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Radiolabeled Antibodies, Albumin and Antimony Sulfide Colloid: A Comparison as Lymphoscintigraphic Agents

RICHARD L. WAHL,* MONICA LIEBERT, BARRY S. WILSON and NEIL A. PETRY

University of Michigan Medical Center, Department of Internal Medicine, Division of Nuclear Medicine, Ann Arbor, MI 48109-0028, U.S.A.

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The kinetics of lymph node and systemic uptake of members of three different classes of lymphoscintigraphic agents were studied in normal laboratory rats. ^{99m}Tc antimony trisulfide colloid (TcSbC), ^{99m}Tc human serum albumin (TcHSA), ^{125}I 5G6.4 (a murine IgG2ak monoclonal antibody), ^{125}I 763.24T (a murine IgG_1), and ^{125}I FT166 (a murine IgM monoclonal) all current or potential lymphoscintigraphic agents, were injected subcutaneously into the hind foot pads of healthy rats. Ipsilateral and contralateral popliteal lymph nodes were sampled up to 4 h post-injection. Subcutaneous injection resulted in far higher nodal uptake for all agents than i.v. delivery with ipsilateral popliteal node/blood ratios 1 h post-subcutaneous injection of: for TcSbC (1900) > ^{125}I IgM (497) > TcHSA (72) > ^{125}I Intact IgG2a or ^{125}I IgG_1 at approximately 10. Thus, while all agents achieve popliteal node/blood ratios far greater than unity, TcSbc has the greatest absolute and relative nodal accumulation, greater than the ^{125}I IgM monoclonal antibody and TcHSA. Uptake of the intact ^{125}I IgG antibodies is lowest. These data suggest that TcSbC in particular, as well as TcHSA and IgM may be most useful as non-specific nodel imaging agents, while the lower background activity of the IgGs may make targeting specific antigen in nodes more feasible.

Introduction

Lymphoscintigraphy, the intradermal or subcutaneous injection of a radiocolloid, has become an increasingly common technique in the past several years for mapping potential sites of nodal metastases and for predicting involvement with tumor (Croll *et al.*, 1983). A considerable number of agents have been evaluated as possible lymphoscintigraphic agents (Bergvist *et al.*, 1983). ^{99m}Tc antimony trisulfide colloid ((TcSbC) has been used extensively clinically, while we have more recently been using ^{99m}Tc human serum albumin (TcHSA) for scanning (Croll *et al.*, 1983; Froelich *et al.*, 1984; Eberbach *et al.*, 1987). Radiolabeled antibodies, and most recently monoclonal antibodies have also been suggested as potentially useful imaging agents and have shown promising results in animal models and in some clinical trials (DeLand *et al.*, 1980; Weinstein *et al.*, 1983; Carrasquillo *et al.*, 1986; Wahl *et al.*, 1987).

*All correspondence should be addressed to: Richard L. Wahl, M.D., University of Michigan Medical Center, Division of Nuclear Medicine, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0028, U.S.A.

While some information is available regarding the rate of egress of monoclonal antibodies injected subcutaneously, little is known regarding the kinetics of uptake of these agents into normal lymph nodes or if the larger IgM is handled differently (Bergqvist *et al.*, 1983; Wahl *et al.*, 1985). Similarly, relatively little is known regarding comparisons of non-colloidal TcHSA, which has been clinically useful in our practice, with the TcSbC product available for investigational use in the U.S. (Bergqvist *et al.*, 1983; Eberbach *et al.*, 1987). In fact, we have recently demonstrated that small molecules such as ^{99m}Tc MDP used for bone scanning can reach relatively high levels in regional lymph nodes (following s.c. administration) suggesting that the colloidal state may not be a necessary prerequisite to achieving significant levels of uptake of radiopharmaceuticals to regional lymph nodes as has been previously suggested (Croll *et al.*, 1983; Wallis *et al.*, 1987). This study was designed to delineate the rate and extent of lymph node and systemic uptake of these current and potential lymphoscintigraphic agents.

