Biodistribution of Radiolabeled Monoclonal Antibody E48 IgG and F(ab')2 in Patients with Head and Neck Cancer

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ABSTRACT

Biodistribution and pharmacokinetics of radiolabeled mAb E48 IgG and E48 F(ab')2 were analyzed and compared in 39 patients with histologically proven squamous cell carcinoma of the head and neck who were included in a radioimmunoscintigraphy study and underwent surgery 44 h after injection. Three groups of patients were distinguished: group 1 (n = 19) received technetium-99m (99mTc)-labeled E48 F(ab')2, group 2 (n = 9) received 99mTc-labeled E48 IgG, and group 3 (n = 11) received 99mTc- and 131I-labeled E48 IgG as well as 125I-labeled F(ab')2. Two patients in group 1 and four patients in group 3 received a high mAb dose (10–50 mg), while all other patients received a low mAb dose (1–4 mg).

From all patients in groups 2 and 3 biopsies from the surgical specimen were obtained 44 h postinjection. Tumor uptake of 99mTc-labeled E48 IgG was high, ranging from 0.007 to 0.082% of the injected dose/g, with a mean of 0.031 ± 0.020% of the injected dose/g. The mean tumor: nontumor ratio of this conjugate was 2.8 for mucosa, 4.6 for bone marrow aspirate, 4.1 for blood, 20.3 for fat, and 21.0 for muscle. Activity uptake in tumor positive lymph nodes was 4.7 times higher as compared to negative lymph nodes.

Sixteen h postinjection radioimmunoscintigraphy revealed activity uptake in the primary tumor, lymph node metastases, oral cavity, and adrenal glands. Using regions of interest, the uptake in the adrenal glands was estimated to be 0.050% of the injected dose/g. If a high mAb dose was used, no adrenal glands were visualized and the uptake in the oral cavity was clearly diminished, while the tumor uptake and tumor:nontumor ratios were increased.

The mean elimination half-lives t1/2 α and t1/2 β in plasma were: for E48 IgG (n = 20) 6.6 ± 2.6 and 54.1 ± 24.3 h and for E48 F(ab')2 (n = 19) 2.3 ± 0.4 and 19.9 ± 4.6 h, respectively. Tumor uptake of 131I-labeled E48 IgG was 49% higher than of 125I-labeled F(ab')2. For most tissues except normal oral mucosa, tumor:nontumor ratios were slightly higher for F(ab')2 than for IgG.

The present study shows that mAb E48 accumulates selectively and to a high level in head and neck squamous cell carcinoma. Although no definite conclusions can be drawn as to which mAb form is more suitable, IgG or F(ab')2, mAb E48 seems to have potential for radioimmunotherapy in head and neck squamous cell carcinoma patients.

INTRODUCTION

Squamous cell carcinoma represents the vast majority of malignant tumors of the head and neck (1). These tumors account for approximately 5% of all malignant neoplasms in northwestern Europe and the United States (2). HNSCC grow locally invasive and have a proclivity to metastasize to regional lymph nodes in the neck rather than to spread hematogenously. Therapeutic management of early stage disease (stages I and II) consists of surgery or radiotherapy alone, whereas advanced stages (stages III and IV) are treated with a combination of these modalities. Such treatment results in a good locoregional control in the early stages. However, in patients with stages III and IV, the failure rate is high. Despite surgery and postoperative radiotherapy locoregional recurrence occurs in 50–60% of these patients. Moreover, 15–25% of these patients develop clinically manifest distant metastases (3). The actual incidence of distant metastases in HNSCC patients may even be much higher as autopsies studies reported on an incidence of 40–57% (4).

The high failure rate in advanced disease warrants the development of adjuvant systemic therapeutic modalities after surgery and radiotherapy. However, the high expectations as to chemotherapy have not become true, and its application is limited to the palliation of recurrent and metastatic disease (5–7). Therefore, development of a more effective adjuvant systemic therapy remains a major challenge in head and neck oncology.

Among other therapeutic approaches the use of mAb for selective delivery of radionuclides to residual disease seems to be promising (8, 9). This may be particularly true for tumors with a high intrinsic sensitivity for radiation, like hematological neoplasms and HNSCC (10). For this application we developed mAb E48 which shows selective reactivity with squamous epithelia and their malignant counterparts (11). The E48 antigen is a surface membrane-bound antigen of 22 kDa located on desmosomes and along the cell membrane. mAb E48 was shown to be highly capable for selective tumor targeting in animal models as well as in patients (12–14). Recently, we reported on the…

3 The abbreviations used are: HNSCC, head and neck squamous cell carcinoma; RIS, radioimmunoscintigraphy; RIT, radioimmunotherapy; p.i., postinjection; %ID/g, percentage of injected dose per g; ROI, regions of interest; ADCC, antibody-dependent cell cytotoxicity.

