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A PUTATIVE MECHANISM FOR THE NON-SPECIFIC UPTAKE OF INTACT RADIOLABELLED MONOCLONAL ANTIBODIES IN THE TESTES AND PROSTATE

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Non-specific testicular accumulation of radiolabelled intact anti-CEA monoclonal antibody (MAb), (A431/26, Behringwerke AG) was observed in 11 out of 12 patients with the testes and prostate included in the examination field at radioimmunoscintigraphy (RIS). Previous studies have shown that placental alkaline phosphatase (PLAP) serves as an Fc-receptor, mediating IgG transport through the placenta. A closely related protein, the germ cell alkaline phosphatase (GCAP), is expressed in the testes. The testicular uptake of IgG is observed only when intact but not fragmented MAbs are used, indicating involvement of Fc-receptors. MDCK cells (dog kidney cell line) transfected with the plasmid pSVT7 containing the GCAP gene were shown to acquire the capacity to both express membrane bound GCAP and to bind IgG on the cell surface. This might indicate that GCAP is responsible for the non-specific accumulation of intact MAb in the testes and prostate often observed when intact murine MAbs are used for radioimmunolocalization (RIL).

Radiolabelled monoclonal antibodies (MAbs) are increasingly used for both radioimmunoscintigraphy (RIS) and radioimmunotherapy (RIT). Following injection of radiolabelled compounds, a rapid distribution and equilibration takes place in different compartments of the body. Radiation damage to normal tissues by high doses of radio-nuclides limits extensive use of these techniques. The distribution of the radiolabelled compound is dependent on the expression and localization of the antigen, abundance of cross-reacting receptors, and the blood supply to different organs. A non-specific uptake renders radiation doses to non-tumour tissues, without contributing to the diagnostic or the therapeutic potential. It has been observed that a significant uptake of radiolabelled intact MAbs occurs in male genitalia and both the testes and the prostate may be visualized at RIS, although no expression of target antigen has been described in these tissues. An example of such non-specific accumulation is the uptake of radiolabelled intact monoclonal anti-carcinoembyronic antigen (anti-CEA) antibody A 431/26 (Behringwerke AG), in the testes and prostate gland (1).

CEA is typically not expressed in these organs and the testicular and prostatic uptake thus takes place as a result of other mechanisms. This non-specific uptake of radiolabelled antibodies in testes and prostate glands has been observed only when intact MAbs have been used (1). This is especially important since the gonads are radiosensitive and amenable to persistent radiation effects. If mechanisms for the non-specific accumulation in the testes and prostate are identified this has to be taken into consideration at RIS and RIT.

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Material and Methods

Patients. Twelve male patients (35–83 years, mean age 67 years), with colorectal carcinomas or metastases from adenocarcinoma of unknown origin, referred to the Department of Radiology, Section for Nuclear Medicine, University Hospital, Umeå, were included in the study. Two patients with tumours expressing CEA were investigated in Erlangen, Germany, by Dr Thomas M. Behr. In his investigation $F(ab')_2$/Fab fragments were compared with intact MAbs (A 431/26, Behringwerke AG), and the results of this investigation with these two patients were kindly put at our disposal.

Monoclonal antibody. A murine intact anti-CEA MAb (A 431/26, Behringwerke AG), labelled with ^{99m}Tc in accordance with the manufacturer's recommendations, was used. This MAb has a preferential affinity for cell-bound CEA, and circulating CEA will therefore not significantly influence the visualization at RIS (2). Each patient received 800–1000 MBq labelled MAb intraveneously.

Imaging methods. The RIS was performed with a General Electric Starcam 3000 large-field gamma camera, using a low-energy, general purpose, parallel-hole collimator. The camera was interfaced to a Starcam 3000 XR/T (General Electric) computer system with a nuclear medicine software package which generated images of the planar and single photon emission computed tomographic (SPECT) studies. Images were acquired digitally in a 256 × 256 word-mode matrix for planar studies and 128 × 128 word-mode matrix for SPECT studies. Planar RIS and SPECT scanning was performed using the same camera, 5 and 12 or 24 h after injection of the labelled MAb for each patient. Planar scans were taken in an anterior view, over the abdomen and pelvis including the prostate and testes with 1 000 000 counts/view. Data acquisition at SPECT was performed at 360° for 30 s per view in 64 projections. Reconstruction of transaxial slices was performed by filtered back projection. Sagittal and coronal slices were created from the transaxial slices. Visual accumulation in the testes and prostate in the planar images was considered positive.

