BNL--37246

DE86 003627

BNL 37246

CONF-8509214--1

RECEIVED BY OSTI DEC 06 1985

CURRENT STATE OF THE ART OF BLOOD CELL LABELING

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ABSTRACT

An update on some recent developments in the area of blood cell labeling is provided. Specific topics covered include red cell labeling with ^{99m}Tc, platelet labeling using an antiplatelet monoclonal antibody, and the labeling of leukocytes with ^{99m}Tc. Mechanistic information, where available, is discussed. A critical evaluation of current techniques, their pitfalls as well as advantages, and the problems that remain to be resolved, is presented. The promise shown by recent results using the antibody approach for cell labeling is emphasized. An assessment of the progress made in these areas is presented.

KEYWORDS

Cell labeling; radiolabeled blood cells; ^{99m}Tc-red blood cells; ^{99m}Tc-leukocytes; radiolabeled platelets; antiplatelet monoclonal antibody; thrombus imaging.

INTRODUCTION

The importance of radiolabeled cellular blood elements in diagnostic nuclear medicine procedures is clearly established and widely recognized. A variety of techniques have been developed for the labeling of red blood cells (RBC), leukocytes (WBC), and platelets with different radionuclides. Technetium-99m labeling of RBC is now a routine procedure widely used in blood pool imaging, for the study of various heart parameters, and a variety of other diagnostic applications (Srivastava and Chervu, 1984). The ^{111}In-oxine method of cell labeling (McAfee and Thakur, 1976) constitutes an important and useful approach, particularly for the labeling of leukocytes and platelets.

Most available techniques, though useful, are not ideal and need further refinement and optimization. Accordingly, research activity in the area of cell labeling has continued with a view to developing newer and better techniques and labels, and in order to eliminate the various pitfalls associated with the methods presently in use.

This article addresses three specific areas: (i) methods and mechanisms of ^{99m}Tc-RBC labeling; (ii) the antibody approach of labeling cells, using as example

results from a recent study of labeling platelets with an antiplatelet monoclonal antibody; and (iii) leukocyte labeling with ^{99m}Tc. A very brief description of the existing labeling methods is presented, followed by a discussion of the newer techniques, their various advantages, mechanisms involved, and the problems that remain to be resolved.

TECHNETIUM-99m-RED BLOOD CELLS

Labeling of RBC with ^{99m}Tc is routinely carried out using either in-vitro or in-vivo techniques (Srivastava and Chervu, 1984). Several years of research have produced methods that provide high labeling yields with good stability of the ^{99m}Tc-RBC. The procedures use Sn^{2+} (stannous) compounds for the in-situ reduction of pertechnetate within the RBC since Tc^{7+} in the form of TcO_4^- does not bind effectively or with sufficient stability to the cells.

In-vitro Methods

In the in-vitro methods, a small sample of blood is incubated with a stannous compound ("tinning" step) and then following centrifugation to remove the plasma (that contains tin not taken up by the RBC), pertechnetate is added. In order to achieve good labeling, all tin outside of the cells must be removed quantitatively before adding pertechnetate. This is accomplished by washing the "tinned" cells with saline or more effectively, with a solution of EDTA in saline (Srivastava and Chervu, 1984). Lyophilized kits containing stannous tin stabilized with a variety of complexing ligands (citrate, pyrophosphate, glucoheptonate, etc.) can be employed for the in-vitro labeling procedure. One such kit, utilizing a stannous citrate formulation, was developed at Brookhaven National Laboratory (BNL) in 1976 (Smith and Richards, 1976). The kit underwent many modifications since then (Srivastava and Chervu, 1984) and its widespread use has continued for the past several years. Schematic representation of the steps involved in the current procedure appears in Fig. 1. The kit contains a freeze-dried mixture of stannous citrate (2 µg tin), 3.7 mg sodium citrate trihydrate, and 5.5 mg anhydrous dextrose. Labeling efficiencies of >98% are routinely obtained.

In-vivo Labeling

In-vivo labeling of RBC is carried out using the procedure developed by Pavel and co-workers (1977). Thirty minutes following the i.v. administration of stannous pyrophosphate (10 - 20 µg tin/Kg body wt) into the patient, $^{99m}TcO_4^-$ in saline is injected. The RBC's are labeled immediately and the label has a clearance half-time in blood of about 50 $\pm$ 4 hr (Srivastava and co-workers, 1982). The method is convenient to carry out in practice and is, therefore, widely utilized. A number of other tin complexes have been shown to work as effectively as tin pyrophosphate (Srivastava and Chervu, 1984). Their superiority over tin pyrophosphate, however, has not been established. Whereas in-vitro methods consistently provide quantitative labeling of the cells, the in-vivo method gives labeling yields that are quite variable. In general, only 60 - 90% of the administered $^{99m}TcO_4^-$ gets incorporated into the red cells. This causes background problems in imaging and often makes the quantification of data difficult. On the other hand, existing in-vitro techniques are more cumbersome than the in-vivo method since they require outside handling of the blood and involve undesirable centrifugation and washing steps.