Methods

The lymphoscintigraphic agents studied included:

TcSbC

This agent, supplied in kit form by Cadema, was prepared by adding 10–15 mCi of [^{99m}Tc]pertechnetate to a kit supplied by Cadema. Fifty to 100 μCi was given per animal. Purity of over 90% was typical by acetone silica gel thin layer chromatography (Gelman, Ann Arbor, Mich.).

TcHSA

This was made by adding 4 mCi of ^{99m}Tc to 7 mg of human serum albumin. This was provided in kit form by Medi-Physics. This reagent was 98% devoid of free TcO_4^- by acetone silica gel thin layer chromatography (Gelman, Ann Arbor, Mich.). One hundred μCi was given per animal (approximately 70 μg).

5G6.4

5G6.4 is a murine IgG2ak reactive with ovarian and other epithelial carcinomas (Wahl *et al.*, 1986). This agent was labeled using the iodogen method in which 100 μg was reacted with 1 mCi of ^{125}I (ICN). Yields of over 70% were typical, with >95% purity following Biogel (Biorad) P-60 chromatography. Nine to ten μCi were given per animal.

Similar iodination conditions were used for FT166, a murine IgM reactive with bladder carcinomas (4–5 μCi injected per animal) (Liebert), and 763.24T (10–11 μCi injected per animal), a murine IgG1k (Wilson *et al.*, 1982). The purity of these antibodies was confirmed by SDS polyacrylamide gel electrophoresis (Laemmli, 1970).

Adult, female Sprague Dawley rats were selected for study. All lymphoscintigraphic agent injections were made either intravenously by the femoral vein or subcutaneously into one hind foot pad. Animals were studied in groups of 3–7. Injection volumes were 100 μL or less. Tissues, including popliteal lymph nodes (ipsilateral and contralateral), ipsilateral and contralateral inguinal lymph nodes, blood, liver and lower leg muscle were obtained at selected intervals post-injection, weighted and counted using standard tissue processing techniques (Wahl *et al.*, 1984). Radioactive decay was corrected for and the results were expressed as % kg-injected dose/g. Lymph node/blood ratios were determined by dividing lymph node uptake (% kg dose/g) by blood uptake (% kg dose/g). 763.24T and FT166 were studied following s.c. administration only.

Statistical analysis was by the Student's *t* test and analysis of variance methods.

Results

^{99m}Tc antimony trisulfide colloid

From 30 min until 4 h after the injection of TcSbC, there was a dramatic increase in the uptake of the agent in the ipsilateral popliteal nodes. By 4 h, the percent kg-dose/g was 231.6 ± 40.2 (SD) (Fig. 1). When given intravenously, the same dose of TcSbC produced nodal uptake of 0.0551 ± 0.0106 (Table 1).

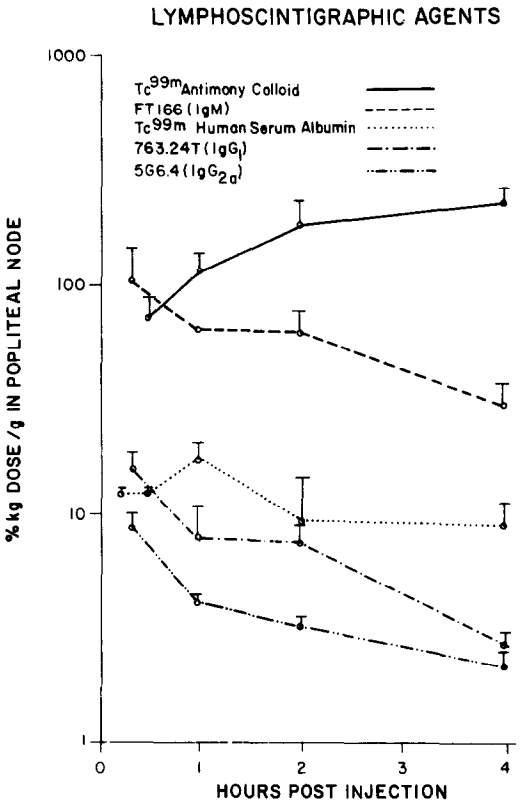

Fig. 1. Semi-log plot of the uptake of five possible lymphoscintigraphic agents over time to the popliteal lymph nodes. Injections were all made subcutaneously to the hind foot pad.

