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Selective Killing of Leukemia and Lymphoma Cells Ectopically Expressing hCGβ by a Conjugate of Curcumin with an Antibody against hCGβ Subunit

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Key Words
MOLT-4 · T-lymphoblastic leukemia · U-937 · Histiocytic lymphoma · Acute myeloid leukemia · Curcumin-antibody conjugate · Human chorionic gonadotropin beta

Abstract
Objective: A variety of cancers ectopically express human chorionic gonadotropin beta (hCGβ). Patients harboring such cancers have poor prognosis and adverse survival. A recombinant chimeric antibody, cPiPP, exhibiting high affinity and specificity for hCGβ/hCG was engineered. This study was designed to determine whether this antibody alone or conjugated to curcumin can selectively kill tumor cells expressing hCGβ. ***Experimental Design:*** The study was carried out on MOLT-4 and U-937 cells expressing hCGβ and on peripheral blood leukocytes of acute myeloid leukemia (AML) patients. The anticancerous compound curcumin was conjugated to cPiPP. The binding of cPiPP and cPiPP-curcumin conjugate to cells was studied by flow cytometry and cytotoxicity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), FACS with propidium iodide staining, trypan blue exclusion assay and microscopy. ***Results:*** The antibody did not impair the growth of MOLT-4 and U-937 cells in culture. Its conjugate with curcumin, however, was lethal to both cell lines. The immunoconjugate killed tumor cells bearing the CD33 marker of an AML patient expressing hCGβ but did not have a similar action on cells of another AML patient with the CD13 marker but who was negative for hCGβ. ***Conclusion:*** A humanized antibody against hCGβ linked to curcumin has potential for therapy of hCGβ-expressing tumors.

Introduction

Trophoblasts are known to produce human chorionic gonadotropin (hCG); hence malignant trophoblastic tumors are expected to produce and secrete hCG [1, 2]. However, a number of nontrophoblastic cancers are reported to express hCG or its subunits ectopically: lung [3], bladder [4], colon [5], gastric [6, 7], pancreatic [8], breast [9], cervical [10], oral [11, 12], head and neck [13], vulva/vaginal [14, 15], prostate [16] and renal cancers [17, 18]. Not only solid tumors but also nonsolid tumors of hematopoietic lineage [19–21] have been reported to express hCGβ. Detection of hCGβ expression in tumors is usually associated with poor prognosis [4, 18]. Lundin et al. [5] reported that survival time of hCGβ-positive colorectal cancer patients was significantly shorter than of hCGβ-negative cancer patients. As normal healthy males and nonpregnant females do not produce hCG in detectable amounts (which is the reason why hCG is em-

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ployed universally as a diagnostic test for pregnancy) antibodies directed against hCG are expected not to react with tissues of normal healthy persons. These would, however, bind with hCGβ or hCG displayed on membranes of tumor cells expressing ectopically the hormone or its subunit. Thus these antibodies could be employed for targeted therapy of hCG synthesizing tumors. To validate the workability of this approach, we examined two tumor cell lines MOLT-4, a T-lymphoblastic leukemia and U-937, a histiocytic lymphoma. Both synthesize and expose hCG on their membranes. A recombinant chimeric anti-hCG antibody, cPiPP, developed earlier [22], binds with over 95% of MOLT-4 cells as determined by flow cytometery [23]. On the other hand, this antibody exhibited no binding to peripheral blood mononuclear cells (PBMC) of normal healthy donors [23]. We report in this communication that this antibody linked to diferuloylmethane (curcumin), a highly safe nontoxic anticancerous compound, can cause selective killing of the hCG-expressing tumor cells bearing the CD33 marker. The action of this conjugate has also been determined on anti-CD33/CD13-reactive cells of 2 acute myeloid leukemia (AML) patients, 1 of them ectopically expressing hCGβ and CD33 and the other in addition to this minor fraction had CD13-positive hCGβ-negative cells.

Materials and Methods

Compounds and Cell Lines

Cell Lines

Human T-lymphoblastic leukemia MOLT-4 cells (ATCC CRL-1582) and human histiocytic lymphoma U-937 (ATCC CRL-1593.2) originally developed by Sundstrom and Nilsson [24] were obtained from the American Type Culture Collection and maintained in suspension culture in a humidified incubator at 37°C under 5% CO_2 and 95% air in RPMI 1640 medium (Hyclone) supplemented with 10% heat-inactivated fetal calf serum (Hyclone) and antibiotic-antimycotic mixture (Gibco/Invitrogen; penicillin 100 U/ml, streptomycin 100 μg/ml and amphotericin B 250 ng/ml working concentration). The cells were subcultured before they reached 70% confluency. All experiments were conducted with log-phase cells.

Human PBMC from healthy adult donors were isolated by centrifugation over a Ficoll-Hypaque density gradient (Amersham-Pharmacia Biotech). The cells were counted using a hemocytometer and viability was determined by trypan blue exclusion.

Blood samples from AML patients were taken with approval of the Ethics Committee of the All India Institute of Medical Sciences, New Delhi and with informed, written consent of the patients. Fourteen patients with AML were enrolled in the study. All patients were new untreated cases. Diagnosis of AML was established by morphological and cytochemical evaluation of blast cells on peripheral blood smear and/or bone marrow smears as per WHO criteria [25]. The blast cells in the peripheral blood smear of these patients constituted more than 80% of the leukocytes. The PBMC were separated on Ficoll-Hypaque density gradient; thus granulocytes were separated and do not figure in the population. Cytotoxicity of the immunoconjugate on PBMC of 2 patients was determined. PBMC of one had high binding with the anti-hCG antibody and the other low binding with the anti-hCG antibody.

