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ORIGINAL ARTICLE

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Anti-(epidermal growth factor) receptor monoclonal antibodies for the induction of antibody-dependent cell-mediated cytotoxicity against squamous cell carcinoma lines of the head and neck

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Abstract Squamous cell carcinomas of the head and neck (SCCHN) frequently display high levels of the epidermal growth factor receptor (EGFR). Since EGFR is expressed on the cell surface it may form a suitable target for anticancer therapy with anti-receptor monoclonal antibodies (mAb). Besides the interference with receptor/ligand interactions, binding of mAb to EGFR leads to immunoglobulin-coated tumour cells that may induce or enhance non-specific immune effector mechanisms like antibody-dependent cell-mediated cytotoxicity (ADCC). In established cell lines of SCCHN (UM-SCC 11B, 14C, 22B, and 8029 NA) we investigated the antitumour activity of allogeneic peripheral blood mononuclear cells (PBMC) in combination with rat (ICR 62), mouse (EMD 55900), and humanized (EMD 72000) anti-EGFR mAb. In addition, autologous PBMC were available for tumour line UD-SCC 4. The EGFR protein content of the tumour cell lines ranged between 170 fmol/mg protein and 8100 fmol/mg protein, and MCF-7 cells served as receptor-negative controls. PBMC activity against SCCHN targets was determined in 96-well microtitre-plate monolayer cultures by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay after coincubation for 4 h, 24 h and 72 h at effector target ratios of 1:1, 5:1, 10:1 and 20:1. PBMC subpopulations were obtained by macrophage depletion (plastic adherence) or natural killer (NK) cell enrichment (magnetic bead negative selection). Prolonged time of exposure and increased effector:target ratios revealed marked antitumour activity of PBMC alone. This non-specific immune destruction was enhanced considerably by humanized and rat, but not mouse anti-EGFR mAb. Increased EGFR protein in tumour cells partly correlated with an intensification of ADCC but was accompanied by decreased primary PBMC cytotoxicity. The utilization of PBMC subpopulations suggested a mainly NK-cell-mediated ADCC, which appeared to benefit directly or indirectly, e.g. via the secretion of cytokines, from other PBMC components. In conclusion, humanized (EMD 72000) and rat (ICR 62) anti-EGFR mAb were able to generate strong antitumour ADCC in target monolayers of SCCHN.

Key words Head and neck carcinoma monolayer · Epidermal growth factor receptor · Monoclonal antibodies · Antibody-dependent cell-mediated cytotoxicity

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Introduction

Modern protein chemistry and recombinant DNA technology have led to the isolation and characterization of a wide array of soluble polypeptide growth factors that are able to modulate growth and differentiation of virtually all cell types through binding to specific cell-surface receptors [28]. Receptor activation induces an intracellular signalling pathway that is formed by a chain of messenger proteins integrating upstream information that is passed on to downstream targets and effectors. Finally, signal transduction ends in the cell nucleus, where activation of transcription factors is responsible for altered gene expression. Since the process of carcinogenesis is associated with the unregulated expression of growth factors, growth factor receptors or components of their intracellular signalling pathways, these molecules are supposed to represent key elements for the determination of malignant behaviour [1, 11, 25].

One of the most carefully studied growth factor receptors is the epidermal growth factor receptor (EGFR). EGFR is a 170-kDa transmembrane phosphoglycoprotein that has been identified in nearly all adult tissues with the exception of haematopoetic cells. The cysteine-rich extracellular domain is responsible for the binding of ligands like epidermal growth factor (EGF), transforming growth factor α (TGFα) and others [2]. Extra- and intracellular portions of the receptor are connected through a single-pass membrane-spanning α helix, and the intracellular domain is

characterized by its intrinsic protein tyrosine kinase activity [27].

Overexpression of EGFR has been reported in a wide range of human tumours, including squamous cell carcinoma of the head and neck (SCCHN) [2, 13]. In addition, there is increasing evidence for autocrine, paracrine and/or juxtacrine stimulatory pathways in EGFR-expressing tumours [25, 29]. Therefore, EGFR may be a potential target for antitumour therapy in SCCHN and, because the receptor is located in the cell-surface membrane, monoclonal anti-EGFR antibodies appear to be suitable agents for the exploration of this approach [3, 14]. Several mouse and rat monoclonal antibodies (mAb) of different isotypes were raised against various epitopes on the external domain of the human EGFR. These antibodies have been shown to exert distinct biological activities in a number of in vitro and in vivo tumour models, including interference with tumour cell proliferation and differentiation [10, 17–20, 24, 30]. Besides inhibition of receptor/ligand interactions, anti-EGFR antibodies may also modulate antitumour effectors of the host's immune system by antibody-dependent cell-mediated cytotoxicity (ADCC). Natural killer (NK) cells have been identified to be the major lymphocyte population mediating ADCC through recognition of the carboxyl-terminal end of antibody molecules via the low-affinity receptor for IgG, FcγRIIIA/CD16 [31, 32]. Hence, NK cells are able to enter into close interaction with antibody-coated tumour cells, and target destruction is considered to be the result of both necrosis and apoptosis [8, 21, 33].