Following i.v. administration, there is high accumulation of TcSbC in the liver and spleen, compatible with the typical behavior of colloid (Table 1). Ipsilateral popliteal node/blood ratios reached 4070/1 by 4 h after s.c. injection.

^{99m}Tc HSA

At 10 min following the injection of TcHSA, popliteal node uptake was 12.359 ± 0.61, with a node/blood ratio of 220.7. The absolute uptake in the right popliteal node increased to peak at 1 h (18.24 ± 7.53), and then fell to 9.33 ± 2.16 by 4 h post-injection. The popliteal node/blood ratio also fell to 38.3/1 by 4 h post-injection (Fig. 1 and Table 2). Blood levels were considerably higher than for TcSbC following s.c. administration. Note that liver uptake following i.v. administration is considerably less than for antimony colloid. When TcHSA was given by the i.v. route, in contrast to the s.c. route, only minimal uptake was seen in popliteal lymph nodes (Table 2).

^{125}I 5G6.4 (intact IgG2ak)

This agent behaved in a similar fashion to TcHSA, in that nodal uptake ipsilateral to the injected foot peaked early and then declined. Maximal node/blood ratios were achieved soon after injection as well. These node/blood ratios fell from 74/1 at 20 min to

Table 1. ^{99m}Tc antimony colloid

	30 min	1 h		2 h		4 h	
	Sq ft pad	Sq ft pad	i.v.	Sq ft pad	i.v.	Sq ft pad	i.v.
Ipsilateral popliteal node	72.17 ± 18.43	114.5 ± 24	0.053 ± 0.003	183.11 ± 52.08	0.0413 ± 0.0006	231.6 ± 40.2	0.0551 ± 0.011
Contralateral popliteal node	0.0186 ± 0.0101	0.068 ± 0.039	0.045 ± 0.002	0.0766 ± 0.0386	0.0444 ± 0.0001	0.0443 ± 0.010	0.0424 ± 0.0033
Ipsilateral femoral node	1.52 ± 1.16	0.0199 ± 0.014	0.091 ± 0.26	0.0149 ± 0.0004	0.0712 ± 0.024	0.011 ± 0.003	0.1066 ± 0.0242
Contralateral femoral node	0.168 ± 0.128	0.009 ± 0.002	0.058 ± 0.005	0.0094 ± 0.0012	0.407 ± 0.0044	0.011 ± 0.002	0.0479 ± 0.0026
Liver	ND	0.153 ± 0.050	1.743 ± 0.69	0.1042 ± 0.011	2.43 ± 1.07	0.1072 ± 0.032	1.94 ± 0.142
Blood	0.0388 ± 0.0052	0.060 ± 0.004	0.154 ± 0.026	0.0658 ± 0.003	0.243 ± 0.110	0.057 ± 0.005	0.1034 ± 0.0044
Ipsilateral leg muscle	0.0265 ± 0.0015	0.023 ± 0.008	0.014 ± 0.011	0.010 ± 0.007	0.005 ± 0.003	0.004 ± 0.002	0.0024 ± 0.0002
Contralateral leg muscle	0.0205 ± 0.0175	0.002 ± 0.002	0.003 ± 0.0003	0.0046 ± 0.003	0.0034 ± 0.002	0.002 ± 0.000	0.0018 ± 0.0001

All organ uptakes are expressed as % kg-injected dose/g ± 1 SD.

