Immunoscintigraphy of small-cell lung cancer xenografts with anti neural cell adhesion molecule monoclonal antibody, 123C3: improvement of tumour uptake by internalisation

HB Kwa1,2, J Wesseling1, AHM Verhoeven1, N van Zandwijk2 and J Hilkens1

Departments of 1Tumour Biology and 2Medical Oncology, The Netherlands Cancer Institute (Antoni van Leeuwenhoekhuis), Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

Summary The efficacy of three murine monoclonal antibodies (MAbs) for immunoscintigraphy of small-cell lung cancer (SCLC) xenografts was studied in a Balb/c nu/nu mouse model. These MAbs, MOC191, belong to cluster 1 of anti-SCLC MAbs and bind to the neural cell adhesion molecule (NCAM) with similar affinity. After intraperitoneal injection of these MAbs, labelled with 125I, the highest uptake in tumour tissue was obtained with MAB 123C3. Seven days after administration of this MAB 13.9% of the injected dose per gram of tumour tissue was retained in the tumour. The corresponding tumour to tissue ratios ranged from 3.97 for blood to 31.03 for colon. The imaging results and the tumour uptake were less favourable for the two other MAbs, 123A8 and MOC191 (fractions of injected dose respectively 6.7% and 9.2%), although affinity, biological activity after labelling and uptake in non-tumour tissues were very similar for all three MAbs. These results may be explained by the differences in the interaction between the MAbs and the tumour cells. MAB 123C3 is internalised into tumour cells, whereas both other anti-NCAM MAbs are not. Internalisation into NCI H69 cells was demonstrated in vitro by a radioimmunoassay, confocal laser scanning microscopy and electron microscopy. The internalisation fraction of MAB 123C3 was 22.3% after 24 h, whereas this fraction was only 7.5% for MAB 123A8. Although the internalised radiolabelled MAbs are usually degraded and dehalogenated intracellularly, the retained radioactivity is high. Apparently, intracellular degradation of radiolabelled MAb 123C3 and subsequent secretion of radioactive iodine did not prevent the accumulation of intracellular radioactivity. In conclusion, accumulation and retention of radioactivity in the tumour tissue, due to internalisation of radiolabelled MAbs, may improve the results of immunoscintigraphy.

Keywords: immunoscintigraphy; small-cell lung cancer; neural cell adhesion molecule; monoclonal antibody; internalisation

Clinically, lung cancer is divided into small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). SCLC accounts for about 25% of the cases and is associated with the worst prognosis of all lung cancers (Yesner, 1985). For the prognosis and treatment of this type of tumour it is very important to determine the initial stage of the tumour (Ihde, 1985). Unfortunately, standard staging procedure is very laborious and inaccurate. In a recent meta-analysis, the best non-invasive diagnostic technique, computerised tomography (CT), achieved an accuracy of only 0.80 for the detection of intrathoracic lesions (Dales et al., 1990). There is clearly a need for new techniques with a higher sensitivity and specificity to replace the multitude of diagnostic procedures used in the initial staging (Ihde, 1985). Monoclonal antibodies (MAbs) have greatly increased the sensitivity in detecting bone marrow metastases in SCLC (Ledermann et al., 1994). Using radiolabelled MAbs in patients with SCLC (Nelp et al., 1990) distant metastases can be detected by immunoscintigraphy in 10% of patients with limited disease, when staged by conventional methods. This finding suggests that immunoscintigraphy has a higher sensitivity than the standard staging procedure.

Monoclonal antibodies (MAbs) raised against SCLC are categorised in clusters by the antigen recognised, according to an international workshop (Beverley et al., 1988). The MAbs belonging to cluster 1 bind to the neural cell adhesion molecule (NCAM) (Moolenaar et al., 1990). Of the existing NCAM isoforms, NCAM-140 and NCAM-180 are the most important in SCLC (Moolenaar et al., 1990) and all three cluster 1 MAbs used in this study recognise these isoforms (Beverley et al., 1988; Hida et al., 1991). As NCAM is expressed by all SCLCs, it seems a suitable target for immunoscintigraphy with these MAbs. The three MAbs investigated in this study showed similar affinities for NCAM and were used for radioimmunodetection in a mouse SCLC xenograft model. We demonstrated that one of the MAbs that is internalised by the SCLC cells shows a significantly higher uptake in tumour tissue.