The objective of the present study was to determine the effects of mouse, humanized and rat anti-EGFR mAb on ADCC with human peripheral blood mononuclear cells (PBMC) in monolayers of established cell lines of SCCHN. Using low-, medium-, and high-EGFR-expressing carcinomas, we determined the antitumour activity of PBMC alone and in combination with mAb at different assay times. In one cell line, we were also able to compare allogeneic and autologous PBMC. Furthermore, the principal immune reactive subset of PBMC was identified.

Materials and methods

Human tumour cell lines

Squamous cell carcinoma lines UM-SCC 11B, UM-SCC 14C and UM-SCC 22B were donated by Dr. T. E. Carey, University of Michigan, Mich., USA. UM-SCC 11B was established from a larynx carcinoma, UM-SCC 14C from the local recurrence of a carcinoma of the floor of mouth, and UM-SCC 22B originated from the lymph node metastasis of a hypopharynx carcinoma [6]. 8029 NA is a recloned population of cell line HLac 79, which had been derived from the neck metastasis of a larynx carcinoma [36], and was a gift from Dr. H.-P. Zenner, University of Tübingen, Germany. Cell line UD-SCC 4 was established in our own laboratory from a squamous cell carcinoma of the oral cavity. EGFR-negative MCF-7 breast cancer cells were kindly provided by Dr. U. Koldovsky, University of Düsseldorf, Germany. All cells were grown as monolayers in plastic culture flasks (Greiner, Solingen, Germany) under standard conditions (37 °C, 5% CO_2 in a fully humidified atmosphere) using RPMI-1640 medium supplemented with 10% (v/v) heat-inactivated fetal calf serum (both Gibco, Eggenstein, Germany), 2 mM L-glutamine, 50 IU/ml penicillin, and 50 µg/ml streptomycin (all ICN, Amsterdam, The Netherlands). To transfer or passage the cell lines, confluent monolayers were detached with 0.05% trypsin/0.02% EDTA solution (Boehringer, Mannheim, Germany). Subsequently, cells were washed twice in medium and resuspended in 96-well flat-bottom microtitre plates (Becton Dickinson, Heidelberg, Germany) or culture flasks.

Determination of EGFR protein

EGFR protein was determined with a quantitative sandwich-type immunoassay using mouse monoclonal capture antibody specific for the extracellular human receptor domain and biotinylated goat monoclonal detector antibody (Oncogene Science, Uniondale, USA). Briefly, on day 6 after subcultivation, log-phase tumour cells growing in complete culture medium were harvested mechanically, resuspended in buffer containing protease inhibitors (5 mM EDTA, 0.5 µg/ml leupeptin, 1.0 µg/ml pepstatin, 0.2 mM phenylmethylsulphonyl fluoride in 50 mM TRIS/HC1 at pH 7.4), and lysed with Triton X-100 (Sigma, Munich, Germany). EGFR of the cell lysates was bound to immobilized capture antibody in 96-well microtitre plates and exposed to detector antibody, which was linked with horseradish-peroxidase-conjugated streptavidin catalysing the conversion of chromogenic tetramethylbenzidine. Absorbance (450/595 nm) was determined with a microplate reader (Ear 400 ATX, SLT-Labinstruments, Crailsheim, Germany). For quantification, a standard curve was set up with known EGFR protein concentrations, and the amount of total protein in cell lysates was measured with the Bio-Rad protein assay (Bio-Rad Laboratories, Munich, Germany). Determination of EGFR protein/mg total protein was performed in three separate experiments in all cell lines except UD-SCC 4 (single determination), and MCF-7 cells served as the receptor-negative control.

Monoclonal anti-EGFR antibodies

Production and biochemical characterization of murine EMD 55900 (Merck KGaA, Darmstadt, Germany), originally developed as mAb 425 at the Wistar Institute, Philadelphia, USA, have been described elsewhere [20]. EMD 55900 is of the IgG2a isotype and binds to a protein determinant on the external domain of the human EGFR, inhibits binding of EGF and TGFα, antagonizes ligand-triggered receptor kinase activity and mitogenesis, does not stimulate EGFR kinase activity on its own, and induces down-regulation of EGFR [3, 24]. EMD 72000 (Merck KGaA) is a reshaped humanized IgG1 version of EMD 55900. Rat anti-EGFR monoclonal antibody ICR 62 has been produced and characterized by Drs. H. Modjtahedi and C. Dean, Cancer Research Campaign, Sutton, England [17]. The IgG2b antibody ICR 62 is also directed to the extracellular domain of human EGFR, and has been found to effectively block the binding of EGF, TGFα, and heparin-binding EGF to the receptor.