Table 2. ^{99m}Tc HSA

	10 min	30 min	1 h		2 h		4 h	
	Sq ft pad	Sq ft pad	Sq ft pad	i.v.	Sq ft pad	i.v.	Sq ft pad	i.v.
Ipsilateral popliteal node	12.359 ± 0.612	12.028 ± 0.882	18.24 ± 7.53	0.0509 ± 0.0059	9.52 ± 5.16	0.0411 ± 0.0031	9.33 ± 2.16	0.0482 ± 0.0162
Contralateral popliteal node	0.061 ± 0.052	0.029 ± 0.008	0.029 ± 0.014	0.0351 ± 0.0037	6.36 ± 6.34	0.0441 ± 0.0075	0.038 ± 0.008	0.364 ± 0.139
Ipsilateral femoral node	0.067 ± 0.023	0.027 ± 0.005	0.926 ± 1.82	0.0751 ± 0.0059	0.544 ± 0.291	0.1073 ± 0.294	0.020 ± 0.004	0.0769 ± 0.0152
Contralateral femoral node	0.020 ± 0.008	0.039 ± 0.005	0.041 ± 0.023	0.359 ± 0.0031	0.016 ± 0.001	0.0317 ± 0.0022	0.022 ± 0.001	0.0284 ± 0.0105
Liver	ND	ND	0.09 ± 0.017	0.3124 ± 0.0085	0.108 ± 0.010	0.3214 ± 0.047	0.143 ± 0.011	0.2362 ± 0.0946
Blood	0.056 ± 0.005	0.330 ± 0.061	0.196 ± 0.041	0.5618 ± 0.0108	0.225 ± 0.014	0.4339 ± 0.021	0.244 ± 0.010	0.0925 ± 0.583
Ipsilateral leg muscle	0.020 ± 0.011	0.017 ± 0.006	0.029 ± 0.038	0.0125 ± 0.0035	0.024 ± 0.009	0.0066 ± 0.0039	0.022 ± 0.010	0.0058 ± 0.0002
Contralateral leg muscle	0.004 ± 0.001	0.006 ± 0.001	0.006 ± 0.002	0.0090 ± 0.0005	0.006 ± 0.002	0.0063 ± 0.0012	0.004 ± 0.000	0.0037 ± 0.0011

All organ uptakes are expressed as % kg-injected dose/g ± 1 SD.

Table 3. 5G6.4 (IgG2ak)

	20 min	1 h		2 h		4 h	
	Sq ft pad	Sq ft pad	i.v.	Sq ft pad	i.v.	Sq ft pad	i.v.
Ipsilateral popliteal node	8.81 ± 1.30	4.07 ± 0.403	0.0325 ± 0.0054	3.34 ± 0.205	0.0397 ± 0.00469	2.24 ± 0.4277	0.04726 ± 0.00493
contralateral popliteal node	0.0159 ± 0.0046	0.0318 ± 0.0032	0.0326 ± 0.0062	0.058 ± 0.0227	0.0353 ± 0.00305	0.0462 ± 0.0103	0.04496 ± 0.00575
Ipsilateral femoral node	0.0171 ± 0.0066	0.932 ± 0.87	0.780 ± 0.0037	0.0697 ± 0.0359	0.0707 ± 0.0033	0.0316 ± 0.00099	0.6714 ± 0.00973
Contralateral femoral node	0.0077 ± 0.0019	0.0313 ± 0.006	0.480 ± 0.0029	0.0297 ± 0.0011	0.05119 ± 0.0021	0.03316 ± 0.00295	0.4485 ± 0.00491
Liver	0.0217 ± 0.0034	0.0628 ± 0.0068	0.1468 ± 0.0060	0.756 ± 0.0039	0.1547 ± 0.0127	0.0762 ± 0.0086	0.08681 ± 0.0035
Blood	0.1188 ± 0.012	0.30 ± 0.026	0.7142 ± 0.275	0.3840 ± 0.0379	0.6535 ± 0.0391	0.3696 ± 0.0329	0.5367 ± 0.0065
Ipsilateral leg muscle	0.0043 ± 0.0015	0.081 ± 0.053	0.0138 ± 0.0013	0.0236 ± 0.0061	0.01297 ± 0.0013	0.0137 ± 0.0021	0.0151 ± 0.0020
Contralateral leg muscle	0.0020 ± 0.0003	0.009 ± 0.00096	0.0128 ± 0.0017	0.0070 ± 0.0007	0.01081 ± 0.00022	0.0074 ± 0.0005	0.0221 ± 0.0075

All organ uptakes are expressed as % kg-injected dose/g ± 1 SD.