Received 9/27/94; accepted 11/2/94.

1 This work was supported by the Dutch Ministry of Economic Affairs and by Centocor Europe, Inc., Leiden, the Netherlands.

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scintigraphical detection of metastatic disease with mAb E48 IgG or F(ab')2 in patients with histologically proven HNSCC and with clinical evidence of cervical lymph node involvement (13, 14). In this study, preoperative findings on lymph node status obtained by RIS were compared to computerized tomography, magnetic resonance imaging, palpation, and finally with the histopathological outcome of the neck dissection specimen. Data revealed that RIS with mAb E48 is as good as the conventional diagnostic methods for the detection of lymph node metastases. Furthermore, these studies showed that E48 IgG and F(ab')2 are equally well suited for tumor detection. We observed an unwanted consistent accumulation of mAb E48 in the oral cavity and adrenal glands.

These data justified further evaluation of mAb E48 for its therapeutic applicability. Armed with 111In or 186Re, E48 IgG was shown to be highly capable of eradicating established human HNSCC xenografts in nude mice (15, 16). Complete ablation of small HNSCC was observed in this animal model by a single bolus injection of 186Re-labeled E48 IgG (17). Results obtained in this animal model, however, have to be interpreted with caution due to, for example, the absence of normal tissue expressing the particular antigen and the high tumor uptake of the mAb in these animals when compared to patients. Of paramount importance for ranking the suitability of a mAb for clinical RIT is its biodistribution in patients comprising the relative uptake in tumor tissues and normal tissues.

The purpose of the present study was to analyze and compare the biodistribution and pharmacokinetics of E48 IgG and F(ab')2 in 39 patients with HNSCC. The RIS results in 28 of these patients were published previously (14). Some patients (n = 11) received IgG and F(ab')2 simultaneously, using different radionuclides. As a bridging study to RIT some patients (n = 6) received a higher dose of mAb E48. We measured the absolute uptake in the primary tumor, lymph node metastasis, mucosa, and several other tissues by taking biopsies from the surgical specimen as well as bone marrow 44 h after injection of the radioimmunoconjugate. Moreover, the accumulation of activity in the oral cavity, tongue, and adrenal glands was estimated with regions of interest on planar images.

PATIENTS AND METHODS

Patient Study. The protocol was approved by the Dutch Health Council and by the Institutional Review Board of the Free University Hospital. Informed consent was obtained from all participants.

Thirty-nine patients with HNSCC participated in this study. Nineteen patients (patients 1–19, group 1) received injections of E48 (F(ab')2), 9 patients (patients 20–28, group 2) of E48 IgG, and 11 patients (patients 29–39, group 3) of E48 IgG and F(ab')2 simultaneously (E48 IgG/F(ab')2). Prior to enrollment a biopsy of the primary tumor had to show positive immunoperoxidase staining with E48 IgG. Patients of groups 1–3 suffered from carcinoma of the larynx [T3N0M0, classification according to the International Union Against Cancer (18)]. The antigen recognized by mAb E48 was found to be expressed by 94% of the primary head and neck tumors (n = 196). In 70% of these tumors the antigen was expressed by the majority of cells within these tumors. A comparable reactivity pattern was observed in 32 tumor-infiltrated lymph nodes from neck dissection specimens (19). Antibody reactivity with normal tissue is restricted to normal stratified squamous epithelium and urothelium of the bladder.

Antibody Preparation. The E48 IgG and F(ab')2 used in this study were supplied by Centocor, Inc. (Leiden, the Netherlands). E48 IgG was purified from a concentrated tissue culture supernatant by affinity chromatography on a protein A-Sepharose column. For virus inactivation, IgG from the protein A eluate was treated for at least 6 h with Tween 80 and tri-n-butyl phosphate. The protein A purified IgG was further purified on Q-Sepharose and subsequently digested to F(ab')2 by pepsin at pH 3.9. The F(ab')2 fragments were further purified by protein A chromatography to remove residual undigested IgG followed by elution over a S-Sepharose column. The purity of F(ab')2 preparations was evaluated by SDS-PAGE under nonreducing conditions and appeared to be more than 95%. This product was filtered through a 0.2-µm filter and dispensed aseptically in a closed environment under anaerobic conditions. The preparation was found to be pyrogenic free.

