Title	131I-labelled Tumour-specific Antibodies : Trial to Concentrate Radioisotopes Specifically in Tumours 3. Improved Radioimaging and Tumour Localization in Nude Mice with a Monoclonal Anti-CEA Antibody by Preincubation of Labelled Antibody with Syngeneic Spleen Cells.(A Rapid Communication)
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^{131}I-labelled Tumour-specific Antibodies: Trial to Concentrate Radioisotopes Specifically in Tumours
3. Improved Radioimaging and Tumour Localization in Nude Mice with a Monoclonal Anti-CEA Antibody by Preincubation of Labelled Antibody with Syngeneic Spleen Cells. (A Rapid Communication)

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放射性同位元素で標識した抗腫瘍抗体による腫瘍の診断と治療の開発
第3報　標識抗CEA抗体と同系脾細胞の投与前培養による腫瘍画像の増強

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我々は約10年前，放射性同位元素で標識したポリクロナール抗腫瘍抗体は腫瘍の画像診断と治療（内照射）に役立てうることを確認した（Ref. 3）．その後細胞融合法の開発により各種モノクロナール抗腫瘍抗体もこの目的に使用出来るようになった．今回は標識モノクロナール抗CEA抗体による（ヌードマウスに移植された）CEA産生直腸癌の検出能をしらべた．そしてさらに血中CEA抗原及びそれと抗体との結合物（免疫複合体）を減少させ，標識抗体による腫瘍の検出能をたかめる方法を検索した．その結果(1) モノクロナール抗CEA抗体は使われた腫瘍に特異的に集積し，したがって腫瘍の画像診断に役立てうること，(2) その際，標識抗体を投与まえに室温で30分間宿主白血球とともに培養すると，より鮮明な画像が得られることが明らかとなった．投与まえの抗体と白血球の培養は新しい試みで，その標識抗体集積増強の機序は，イムノグロブリンFc受容体をもつ細胞上で免疫複合体が出来るため，抗原及び免疫複合体の血中からの除去が促進されるためと推定している．また，細胞に結合した状態の抗体による，より積極的な腫瘍内集積のメカニズムも考えられるが，これ等の点に関してはさらに実験動物を増やして検討することとしている．

Introduction

Radioisotope labelled antibodies have proved useful as tracers for tumour localization since Pressman and Bale first succeeded in detecting Wagner Osteosarcoma or Walker carcinoma by external scintiscanning using labelled antibodies to these tumours[1)~2)]. In our previous study, we also demonstrated the localization of a murine spontaneous hepatoma MH134 by using labelled rabbit antibody to the tumour[3)]. The recent development of cell fusion technology has enabled use of monoclonal anti-tumour antibodies in this field as well. These offer promise for the improvement in tumour imaging using labelled antibodies because of their superior characteristics over polyclonal ones. Their exquisite specificity and unlimited availability are examples. It was also shown that $F(ab')_2$ fragments of monoclonal antibody were cleared more rapidly from the circulation than intact antibody or F(ab) fragments[4)]. We have examined monoclonal anti-CEA antibodies for their abilities to detect human rectal carcinoma grafted in nude mice. Here, a circulating tumour antigen interferes with binding the antibody to the tumour. The following blocking methods were also combined in an injection of labelled antobody to enhance tumour imaging: (1) injection of unlabelled antibody, and (2) incubation of labelled antibody with syngeneic spleen cells. Preincubation of antibody with spleen cells, the mixture then being injected, greatly reduced the blood-tumour ratio and enhanced tumour scanning images. This new blocking method is described here, and possible mechanisms of enhancement are discussed.