Materials and methods

Cell lines

The NCI H69 cell line (hereafter referred to as H69 cells), derived from a small cell lung carcinoma, was kindly provided by Dr D Carney (Gazdar et al., 1980) and was grown in Dulbecco’s modified Eagle medium supplemented with 1 mM glutamine, 10% fetal calf serum and antibiotics. This cell line expresses high levels of NCAM when grown in vitro and as xenografts in nude mice (Rygaard et al., 1992).

Monoclonal antibodies

From a panel of nine cluster 1 MAbs, MAbs 123C3, 123A8 and MOC191 were selected for this study, on the basis of their affinity for NCAM and biological activity after labelling with radioactive iodine (Beverley et al., 1988; Moolenaar et al., 1990). MAbs 123C3 and 123A8 are IgG1 antibodies raised at our institute against a membrane fraction of a fresh SCLC specimen. Both MAbs recognise epitopes on the protein backbone of NCAM close to the attachment site of the polysialic acid side-chains (Gerardy-Shahn and Eckhardt, 1994) and bind to all NCAM isoforms (Moolenaar et al., 1990). The tissue distribution of MAB 123C3 in human tissues was described previously (Schol et al., 1988). MAB 123A8 showed a very similar tissue distribution (DJ Schol and Ph C Hageman, unpublished data). MAB MOC191 is an
IgG2a antibody and was kindly provided by Dr LF de Ley, University Hospital of Groningen. The epitope recognised by MAb MOC191 is probably located at the third immunoglobulin loop (Hida et al., 1991; Gerardy-Shahn and Eckhardt, 1994). MAb M6/1, and IgG1 MAb raised against melanoma cells, detects a high molecular weight proteoglycan and did not bind to H69 cells in vitro. All MAbs were affinity purified from ascitic fluid by protein-A-Sephadex column chromatography and were eluted with a citrae buffer (pH 4.5) (Jones et al., 1985).

Antibody labelling
The MAbs were labelled with 125I (Amersham) using the chloramine-T method (Hunter and Greenwood, 1962). MAb (50 µg) was labelled with 50 µCi of 125I. Free iodine was removed from the labelled MAbs by ion-exchange column chromatography (Dowex G25). For immunofluorescence experiments the MAbs were labelled with fluorescein isothiocyanate (FITC) (The and Felkamp, 1970). Briefly, 1 mg of FITC in dimethylsulphoxide (DMSO) was added to 1 mg of MAb solution in 0.1 M sodium carbonate buffer (pH 9.3) and incubated overnight at 4°C. Free FITC was removed over a Dowex G25 column.

Determination of immunoreactivity and in vitro affinity
The immunoreactivity of the MAbs after labelling with 125I, was assessed according to the method described by Lindmo et al. (1984) with slight modifications. Serial dilutions of H69 cells in a volume of 200 µl of medium, starting at a cell concentration of 2.5 x 10^6 ml^-1, were incubated with an equivalent volume of 125I-labelled MAb in phosphate-buffered saline (PBS) at a concentration of 50 ng ml^-1 for 4 h at 4°C. After centrifugation of the cell suspension 200 µl of supernatant was taken and the fraction with and without the pellet was measured separately in a gamma counter to determine the amount of bound and free radiolabelled antibody. From the results the immunoreactive fraction after iodination was calculated and the immunoreactive fraction was used to determine the affinity using the Scatchard method (Lindmo et al., 1984). Briefly, 200 µl of a cell suspension in medium containing 2.5 x 10^6 H69 cells was incubated for 4 h at 4°C with 200 µl of a serial dilution of the radiolabelled MAb in PBS starting at a concentration of 1 µg ml^-1. The amount of bound and free radioactivity was determined in the same way as described above. After correction for the immunoreactive fraction the association constant Kd and the number of binding sites per cell were calculated.