Antibodies

The antibody, cPiPP, is a chimeric recombinant anti-hCGβ antibody engineered from a mouse monoclonal antibody raised against hCGβ [22]. The heavy chain variable region gene of a mouse monoclonal antibody [26] was fused to the human IgG_1 constant region, while the light chain variable region gene was fused to the human kappa constant region. The antibody was expressed at high yield in tobacco leaves (*Nicotiana tabacum*, cultivar Petit Havana SR1), as described earlier [27], and purified by Protein-A affinity chromatography. The association constant of the antibody for hCG is 3×10^{10} M^{-1}. The antibody is specific to β subunit of hCG and does not recognize human thyroid-stimulating hormone and human follicle-stimulating hormone which share the α chain with hCG. It has <5% cross-reactivity with human luteinizing hormone. Another monoclonal antibody 730 against prostate-independent cancer cell lines [28], not binding to hCG or its subunits, was taken as negative control.

Fluorescent isothiocyanate (FITC)-conjugated goat anti-human IgG Fc (γ) was obtained from Jackson Immuno Research Laboratories (West Grove, Pa., USA). Phycoerythrin (PE)-labeled anti-CD13 and anti-CD33 antibodies were obtained from eBioscience (San Diego, Calif., USA).

95% pure curcumin powder was obtained from Sanat Products (New Delhi, India). The content of curcumin was determined by dissolution in acetone and measurement of the absorption coefficient at 426 nm. Its molecular mass was cross-checked by mass spectrophotometry.

Linking of Curcumin to the Antibody

The conjugation of cPiPP with curcumin via the amino group was carried out in three steps as follows: an aliquot of purified anhydrous curcumin was reacted with butoxycarbonyl glycine (BOC-glycine) using dicyclohexylcarbodiimide in the presence of 4-dimethylaminopyridine, all dissolved in anhydrous dichloromethane (DCM) at room temperature. The appearance of turbidity signified the formation of the product. The reaction was carried out in the dark with gentle stirring. Completion of reaction was assessed by thin layer chromatography, and the reaction mixture concentrated by vacuum evaporation at ambient temperature. The concentrated product was loaded on a silica gel for purification. Elution was carried out with varying ratios of DCM-methanol, to obtain the product BOC-glycine-curcumin (fig. 1a). The purified BOC-glycine conjugated to curcumin had a molecular mass of 526 Da which was characterized by mass spectroscopy and indicated a single molecule of BOC-glycine linked to one molecule of curcumin. The BOC-protected group was removed with a solution of trifluroacetic acid in DCM at 0°C, leading to the intermediate compound curcumin-glycine (fig. 1b). Chimeric recombinant antibody cPiPP [dissolved in and dialyzed against 10 mM phosphate-buffered saline (PBS) at pH 7.6] was mixed with curcumin-glycine (fig. 1c). The mixture was cooled in an ice bath and 1-ethyl-3-(3-

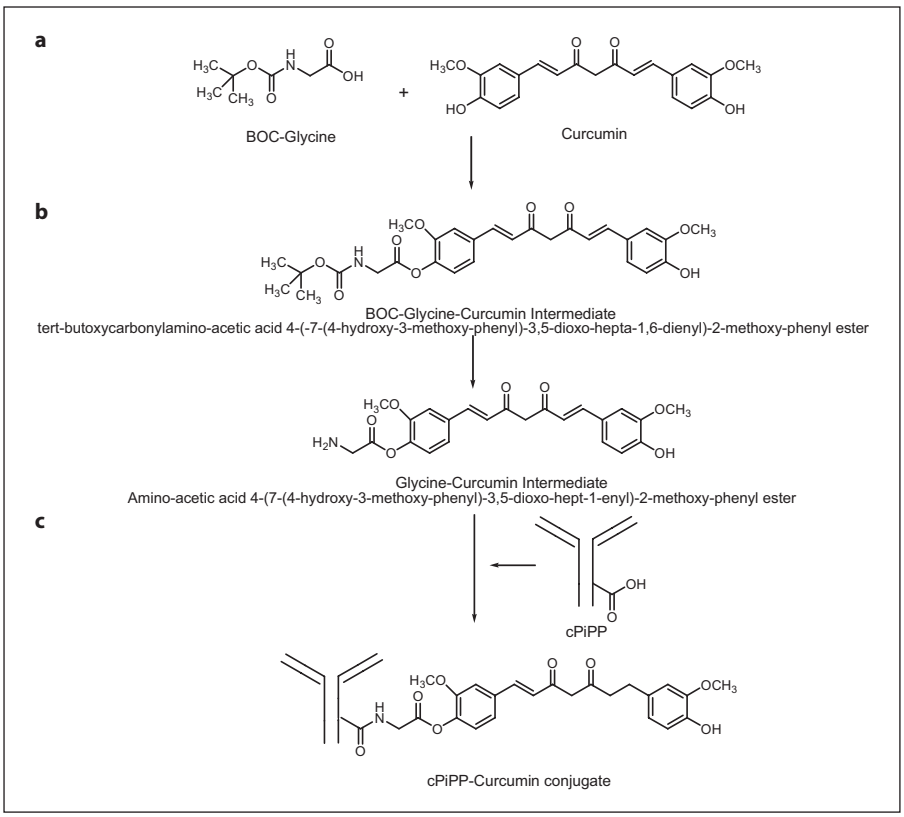


Fig. 1. Conjugation of curcumin to antibody. **a** Generation of an amine group on curcumin by coupling it to BOC-glycine. **b** Removal of BOC group and activation of free amine group. **c** Coupling of curcumin-glycine to exposed acidic amino acids (glutamic and aspartic acid) on the antibody by carbodiimide.

dimethylaminopropyl)carbodiimide hydrochloride (EDC) was added to the solution with gentle stirring at 4°C. After 1 h, additional EDC was added and stirred for 1 h in an ice bath. The reaction mixture was then stirred at room temperature for 1–2 h. Non-linked curcumin-glycine was separated from the conjugated antibody product by passing the reaction mixture through a Sephadex G-50 column (Sigma-Aldrich) equilibrated with PBS. The conjugate was collected in the void volume, dialyzed against PBS employing a 10-kDa membrane, concentrated and stored at –20°C. The linkage of curcumin-glycine to the cPiPP was confirmed by UV absorption spectrum (UV-Vis spectrophotometer, Shimazdu). Whereas antibody, like other proteins, has an absorption maximum at 280 nm, very little absorption is observed at 426 nm, at which curcumin has its maximum, thereby permitting calculation of stoichiometry; 40 mol curcumin were present per mole of antibody. An irrelevant monoclonal antibody 730 was also linked to curcumin by the same method as described above.