Preparation of effector cells

PBMC were obtained by Ficoll-Hypaque density gradient centrifugation (ICN Biomedicals, Meckenheim, Germany) from freshly harvested venous blood of healthy volunteers and the patient from whom tumour line UD-SCC 4 had been established. Complete PBMC fractions were employed in the majority of experiments, whereas subpopulations of PBMC helped to identify relevant effector cells. Monocyte-rich PBMC were separated by adherence to plastic culture flasks (45 min at 37 °C) and subsequent detachment [22]. Accordingly, non-adherent cells represented a monocyte-depleted fraction of PBMC.

For NK cell enrichment, non-NK cells in PBMC were labelled with a cocktail of hapten-modified anti-CD3, -CD4, -CD19 and -CD33 IgG1 mouse monoclonal antibodies (Miltenyi Biotec, Bergisch Gladbach, Germany). In a second reaction, these antibodies were magnetically labelled with an anti-hapten antibody microbead, and NK cells were obtained by single negative immunoselection with a magnetic bead separator (Separation Column AS, VarioMACS, Miltenyi Biotech).

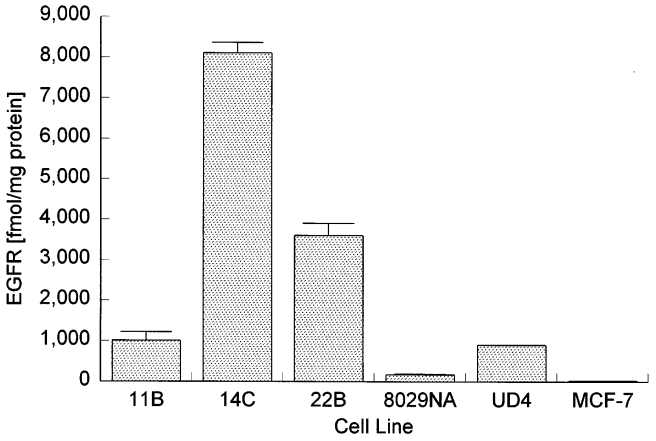

Fig. 1 Epidermal growth factor receptor (*EGFR*) protein expression in squamous cell carcinoma lines of the head and neck (mean ± SEM, $n = 3$, UD-SCC 4: $n = 1$)

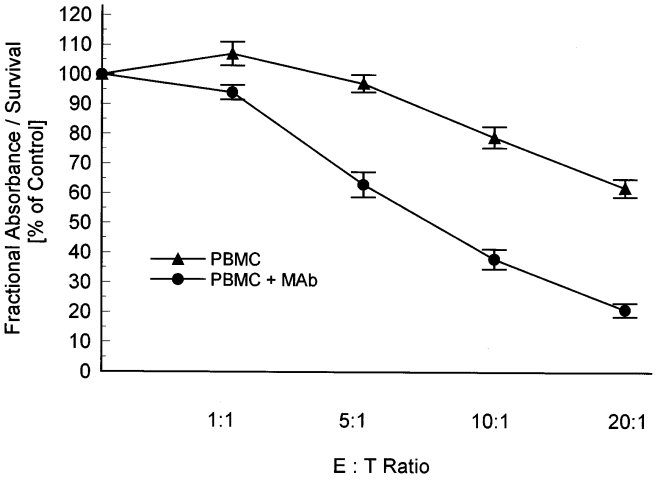

Fig. 2 Dependence of antibody-directed cell-mediated cytotoxicity (ADCC) against UM-SCC 14C target cell monolayers on the amount of peripheral blood mononuclear cells (*PBMC*) effectors. E:T ratios were 1:1, 5:1, 10:1, 20:1, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were performed ± mAb EMD 72000/ICR 62 after 72 h (mean ± SEM, $n = 15$, repeats = 6, CV ≤ 10%). At all E:T ratios, both mAb led to significantly enhanced PBMC activity (E:T = 1:1 $P < 0.005$; E:T = 5:1, 10:1, and 20:1 $P < 0.001$)

The purity of NK cell preparations was confirmed by flow cytometry (FACScan, Becton Dickinson, San Jose, USA), using fluorescein-isothiocyanate-conjugated anti-CD3 and phycoerythrin-conjugated anti-CD56 IgG1 mouse monoclonal antibodies (Immunotech, Hamburg, Germany). NK-cell-rich PBMC fractions contained between 79% and 96% $CD56^+$ $CD3^-$ cells. Prior to their use as effector cells, the viability of all PBMC preparations was tested by 0.04% trypan blue exclusion and always exceeded 95%.