H69 xenograft model
Human xenografts of H69 cells were established in Balb/c-nu/nu mice by subcutaneous injection of a cell suspension in PBS containing 10^7 cells from in vitro cultures. Within 3 to 4 weeks the tumours reached a volume of 150–500 mm^3 (diameters between 5 mm and 10 mm), suitable for in vivo studies.

Immunoscintigraphy and biolocalisation
For imaging purposes, groups of five tumour-bearing mice were injected with radiolabelled MAbs 123C3, 123A8 or MOC191 and a group of three mice received an injection of radiolabelled MAb M6/1. A dose varying between 50 and 100 µg of MAb labelled with 50 µCi 125I was injected intraperitoneally in each mouse. No potassium iodide was given to the animals to block the iodine uptake in the thyroid gland. Images were made on days 2, 4 and 7 after administration of the radiolabelled MAb. From the images the counts from the tumour area and the background are used for quantification of the tumour to background ratio. The animals were killed after the last image had been made, and the tissue samples were collected. Wet tissue weight was determined and the retained radioactivity in the samples was measured in a gamma counter. Percentages of injected dose per g of tissue and tumour to tissue ratios for the samples were calculated from these results.

Internalisation assay
A cell suspension containing 1 x 10^6 H69 cells in 200 µl of medium was incubated with 10–20 µg of each MAb in 200 µl of PBS, labelled with 50 µCi 125I, at 4°C for 60 min. After removing unbound antibodies by washing the cells three times in PBS the bound radioactivity was determined in a gamma counter. Subsequently, the cells were incubated at 0°C or 37°C for various time periods and washed three times in PBS. The antibodies still present on the cell surface were removed by incubation of the cells with a buffer containing 0.1 M glycine hydrochloric acid and 0.1 M acetic acid (pH 3) for 5 min at 0°C (modified from Matzku et al., 1986). After washing the cells three times in PBS, the remaining radioactivity was measured. The fraction of internalised antibody was calculated from the remaining radioactivity divided by the initially bound radioactivity.

Immunofluorescence
To determine the internalisation of MAb 123C3 by immunofluorescence, 1 x 10^6 H69 cells in 1 ml of medium were incubated with 20 µg FITC-labelled MAb 123C3 for 120 min at 37°C. Subsequently, the cells were washed with PBS and divided into two fractions. One fraction did not receive any additional treatment and from the other fraction the surface-bound antibody was removed by treatment with low-pH buffer as described above to improve visibility of the intracellular fraction. Then, the cells were attached to a glass slide coated with poly-t-lysine and fixed with 4% paraformaldehyde for 10 min. As control, the same experiment was done with FITC-labelled MAb 123A8 and with MAb 123C3 in the presence of 50 mM 2-deoxy-D-glucose and 0.01% sodium azide to block energy-dependent processes, including internalisation. The cells were imaged with a confocal laser scanning fluorescence microscope (CLSM) with tomographic slices of 1 µm thickness.

Electron microscopy
To investigate the internalisation process at the ultrastructural level, a suspension of 1 x 10^6 H69 cells in 1 ml was incubated with 1 mg of MAb 123C3 for 24 h. Unbound MAb was removed by washing the cells three times in PBS. The cells were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer for 60 min at room temperature and embedded in 10% gelatine. After impregnation with 20% PVP-10 and 1.8 M sucrose for 120 min, the cells were frozen in liquid nitrogen and 60 nm sections were cut. Subsequently, the sections were incubated with a 1:40 dilution of rat-anti-mouse immunoglobulin [RAM/Ig (Nordic)] for 30 min, followed by incubation a 1:40 dilution of a conjugate of goat anti-rat immunoglobulin with gold particles [GAR/G10 (Amersham)] for 20 min. After washing, the sections were covered with 1.5% methylcellulose and 0.3% uranylacetate. The sections were then examined with a Philips CM10 electron microscope.