In-vitro Method for Selective RBC labeling in Whole Blood

A new in-vitro technique was recently developed (Srivastava and co-workers, 1983;

1984a) in an attempt to circumvent the above-mentioned methodological problems and in order to achieve simplicity and convenience comparable to the in-vivo method. Using this procedure, quantitative (>95%) and selective labeling of RBC can be obtained using small (~1 ml) whole blood samples, in a closed one-vessel operation. The kit consists of a stable, lyophilized mixture of stannous citrate (50 µg tin), 3.7 mg trisodium citrate, 5.5 mg dextrose, and <2 mg sodium chloride. The labeling protocol is described in Table 1.

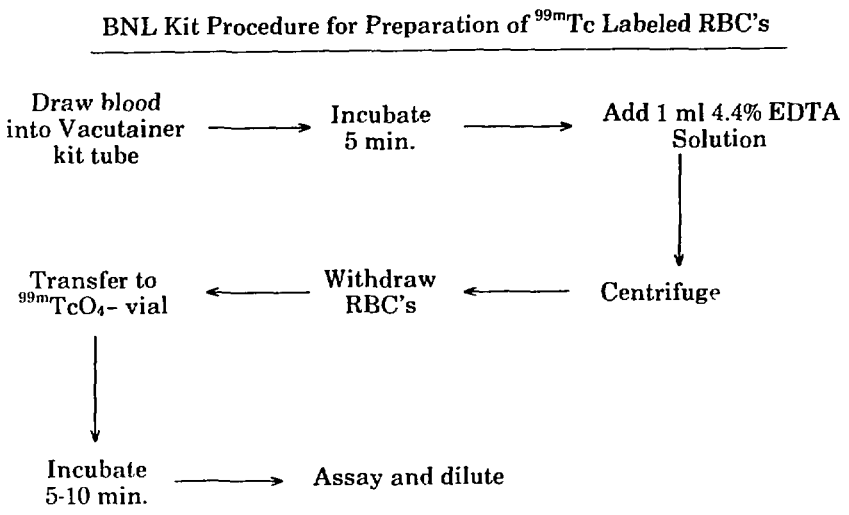


Fig. 1. Schematic representation of steps involved in the currently used BNL kit method for the in vitro labeling of red cells.

Table 1 BNL Kit Procedure for Labeling RBC in Whole Blood with ^{99m}Tc

(1) Draw 1 ml blood into heparinized syringe and add to the kit tube (or vial).

(2) Incubate 5 min with gentle mixing.

(3) Add 0.6 ml of 0.1% sodium hypochlorite solution, and mix.

(4) Add 1 ml 4.4% calcium disodium EDTA solution, or 1 ml ACD solution, and mix.

(5) Add the desired quantity of ^{99m}Tc-pertechnetate in 0.5 - 3.0 ml saline.

(6) Incubate 15 min with gentle occasional mixing.

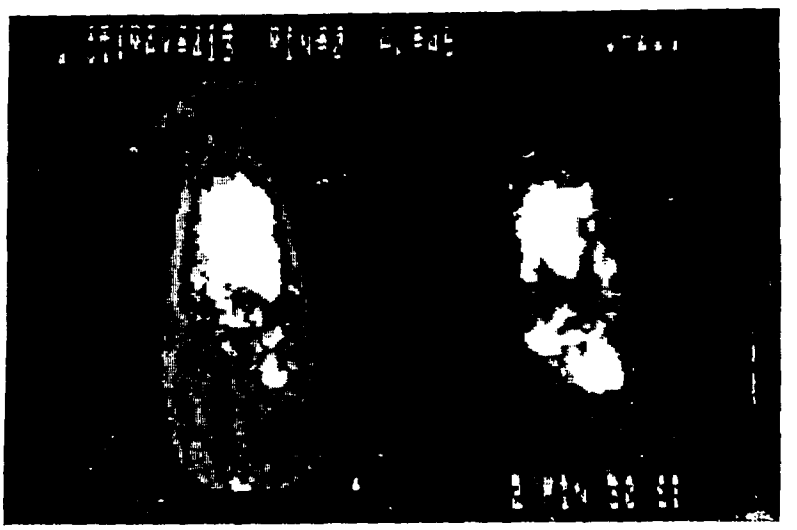
(7) Assay and dilute appropriately for injection.

(8) Incubate for 15 min at 49°C in place of step (6) above, if a splenic agent (^{99m}Tc-RBC) is desired.