Transfection. MDCK cells, not expressing GCAP, were transfected by electroporation with the plasmid pSVT7-GCAP 8 (20 $\mu g/10^7$ cells) (Fig. 1), linearized by the enzyme PvuI (at the β-lactamase encoding region). The cells were cultured on glass microscopic slides in a Petri dish. The transfected cells were incubated in human IgG-SASD solution (7.5 mg/ml) and in the anti-PLAP MAb H7 (3) in complete medium and then washed. The cells were double-stained with FITC-conjugated goat anti-human IgG (gamma chain specific) (DAKO, Denmark) in PBS containing 0.5% BSA and 0.1% saponin, and with rhodamine-labelled rabbit anti-mouse IgG (DAKO, Denmark).

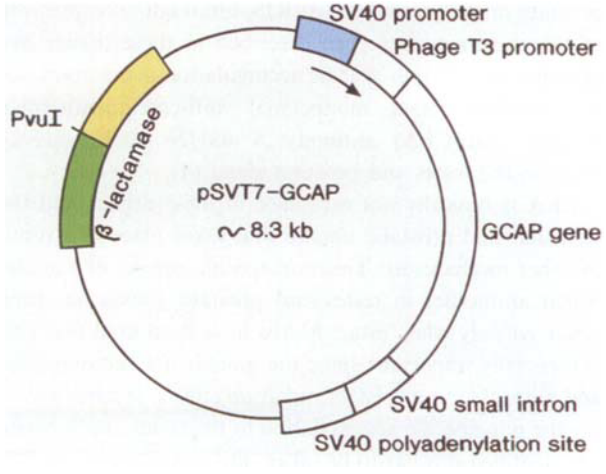


Fig. 1. The plasmid pSVT7-GCAP

Results

Eleven out of twelve patients had a significant accumulation of the labelled anti-CEA MAb (A 431/26) in their testes and prostate glands (Table). The uptake could be seen within 5 h after injection and was still pronounced after 12 and 24 h (Fig. 2). One patient (No. 2) had no accumulation in the testicular region due to an orchidectomy six years prior to RIS, following a prostatic cancer. He had a significant accumulation in his prostate at both 5 and 24 h after injection. Furthermore, the seminal ducts were visualized in two patients (Nos. 6 and 8), at RIS (Fig. 2). No adverse reactions at RIS were observed.

The transfection experiments demonstrate one single cell positive for expression of placental alkaline phosphatase (PLAP), as visualized by the murine anti-PLAP MAb H7 and rhodamine-labelled rabbit anti-mouse IgG (Fig. 3a).

Table

Results from RIS at 5, 12 and 24 h after injection of ^{99m}Tc-labelled intact anti-CEA-MAb

Patient No.	Years	Testes			Prostate		
		5 h	12 h	24 h	5 h	12 h	24 h
1	84	+	nd	+	+	nd	+
2	78	–	nd	–	+	nd	+
3	76	ni	nd	+	+	nd	+
4	73	+	nd	+	+	nd	+
5	73	+	nd	ni	+	nd	ni
6	69	+	+	+	+	+	+
7	66	ni	+	+	+	+	ni
8	65	ni	+	nd	ni	+	nd
9	64	ni	+	nd	ni	+	nd
10	62	ni	nd	+	+	nd	+
11	59	+	nd	ni	+	nd	ni
12	36	ni	nd	+	+	nd	ni

+ = accumulation, – = no accumulation, nd = not done, ni = not included in examination field.