Table 4. ^{125}I 763.24T (IgG_1)

	20 min	1 h	2 h	4 h
Ipsilateral popliteal node	15.82 ± 2.73	7.86 ± 2.99	7.60 ± 1.58	2.82 ± 0.40
Contralateral popliteal node	0.058 ± 0.027	0.057 ± 0.020	0.085 ± 0.026	0.052 ± 0.008
Ipsilateral femoral node	1.34 ± 1.32	0.426 ± 0.247	0.239 ± 0.180	0.584 ± 0.328
Contralateral femoral node	0.033 ± 0.006	0.031 ± 0.008	0.036 ± 0.001	0.048 ± 0.003
Liver	0.03 ± 0.007	0.089 ± 0.004	0.130 ± 0.012	0.152 ± 0.015
Blood	0.190 ± 0.042	0.484 ± 0.044	0.682 ± 0.088	0.761 ± 0.190
Ipsilateral leg muscle	0.005 ± 0.000	0.006 ± 0.001	0.009 ± 0.002	0.011 ± 0.002
Contralateral leg muscle	0.033 ± 0.006	0.007 ± 0.001	0.008 ± 0.000	0.011 ± 0.002

All organ uptakes are expressed as % kg-injected dose/g ± 1 SD (following s.c. administration to a hind foot pad)

6.1/1 at 4 h post-injection (Table 3). Absolute popliteal node activity also dropped with time. At 20 min post ^{125}I 5G6.4 injection the ipsilateral popliteal node activity was 8.81 ± 1.30, but fell to 2.24 ± 0.43 at 4 h after injection.

^{125}I 763.24T (IgG1k)

This 150,000 dalton mouse monoclonal behaved very similarly to the ^{125}I 5G6.4 antibody. At 20 min post-s.c. injection 15.82 ± 2.73% kg-dose/g was located within the ipsilateral popliteal lymph node, while by 4 h post-injection, this had dropped to 2.82 ± 0.40. Ipsilateral popliteal node/blood ratios fell from 83.4/1 to 3.71/1 at 4 h post-injection (Table 4).

^{125}I FT166 (IgM)

This 900,000 dalton mouse monoclonal behaved differently from the ^{125}I IgG's in terms of nodal uptake. For example, at 20 min after s.c. injection, the ipsilateral popliteal node harbored 102.1 ± 40.3% kg-dose/g and at 4 h still retained 32.4 ± 6.4% kg-dose/g (Fig. 1). Ipsilateral node/blood ratios also were much higher than with the ^{125}I IgG antibodies, with node/blood ratios of 1173/1 at 20 min, and 224.7/1 at 4 h post-injection (Table 5).

Discussion

Our study compares several diverse colloidal and non-colloidal lymphoscintigraphic agents in the same animal system. These data shows that TcSbC is taken up to a much higher level in lymph nodes than are ^{125}I IgG antibodies or TcHSA. In fact, node/blood ratios in excess of several thousand are seen with TcSbC. The ^{125}I IgM, FT166, is taken up in the popliteal nodes far more than TcHSA or either the ^{125}I IgG_1 or the ^{125}I IgG2a intact antibodies. At early time points, the ^{125}I IgM has slightly higher nodal uptake than the TcSbC. The TcHSA has a behavior intermediate between the ^{125}I IgM and the ^{125}I IgG's. This was somewhat surprising, in that monomeric allumin would have a molecular weight in the 70,000 range, smaller than an intact antibody and might be expected to be less highly retained than the larger IgG's. Radiochromatographic TSK 3000 HPLC tracings of the TcHSA product we use routinely show an elution volume slightly greater than that of IgG, in the range expected for monomeric albumin. This indicates that the TcHSA is not aggregated into a larger moiety.