Materials and methods

Tumour was obtained from liver metastases from rectal carcinoma in a man (M.K.) and grafted in male Balb/c nude mice. The blood CEA levels in mice averaged 35 ng/ml, when tumours attained a size of approximately 1.0 cm^3. After screening, one lot of monoclonal anti-CEA antibody (29B), specifically bound to the tumour, was used in this study. Antibodies were iodinated with ^{131}I or ^{125}I using Chloramin-T method[5)] with minor modifications. Syngeneic spleen cells, RBC being lysed, were labelled with ^{111}In-oxine following the manufacturer's instructions (Amersham, U.K.). 1 mCi was used to label 25×10^6 spleen cells.Scintiphotoscanning was performed using gamma camera with a pinhole collimator (Toshiba GCA-192, JAPAN).

For radioimaging, four mice bearing tumours of nearly the same size (1.0 cm^3) in their right flanks were injected intravenously with approximately 20 μg ^{131}I-labelled antibody or control normal mouse IgG (column purified). Mouse No. 1 received only labelled 29B antibody. Mouse No. 2 received unlabelled polyclonal (rabbit) anti-CEA antibody 1 hour prior to the injection of labelled 29B. Mouse No. 3 was injected with labelled 29B and ^{111}In-labelled syngeneic spleen cells, the mixtures having been incubated at room temperature for 30 min beforehand. Labelled normal mouse IgG was injected in to a control mouse (No. 4). Serial scintiphotoscanning was performed at 24, 48, 72 and 96 hours after the injection of labelled antibody or normal mouse IgG. To estimate the blood pools in the mice ^{99m}Tc-HSA, 100 μCi was injected intravenously 96 hours after the injection of labelled antibody and they were scanned 15 minutes after injection.

Immediately after completion of the 96 hour scans, the animals and another mouse (No. 5), which had the same tumour and received ^{125}I-labelled antibody and spleen cells 96 hours previously (compatible with mouse No. 3), were exsanguinated and the radioactivity in the blood, tumours, and tissues was assessed. The organs were weighed and after correcting for the physical decay rates of ^{131}I, the counts per minute (cpm)/g and % of injected dose/g were calculated for each tissue (Table 1). The tissue-tumour ratios were then obtained from dividing the specific counts per minute per gram of each tissue by the specific counts per minute per gram of tumour (Fig. 1).

Abbreviations: CEA (carcino-embryonic antigen), HSA (human serum albumin), RBC (red blood cells)