METHODS

All patients received 1–2 mg E48 IgG or F(ab')2 radiolabeled with 99mTc (mean dose, 722 ± 74 MBq and 722 ± 81 MBq, respectively) by i.v. injection in 5 min for imaging and biodistribution purposes. Two patients in group 1 (patients 18 and 19) were additionally injected i.v. with 10 mg unlabeled E48 F(ab')2 1 h before administration of the radiolabeled dose of E48 F(ab')2. Four patients in group 3 received additionally 10 mg (patient 36) or 50 mg (patients 37–39) unlabeled E48 IgG at the time of injection of the radiolabeled dose of E48 IgG. Patients in group III received 99mTc-labeled E48 IgG and additionally a low dose of 125I-labeled E48 IgG (2.5 ± 0.9 MBq) and 111In-labeled E48 F(ab')2 (2.5 ± 0.8 MBq) to compare the biodistribution of whole IgG and its F(ab')2 fragment in biopsies in the same patient. These patients received sodium perchlorate to prevent uptake of radioactive iodine in the thyroid gland. Patients in group 1 were operated on 2–5 days p.i. All patients in groups 2 and 3, receiving injections of E48 IgG or E48 IgG/F(ab')2, were operated on 44 h after injection, except for patient 39, who was operated on 9 days p.i. Trying to diminish the activity uptake in the adrenal glands by suppression of its endocrine function, 4 patients (patients 32–35) in group 3 received 4–8 mg dexamethasone p.o. 5 h prior to injection. In one patient this administration was repeated after 12 h.

Prior and up to 7 days after administration of 99mTc-labeled mAb E48, urine and blood samples were obtained for analysis. Electrolytes, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, γ-glutamyl transferase, lactate dehydrogenase, urea nitrogen, creatinine, and uric acid were determined in serum. Hematological determinations included hemoglobin, hematocrit, platelet count, WBC count and differential, and sedimentation rate. Skin tests were not performed. Vital signs were recorded before and up to 3 h after injection.

mAb E48. mAb E48 was derived from mice immunized with cells from a metastasis of a moderately differentiated squamous cell carcinoma of the larynx [T3N0M0, classification according to the International Union Against Cancer (18)]. The antigen recognized by mAb E48 was found to be expressed by 94% of the primary head and neck tumors (n = 196). In 70% of these tumors the antigen was expressed by the majority of cells within these tumors. A comparable reactivity pattern was observed in 32 tumor-infiltrated lymph nodes from neck dissection specimens (19). Antibody reactivity with normal tissue is restricted to normal stratified squamous epithelium and urothelium of the bladder.

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Preparation of Radiolabeled E48 IgG and F(ab')2. All radiolabeling procedures were performed under aseptic conditions in a shielded laminar flow hood. All glassware, plastics, and solutions were sterile and pyrogen free.

For labeling mAb E48 IgG or E48 F(ab')2 with 99mTc, a modification of the multistep procedure as described by Fritzberg et al. (20) was followed using a 5-benzoylmercapto-glucyglycylglycine chelator which was a gift from Mallinckrodt (Petten, the Netherlands). The purified E48 IgG and E48 F(ab')2 were labeled with a specific activity of 556 ± 168 MBq/mg and 641 ± 178 MBq/mg protein, respectively. A mean of 98.7 ± 0.7% and 98.2 ± 1.1% of the 99mTc was bound to E48 IgG and E48 F(ab')2, respectively, as determined by chromatography on ITLC-SG strips (Gelman Sciences, Ann Arbor, MI) with 0.1 M citrate buffer, pH 5.0. Every radiolabeled E48 IgG and E48 F(ab')2 preparation was assayed for immunoreactivity by measuring the binding to gluteraldehyde-fixed cells of the vulva SCC cell line A431 (21). As determined by modified Lineaweaver-Burk plot, the immunoreactive fractions of 99mTc-labeled E48 IgG and E48 F(ab')2 at infinite antigen excess were 79 ± 13% and 79 ± 10%, respectively.

Labeling of E48 IgG and E48 F(ab')2 with 131I and 125I for biodistribution measurements was carried out using a one-vial method as described by Haisma et al. (22). The purified E48 IgG and E48 F(ab')2 were 131I- and 125I-labeled with a specific activity of 3.6 ± 0.9 MBq/mg and 2.9 ± 1.0 MBq/mg protein, respectively. Using the same methods as described above, the mean 131I and 125I incorporation percentages were 97.2 ± 1.6% and 95.8 ± 2.0%, respectively. The immunoreactive fractions of 131I-labeled E48 IgG and 125I-labeled E48 F(ab')2 at infinite antigen excess always exceeded 70%. The affinity constants were 1.5 × 10^10 M^-1 for E48 IgG and 1.2 × 10^10 M^-1 for E48 F(ab')2 as determined by the Scatchard plot.