Proliferation/cytotoxicity assay

On day 0, log-phase tumour cells harvested from culture flasks were resuspended in 96-well flat-bottom microtitre plates according to their growth characteristics at 2000 cells (UM-SCC 14C) or 6000 cells (UD-SCC 4, MCF-7, UM-SCC 11B and 22B) in 200 µl medium/well. After consolidation of monolayers, medium was replaced on day 3 by PBMC preparations at different effector:target ratios (E:T = 1:1, 5:1, 10:1, 20:1). A 10-ng sample of anti-EGFR mAb was added to each well since previous experiments had shown no antiproliferative effect in the present tumour panel at this concentration [10]. Controls remained untreated (200 µl medium) or received PBMC preparations only. Subsequently microcultures were incubated for 4 h, 24 h or 72 h, and control monolayers in medium alone almost reached confluence after 3 days. Non-adherent cells were removed from microtitre plates by vigorous pouring away of the supernatents and careful washing with phosphate-buffered saline (ICN), and proliferation/cytotoxicity was determined with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) colorimetric assay [4, 33]. The assay is based on the reduction of a non-toxic water-soluble yellow tetrazolium salt to a purple-coloured water-insoluble formazan precipitate by the reductive capacity of cytoplasmatic and mitochondrial dehydrogenases present only in living metabolically active cells. MTT solution was added to each well at 5 µl, and left to react for 4 h; after the complete dissolving of formazan precipitates in 150 µl dimethylsulfoxide (Sigma), absorbance was measured at 570 nm in a microplate reader (Ear 400 ATX). Wells with all components of the mixtures except cells served as blanks. In previous investigations, this experimental procedure has been identified to produce a good correlation between absorbance and the number of viable tumour cells [4, 10]. Therefore, cell survival as a percentage of the control (fractional absorbance) was calculated according to the formula:

$$\text{Fractional absorbance (survival)} = \frac{\text{absorbance test} - \text{absorbance blank}}{\text{absorbance control} - \text{absorbance}} \times 100$$

All MTT assays were performed as sixfold determinations, and data are mean values (x). Since the coefficient of variation (CV = standard deviation × 1/x × 100) of repeats never exceeded 10%, the presentation of SEM is omitted. Figures 1–3 show the results of several independent experiments as means ± SEM. The relationship between the effects of PBMC ± mAb was examined using the Wilcoxon signed-rank test. Differences with $P < 0.05$ were considered significant. Representative results obtained in single experiments, using one freshly harvested PBMC charge, are shown in Figs. 4–9.

Results

EGFR protein

In the tumour panel employed (Fig. 1), cell line UM-SCC 14C showed the highest amount of EGFR protein with 8100 fmol/mg total protein, followed by UM-SCC 22B exhibiting less than half as much receptor protein (3600 fmol/mg total protein). EGFR values for lines UM-SCC 11B (1000 fmol/mg total protein) and UD-SCC 4 (900 fmol/mg total protein) were similar, whereas 8029 NA cells had only 170 fmol/mg total protein. Receptor-negative MCF-7 cells served as the control (20 fmol/mg total protein or less).

Dependence of ADCC against tumour cell monolayers on E:T ratio, exposure time, anti-EGFR mAb and EGFR expression

In all experiments, the increase of E:T ratios (1:1, 5:1, 10:1, 20:1) caused enhanced tumour cell killing. Results obtained in UM-SCC 14C cells after a 72-h coincubation with PBMC from different donors ± mAb EMD 72000/ICR 62 are shown in Fig. 2 ($n = 15$). Survival fractions for the

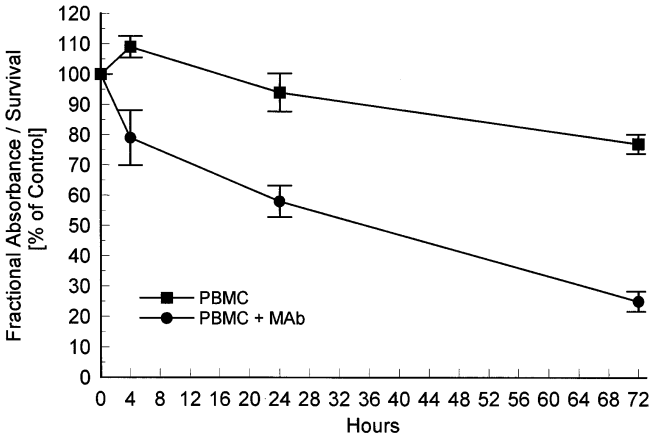

Fig. 3 Dependence of ADCC against UM-SCC 14C target cell monolayers on the exposure time to PBMC effectors. MTT assays were performed ± mAb EMD 72000/ICR 62 after 4 h, 24 h, and 72 h at E:T ratio 20:1 (mean ± SEM, n = 5, repeats=6, CV≤10%). After 24 h and 72 h, differences between PBMC activities ± mAb were significant ($P < 0.05$)