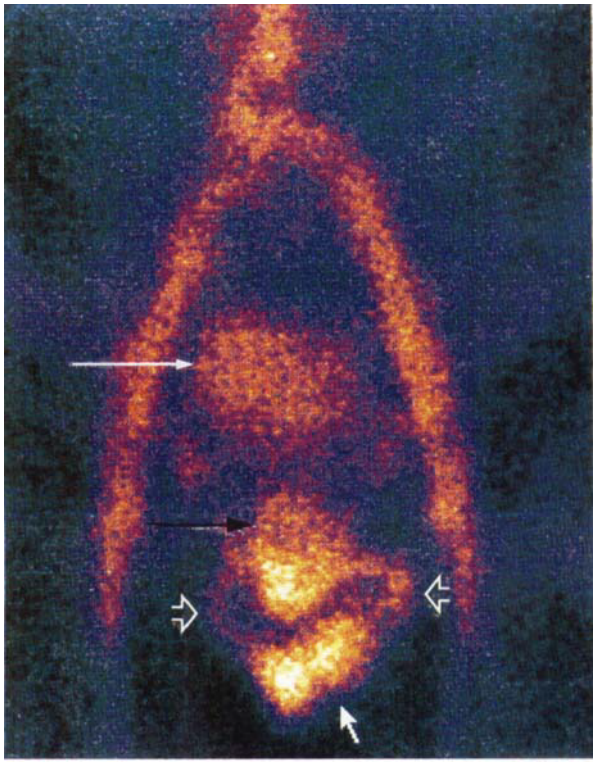
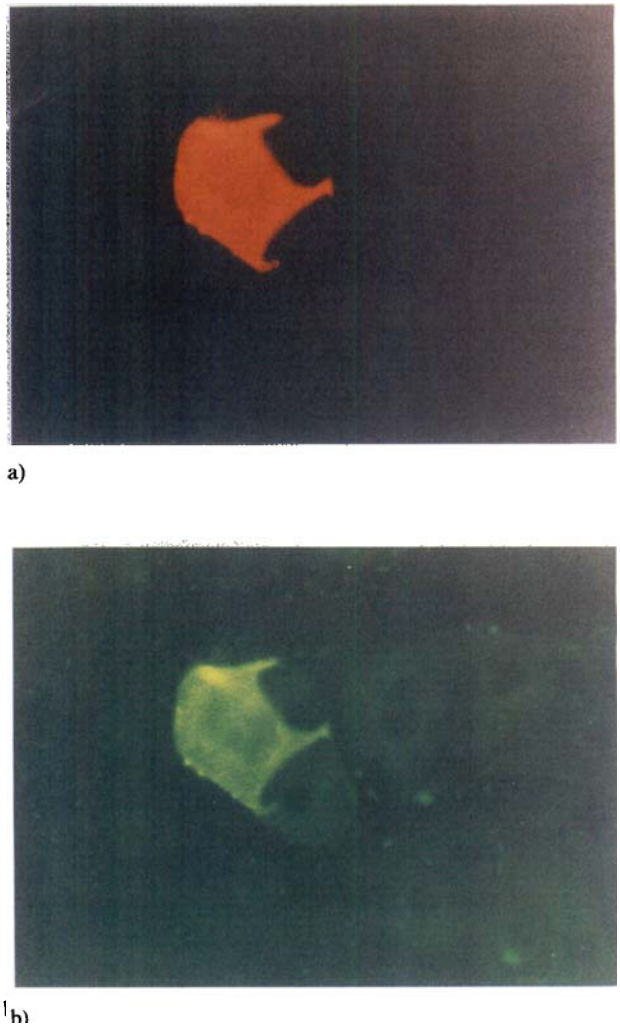


Fig. 2. Accumulation of ^{99m}Tc-labelled intact anti-CEA MAb, A 431/26 (Behringwerke AG) in testes (white short arrow), prostate, and the seminal ducts (open arrows) in patient No. 8, 12 h after injection. White long arrow indicates activity in the urinary bladder. Black thin arrow indicates activity in the prostate.

The preparation was double-stained for IgG and the FITC-conjugated goat anti-human IgG stains only the same cell as mentioned above (Fig. 3b).



a)

b)

Fig. 3. MDCK cells transfected with the pSVT7-GCAP plasmid. Phase contrast microscopy of the cells is shown; in section a) stained for GCAP, and in section b) stained for human IgG.

Discussion

It has been shown that PLAP serves as an Fc-receptor, mediating IgG transport in normal placenta, from the mother to the foetus (4–7). Only species having PLAP are capable of placental transmission of IgG molecules. A closely related protein, GCAP, is expressed in the testes (8). The PLAP and GCAP are structurally related with a 98% homology on the amino acid sequence level (9).

The present investigation demonstrates that transfection with a plasmid harbouring the GCAP gene confers the ability to bind IgG, as well as to express membrane-bound GCAP. Mainly membrane fluorescence is observed (Fig. 3a and b), indicating that binding, but not necessarily internalization, takes place.

Behr et al. (1), compared RIS with intact and fragmented ($F(ab')_2$/Fab') MAbs against CEA with respect to antigen targeting, tumour uptake kinetics, sensitivity and diagnostic accuracy in patients with colorectal carcinomas. At RIS one hour after injection of intact MAbs, an intense uptake in the testes was observed in both patients, one of whom is featured in Fig. 4. At RIS one hour after injection of fragmented MAbs, (in the same patients), no activity accumulation in the testes was observed in any of these patients, one of whom is presented in Fig. 5. (Figures reproduced with the kind permission of Dr. Behr). This observation, that only intact but not fragmented MAbs accumulate in the testes and prostate, supports the hypothesis of Fc mediated uptake of non-specific antibodies in these organs.

Activity accumulation solely dependent on the blood supply to the male genitalia is another mechanism that has been suggested as an explanation for this non-specific testicular uptake of labelled MAbs. The vascularization of this organ is, however, quite low and cannot itself be responsible for the relatively high amount of radioactivity that accumulates in the testes. Furthermore, ejaculate following RIS has been shown to contain significant radioac-