Binding Assay

To assess binding of the cPiPP-curcumin conjugate, MOLT-4 cells (0.2×10^6) were incubated for 60 min at 4°C with 5 μg of cPiPP-containing conjugate or 5 μg cPiPP alone in 100 μl of FACS buffer (1% bovine serum albumin, 0.02% sodium azide in PBS). After washing thrice with FACS buffer, cells were stained with a 1:100 dilution of FITC-conjugated goat anti-human IgG Fc (γ) for 60 min at 4°C. FACS analysis was carried out on a BD LSR (Becton Dickinson, San Jose, Calif., USA) and analyzed by WinMdi software (version 2.9).

Cytotoxicity Assay

Cytotoxicity of Antibody-Linked Curcumin

MOLT-4 and U-937 in log phase were cultured in 96-well plates (Nunc). Fresh medium, cPiPP at 50 μg/ml concentration or the cPiPP-curcumin conjugate at concentrations of the antibody ranging from 10 to 100 μg/ml of the medium was added and the cells were incubated for 48 h at 37°C. Viable and dead cells were distinguished by trypan blue staining (in triplicate) and analyzed by FACS subsequent to propidium iodide (PI) staining.

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide Assay

Cellular proliferation was measured via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay using the Cell Proliferation Kit 1 (Roche Applied Sciences). Briefly, after incubation of cells for 48 h with the immunoconjugate or antibody alone, cells were incubated at 37°C for additional 2 h in the presence of Hanks' balanced salt solution (HBSS, Gibco, Invitrogen) along with the MTT reagent as described by the manufacturer. Cells were lysed and absorbance measured at 560 nm. The percentage cytotoxicity (X′) was calculated by the formula:

$$X' = 1 - (X/R1) \times 100$$

where X = optical density of test wells and R1 = optical density of control wells containing only cells with HBSS. All studies were done in replicates of five.

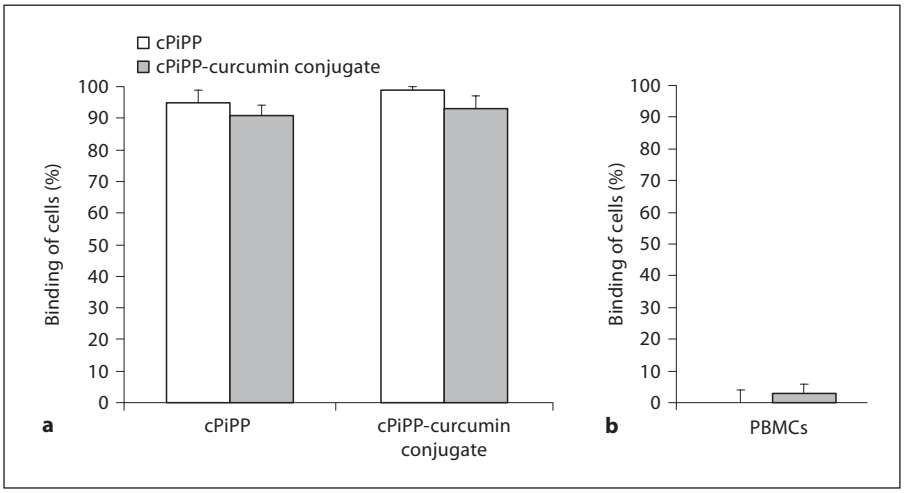


Fig. 2. Binding of cPiPP and cPiPP-curcumin conjugate. **a** With MOLT-4 and U-937 cells. **b** With PBMCs of normal healthy donors as determined by FACS. The values are mean ± SD of 4 assays.

Analysis of Cell Morphology

MOLT-4 cells were grown on poly-L-lysine-treated microwells for 24 h. The cells were then incubated with or without the cPiPP-curcumin conjugate at antibody equivalent concentrations of 10, 50 and 100 μg/ml for 48 h at 37°C. Digital images were recorded on a phase-contrast light microscope (Nikon, Eclipse TE2000-U).

Results

Conservation of Binding of cPiPP-Curcumin Conjugate to MOLT-4 and U-937 Cells

The manner in which curcumin was linked to recombinant chimeric antibody, cPiPP, did not appreciably mask the ability of the conjugate to bind to cells expressing hCGβ or hCG on the membranes. Data in figure 2a show that 91% of MOLT-4 cells bound to the conjugated antibody cPiPP compared to 95% to the antibody alone. Similarly, 93% of U-937 cells bound to the conjugated antibody whereas cPiPP alone bound 99% of tumor cells.

Neither the cPiPP nor the cPiPP-curcumin conjugate demonstrated any significant binding with PBMCs of normal healthy donors.

Effect of cPiPP and cPiPP-Curcumin Conjugate on MOLT-4 Cells

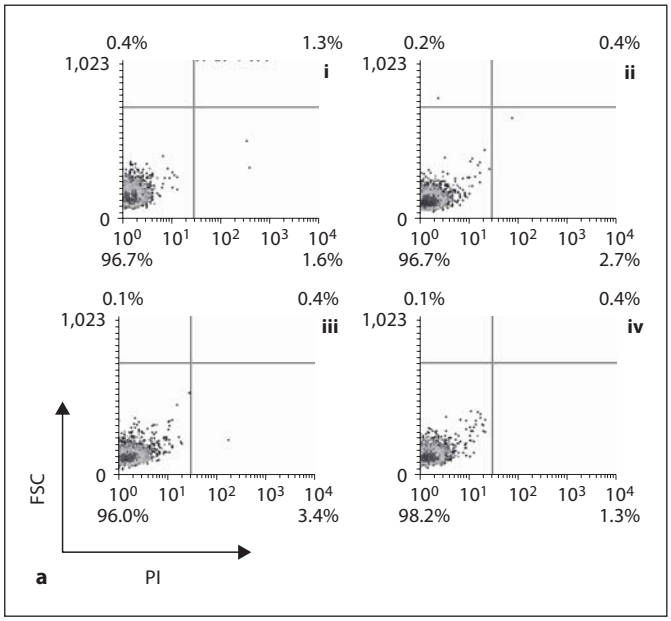
The antibody cPiPP alone did not have any cytotoxic effect on MOLT-4 cells in vitro as shown by results in figure 3a. FACS analysis after incubation with cPiPP and cPiPP-curcumin conjugate showed that cells cultured in the presence of an increasing concentration of cPiPP alone did not incorporate PI. This was, however, not the case when cells were cultured in the presence of cPiPP-curcumin conjugate (fig. 3b). At 10 μg/ml cPiPP-curcumin conjugate, 68% cells were killed as reflected by staining with PI. Almost 100% killing was observed at concentrations of 50 μg/ml and above of the conjugate (fig. 3b, iv). Similar results were obtained employing the trypan blue exclusion assay. No cytotoxicity was observed on these cells on incubation with 100 μg/ml of conjugate of the irrelevant antibody 730 with curcumin (fig. 3c).