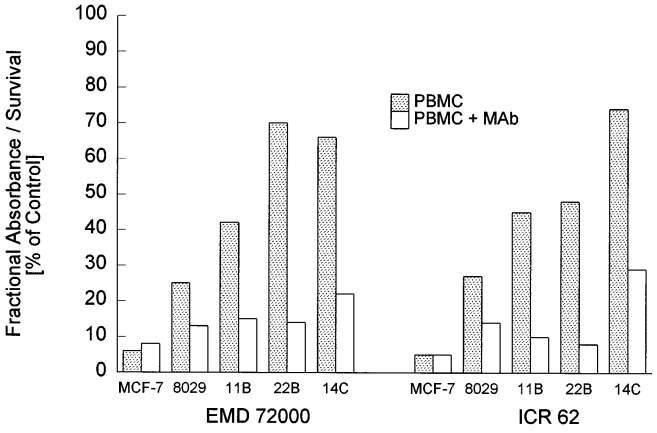

Fig. 5 Dependence of ADCC against tumour target cell monolayers on the EGFR expression. EGFR protein content of the cell lines used was less than 20 fmol (MCF-7), 170 fmol (8029 NA), 1000 fmol (UM-SCC 11B), 3600 fmol (UM-SC 22B), and 8100 fmol (UM-SCC 14C)/ mg protein. MTT assays were performed ± mAb EMD 72000/ICR 62 after 72 h at an E:T ratio of 20:1 (repeats = 6, CV≤10%)

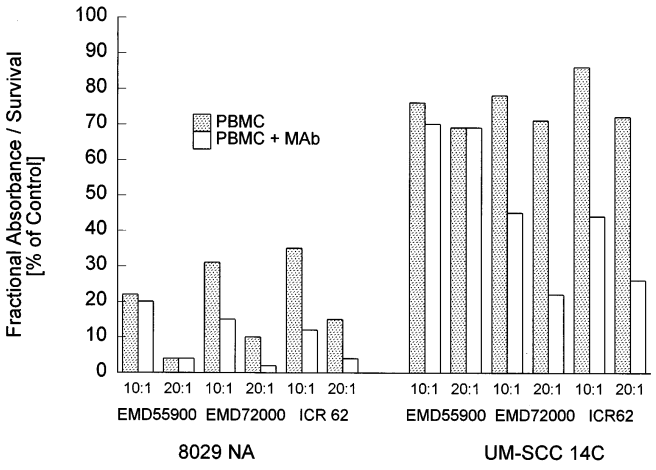

Fig. 4 Dependence of ADCC against 8029 NA and UM-SCC 14C target cell monolayers on the type of anti-EGFR mAb. mAb employed were mouse EMD 55900, humanized EMD 72000 and rat ICR 62. MTT assays were performed after 72 h at E:T ratios 10:1 and 20:1 (repeats = 6, CV≤10%)

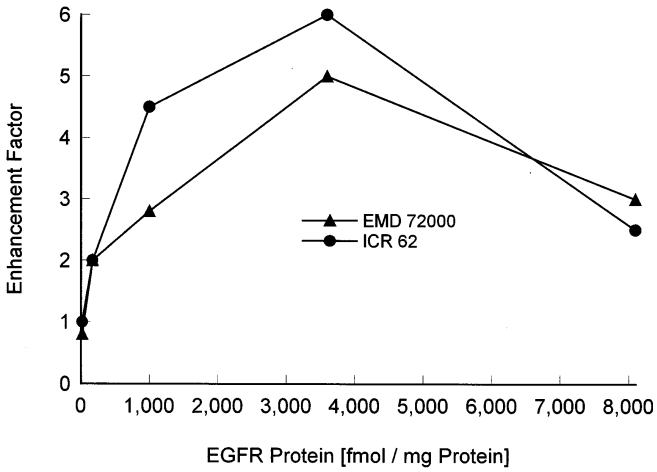

Fig. 6 Correlation between EGFR protein content of tumour target cells and anti-EGFR-mAb-mediated enhancement of PBMC cytotoxicity. Enhancement factors were calculated from the results shown in Fig. 5 according to the formula: survival fraction PBMC alone/survival fraction PBMC+mAb

respective E:T ratios were 107%/97%/79%/62% (–mAb), and 94%/63%/38%/21% (+mAb). At all E:T ratios, both humanized and rat mAb led to a significant enhancement of effector cell activity (E:T = 1:1 $P < 0.005$; E:T = 5:1, 10:1, 20:1 $P < 0.001$).