Radioimmunoaonography. The radioimmunoaonograms were obtained with a large field of view gamma camera (Gemini; General Electric, Milwaukie, WI) equipped with a low-energy parallel hole collimator and connected to a computer (Bartec, Farnborough, United Kingdom). Whole-body images (anterior and posterior views) and planar images of the head and neck (anterior views) were obtained immediately, 16 h, and 21 h after injection. Planar images included the following acquisition parameters: matrix size 256 × 256 (head and neck) or 256 × 256 (whole body) and at least 100 kilocounts/view during 5–20 min. Single-photon emission computerized tomography images of the head and neck were acquired 16 h p.i., while lateral views of the head and neck were obtained 21 h p.i.

For topographical evaluation the findings on RIS and the pathological outcome (the gold standard) were recorded and compared per side as well as per level according to the Memorial Sloan Kettering Classification (23) as previously illustrated (14).

Biodistribution. In all patients receiving injections of E48 IgG (group 2) or E48 IgG/F(ab')2 (group 3), biopsies of the primary tumor and several other tissues were taken from the surgical specimen. In these patients blood and bone marrow aspiration and biopsy were taken under general anesthesia just before surgery. All biopsies were weighed and the amounts of 99mTc, 125I, and 131I were measured by differential counting methods in a well counter (1282 Compugamma; LBK Wallac, Turku, Finland) to compare biodistribution of E48 IgG and E48 F(ab')2. The effect of self-absorption by volume effects was corrected by comparison of the sample with a set of reference samples, prepared by diluting an equal amount of the standard in different volumes of saline. All data were corrected for decay and converted to %ID/g tissue. Tumor:nontumor ratios were calculated using matched uptake values of one patient. If in a patient several biopsies of one kind of tissue were taken, the mean uptake in this tissue was calculated and used for further analysis. After counting, all biopsies were assessed histopathologically to determine the presence or absence of HNSCC.

ROI. To get information on the activity uptake at sites, which were not included in the surgical specimen, ROI were drawn on the planar views for all 19 E48 F(ab')2, and for 9 E48 IgG patients to obtain the 99mTc counts within these ROI. In the patients of group 3 (E48 IgG/F(ab')2) these measurements could not be performed because of the substantial contribution to the late images of the additionally injected 131I. ROI were drawn around the primary tumor, mouth, and tongue on the anterior view of the head and neck at 16 h p.i. Correction for background was done by measuring counts in a region as much as comparable to the chosen ROI (Fig. 1A). These corrected counts were compared to the counts of a standard obtained directly after the imaging of the head and neck.

From the posterior whole-body views at 0, 16, and 21 h p.i. counts in the whole body were corrected for background. ROI were drawn around the visible adrenal glands on these views at 16 h p.i. The region for background activity was chosen below the left kidney. The counts in the whole body and adrenal glands were compared to a standard located between the legs (Fig. 1B).

The counts were not corrected for attenuation and scatter. To allow for comparison of IgG and F(ab')2 uptake in the mouth, tongue, and adenral glands, the activity values were expressed as %ID after correction for decay and camera efficiency. For comparison of tumor uptake in different patients the %ID was divided by the surface area of the ROI.

Pharmacokinetics. Blood samples were obtained from the arm opposite to the injection site for the determination of the activity uptake at sites, which were not included in the surgical specimen. Aliquots of blood samples were measured for 99mTc, 131I, and 125I activity in a well counter, compared to an aliquot retained from the conjugate preparation, and corrected for decay. Blood activity was expressed as %ID/g. HPLC analysis of the serum samples up to 21 p.i. revealed that more than 95% of the radioactivity was bound to the mAb. The pharmacokinetics was analyzed modeling a time versus radioactivity curve for each infusion. A MW/Pharm program (MediWare, Groningen, the Netherlands) was used for nonlinear Bayesian estimation of pharmacokinetic parameters. One-, two-, and three-compartment models were fit to the data. The peeling algorithm was used to estimate initial parameters. A Bayesian least-square method was used to estimate the final parameters: the initial (t1/2 α) and final half-lives (t1/2 β).

Statistical Analysis. Student’s t test for paired and unpaired data was used to test the statistical significance of the difference between the uptake of E48 F(ab')2 and E48 IgG as assessed by biopsies and ROI. Statistical differences was reached at P < 0.05.
RESULTS

No adverse reactions were observed which could be related to the injection of the antibody and no significant changes were noted in blood and urine.