Internalisation and degradation
To investigate the fate of the radiolabelled antibody after internalisation, 2.5 µg MAB 123C3 or 123A8 in PBS, labelled with 25 µCi 125I, were incubated with 1.4 x 10^6 H69 cells in 0.5 ml of culture medium at 4°C for 1 h. After removal of the unbound MAbs, the total amount of radioactivity in the cell suspension was determined and the cells were cultured at 37°C. After several periods the culture medium was collected and the cell suspensions were washed twice in PBS. The amount of radioactivity in the culture medium and the cell-associated radioactivity were determined in a gamma counter.
Cell-surface bound and intracellular radioactivity were determined by measuring the cell-associated radioactivity before and after treatment with low-pH buffer (see above). The amount of free $^{125}$I in the culture supernatant was determined by adding 0.3 ml of 10% trichloroacetic acid (TCA) and counting the radioactivity in the supernatant after centrifugation.

Results

We determined the immunoreactive fraction of a panel of nine cluster 1 MAbs after labelling with $^{125}$I according to the method described by Lindmo et al. (1984). The affinity of the MAbs for NCAM was determined by Scatchard assay. For these assays, H69 SCLC cells, expressing high levels of NCAM, were used (Rygaard et al., 1992). MAbs 123C3, 123A8, and MOC191, which showed the highest immunoreactivity and affinity, were selected for further study. The immunoreactivity of these MAbs were respectively 0.94, 0.68 and 0.85 and the association constants ($K_a$), corrected for the immunoreactive fraction, were respectively 1.04, 0.43 and 1.16.10$^{6}$ M$^{-1}$. The results of 3–5 experiments were shown. From the results of the Scatchard analysis we calculated that for each MAb approximately the same numbers of antibody binding sites were available per H69 cell ($5 \times 10^6$ per cell).

All three selected MAbs and the control MAb, M6/1, labelled with $^{125}$I, were injected intraperitoneally into mice bearing H69 xenografts and after 2, 4 and 7 days scintigrams were made. The images made on days 2 and 4 showed a high background and a relatively low tumour uptake, whereas the images made on day 7 had a relatively low background and higher tumour uptake and were judged to be optimal (Figure 1). These results were confirmed by quantifying the counts from the images. The mean tumour to background ratio obtained from the images made with MAb 123C3 showed an increase from 1.03 on day 2 to 1.99 on day 7, whereas for MAbs MOC191 and 123A8 the values remained constant at 1.13 and 1.05. Images made with radiolabelled MAb 123C3 showed a higher tumour uptake, resulting in a more distinct localisation of the tumour than the images produced with MAbs MOC191 and 123A8. The larger tumours showed a higher total radioactive count than the smaller ones. Images made with M6/1, the control antibody, showed no tumour at all, and a higher background activity than the cluster 1 MAbs. The thyroid gland was not blocked in order to facilitate the orientation of the scan and was clearly visible on all scintigraphic images.

On day 7 the mice were dissected and the radioactivity in each tissue was measured. The total radioactive counts varied greatly with tumour size, but all calculations for tumour tissue ratios and fraction of injected dose were done on the counts per g of tissue, which showed less variation. The mean tumour to tissue ratios 7 days after administration of the radiolabelled MAbs are shown in Figure 2. The highest tumour to tissue ratios were observed with MAb 123C3. The tumour to blood ratio achieved with this MAb was the highest (3.97, $P=0.05$, Kruskal–Wallis test), twice the ratio for MAbs MOC191 and 123A8 (respectively 1.99 and 2.00). The control MAb, M6/1, showed very low ratios for all tissues tested (tumour to blood ratio 0.29). The mean fractions of the injected dose retained in the tissues on day 7 after administration of the MAbs are shown in Figure 3. MAB 123C3 revealed the highest uptake in the tumour tissue (13.9%, $P=0.04$, Kruskal–Wallis test), whereas the values for MAbs MOC191 and 123A8 were significantly lower (9.2% and 6.7%). The fraction of the injected dose in the non-tumour tissues was very similar for all three tested

