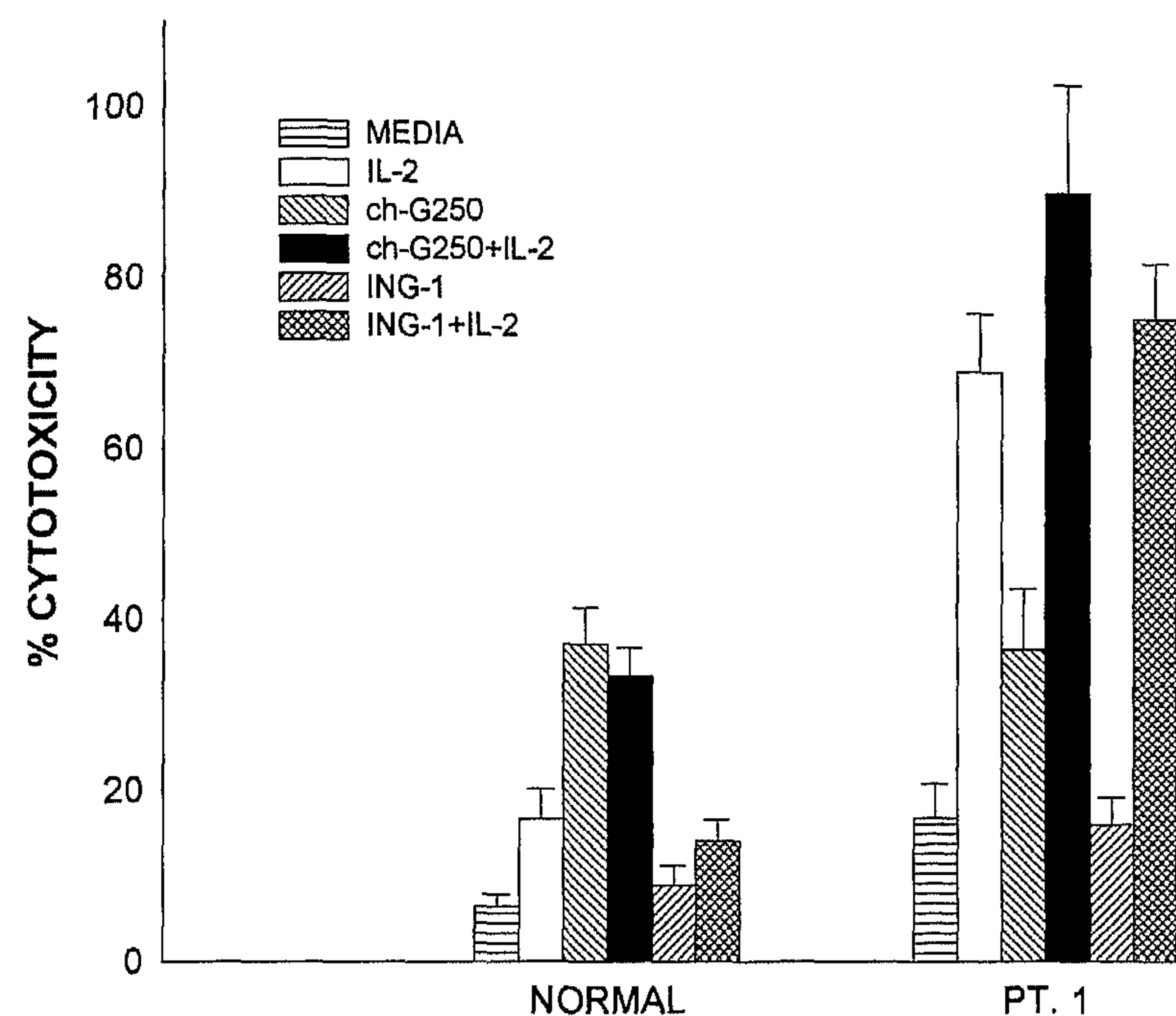


FIG. 3. Antibody-dependent cellular cytotoxicity mediated by freshly obtained peripheral blood mononuclear cells from a healthy control donor and a cancer patient after interleukin-2 (IL-2) therapy. The SK-RC-13 renal carcinoma line was used in a 4-h ^{51}Cr release assay. Assay conditions included standard tissue culture media, media supplemented with IL-2 at 100 U/ml, ch-G250 at 0.5 $\mu\text{g}/\text{ml}$, ING-1 at 1 $\mu\text{g}/\text{ml}$, and these antibody concentrations plus IL-2 at 100 U/ml. Results shown are the 100:1 effector:target ratio. Error bars indicate 1 SD.

bled over that obtained in medium. This lytic activity was increased further when IL-2 was added in combination with the ch-G250 antibody. Thus, as seen previously (14), PBMC obtained from a patient after IL-2 treatment show a striking increase over pretreatment PBMC in their ability to mediate LAK and ADCC against a tumor target. The fresh normal control PBMC tested in Fig. 4 showed LAK and ADCC activity against the SK-RC-13 target, as noted in Figs. 2 and 3.