Radioimmunoscintigraphy. In 39 patients all 34 primary tumors were visualized. RIS was correct in 215 of 243 levels and 38 of 51 sides of the neck. The RIS results for the first 28 patients have been described previously (14). Whole-body images up to 21 h p.i. showed decreasing blood pool activity with visualization of liver, lungs, heart, spleen, kidneys, and nose. Blood pool activity was less pronounced for E48 F(\(\text{ab}'\))\(_2\) as compared to E48 IgG. Uptake of activity was also seen in the adrenal glands, mouth, scrotal area, and sometimes intestine and gallbladder at 16 and 21 h p.i. (Fig. 2), as described previously (14). In one patient given an injection of 1.2 mg E48 F(\(\text{ab}'\))\(_2\), one patient given 1.1 mg E48 IgG, and in four patients given 2–4 mg E48 IgG/F(\(\text{ab}'\))\(_2\), the adrenal glands were not clearly visualized. In the first patient who received 4 mg dexamethasone 5 h prior to injection no adrenal glands were visualized. However, in the next three patients the adrenal glands were clearly visualized, despite an increase of dexamethasone dose and repetition of administration. In three of these patients dexamethasone administration resulted in almost complete adrenal depression of its endocrine function (cortisol < 30 nmol/liter). In contrast, on additional administration of unlabeled mAb E48 (10 or 50 mg) in six patients, in five patients the adrenal glands were not visualized, while the mouth uptake also was less pronounced (Fig. 3).

Biodistribution. The activity uptake in biopsies from the surgical specimen of group 2 and 3 patients are shown in Fig. 4. The counts in biopsies 9 days p.i. (patient 39) were too low for a reliable evaluation. Uptake of \(\text{\(^{99m}\)Tc}\)-labeled E48 IgG in all patients of groups 2 and 3 was the highest in tumor tissue: 0.031 ± 0.020 (mean ± SD); range, 0.007–0.082%ID/g. High \(\text{\(^{99m}\)Tc}\) activity was also seen in normal mucosa (0.014 ± 0.007%ID/g) and tongue tissue (0.011 ± 0.002%ID/g), but was...
Fig. 2 Planar (A) anterior image of the head and neck and (B) posterior image of the whole body 16 h after injection of 2 mg $^{99m}$Tc-labeled E48 IgG. Note the intense uptake of activity in the primary tumor of the supraglottic larynx (arrow), the mouth, and the adrenal glands (arrowheads).

significantly ($P < 0.01$) lower than in tumor tissue. Tumor-positive lymph nodes contained significantly ($P < 0.02$) more $^{99m}$Tc activity than tumor-negative lymph nodes: $0.007 \pm 0.006\%$ID/g and $0.007 \pm 0.001\%$ID/g, respectively, with a mean ratio of $4.7 \pm 4.3$. Low activity was seen in bone marrow biopsies: $0.002 \pm 0.001\%$ID/g. Bone marrow aspiration showed a mean $^{99m}$Tc activity of $0.007 \pm 0.002\%$ID/g, while the activity in blood was slightly higher ($0.008 \pm 0.001\%$ID/g, mean ratio $0.9 \pm 0.1$). The activity in the bone marrow aspirate was mainly located in the plasma (supernatant). The mean plasma activity of the bone marrow aspirate and blood were similar ($0.013$ and $0.013\%$ID/g, respectively, mean ratio $1.0 \pm 0.1$). Mean tumor:nontumor ratios varied between $2.8$ for mucosa and $99.9$ for parotid gland tissue (Fig. 5). For muscle, fat, blood, and bone marrow aspirate these ratios were $21.0, 20.3, 4.1$, and $4.6$, respectively.

Mean uptake levels of $^{99m}$Tc-labeled E48 IgG was also calculated at low (1–4 mg) and high (12–51 mg) mAb dose separately. With the addition of 10–50 mg unlabeled E48 IgG tumor uptake increased significantly ($P < 0.05$) from $0.026 \pm 0.018\%$ID/g to $0.054 \pm 0.016\%$ID/g, whereas the uptake in normal mucosa and other tissues was not influenced ($P > 0.2$), resulting in higher tumor:nontumor ratios. The mean tumor:nontumor ratios increased for mucosa from $2.5$ to $3.7$, for muscle from $15.9$ to $46.7$, for fat from $17.2$ to $34.7$, for blood from $3.2$ to $7.6$, and for bone marrow aspirate from $4.2$ to $8.4$.