The immunoconjugate prepared and purified as described is not expected to contain free curcumin, which is hydrophobic and insoluble in aqueous medium. The lack of cytotoxicity of curcumin alone dispersed at various concentrations in PBS is shown in figure 4. However, when dissolved in DMSO, curcumin does have a cytotoxic effect on MOLT-4 cells (fig. 4). Conjugation with the antibody helps to carry it to target cells in aqueous medium.

The lethal action of cPiPP-curcumin conjugate is not only confined to MOLT-4 cells but is also manifest in other cell lines expressing hCGβ on their membranes. Figure 5 shows that nearly 89% of U-937 cells were killed by incubation with 100 μg/ml of cPiPP-curcumin conjugate.

Lack of Effect of cPiPP-Curcumin Conjugate on PBMC of Healthy Donors

The binding of cPiPP and of cPiPP-curcumin conjugate with PBMC of 4 healthy donors was 2.3 ± 3 to 9.4 ± 4% (fig. 2b). In experiments conducted to see the possible cytotoxicity of the conjugate on these cells, viable cells were counted after 48 h of exposure in culture medium to 50 and 100 μg/ml concentration of cPiPP and cPiPP-curcumin conjugate. Data in figure 6 show that the viable cells by trypan blue exclusion assay were 19.6 ±



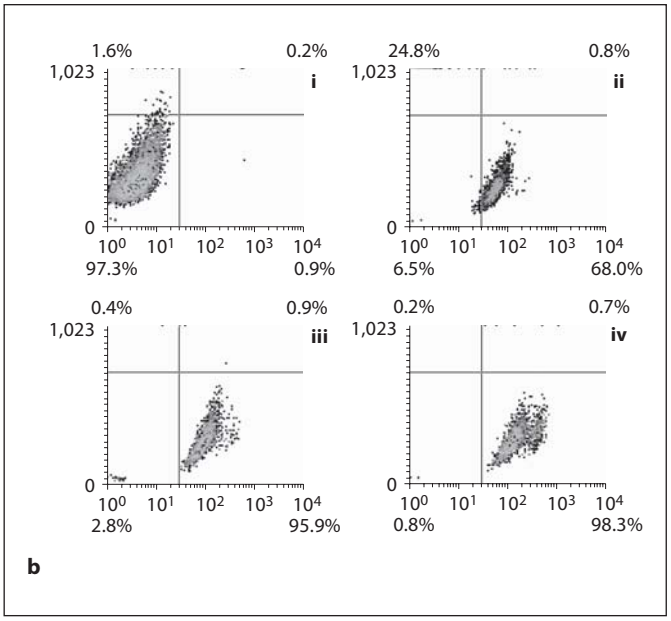
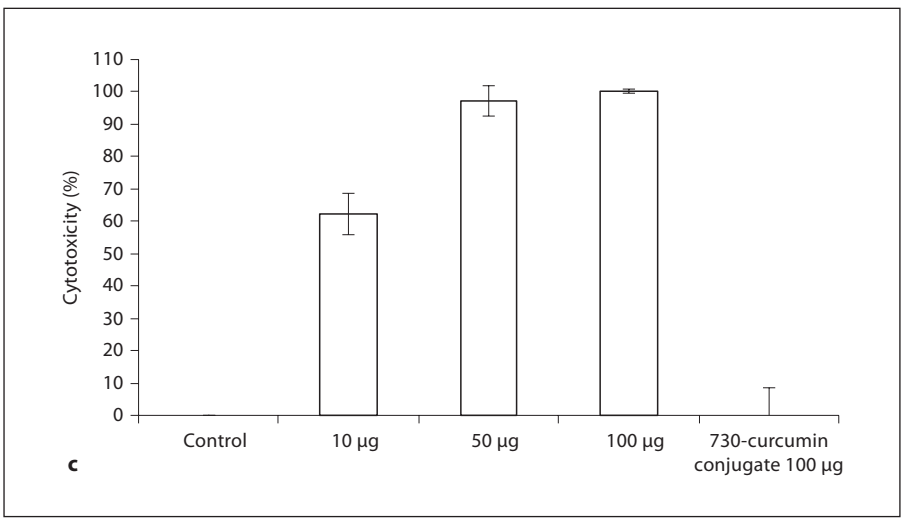


Fig. 3. Cytotoxic effect of cPiPP on MOLT-4 cells. **a** 0.1 million cells were cultured with RPMI 1640 supplemented with 0 μg (**i**), 10 μg (**ii**), 50 μg (**iii**) and 100 μg/ml (**iv**) of the antibody equivalent, for 48 h. FACS analysis of cells was carried out after staining with PI. **b** Cytotoxic effect of cPiPP-curcumin conjugate on MOLT-4 cells by FACS analysis of PI-stained cells at 0 μg (**i**), 10 μg (**ii**), 50 μg (**iii**) and 100 μg/ml (**iv**). Percentages of dead cells appearing in right lower quadrant were 0.9, 68, 96 and 98.3%, respectively. **c** The cytotoxic effect of the immunoconjugate was confirmed by trypan blue exclusion assay. Curcumin conjugated to an irrelevant antibody (MoAb 730) was devoid of cytotoxicity on MOLT-4 cells.