DISCUSSION

Although IL-2 therapy can provide clinically significant antitumor responses for some patients with RCC, these are seen in only 10–20% of treated patients, whereas virtually all patients show the dose-dependent toxicities arising from the systemic activation of IL-2-responsive cells (5). Increasing the IL-2 dose or combining IL-2 with in vitro activated LAK cells appears to increase the toxicity with little added antitumor benefit (15,16). Enhanced antitumor effects might be possible if the IL-2-activated effector cells were more selective in their recognition and destruction of RCC.

In murine tumor models, the in vivo combination of IL-2 and tumor-reactive mAb (which mediate ADCC in vitro) provide better antitumor effects than treatment with IL-2 or the mAb alone (17–19).

The Fc component of the mAb is essential in this antitumor effect, because the Fab'2 component of the mAb does not provide tumor protection (19). These data suggest that the in vivo combination of tumor-reactive mAb capable of facilitating ADCC, plus IL-2 allows in vivo ADCC, mediating an antitumor effect in appropriate mouse models.

This concept has now been extrapolated to clinical trials in human cancer patients. After in vivo IL-2 treatment, the circulating PBMC are able to show much greater in vitro ADCC capability than are PBMC obtained before IL-2 treatment (14,20). Clinical protocols are testing the combination of IL-2 with murine and chimeric mAb able to mediate ADCC in vitro (21–23). We have recently shown that patients receiving combined treatment with IL-2 and the GD2 reactive 14.G2a mAb have serum levels of mAb that can facilitate in vitro ADCC with autologous freshly obtained PBMC. This result indicates that conditions compatible with antibody-specific ADCC have been achieved by this combination in vivo (21).

To test this concept in patients with RCC, it was necessary to identify a mAb able to mediate ADCC of human RCC with human effectors. The murine G250 mAb (IgG₁) has been used clinically in a Phase I antibody localization study and demonstrated highly specific in vivo tumor targeting (2). However, this antibody is not capable of facilitating