Prolonged interaction between tumour cells, PBMC and mAb led to increased ADCC. Using different PBMC charges in combination with mAb EMD 72000/ICR 62 in tumour line UM-SCC 14C (n = 5), survival fractions (E:T = 20:1) dropped from 79% after 4 h and 58% after 24 h to 25% after 72 h (Fig. 3). Again, the differences between PBMC ± mAb to bring about tumour cell destruction after 24 h and 72 h were significant ($P < 0.05$).

When different anti-EGFR mAb were tested in tumour target cells with low (8029 NA) and high (UM-SCC 14C) receptor expression, humanized mAb EMD 72000 and rat mAb ICR 62 proved to be potent inducers of ADCC (Fig. 4). Enhancement factors (survival fraction PBMC alone/survival fraction PBMC+mAb) for E:T = 20:1 were 5/3.75 (8029 NA) and 3.2/2.8 (UM-SCC 14C). On the other hand, no enhancement of PBMC cytotoxicity occurred with murine mAb EMD 55900 (factor 1 in both cell lines).

The impact of EGFR expression on tumour cell susceptibility to ADCC was investigated for antibodies EMD 72000 and ICR 62 (Fig. 5). In the present tumour panel covering a wide range of EGFR protein concentrations, both mAb led to comparable enhancement of antitumour PBMC activity (E:T = 20:1, MTT after 72 h). Enhancement factors (survival fraction PBMC alone/survival fraction PBMC+mAb) for mAb EMD 72000/ICR 62 were: 2/2

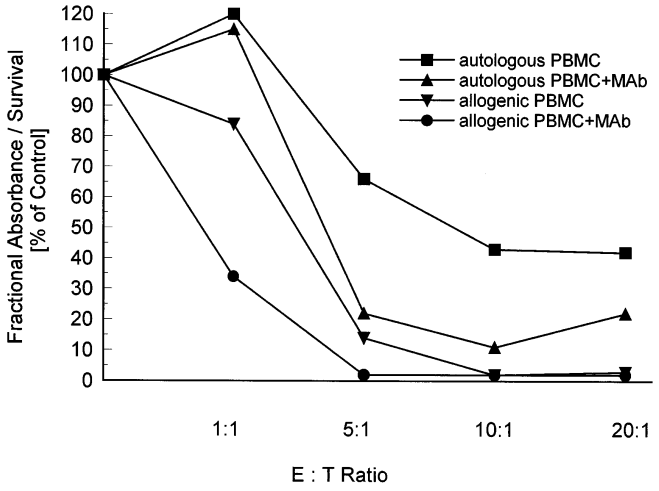

Fig. 7 Dependence of ADCC against UD-SCC 4 target cell monolayers on the utilization of autologous and allogeneic PBMC. MTT assays were performed ± mAb EMD 72000 after 72 h (repeats = 6, CV≤10%)

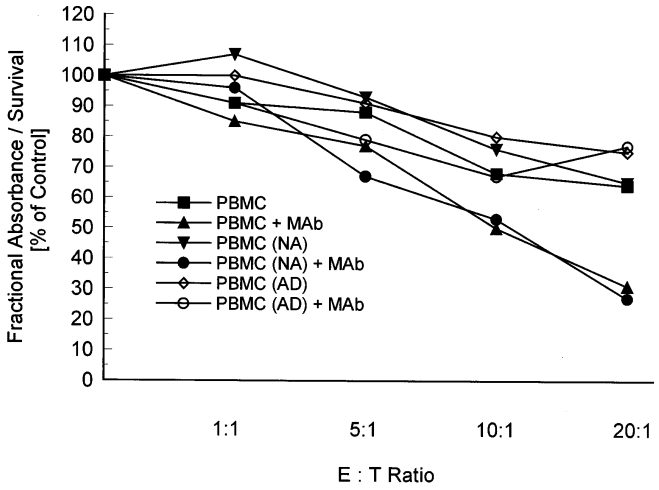

Fig. 8 Dependence of ADCC against UM-SCC 14C target cell monolayers on plastic-adherent macrophages. Effector cells were complete PBMC, non-adherent PBMC (*NA*), and adherent PBMC (*AD*). MTT assays were performed ± mAb ICR 62 after 72 h (repeats = 6, CV≤10%)