![Figure 1 Immunosintigram of H69 xenografts in Balb/c nu/nu mice using three anti-NCAM MAbs and the control antibody M6/1. The images were made 7 days after intraperitoneal administration of $^{125}$I-labelled MAbs 123C3 (a), MOC191 (b), 123A8 (c) and MAb M6/1 (d). The images of three mice are shown except in (b), on which only two mice are shown. On the scans the heads of the mice are directed upwards. The xenografts are indicated by arrows in (a–c) and are located in the side of the mouse. In (d) the arrow indicates the location of the thyroid, which is not blocked to facilitate the orientation of the images.](image-url)
Immunoscintigraphy of SCLC xenografts with anti-NCAM MAb 123C3

H8 Kwa et al

Figure 2 The tumour to tissue ratios in Balb/c nude mice carrying H69 xenografts 7 days after administration of the radiolabelled anti-NCAM MAbs. The mean values and the standard error of the mean are shown. The ratios for each MAb are all statistically different from each other ($P=0.05$, Kruskal–Wallis test). ■, 123C3; □, MOC191; ☐, 123A8; □, M6/1.

Figure 3 The fractions of the injected dose per g of tissue, retained in the various tissues 7 days after administration of the radiolabelled MAbs to the mice carrying an H69 xenograft. The mean values and the standard error of the mean are shown. The tumour uptake for each MAb is statistically different from all other MAbs ($P=0.04$, Kruskal–Wallis test). The non-tumour uptake is similar for all anti-NCAM MAbs. ■, 123C3; □, MOC191; ☐, 123A8; □, M6/1.
cluster 1 MAbs, suggesting similar pharmacokinetic behaviour. The control MAb showed very low uptake in the tumour tissue (fraction ID g⁻¹ is 3.3%), but a high retention in blood compared with the cluster 1 MAbs.

Since MAbs 123C3 and MOC191 had similar immuno-reactivity and affinity, whereas MAb 123A8 had only slightly lower values, these parameters are unlikely to be responsible for the difference in tumour uptake between the MAbs. Therefore, other factors must play a more important role in causing the difference between MAb 123C3 and both other MAbs.

We investigated the possibility of internalisation of the bound MAbs as the cause of the difference in the in vivo tumour uptake. Since the cell-surface bound MAbs can be released by treatment of the cells with a low pH buffer (Matzku et al., 1986), whereas internalised antigen–antibody complexes are not affected by this treatment, we used this property in a radioimmunossay to measure internalisation. H69 cells were loaded with ¹²⁵I-labelled MAbs at 0°C and, subsequently, the cells were incubated at 37°C or at 0°C. Since internalisation is an energy-dependent process, it will not take place at 0°C. Figure 4 shows that the fraction of MAb 123C3 that remained associated with the cells after treatment with a low pH buffer increased with longer incubation periods at 37°C, whereas most MAb could be removed from the cell surface when the cells were incubated at 0°C. In contrast, the acid-resistant fractions of both the other MAbs did not increase with time at both incubation temperatures. This finding suggests that MAb 123C3 is internalised by the tumour cells, whereas MAbs 123A8 and MOC191 remain at the cell surface. The process of internalisation of MAb 123C3 is slow compared with that of MAb bound to other molecules, for example receptor molecules (Matzku et al., 1986; Press et al., 1989), since only a relatively small fraction of the bound MAb (<25%) was internalised after 2 h of incubation at 37°C.