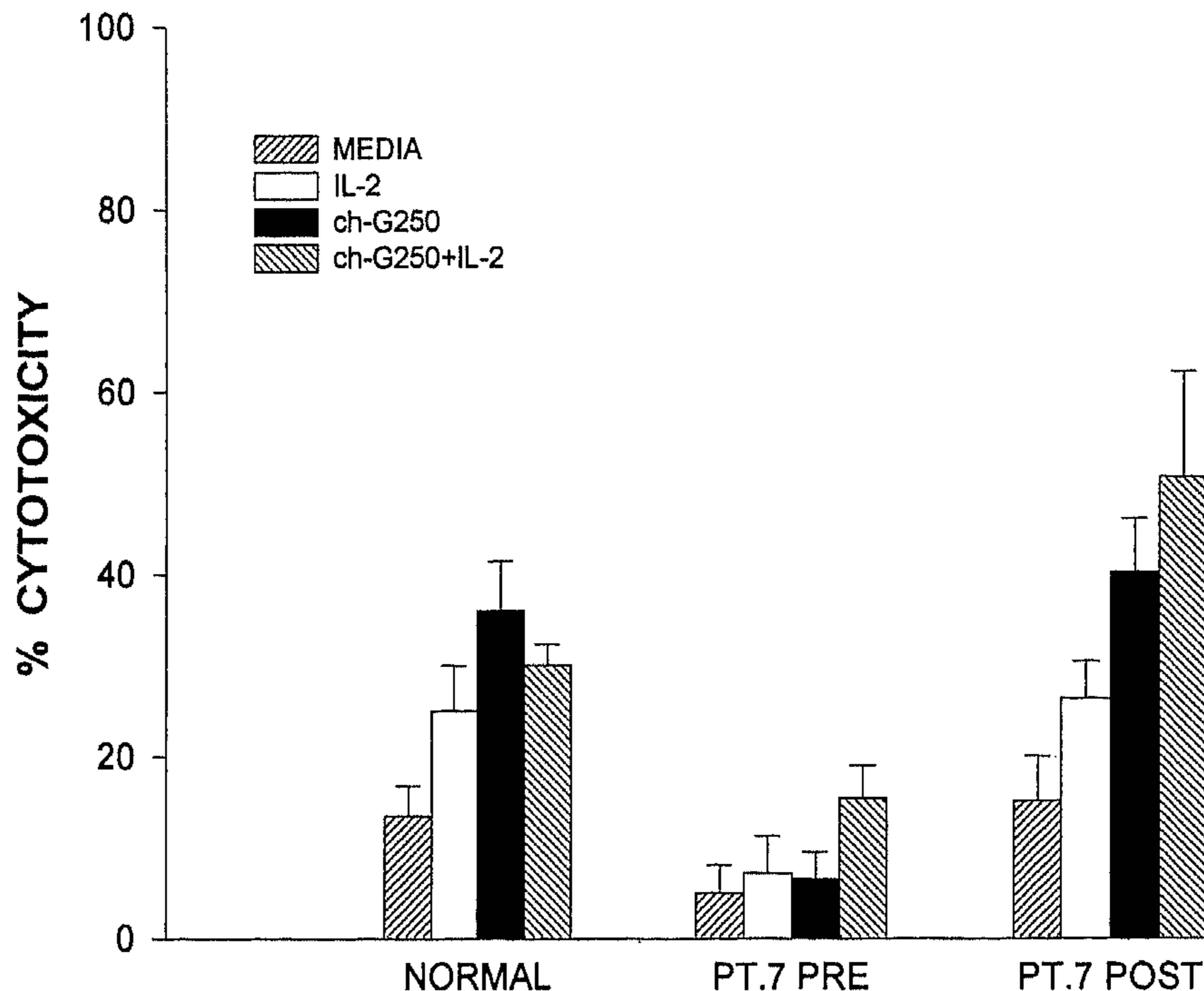


FIG. 4. Comparison of antibody-dependent cellular cytotoxicity seen with fresh peripheral blood mononuclear cells (PBMC) from a normal control donor and with freshly thawed PBMC samples obtained from a patient just before (Pt.7 pre) and 24 h after (Pt.7 post) a 96-h interleukin-2 (IL-2) infusion. SK-RC-13 was used as the target in a 4-h chromium release assay. Assay conditions included media alone, IL-2 at 100 U/ml, ch-G250 at 0.5 μ g/ml, or ch-G250 plus IL-2. The effector:target ratio was 100:1. Error bars indicate 1 SD.

ADCC with human effector cells. An IgG2a isotype switch variant of the murine G250 antibody was used in an RCC xenografted nude mouse model in which antitumor activity was detected, and this activity was enhanced by concomitant treatment with interferon and TNF- α (3). Interferon is known to enhance the ADCC activity of both macrophages and NK cells, whereas TNF enhances macrophage ADCC. Histopathology of regressing lesions showed an infiltration of mononuclear cells with a significant macrophage component, suggesting that the tumor inhibition may have included a component of in vivo ADCC. In this report, we used a human/murine chimeric variant of the G250 antibody to assess the ADCC functions of the human isotype with human effector cells.

The data presented here document that ADCC was mediated by the ch-G250 mAb on human RCC lines with human effector cells. The level of ADCC was dramatically enhanced when effector cells were obtained after in vivo IL-2 treatment, as shown previously for other mAb capable of facilitating ADCC (14,21). In these current in vitro studies, the level of ADCC corresponded to the level of expression of the G250 molecule on the different RCC lines.

The majority of human RCC tumors show homogeneous expression of the G250 antigen in vivo, with little expression on normal tissues (1). Furthermore, the murine mG250 and the chimeric ch-G250 antibodies have shown little toxicity in their initial clinical testing as single-agent treatment in radiolocalization or Phase I testing (2). Therefore, these data suggest that the ch-G250 mAb should be tested in a Phase I clinical trial in combination with IL-2 to determine their combined toxicity and to attempt to induce effective in vivo ADCC to RCC.

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REFERENCES

1. Oosterwijk E, Ruiters DJ, Goedemaeker PHJ, et al. Monoclonal antibody G250 recognizes a determinant present in renal-cell carcinoma and absent from normal kidney. *Int J Cancer* 1986;38:489-94.
2. Oosterwijk E, Bander NH, Divgi CR et al. Antibody localization in human renal cell carcinoma: a phase I study of monoclonal antibody G250. *J Clin Oncol* 1993;11:738-50.
3. Van Dijk J, Uemura H, Beniers AJ, et al. Therapeutic effects