(8029 NA), 2.8/4.5 (UM-SCC 11B), 5/6 (UM-SCC 22B), and 3/2.5 (UM-SCC 14C) Anti-EGFR mAb had no effect in receptor-negative MCF-7 cells (enhancement factors: 0.8/1). Figure 6 shows the correlation between the EGFR protein content of tumour target cells and enhancement factor. Increasing amounts of EGFR helped to intensify ADCC; however, very high receptor expression in UM-SCC 14C target cells was accompanied by only average mAb-dependent enhancement of cytotoxicity. Furthermore, low (8029 NA) or absent (MCF-7) EGFR expression appeared to be associated with considerably higher primary (i.e. not mAb-dependent) susceptibility to PBMC cytotoxicity (Fig. 5).

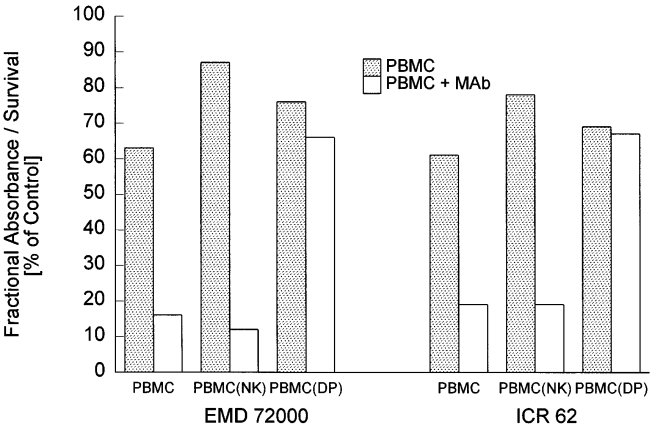

Fig. 9 Dependence of ADCC against UM-SCC 14C target cell monolayers on NK cells. Effector cells were complete PBMC, NK cells isolated from PBMC (*NK*), and NK-cell-depleted PBMC (*DP*). MTT assays were performed ± mAb EMD 72000/ICR 62 after 72 h at E:T ratio 20:1 (repeats = 6, CV≤10%)

Dependence of ADCC against tumour cell monolayers on source and subpopulation of PBMC

ADCC was tested with autologous and allogeneic effector cells in tumour line UD-SCC 4 (Fig. 7). Although considerable enhancement of autologous PBMC cytotoxicity was obtained with mAb EMD 72000, allogeneic effectors showed superior activity. When PBMC of cancer patient UD-SCC 4 were employed against other allogeneic tumour targets, again they exhibited less cytotoxic activity than did effector cells obtained from healthy volunteers (data not shown). PBMC fractions either depleted or enriched in monocytes by plastic adherence were used against UM-SCC 14C cells in combination with either mAb EMD 72000 (data not shown) or ICR 62 (Fig. 8). ADCC was equally effective for complete and non-adherent PBMC, whereas monocyte-rich effectors exerted only weak anti-tumour cytotoxicity, which was hardly enhanced by mAb.

NK cell preparations and NK-cell-depleted fractions of PBMC were also tested against UM-SCC 14C targets (Fig. 9). NK cells mediated ADCC with mAb EMD 72000 and ICR 62 to the same degree as did complete PBMC. NK-cell-depleted PBMC showed similar primary cytotoxic activity; however, no mAb-dependent enhancement was observed.

Discussion

Both frequency and a high level of expression of the EGFR in SCCHN and the important role of the receptor in signal transduction make it potentially an excellent target for antibody-directed therapy. Using in vitro and in vivo models, several reports have described antitumour activity of anti-EGFR mAb alone or in combination with antineoplastic drugs [10, 17–19, 30]. These effects are mainly attributed to the interference with ligand/receptor interac-

tions resulting in tumour cell deprivation of proliferative stimuli or survival signals, and induction of terminal differentiation. In addition, anti-EGFR-mAb-coated tumour cells should recruit and activate cytotoxic host effector cells and, relating to this mechanism, ADCC is believed to play an important role in cancer immunotherapy with unconjugated antibody [31, 32].

In the present paper, we have investigated ADCC with human PBMC in target cell monolayers of established SCCHN lines employing murine (EMD 55900), humanized (EMD 72000) and rat (ICR 62) anti-EGFR mAb. Humanized IgG1 and rat IgG2b antibody proved to be equally potent inducers of ADCC, whereas murine EMD 55900 was not able to enhance PBMC cytotoxicity. This result is in accordance with other reports on the poor activation of human immune effector functions by mouse antibodies as compared to chimeric or rat antibodies [9, 17, 32]. ADCC is also known to be dependent on the antibody isotype, and therefore consideration of optimal antibody characteristics gains major relevance in mAb immunotherapy [17].