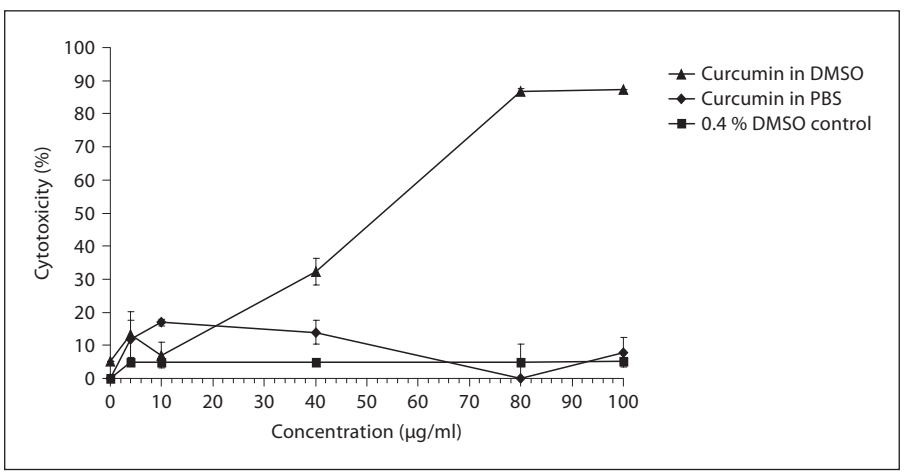


Fig. 4. Cytotoxic effect of curcumin on MOLT-4 cells, determined as dispersed at various concentrations in the medium or from a solution of curcumin in DMSO. Cells were cultured in RPMI 1640 medium for 48 h and the cytotoxicity determined by MTT assay.

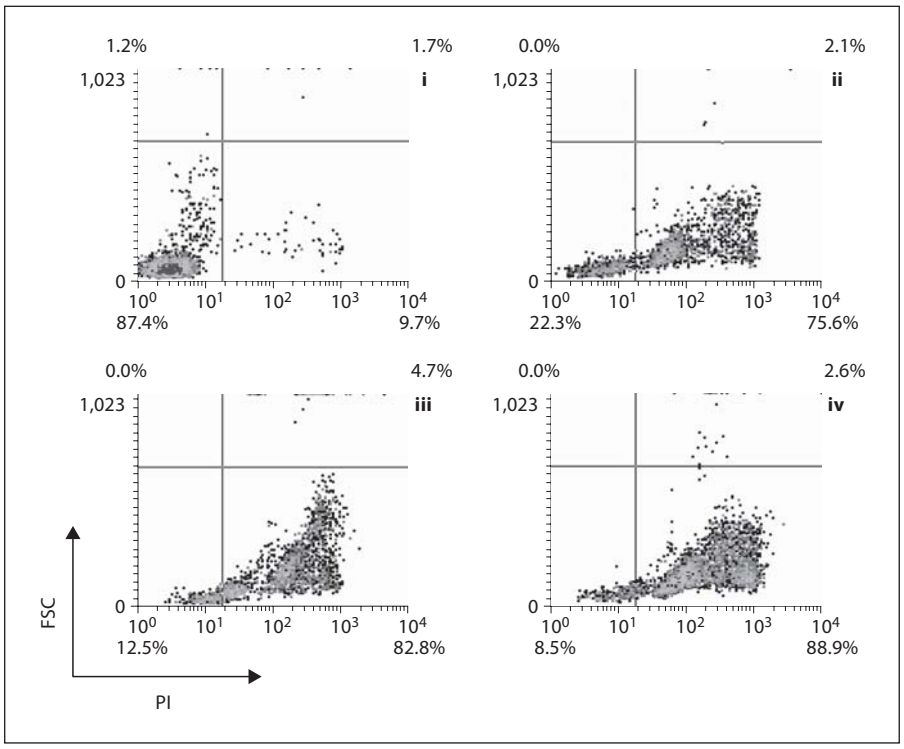


Fig. 5. Cytotoxic effect of cPiPP-curcumin conjugate on U-937 lymphoma cells as determined by PI staining. At antibody equivalent concentration of 10 μg (**ii**), 50 μg (**iii**) and 100 μg/ml (**iv**), the percentages of dead cells were 75, 82.8 and 88.9%, respectively. In control cultures without conjugate (**i**) the dead cells were 9.7%.

0.92×10^6 in control compared to $19.6 \pm 0.98 \times 10^6$ in the presence of 50 μg/ml cPiPP, $19.5 \pm 0.87 \times 10^6$ in wells with cPiPP-curcumin conjugate and $19.6 \pm 1.08 \times 10^6$ with 100 μg/ml cPiPP-curcumin conjugate.

Effect of cPiPP-Curcumin Conjugate on Leukocytes of AML Patients

We investigated 14 patients with AML. PBMCs from 7 patients bound the anti-hCGβ antibody to a significant extent while PBMCs from the other 7 had either none or a small percentage of cells expressing hCGβ. The cytotoxicity of the conjugate on cells from 1 patient from each category was studied.

Patient A.D., a 45-year-old female, presented with a history of fever and vaginal bleeding of 2 months' duration. On examination, she was pale, but had no lymphoadenopathy or organomegaly. Laboratory investigations revealed a hemoglobin content of 9 g/dl, total leukocyte count of 4,200/μl, and platelet count of 40,000/μl. Peripheral blood smear and bone marrow aspirate findings were consistent with the AML M3 subtype. 48.5% of PBMCs bound with the cPiPP antibody. Nearly the same percentage of cells (46.7%) carried the CD33 marker (fig. 7b). At the end of a 48-hour culture, cytotoxicity of the cPiPP-curcumin conjugate was determined by PI staining. It was observed that the immunoconjugate killed 48% of cells (fig. 7c).

Another AML patient (R.D.), a 72-year-old male, presented with a history of fever, coughing and loss of weight and appetite for a period of 3.5 months. No organomegaly was observed. Laboratory investigations revealed the following: hemoglobin content of 3.6 g/dl, total leukocyte count of 1,800/μl, platelet count of 98,000/μl, peripheral blood smear and bone marrow aspirate revealed myeloblasts. Based on these findings, a diagnosis of AML M2 was made.