Simultaneous measurements of $^{131}$I-labeled E48 IgG and $^{125}$I-labeled E48 F(ab')$_2$ 44 h p.i. resulted in a mean tumor uptake of $0.028 \pm 0.019\%$ID/g and $0.019 \pm 0.011\%$ID/g ($P < 0.2$), respectively. The mean tumor:nontumor ratio of $^{131}$I-labeled E48 IgG was lower, however not significant, as compared to $^{125}$I-labeled E48 F(ab')$_2$: for muscle $18.1$ and $22.9$, for fat $18.0$ and $27.0$, for blood $4.3$ and $7.0$, and for bone marrow aspirate $3.3$ and $5.2$, respectively. An exception formed the normal mucosa in which the mean ratio of $^{131}$I-labeled E48 IgG was similar to $^{125}$I-labeled E48 F(ab')$_2$: $2.1$ and $1.9$, respectively. When additionally 10–50 mg unlabeled E48 IgG was administered also, the tumor uptake of $^{125}$I-labeled E48 F(ab')$_2$
increased significantly ($P < 0.05$) from $0.014 \pm 0.009$%ID/g ($n = 7$) to $0.030 \pm 0.009$%ID/g ($n = 3$).

ROI. Planar images obtained in 28 patients given injections of E48 F(ab')$_2$ or IgG (groups 1 and 2) were used for biodistribution estimations of sites, which were not included in the surgical specimens. In some patients the activity uptake in the tumor could not be assessed due to the lack of a suitable background region. Particularly, in patients with a tumor of the oral cavity no reliable ROI for the tumor, mouth, and tongue could be drawn. Although the adrenal glands in almost all patients given injections of 1–2 mg mAb E48 were visualized, in some patients a reliable ROI could not be drawn, due to overprojection of other abdominal structures.

Retention of activity in the whole body was significantly higher for $^{99m}$Tc-labeled E48 IgG as compared to $^{99m}$Tc-labeled E48 F(ab')$_2$; after 16 h the percentage of the whole-body activity directly after injection was $97.9 \pm 12.5$% and $89.1 \pm 6.0$% ($P < 0.05$), respectively (Table 1). With the ROI technique there was no significant difference in tumor uptake per area between E48 IgG and E48 F(ab')$_2$ at 16 h p.i. The uptake of E48 IgG in the mouth was almost half of the E48 F(ab')$_2$ uptake ($P < 0.1$). The uptake in the central area of the mouth, the tongue region, was also less for E48 IgG as compared to E48 F(ab')$_2$, but did not reach statistical significance. In the adrenal glands the uptake of E48 IgG was similar to E48 F(ab')$_2$. The uptake of E48 F(ab')$_2$ in the adrenal glands was lower if unlabeled E48 F(ab')$_2$ was given before, while the tumor uptake per area was similar (Table 1).

Pharmacokinetics. The time versus radioactivity curves of E48 IgG and F(ab')$_2$ best fitted a two-compartment model. Significant ($P < 0.001$) faster elimination from the blood was observed for $^{99m}$Tc-labeled E48 F(ab')$_2$, as compared to E48 IgG: at 1–2 mg, $t_{1/2\alpha}$ and $t_{1/2\beta}$ were for E48 F(ab')$_2$, 2.3 ± 0.4 and 19.9 ± 4.6 h and for E48 IgG 6.6 ± 2.6 and 54.1 ± 24.3 h, respectively. An increase in mAb dose did not influence the clearance from the blood: $t_{1/2\alpha}$ and $t_{1/2\beta}$ were for $^{99m}$Tc-labeled
E48 IgG at 10 mg 8.6 and 48.2 h and at 50 mg 7.3 ± 0.1 and 56.4 ± 5.9 h, respectively.

**DISCUSSION**

In earlier reports (13, 14) we demonstrated that ⁹⁹ᵐTc-labeled mAb E48 IgG or F(ab')₂, can be used for tumor detection with radioimmunoscintigraphic. In these studies mAb E48 was able to visualize all primary tumors (n = 31) as well as 68% of the tumor-involved lymph nodes. The missed nodes contained only a small amount of tumor cells. These findings indicate that mAb E48 may also be suitable for RIT, especially because HNSCC is known to be a radiosensitive tumor type. For RIT it is necessary that a mAb, after administration to the patient, accumulates selectively and to a high level in the tumor.

In the present study we compared the uptake of radiolabeled mAb E48 IgG and F(ab')₂ in HNSCC and some normal tissues 44 h after injection. The uptake of ⁹⁹ᵐTc-labeled E48 IgG in HNSCC was high (0.031 ± 0.020%ID/g), as compared to the uptake of other mAbs in other types of tumors. For instance, the mean uptake for AUA1 IgG and HMFG2 IgG in breast, ovarian, and gastrointestinal cancer 1 day p.i. was 0.015%ID/g (24), for OC 125 IgG in large ovarian cancer 2 days p.i. was 0.003%ID/g (25), for 81C6 IgG in intracranial cancers 29–77 h p.i. was 0.002%ID/g (26), for OKB7 IgG in non-Hodgkin’s lymphomas 3–5 days p.i. was 0.002%ID/g (27), for B72.3 IgG in colon cancer 4–14 days p.i. was 0.008%ID/g (28), for BW431 IgG in colorectal cancer 4–14 days p.i. was 0.008%ID/g (29), for G250 IgG in renal cell carcinoma 8 days p.i. was 0.014%ID/g (30), for chimeric MOv18 IgG in ovarian cancer 2 days p.i. was 0.009%ID/g (31), and for K928 IgG in HNSCC 2 days p.i. was 0.008%ID/g (19).