In a similar experiment, internalisation of FITC-labelled MAb was monitored by confocal laser scanning fluorescence microscopy (CLSM). The results with FITC-labelled MAb 123C3, after removing the surface-bound MAb for visibility reasons, are shown in Figure 5a. The tumour cells showed evident cytoplasmic fluorescence in different tomographic planes, indicating the presence of internalised antibody. The images also showed that treatment of the cells with low pH buffer indeed removed the antibody bound to the cell surface very effectively (Figure 5a). In Figure 5b the results of the experiment with FITC-labelled MAb 123A8, without the treatment with the acidic buffer, are shown. The H69 cells showed only fluorescence at the cell surface and failed to show intracellular fluorescence in any of the planes, indicating that this MAb does not induce internalisation of the NCAM–MAb complex.

Active internalisation of an antigen–MAb complex requires adenosine triphosphate (ATP). Therefore, incubation of the cells at 37°C in the presence of 2-deoxy-d-glucose and sodium azide, which will deplete the cells of ATP, is expected to prevent internalisation. Indeed, when H69 cells were incubated with FITC-labelled MAb 123C3 in the presence of these drugs no intracellular fluorescence could be detected, whereas the binding of the labelled MAb to the cell surface was not affected (not shown). These results confirm the notion that the MAb 123C3–NCAM complex is actively internalised.

We studied the processing of the internalised NCAM–MAb 123C3 complexes in more detail by electron microscopy. H69 cells were incubated with MAb 123C3 for 24 h at 37°C, fixed and indirectly stained with an immunogold conjugate. Using a dose of 1 mg, MAb 123C3 could not only be detected on the cell surface and in coated pits, but it was also present intracellularly, in coated vesicles and multilamellar bodies (Figure 6). This suggests that at least part of the internalised NCAM–MAb 123C3 complexes follows a pathway that is likely to end in the lysosomes. There were no gold particles found in other parts of the cytoplasm.

To investigate the fate of the radiolabelled MAb and radiolabel after internalisation we incubated ¹²⁵I-labelled MAb 123C3, and MAb 123A8 for comparison, with H69 cells in culture at 37°C for various periods. By removing the unbound antibody from the culture medium we used a fixed amount of radiolabelled MAb for this experiment. The amount of cell-surface bound and intracellular radiolabel could be determined separately after treatment with a low pH buffer (see above). The cell-surface bound fraction of MAb 123C3 decreased in time owing to internalisation, whereas the amount of cell-surface bound MAb 123A8 remained constant (Figure 7a). The intracellular fraction of MAb 123C3 increased to 22.3% at 24 h, whereas the intracellular fraction of MAb 123A8 remained low (7.5%), confirming that MAb 123C3 is internalised. However, the amount of surface-bound MAb 123C3 decreased more than was recovered from the intracellular compartment. The discrepancy may be the result of degradation of the radiolabelled antibody in the lysosomes and subsequent secretion of the radiative iodine (Press et al., 1989). To investigate the catabolism of radiolabelled MAb, free ¹²⁵I was determined after precipitation of the protein-bound iodine with TCA. The amount of free iodine showed a greater increase than for MAb 123C3 and MAb 123A8. The results indicate that catabolism at least partly explains the loss of cell-associated radioactivity. However, despite the degradation of radiolabelled MAb 123C3 and the subsequent release of radiolabel, there is still an accumulation...
of intracellular radioactivity when using radiolabelled MAb 123C3. In contrast, when radiolabelled MAb 123A8 is allowed to bind to the cells, there is no intracellular accumulation of radioactivity.