Considering the initial clinical data, we were particularly interested in the consequence of prolonged exposure of tumour cells to mAb and PBMC on ADCC; a single intravenous administration of murine mAb EMD 55900 in patients with advanced squamous cell carcinomas of the larynx or hypopharynx led to complete and homogeneous occupation of cell-surface EGFR in primary and metastatic lesions for at least 3 days [2]. We therefore measured ADCC at different times up to 72 h. Since the chromium-release assay (the classical in vitro test for ADCC) allows short-term experiments only, we modified the reliable colorimetric MTT assay for long-term assessment of ADCC. Recently the MTT assay has been successfully introduced for the determination of ADCC by Sung et al. [33]. They were able to demonstrate that apoptosis appears to be the major mechanism of cell death in target cell monolayers, while membrane-mediated lysis predominates in 4-h chromium-release assays in cell suspensions. In our experiments, 72 h cocultivation of tumour cell monolayers and PBMC in the presence of anti-EGFR mAb resulted in remarkable enhancement of ADCC as compared to exposure for merely 4 h or 24 h. Furthermore, 72-h assays revealed considerable ADCC even at a very moderate E:T ratio of 10:1. Thus, the long-lasting retention of mAb by EGFR observed in vivo is a promising prerequisite for sufficient activation of ADCC.

Another purpose of this paper was to explore the dependence of ADCC on the EGFR expression in tumour target cells. The intensity of ADCC, as determined by mAb-induced enhancement of effector cell activity, improved from cells without EGFR expression (MCF-7) to cells expressing considerable levels of EGFR (UM-SCC 22B), while only moderate ADCC was obtained in the carcinoma line UM-SCC 14C bearing very high levels of receptors. On the other hand, there was a clear correlation between rise in receptor expression and increase of tumour cell resistance to primary PBMC cytotoxicity. One explanation for these observations could be that EGFR provides tumour cells with anti-apoptotic signals [15]. But since all the anti-EGFR mAb used interfere with functional ligand/receptor interactions, murine EMD 55900, although a poor activator of ADCC, should have produced at least some improvement of PBMC cytotoxicity through facilitation of apoptosis by blocking the receptor. If receptor activation is not required for protection from PBMC cytotoxicity, EGFR may positively or negatively interfere with recognition sites on target cells that are responsible for the inhibition or initiation of ADCC [7, 31]. Recent findings have emphasized the important role of clonally distributed NK cell receptors recognizing polymorphic MHC class I molecules for positive and negative regulation of NK cell cytotoxicity. Furthermore, several costimulatory and/or adhesion molecules have been identified to modulate NK cell activity [12]. However, no relationship between NK cell activity and growth factor receptor expression has been described so far.

As expected for MHC-unrestricted ADCC, both autologous and allogeneic effector cells showed increased cytotoxic activity in combination with anti-EGFR mAb. The impression that PBMC from tumour patients might be less active than those obtained from healthy donors needs to be confirmed in a larger series of experiments.

Several types of effector cell can mediate ADCC, including NK cells, monocytes, macrophages, activated T lymphocytes and granulocytes, all of which express various Fc receptors on the cell surface. In our experiments, NK cells separated from PBMC by negative immunoselection proved to be the best effectors for ADCC with humanized mAb EMD 72000 and rat mAb ICR 62. Although the number of these effectors was five- to tenfold increased in NK cell preparations as compared to the respective PBMC samples, both effector populations exerted similar activity against tumour cell monolayers. This observation may suggest an important role for non-NK cells in the induction or enhancement of ADCC, probably because of the release of promoting cytokines [23, 34].

In conclusion, our results emphasize the potential relevance of immunological effector mechanisms in the course of antitumour mAb therapy directed against growth factor receptors. Both humanized and rat anti-EGFR antibodies proved to be potent inducers primarily of NK-cell-mediated ADCC against monolayers of SCCHN. However, we have to realize that, in SCCHN, tumour-infiltrating lymphocytes consist of less than 5% NK cells, and they appear to have a functional impairment, the cause of which is unknown [5, 16, 26, 35].

Further preclinical experiments will focus on the relation between PBMC sensitivity and EGFR expression of tumour cells, and on the identification of factors that are able to improve ADCC. Currently, a clinical phase I trial with humanized anti-EGFR mAb in patients with advanced carcinomas of the larynx and hypopharynx is being performed. The data from these in vitro and in vivo experiments might help the design of novel strategies for the adjuvant treatment of SCCHN.

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