- of monoclonal antibody G250, interferons and tumor necrosis factor in mice with renal-cell carcinoma xenografts. *Int J Cancer* 1994;56:262-8.
4. Divgi CR, Bander NH, Welt S, et al. Phase I/II trial with I-131 labeled monoclonal antibody (MAB) G250 in metastatic renal cell cancer (RCC). *Proc Am Soc Clin Oncol* 1995;14:423.
 5. Rosenberg SA, Yang JC, Topalian SL, et al. Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high dose bolus interleukin-2. *JAMA* 1994;271:907-13.
 6. Phillips JH, Gemlo BT, Myers WW, et al. *In vivo* and *in vitro* activation of natural killer cells in advanced cancer patients undergoing recombinant interleukin-2 and LAK cell therapy. *J Clin Oncol* 1987;5:1933-41.
 7. Hank JA, Kohler PC, Weil-Hillman G, et al. *In vivo* induction of the lymphokine-activated killer phenomenon: interleukin 2-dependent human non-major histocompatibility complex-restricted cytotoxicity generated *in vivo* during administration of human recombinant interleukin 2. *Cancer Res* 1988;48:1965-71.
 8. Sondel PM, Kohler PC, Hank JA, et al. Clinical and immunological effects of recombinant interleukin-2 given by repetitive weekly cycles to patients with cancer. *Cancer Res* 1988;48:2561-7.
 9. Oosterwijk E, Debruyne FM, Schalken JA. The use of monoclonal antibody G250 in the therapy of renal-cell carcinoma. *Semin Oncol* 1995;22:34-41.
 10. Velders MP, Litvinov SV, Warnaar SO, et al. New chimeric anti-pancarcinoma monoclonal antibody with superior cytotoxicity-mediating potency. *Cancer Res* 1994;54:1753-60.
 11. Vessella RL, Moon TD, Chiou R-K, et al. Monoclonal antibodies to human renal cell carcinoma: recognition of shared and restricted tissue antigens. *Cancer Res* 1985;45:6131-9.
 12. Robinson RR, Chartier J Jr, Chang CP, Horwitz AH, Better M. Chimeric mouse-human anti-carcinoma antibodies that mediate different anti-tumor cell biological activities. *Hum Antibodies Hybridomas* 1991;2:84-93.
 13. Sondel PM, Kohler PC, Hank JA, et al. Clinical and immunological effects of recombinant interleukin 2 given by repetitive weekly cycles to patients with cancer. *Cancer Res* 1988;48:2561-7.
 14. Hank JA, Robinson RR, Surfus J, et al. Augmentation of antibody dependent cell mediated cytotoxicity following *in vivo* therapy with recombinant interleukin 2. *Cancer Res* 1990;50:5234-9.
 15. Albertini MR, Sosman JA, Hank JA, et al. The influence of autologous lymphokine-activated killer cell infusions on the toxicity and antitumor effect of repetitive cycles of interleukin-2. *Cancer* 1990;66:2457-64.
 16. Sosman JA, Hank JA, Moore KH, et al. Prolonged interleukin-2 (IL-2) treatment can augment immune activation without enhancing antitumor activity in renal cell carcinoma. *Cancer Invest* 1991;9:35-48.
 17. Eisenthal A, Cameron RB, Uppenkamp I, Rosenberg SA. Effect of combined therapy with lymphokine-activated killer cells, interleukin-2 and specific monoclonal antibody on established B16 melanoma lung metastases. *Cancer Res* 1988;48:7140-5.
 18. Kawase I, Komuta K, Hara H, et al. Combined therapy of mice bearing a lymphokine-activated killer-resistant tumor with recombinant interleukin-2 and an antitumor monoclonal antibody capable of inducing antibody-dependent cellular cytotoxicity. *Cancer Res* 1988;48:1173-9.
 19. Berinstein N, Starnes CO, Levy R. Specific enhancement of the therapeutic effect of anti-idiotypic antibodies on a murine B cell lymphoma by IL-2. *J Immunol* 1988;140:2839-45.
 20. Munn DH, Cheung NKV. Interleukin-2 enhancement of monoclonal antibody mediated cellular cytotoxicity against human melanoma. *Cancer Res* 1987;47:6600-5.
 21. Hank J, Surfus J, Gan J, et al. Treatment of neuroblastoma patients with antiganglioside GD₂ antibody plus interleukin-2 induces antibody dependent cellular cytotoxicity against neuroblastoma detected *in vitro*. *J Immunother* 1994;15:29-37.
 22. Ziegler LD, Palazzolo P, Cunningham J, et al. Phase I trial of murine monoclonal antibody L6 in combination with subcutaneous interleukin-2 in patients with advanced carcinoma of the breast, colorectum, and lung. *J Clin Oncol* 1992;10:1470-8.
 23. Bajorin D, Chapman P, Wong G, et al. Phase I evaluation of a combination of monoclonal antibody R24 and interleukin-2 in patients with metastatic melanoma. *Cancer Res* 1990;50:7490-5.