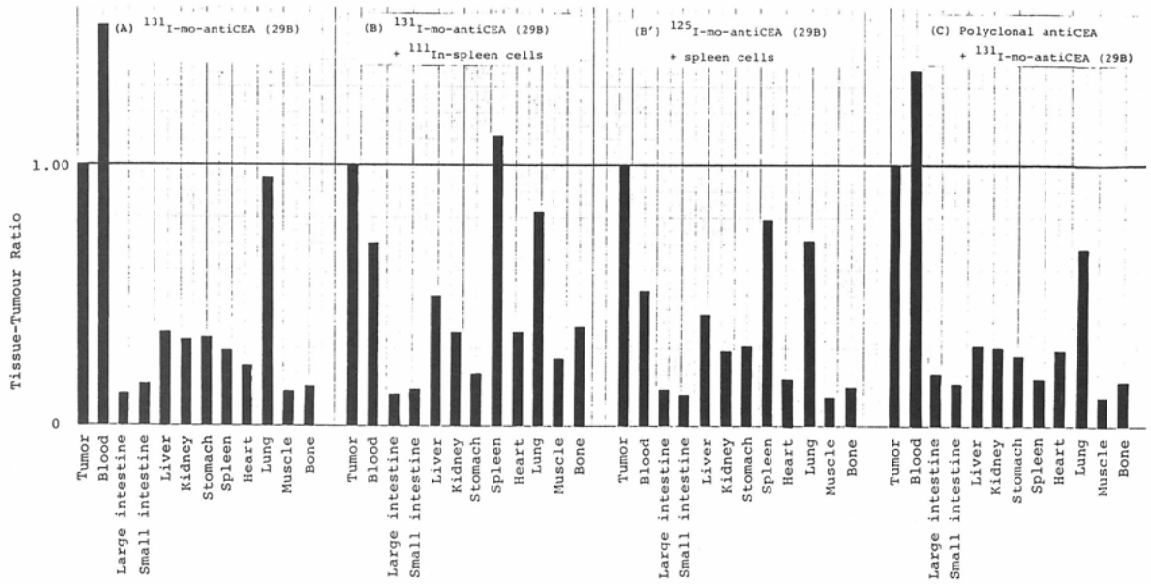

Fig. 1 Relative ^{131}I (^{125}I)-Radioactivity in tissues.

Results

Scans with good tumour localization were obtained at 72 and 96 hours for mouse No. 3, which received labelled antibody and ^{111}In-labelled spleen cells (Fig. 2). Labelled 29B alone did not provide satisfactory tumour images. Unlabelled polyclonal antibody injected 1 hour before the injection of labelled antibody also had virtually no effect on the accumulation of labelled antibody in the tumour. In the normal control mouse, IgG was also concentrated in the tumour (Table 1). This non-specific accumulation, however, never exceeded

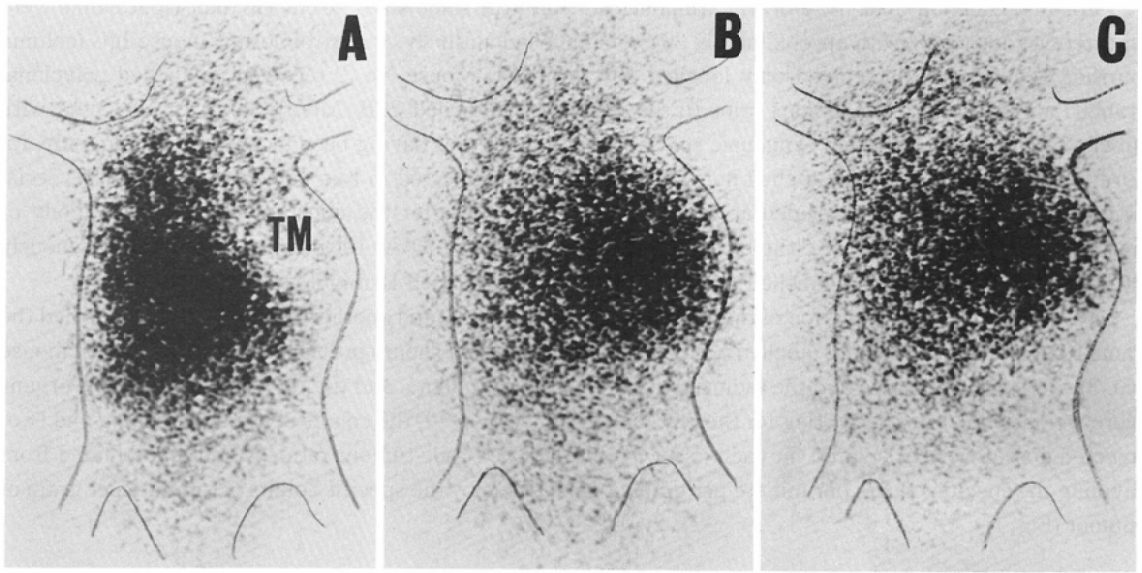

Fig. 2 Scintigraphic images of mouse No. 3, injected with ^{131}I-monoclonal anti-CEA (^{131}I-29B) and ^{111}In-labelled spleen cells. (A) Image of blood pool with ^{99m}Tc-HSA at 15 min. after injection. (B) Image at 72 hrs after ^{131}I-29B injection. (C) Image at 96 hrs after ^{131}I-29B injection.