FACS analysis of cells showed nonsignificant expression (13.5%) of hCGβ on the cell surface. The CD13 marker was expressed on 52% of the cells and CD33 on 12%. The lethal effect of cPiPP-curcumin conjugate on these cells was investigated. After 48 h of incubation, 15–17% cells were killed as revealed by the MTT assay (fig. 8).

Discussion

The use of recombinant antibodies for therapy of cancers and other chronic disorders is well accepted and is on the increase. A large number of antibodies are in clinical use after obtaining the approval of drug regulatory

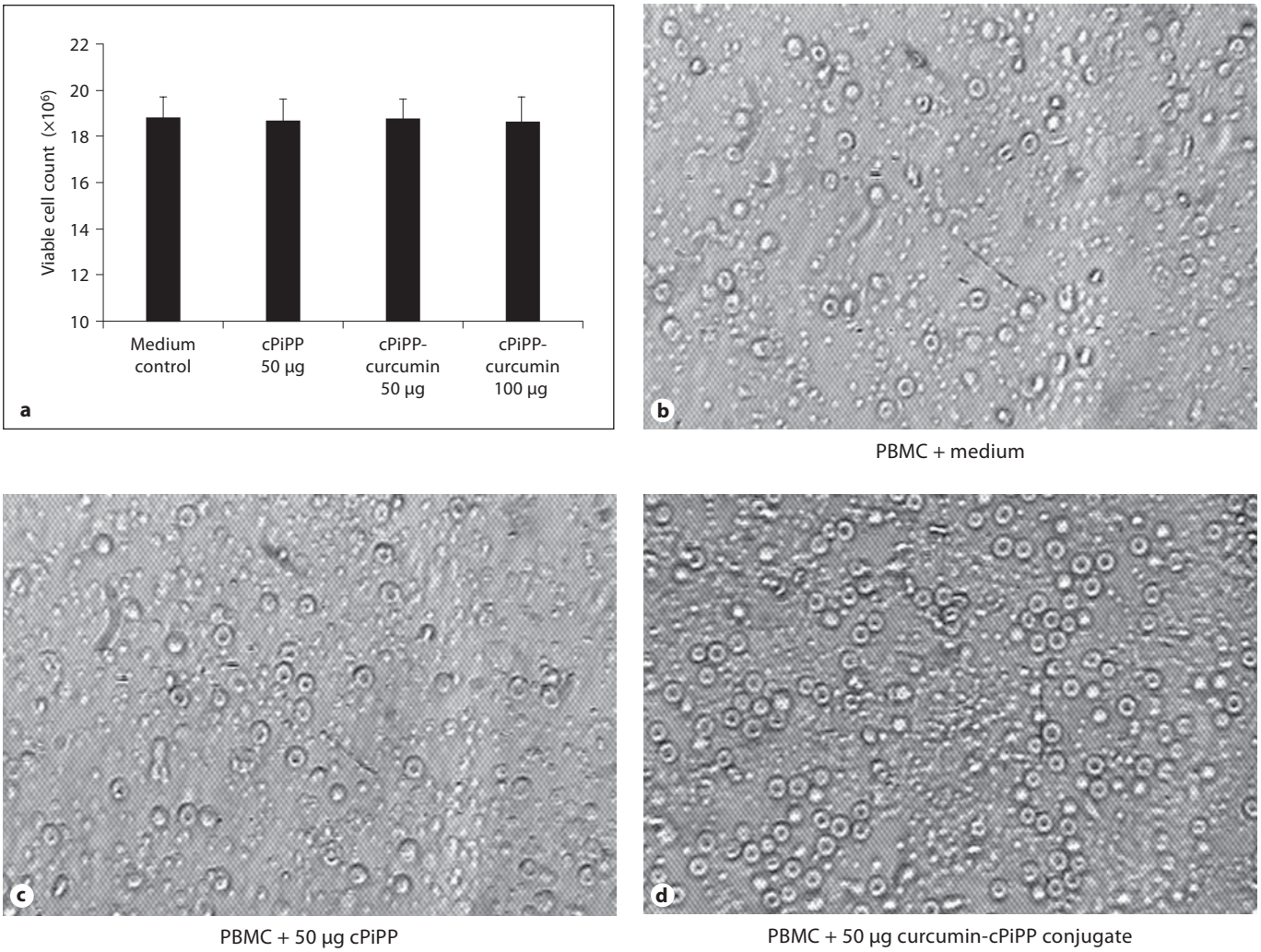
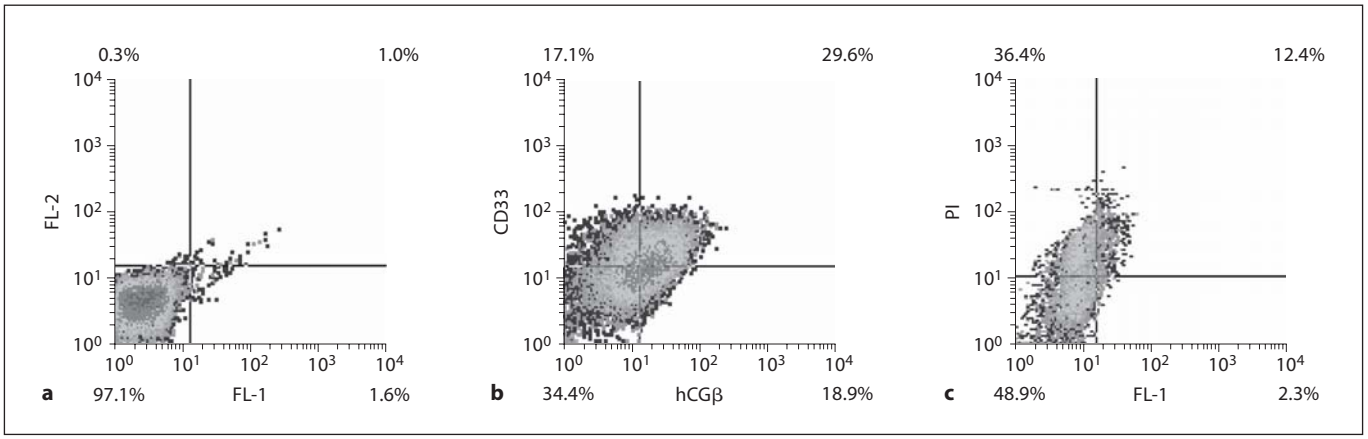
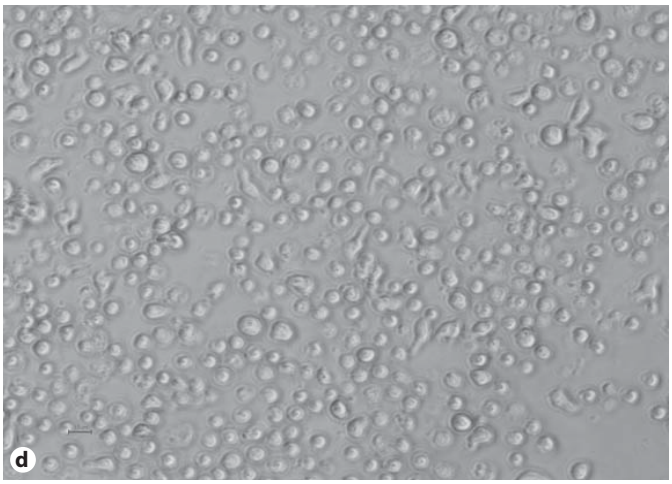