Discussion

We have investigated the efficacy of three anti-NCAM MAbs for immunoscintigraphy of H69 SCLC xenografts in nude mice in order to design new diagnostic tools. The best images and the highest tumour to tissue ratios were obtained with MAb 123C3 7 days after administration of this MAb. Comparison of the biodistribution of the three MAbs on day 7 revealed that the specific uptake of radiolabelled MAb 123C3 in the tumour was much higher than the two other anti-NCAM MAbs, whereas the non-specific uptake in normal tissues was the same for all three MAbs. This finding was rather unexpected and could not be explained by differences in binding properties, as the affinities of all MAbs for the targeted antigen were similar. Furthermore, the biological activity was not strongly affected by radiolabelling and the number of binding sites per cell was the same for all MAbs. A possible explanation for the difference in retention in tumour tissue between MAb 123C3 and the two other anti-NCAM MAbs could be a specific interaction between this MAb and NCAM molecules, inducing internalisation of the NCAM–MAb 123C3 complex into the tumour cells. Indeed, radiolabelled MAb 123C3, bound to the cell surface, was internalised in contrast to two other anti-NCAM MAbs. Immunofluorescence studies confirmed the difference in interaction of MAbs 123C3 and 123A8 with NCAM. Electron microscopy findings suggest that the internalisation pathway of the NCAM–MAb 123C3 complex starts with endocytosis through coated pits, via multilamellar bodies, and may finally end in the lysosomes. MAb-induced internalisation of antigens through coated pits has been described previously (Matzku et al., 1986; Press et al., 1989).

Internalisation of the NCAM–MAb complex, which was briefly reported previously by our group (Michalides et al., 1994), may lead to a long-lasting association of the radiolabelled MAb with the cell in contrast to non-internalising MAbs that might easily dissociate from the cell surface after binding to the antigen. However, it is necessary for the retention of radioactivity that the 125I-labelled MAb is not degraded and dehalogenated immediately after internalisation. Our results show that internalised radiolabelled MAb 123C3 is only slowly catabolised. Although the internalisation rate of NCAM–MAb 123C3 complex is relatively slow compared with the fast internalisation rate of MAbs binding to receptor molecules at the cell surface (Matzku et al., 1986; Press et al., 1989), the degradation of 125I-MAb 123C3 is still slower than the internalisation process resulting in accumulation of radioactivity in the cell. These results are in agreement with the finding reported by Press et al. (1989), who showed that slow internalisation of MAbs is associated with slow degradation. The fast internalising MAbs followed a pathway through the tubular endocytic compartment and the lysosomes, leading to a fast degradation. In contrast, the slow internalising MAbs showed only limited presence in these cell compartments. Apparently, antibody molecules may follow several pathways following internalisation. The result of the slow internalisation and degradation of MAb 123C3 is the accumulation of this MAb in tumour cells and the high tumour retention in vivo, even on day 7 after administration. The in vivo tumour uptake achieved with MAb 123C3 compared favourably with those of other MAbs with similar affinity for the targeted antigen reported in the literature (Boerman et al., 1991; Waibel et al., 1993). However, the results of the in vitro studies cannot be directly translated into in vivo results, but only indicate that there may be a difference in the in vivo
uptake. Other factors, such as the pharmacokinetic properties of the conjugate and the interaction of the radiolabelled MAb with normal tissues, play a role in vivo in determining the final tumour uptake.

The absence of internalisation after binding of MAb 123A8 to an epitope close to the binding site of MAb 123C3 (Gerardy-Shahn and Eckhardt, 1994) suggests that MAb 123C3 might induce conformational changes of the NCAM molecule causing internalisation of the Ag-MAb complex. It is remarkable that the only antibody known to cause a change in NCAM function, ERIC-1 (Dickson et al., 1990), binds to the same domain (Gerardy-Shahn and Eckhardt, 1994). The internalisation of NCAM-MAb 123C3 complex does not induce internalisation of a non-internalising MAb, which binds to another epitope (unpublished data). The absence of co-internalisation of MAbs is in analogy to the findings of other investigators (Matzku et al., 1990; Casalini et al., 1991).

In conclusion, this study suggests that the relatively high uptake of MAb 123C3 in the tumour can be attributed to internalisation. Closer attention should be paid to this property when screening antibodies for immunoscintigraphy. Our results with radiolabelled MAb 123C3 justify the use of this MAb in human studies. However, the binding to normal human tissues, such as neural tissue and natural killer cells, may cause unwanted side-effects (Moolenaar et al., 1990; Schol et al., 1988; Goldman et al., 1984), although earlier studies with these MAbs did not report neurotoxicity or leucopenia as side-effects (Goldman et al., 1984; Lashford et al., 1987).

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