The ^{125}I IgG antibodies, an IgG2a 5G6.4 (reactive with ovarian cancer) (Wahl *et al.*, 1986) and the IgG_1 763.24T (reactive with melanoma) (Wilson *et al.*, 1982) both have kappa light chains, and both behave similarly *in vivo*. While non-specific uptake of antibodies has been described in human imaging studies, the non-specific retention of these IgG agents appears relatively low, as at 4 h the popliteal nodes had less than 1/100th the uptake that antimony colloid possessed (Carrasquillo *et al.*, 1986; Engelstad *et al.*, 1986). TcHSA was retained to a higher degree than the ^{125}I IgG antibody in the nodes. The mechanism for these differences is unclear at present. Certainly, some antibody binding to lymph nodes could be due to F–C receptor binding to mononuclear cells in the node, however the reason for higher TcHSA uptake is unclear (Hopf *et al.*, 1976).

Presumedly, much of the activity we see in lymph nodes over time is related to transient passage of the ^{125}I IgG antibodies through the nodes. The TcSbC appears to be actively sequestered by nodes, while the ^{125}I IgM and TcHSA appear to be taken up and retained to a greater extent than the intact ^{125}I IgG's.

These differences in uptake kinetics suggest that for delayed imaging of small lymph nodes, TcSbC would

Table 5. ^{125}I FT166 (IgM)

	20 min	1 h	2 h	4 h
Ipsilateral popliteal node	102.07 ± 40.3	64.20 ± 3.76	62.93 ± 17.63	32.35 ± 6.45
Contralateral popliteal node	0.128 ± 0.077	0.051 ± 0.008	0.243 ± 0.158	0.056 ± 0.008
Ipsilateral femoral node	0.387 ± 0.201	2.08 ± 1.57	0.785 ± 0.659	0.111 ± 0.036
Contralateral femoral node	0.074 ± 0.042	0.051 ± 0.011	0.0676 ± 0.028	0.044 ± 0.006
Liver	0.037 ± 0.003	0.061 ± 0.006	0.050 ± 0.006	0.045 ± 0.004
Blood	0.087 ± 0.003	0.129 ± 0.010	0.134 ± 0.015	0.144 ± 0.015
Ipsilateral leg muscle	0.008 ± 0.001	0.015 ± 0.002	0.015 ± 0.003	0.013 ± 0.002
Contralateral leg muscle	0.006 ± 0.0002	0.013 ± 0.001	0.014 ± 0.002	0.012 ± 0.001

All organ uptakes are expressed as % kg-injected dose/g ± 1 SD (following s.c. administration to a hind food pad).