Fig. 6. Lack of cytotoxic action of cPiPP and cPiPP-curcumin conjugate on PBMC of normal healthy donors. **a** Viable cells counted by trypan blue exclusion assay after 48 h of exposure to 50 µg/ml of cPiPP or 50 and 100 µg/ml cPiPP-curcumin conjugate. **b** PBMC in RPMI 1640 medium. In medium with cPiPP antibody (**c**) and with cPiPP-curcumin conjugate (**d**). **b–d** ×400.

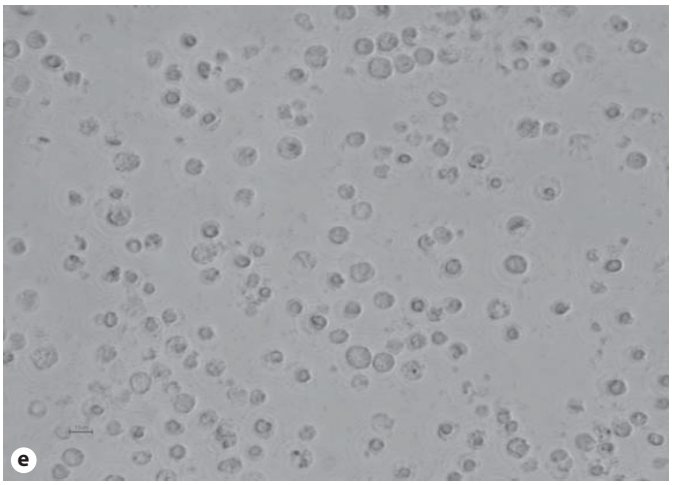
bodies [29]. Many are against cytokines such as anti-TNF-α, IL-5 and cytokine receptors like IL2Rα (Tac or CD25) or against growth factors like vascular endothelial growth factor or their receptors, for example, epidermal growth factor receptors. These target molecules are no doubt overexpressed on cancerous cells, but they are not unique to them and are also present on normal healthy cells. Thus, immunotherapy with recombinant antibodies against such molecules, though beneficial, is not totally devoid of side effects [30, 31].

Our choice of chimeric recombinant antibody, cPiPP, to target tumor cells expressing ectopically hCG or its subunits has the advantage of targeting a molecule expressed only in pregnancy or by tumors at an advanced stage. A large number of reports have appeared on the unexpected expression of hCG or its subunits by tumors of diverse origin [32] over and above the metastasis of trophoblastic carcinomas. Moreover, it is observed that the survival of patients with tumors expressing hCG is poorer than that of those not expressing hCG or its subunits. Thus, a therapeutic approach which targets selectively the tumor cells expressing hCG or its subunits without affecting the normal healthy cells could be of great utility.