Biodistribution data on ⁹⁹ᵐTc-labeled mAb E48 IgG revealed that tumor-positive lymph nodes contained 4.7 times more activity than tumor-negative lymph nodes. The uptake in tumor-positive lymph nodes was much less than in primary tumors. However, this difference should be interpreted with caution, because the proportion of tumor cells present in the lymph nodes is difficult to assess and shows great variation.
some positive lymph nodes contain only a few tumor cells, whereas other nodes are totally occupied by tumor tissue. As known from extensive immunohistochemical screening, the mAb E48 defined antigen is also expressed in normal squamous epithelium like oral mucosa and skin (11). Despite the fact that the level of E48 antigen expression is apparently the same in these normal tissues as in HNSCC, 99mTc-labeled mAb E48 IgG uptake 44 h p.i. was significantly higher in HNSCC than in oral mucosa and tongue mucosa. Since biopsies show variance in amount of normal and malignant squamous cells and other cells, the exact extent of the difference in uptake levels is difficult to assess. Moreover, RIS studies did not reveal marked uptake of the mAb in the skin. The higher uptake of mAb E48 IgG in HNSCC may be due to the better accessibility of the E48 antigen in malignant tissue than in normal tissue. Penetration of mAbs into the tumor is facilitated by the presence of fenestrated endothelium and the absence of a basement membrane. On the other hand, antigens present in normal squamous epithelium are particularly poorly accessible for mAbs. In this tissue, mAbs must pass across the endothelial cells by intracytoplasmic vesicles and transendothelial cell channels (32).

An unexpected finding was the high uptake of activity in the adrenal glands. Using ROI in patients given injections of 1-2 mg 99mTc-labeled mAb E48 IgG, it is estimated that 0.3%ID was present in an adrenal gland 16 h p.i. Assuming that one adrenal gland weighs 6 g, the uptake in adrenal tissue is 0.050%ID/g, about 2 times higher than in tumor tissue 44 h p.i. Immunohistochemical evaluation showed no reactivity of E48 with five frozen adrenal tissues. Four other mAbs (323/A3 F(ab')2, chimeric SF-25 IgG, K928 IgG (19), and U36 IgG) labeled in the same manner as mAb E48 showed no uptake of activity in the adrenal glands. Therefore, the adrenal uptake does not seem to be related to the labeling technique used. The fact that no adrenal glands were visualized after administration of a higher mAb dose (10-50 mg) indicates that a limited number of good accessible binding sites may be present in the adrenal glands which become saturated after a higher dose. In contrast, administration of dexamethasone did not decrease the activity uptake. We recently cloned the E48 encoding cDNA which may open new avenues for analyzing E48 antigen expression in

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<tr>
<td>Whole body</td>
<td>%*</td>
<td></td>
<td>97.9</td>
<td>12.5</td>
<td></td>
<td>8*</td>
<td></td>
</tr>
<tr>
<td>Tumor</td>
<td>%ID/A</td>
<td>0.013</td>
<td>0.003</td>
<td>6*</td>
<td>0.013</td>
<td>0.006</td>
<td>9*</td>
</tr>
<tr>
<td>Mouth</td>
<td>%ID</td>
<td>0.131</td>
<td>0.023</td>
<td>3*</td>
<td>0.201</td>
<td>0.054</td>
<td>9*</td>
</tr>
<tr>
<td>Tongue</td>
<td>%ID</td>
<td>0.048</td>
<td>0.007</td>
<td>3*</td>
<td>0.067</td>
<td>0.023</td>
<td>9*</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>%ID</td>
<td>0.312</td>
<td>0.091</td>
<td>14*</td>
<td>0.301</td>
<td>0.195</td>
<td>23*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1-2 mg E48 F(ab')2</td>
<td></td>
<td>11-12 mg E48 F(ab')2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor</td>
<td>%ID/A</td>
<td>0.013</td>
<td>0.006</td>
<td>9*</td>
<td>0.013</td>
<td>0.013</td>
<td>2*</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>%ID</td>
<td>0.301</td>
<td>0.194</td>
<td>23*</td>
<td>0.037</td>
<td>0.013</td>
<td>2*</td>
</tr>
</tbody>
</table>

* Percentage of whole body activity at 0 h p.i.
* Number of patients.
* Percentage of the injected dose per pixel.
* Number of visible adrenal glands.