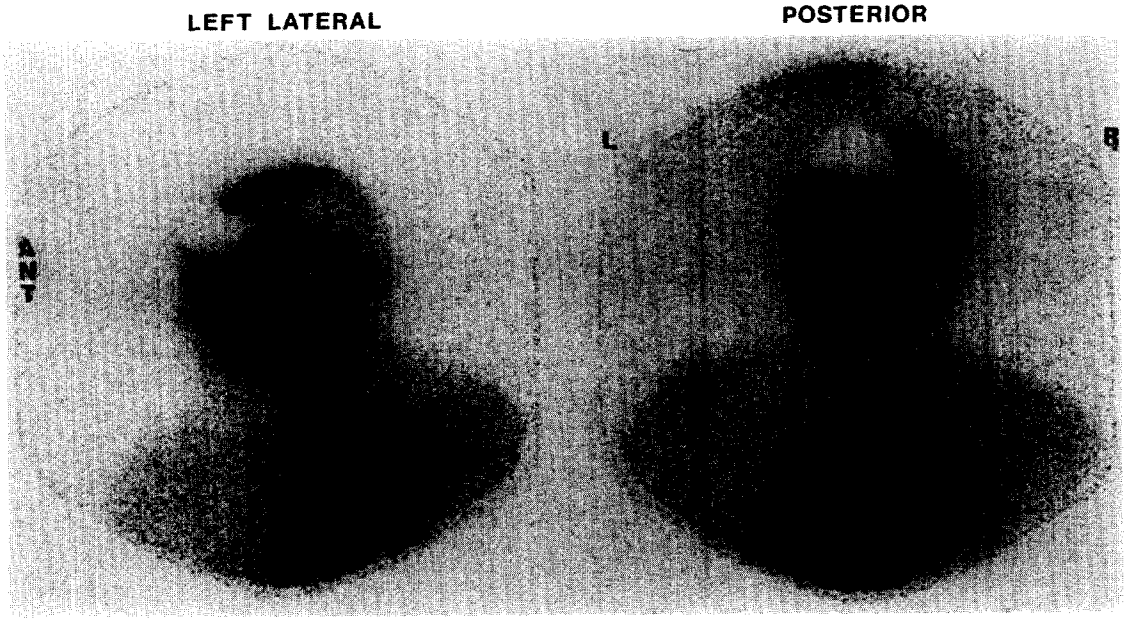

Fig. 2. Left lateral and posterior gamma camera images from approximately 20 min post-injection of 4 mCi of TcHSA about the excisional biopsy site of an anterior scalp melanoma. Note the multiple drainage routes shown on this image. The injection site is shielded with lead.

be the agent of choice. Despite the higher node/background ratios for TcSbC, TcHSA in our hands has produced clinically usable lymphoscintigrams of high quality at very early time points (minutes following injection) in patients. While node/blood ratios with TcHSA are lower than with TcSbC, the early node/blood ratio of >200 for TcHSA and our excellent clinical images at early times indicate it has considerable utility as a non-specific nodal scanning agent (Eberbach *et al.*, 1987). An example of a clinical scan with TcHSA is shown in Fig. 2. Note that the TcHSA scan shows obvious nodal as well as draining lymphatic channel visualization minutes following the s.c. injection of 4 mCi of TcHSA (Fig. 2). TcSbC scans show mainly the nodes themselves on diagnostic images (Croll *et al.*, 1983; Froelich *et al.*, 1984).

It is of interest that in the rat, the popliteal node uptake of TcSbC is greater than that of TcHSA at all times. In patients, our clinical impression is that at early time points the uptake of TcSbC to draining nodes is inferior to that of TcHSA (Eberbach *et al.*, 1987). This apparent discrepancy between the rat data and our clinical observations may be due to the larger distance it is necessary for the scanning agent to traverse from injection site to nodes in man than in rats, or to slower mobilization from the injection site following intradermal or subcutaneous administration.

The relatively high initial and the prolonged retention of the non-specific ^{125}I IgM suggests that for purposes of tumor-specific lymphoscintigraphy (i.e. detecting antigen positive tumors in lymph nodes), IgM's may have unacceptably high levels of background activity. Naturally, several other IgM's would need to be evaluated to more definitively address this issue. For the IgG's, however, the background nodal uptake is considerably less, at least at later time points, and the possibility of detecting tumor-specific binding, if present, is correspondingly higher. Despite the lower background with the IgG's, some clinical studies with intact IgG have shown problematically high nodal background levels (Engelstad *et al.*, 1986). Our recent data with ^{131}I antimelanoma antibodies suggests this is less problematic (Wahl *et al.*, 1987).

In conclusion, TcSbC, TcHSA, and radioiodinated monoclonal antibodies are all taken up into normal lymph nodes after s.c. injection and at early times post-injection this results in high lymph node/background ratios. The uptake of the TcSbC is highest, the ^{125}I IgM next, followed by TcHSA and the ^{125}I IgG antibodies. To non-specifically detect lymphatic drainage to lymph nodes, the TcSbC, the iodinated IgM, and TcHSA appear preferable to the iodinated IgGs. The TcSbC is best suited, based on the animal data, to the detection of small draining lymph nodes due to the high lymph node/background ratio. As agents with lower long-term nodal retention, but definite transport to nodes, the iodinated IgG's appear superior to the other agents. These experimental observations should be useful in rationally planning clinical and experimental studies with lymphoscintigraphic agents.

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