Table 1 Localization of Radioactivity in Tissues

Tissue	Normal IgG	Monoclonal Anti-CEA(29B)	^{131}I Monoclonal Anti-CEA(29B) + ^{111}In-spleen cells	Polyclonal Anti-CEA + Monoclonal Anti-CEA(29B)	^{111}In Monoclonal Anti-CEA(29B) + ^{111}In-spleen cells
Tumour	0.6*	3.4	7.7	2.1	8210**
Blood	1.3	7.4	4.8	3.2	5529
Large intestine	0.1	0.6	0.8	0.5	1096
Small intestine	0.1	0.8	1.3	0.4	1572
Liver	0.3	1.8	3.3	0.7	9326
Kidney	0.2	1.6	2.5	0.7	3451
Stomach	0.3	1.6	1.4	0.6	1939
Spleen	0.1	1.4	6.5	0.4	75658
Heart	0.2	1.1	2.5	0.7	3115
Lung	0.9	4.6	5.7	1.6	6083
Muscle	0.1	0.6	1.8	0.3	2137
Bone	0.2	0.7	2.6	0.4	4429

* The data are given as % injected dose/g tissues.
**The data are given as cpm/g tissues.

that of specific 29B antibody. All tissue tumour ratios in mice No. 1 and 2 also suggested that images without background subtraction would be subopitmal, due to the high blood pool of radioactivity (Fig. 1, A and C). However, the blood-tumour ratio was reduced to less than 1 in mice No. 3 and 5 which received preincubated antibody and spleen cells, although a great increase of radioactivity in the spleen was observed (Fig. 1, B and B'). Radioactivity of ^{111}In, which was used to monitor injected spleen cells was greatest in the spleen, liver and tumour, followed by lung and blood in mouse No. 3 (Table 1).

Discussion

These findings indicated that preincubating antobody with spleen cells enhanced the clearance of CEA and its immune complexes (ICs) from the bloodstream, and they suggest that this phenomenon is mediated by Fc receptor bearing cell populations, on which the immune complexes were made. A wide variety of cells of the lympho-myeloid series bind immunoglobulins via Fc receptors: Polymorphonuclear leucocytes, monocytes, macrophages, NK cells and lymphocyte subpopulations all express Fc receptors[6]. Thus, ICs can be cleared rapidly from the circulation while the cell migrates to various organs, especially to the liver and spleen. This is suggested by the higher levels of ^{131}I (^{125}I) and ^{111}In radioactivity in these organs. Preliminary studies in a similar fashion, with the F(ab) fragment of 29B have shown that use of spleen cells or peripheral white blood cells had little or no enhancing effect on the localization of the labelled F (ab) fragment of antibody in the tumour. This also supports this hypothesis.

It is also logical to assume that significant radioactivity of ^{131}I (^{125}I) and ^{111}In in the tumour, and the well-defined tumour images in mouse No. 3, were induced by a more active mechanism for antibody accumulation in the tumour; i.e. a mechanism inhibiting migration of antibody and/or ICs-bearing cells to the outside of the tumour, similar to that of antibody-dependent cell mediated cytotoxicity (ADCC). Here, the antibody binds to the white blood cells via Fc receptor on one side and to the antigen on the target cells on the other, via antigen-binding site of immunoglobulins[7].

Further studies to determine whether preincubation of unlabelled antiody with spleen cells and injection of the mixture before the labelled antibody can also enhance the accumulation of labelled antibodies are planned. If so, the former mechanism may be suggested more likely than the latter or both.

F(ab) and F(ab′)$_2$ fragments, lacking the Fc fragment of antibody, may have less non specific tissue binding than intact antibody in the liver, lung and spleen where large populations of Fc receptor positive cells reside, and seem to be better agents than intact antibodies in obtaining tumour images[4]. There is, however, some evidence that intact antibodies are better localized by the tumour than fragments[8]. Although findings presented here were obtained using 1—2 mice each for different protocols, results were reproducible in identical experiments using human hepatoma and anti-alpha-fetoprotein antibody (to be published). Using intact antibodies this simple technique may be useful to enhance tumour images relative to normal tissues.

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