Adrenal tissue by Northern blotting and PCR-techniques. Since mAb doses of 10-50 mg are recommended for clinical RIT, uptake in the adrenal glands probably will not hamper RIT. A high mAb dose administration may provide even more advantages in comparison to low-dose administration as indicated by the significant higher tumor uptake of the radioimmunoconjugate and the probably higher tumor:nontumor ratios in patients receiving a high E48 IgG dose.

No selective accumulation of radioactivity was observed in any other normal tissue. For example, the activity in the bone marrow aspirate 44 h after administration of 99mTc-labeled mAb E48 was almost the same as in blood and 4.6 times less than in the tumor. Radioactivity in the bone marrow aspirate was not confined to the cellular compartment as became apparent on centrifugation. This is important since, in general, bone marrow is the dose-limiting organ in RIT.

One of the aims of this study was to analyze whether mAb E48 IgG or F(ab')2 is better suited for RIT. For this purpose we administered E48 IgG and F(ab')2 simultaneously, both in an radioiodinated form. This is important for a proper comparison since tissue uptake values may depend on the kind of radioiodinated conjugate used. In this study, tissue uptake levels of 131I-labeled E48 IgG were lower than those of coadministered 99mTc-labeled E48 IgG, probably due to the process of dehalogenation.

Unfortunately, no definite answer has been obtained as to which antibody form is better for RIT, IgG or F(ab')2. On one hand, tumor uptake levels were on average 49% higher for IgG than for F(ab')2. On the other hand, tumor:nontumor ratios were in general lower for IgG. However, the opposite was true for oral mucosa including the tongue, sites with relatively high activity uptake. The analysis of biodistribution on images obtained 16 h p.i., as well as measurement of biopsies from the surgical specimens obtained 44 h p.i. revealed relatively lower uptake in the mouth region for IgG in comparison to F(ab')2. Also other considerations might indicate the advantage of whole IgG. The use of IgG in RIT may provide an additional advantage because of its ability to mediate ADCC. As stated previously we

Manuscript in preparation.
intend to use $^{188}$Re-labeled chimeric mAb E48 for RIT of minimal residual disease. For this purpose we recently developed a technical protocol for labeling of mAbs with $^{188}$Re using the MAG$_3$ chelate (33). For the same purpose, we constructed a chimeric (mouse/human) mAb E48 containing the human $\gamma_1$ heavy chain. This chimeric mAb appeared to be highly effective in mediating ADCC even when loaded with 16 MAG$_3$ groups. When using this chimeric mAb for RIT of minimal residual disease it can be anticipated that ADCC activity may be supportive to irradiation, especially in eradicating single disseminated cells or small cell aggregates.

When combining the biodistribution data obtained herein with data obtained from previous animal studies, we might be able to speculate about the potential of $^{188}$Re-labeled E48 IgG for eradicating HNSCC in patients. As shown before (16), it is possible to eradicate 50% of large HNSCC xenograft-bearing nude mice by treatment with 18.5 MBq $^{188}$Re-labeled mAb E48 IgG. Because the mean maximum uptake in these tumors was assessed to be 10%ID/g, it can be calculated that the amount of $^{188}$Re in the xenografts was 1.85 MBq/g. As shown in the present study in patients the average uptake of mAb E48 IgG, 44 h p.i., in tumors of 0.5–6 cm was 0.031 ± 0.020%ID/g. Assuming that the maximum-tolerated radiation dose of $^{188}$Re-labeled E48 IgG is about 7400 MBq, the same as found for other $^{188}$Re-labeled mAbs (34), we expect that in clinical RIT studies with $^{188}$Re-labeled mAb E48 it will be possible to reach an average uptake level in the tumor of 2.26 MBq/g (0.031% of 7400 MBq/g). This is higher than the level obtained in xenografts responding to RIT. Taking into account the higher uptake by small tumors in comparison to large tumors (25), we think that sufficient high radiation doses can be achieved to eliminate minimal residual disease.

A main limitation of the aforementioned calculation is that no data are available on mAb E48 IgG tumor retention in HNSCC patients. Before starting clinical RIT studies we will obtain imaging and biodistribution data up to 7 days p.i. using $^{131}$I as imaging radionuclide instead of $^{99m}$Tc. The efficacy of adjuvant RIT with mAb E48 will be first evaluated in HNSCC patients who are at high risk for developing distant metastases and locoregional recurrences.

ACKNOWLEDGMENTS

We thank Drs. Hein Leverstein and Richard P. Wong Chung for clinical support, Henri Greuter for laboratory support, and Dr. Jacqueline E. van der Wal for pathological support.

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