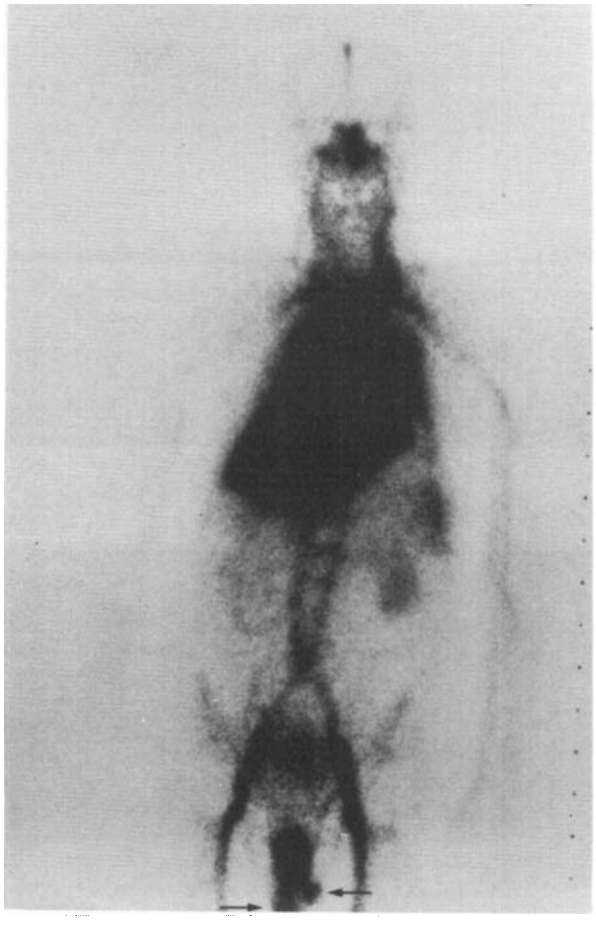


Fig. 4. RIS one hour after injection of intact labelled anti-CEA, A 431/26 (Behringwerke AG), showing a planar scintigram over head, thorax, abdomen, and pelvis with significant activity accumulation in the testes, indicated by arrows.

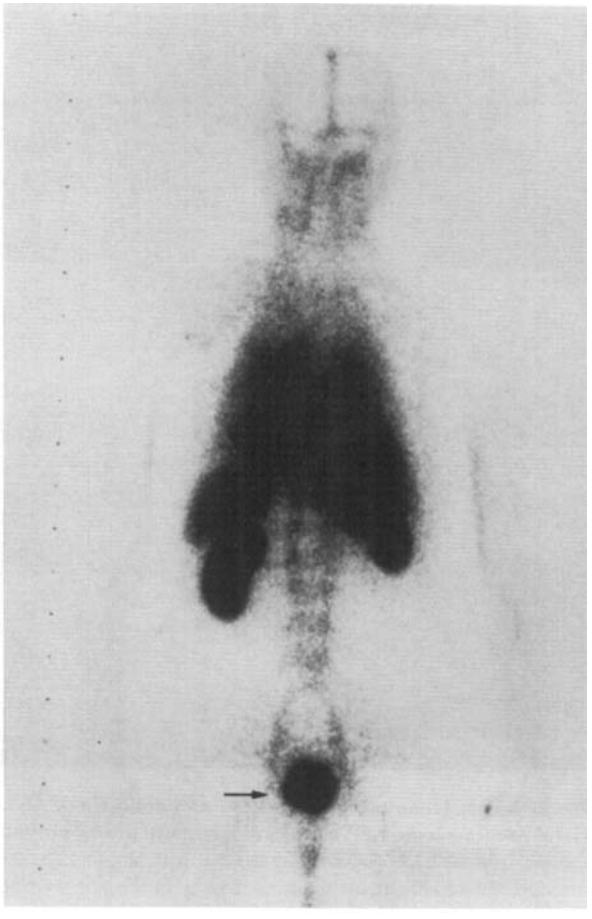


Fig. 5. RIS one hour after injection of fragmented ($F(ab')_2$/Fab') labelled anti-CEA antibodies, showing a planar scintigram over head, thorax, abdomen, and pelvis with no activity accumulation in the testes. Arrow indicates activity in the urinary bladder.

tivity confirming the transfer of radiolabelled IgG into the seminal plasma (Dr Behr, pers. comm.). It should, however, be noted, that the prostate alone is capable of accumulating MAbs, since vasectomy of patients will not significantly affect the levels of IgG in the prostate-derived seminal plasma. Seminal plasma has been shown to contain other IgG receptors and one 16 kD component purified from human seminal plasma has been shown to bind IgG (10). Seminal plasma is also known to contain IgG in higher concentrations than IgA, which is contrary to all other human secretions.

The present investigation indicates that the non-specific accumulation of radiolabelled MAbs to the testes might be due to the presence of testicular Fc-receptors. This antigen independent accumulation delivers a radiation dose to the radiosensitive sperm and germinal cells, mainly when intact MAbs are used. This should be taken into consideration when RIS and RIT are performed. Alternatively, the identification of this mechanism could be used for scintigraphic investigations of male genitalia, offering a new concept to explore the pathology in these organs.

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