Cells + cPiPP antibody

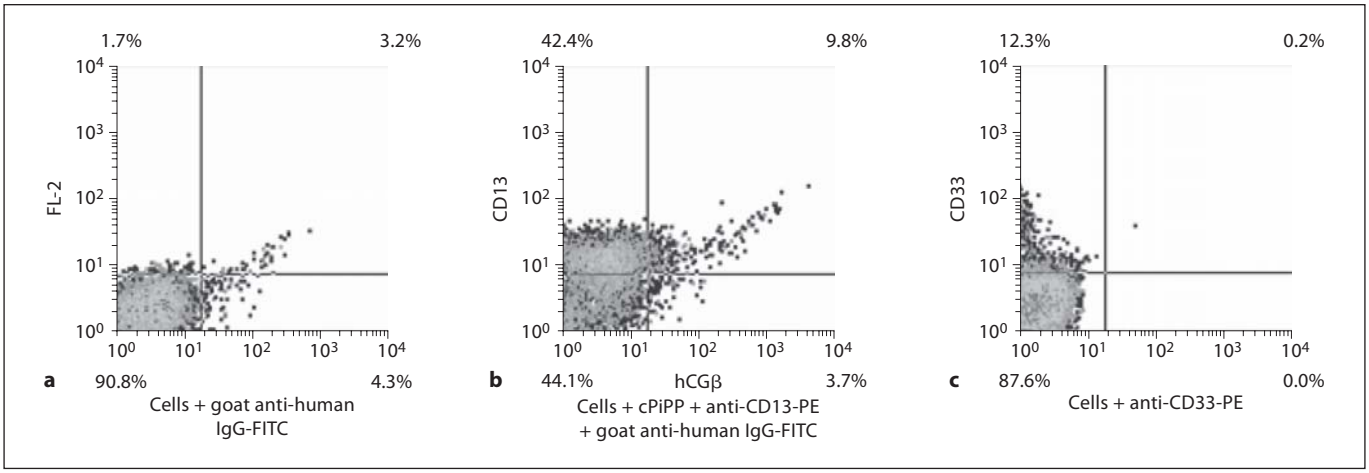


Cells + cPiPP-curcumin conjugate

Fig. 7. Binding with cPiPP and anti-CD33 antibodies and effect of cPiPP-curcumin conjugate on PBMCs of an AML patient (A.D.) as determined by PI stain by FACS. **a** Control with secondary antibody IgG (goat anti-human) linked to FITC. **b** Profile of cells incubated with both anti-CD33-PE and anti-hCG antibody cPiPP. **c** FACS obtained after incubation with 50 μg/ml of cPiPP-curcumin conjugate followed by staining with PI. Cells incubated with cPiPP (**d**) or with cPiPP-curcumin conjugate (**e**), respectively.

Initial work was undertaken on two cell lines, MOLT-4, a T-lymphoblastic leukemia cell line derived from a patient in relapse and U-937, a histiocytic lymphoma cell line. Both cells express β-hCG/hCG on membranes and have the ability to bind to cPiPP, an antibody of high affinity and specificity for hCG [23]. cPiPP did not cause the lysis of MOLT-4 cells in presence of complement or inhibit the growth of these cells in culture. It cannot be ruled out that the antibody is cytotoxic in these cells in vivo by ADCC, a modus observed for antibodies such as mouse-anti-Ep-CAM, anti-CD52 (alemtuzumab), anti-HER2/neu (trastuzumab), or chimeric anti-CD20 (rituximab) [33].

To benefit from the selective binding of cPiPP to the tumor cells (and not to healthy cells of the same lineage) (fig. 2b), we considered delivery to tumor cells of an anticancerous compound diferuloylmethane (curcumin) linked to the antibody. Curcumin is a nontoxic compound with anti-inflammatory properties [34–36]. It blocks the cancer pathway by downregulating the NFκB activation pathway [37], and suppression of IκBα kinase and Akt activation [38]. In phase I human safety trials, curcumin up to 8 g/day had no perceptible side effects [36]. Another advantage of loading curcumin on the antibody was to render it hydrophilic. Curcumin itself is hardly soluble in aqueous medium. Oral intake of cur-



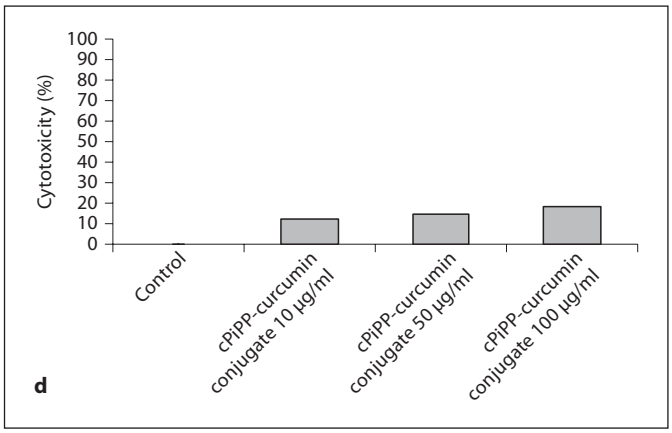


Fig. 8. Lack of cytotoxic action of cPiPP-curcumin conjugate on PBMCs bearing CD13 marker of an AML patient (R.D.). **a** Control. **b** FACS after incubation with anti-CD13-PE, anti-hCGβ antibody cPiPP. **c** With anti-CD33-PE antibody. 52.4% cells were expressing CD13 tumor marker. 13% cells were positive for hCGβ and 12.3% carried CD33 marker. **d** 15–17% cytotoxicity was observed after 48 h of incubation with cPiPP-curcumin at 50 and 100 μg/ml concentrations, respectively, by MTT assay.

cumin has low bioavailability [39–41] and low efficiency due to its random distribution in the body.

The cPiPP-curcumin conjugate killed nearly all tumor cells of the two types of tumor cells tested. This was determined by employing more than one method, for example, PI staining by FACS, MTT assay and trypan blue exclusion assay. Phase contrast microscopy of cells cultured with increasing concentration of the immunoconjugate confirmed evidence of the lethal action of the cPiPP-curcumin on these cells. Interestingly, no such untoward effect of the cPiPP-curcumin conjugate was noted on the PBMC of normal healthy donors (fig. 6).

Hydrophobic curcumin is not expected to be present in the free state in the purified antibody-curcumin conjugate. Curcumin suspended in PBS was tested for possible cytotoxicity at and above the molar concentrations of curcumin present in the conjugate. No such cytotoxicity was observed. On the other hand, curcumin dissolved in a subtoxic concentration of DMSO was cytotoxic (fig. 4).

The findings on the MOLT-4 and U-937 suggested that it should be checked in actual leukemia patients whether there were cells in circulation expressing hCGβ and, furthermore, whether the antibody linked to curcumin had any cytotoxic effect on these cells. In an ongoing study, 14 AML patients were examined, 7 of whom had a fair number of cells immunoreactive with anti-hCGβ antibody and 7 others had a low or negligible number of such cells in circulation. Data in figures 7 and 8 show that the cells killed in culture by anti-hCGβ-curcumin were hCGβ-positive also with the CD33 marker in patient A.D. This patient was diagnosed with AML M3 subtype. Another patient (R.D.) diagnosed with AML M2 had about 13% of hCGβ-positive cells, nearly equivalent to those which had the CD33 marker and those killed with cPiPP-curcumin conjugate were also of the same order. The bulk of tumor cells (52%) in this patient had the CD13 marker which was apparently negative for hCGβ; they were spared by the immunoconjugate.

The selective lethal action of cPiPP-curcumin conjugate on hCGβ-positive T-lymphoblastic leukemia MOLT-4 and U-937 histiocytic lymphoma cells as well as on cells expressing hCGβ and CD33 of AML patients suggests the potential utility of this conjugate for immunotherapy of hCGβ-expressing tumor cells, for which further studies are indicated.

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