This new "whole-blood" method utilizes chemical inactivation (oxidation) of extracellular Sn^{2+}, and thus obviates the need for cell separation by centrifugation, prior to the addition of pertechnetate. Since the cells stay in their native plasma environment and since there is less handling and manipulation, technique-associated damage to the RBC does not occur. In addition, since no

transfers are involved, aseptic conditions are easy to maintain. Finally, the cellular uptake of tin is much higher than that obtained in other procedures and this virtually eliminates the common drawback of other in-vitro methods, namely, their limited capacity for handling certain ^{99m}Tc samples (due to their high ^{99}Tc content).

Preliminary heart blood pool images in dogs (Fig. 2) and in man (Fig. 3) demonstrate the effectiveness of the whole blood labeling method for ^{99m}Tc-RBC imaging applications. Further evaluation will, however, be necessary to establish its ultimate clinical potential and superiority.



A

B

Fig. 2. Blood pool images in an asplenic dog obtained 30 min (left) and 5 hr (right) after the injection of ^{99m}Tc-RBC prepared using (A) control, current BNL procedure employing 6 ml saline wash after tinning or (B) the new BNL whole blood labeling procedure.

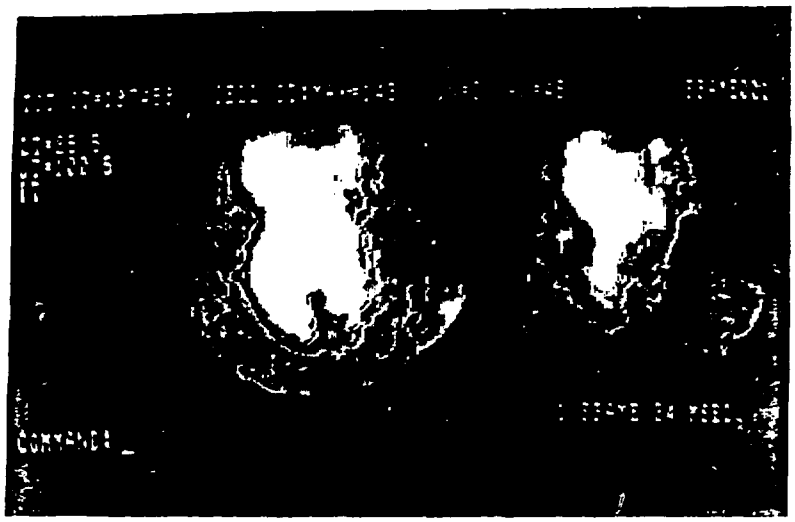


Fig. 3. LAO projection in end diastole (left) and end systole (right) obtained in a patient following the administration of ^{99m}Tc-RBC prepared using the new BNL whole blood labeling procedure.

Mechanisms

Studies were undertaken to define the mechanisms involved in the in-vitro (Srivastava, Straub, and Richards, 1984; Straub and co-workers, 1985) and the in-vivo (Srivastava and co-workers, 1982) methods for labeling RBC with ^{99m}Tc. Factors such as (i) uptake and distribution of Sn^{2+} in blood components, using various stannous compounds; (ii) role of oxidation and chelation in the chemical inactivation of extracellular Sn^{2+}; (iii) uptake kinetics and distribution of ^{99m}Tc in "tinned" RBC; (iv) effect of carrier ^{99}Tc; (v) effect of plasma and other suspending media; (vi) sites of binding of Sn^{2+} and ^{99m}Tc within the RBC; and (vii) long-term retention of tin in RBC following in-vivo labeling, were investigated. Results of these studies are summarized in Tables 2-6, and Figures 4-5.

TABLE 2 Effect of Complexing Ligands on the Percent Distribution of Sn^{2+} in Whole Blood (n = 10)[a]

	Glucoheptonate	Citrate	Pyrophosphate[b]	DTPA[b]
RBC	49.8 ± 2.5	34.5 ± 1.3	1.1 ± 0.2	4.0 ± 0.4
Plasma	44.8 ± 3.0	59.1 ± 1.1	91.5 ± 1.0	90.4 ± 0.3
Washes, 2x2 ml saline	5.5 ± 0.9	6.5 ± 1.2	7.5 ± 0.8	5.5 ± 0.1

[a]Lyophilized kits, labeled with ^{117m}Sn or ^{113}Sn were used. The kits contained 15 µg Sn^{2+}, a 100-fold molar excess (over tin) of the appropriate ligand, and 5.5 mg dextrose. The kits were incubated for 5 min with 4 ml blood, and then the phases separated to determine the activity distribution.

[b]n = 3

TABLE 3 Tin Uptake in 1 ml Blood Following ^{99m}Tc-Labeling Using Stannous Citrate (^{113}Sn or ^{117m}Sn) Kits (n = 10)[a]

Tin(2+) Content of kit, µg	RBC			% in Plasma[c]
	Total Bound µg	% Membrane Associated	% Non-membrane Associated[b]	
2	.43	4.0 ± 2.0	17.5 ± 9.6	77.1 ± 12.1
5[d]	2.1	8.3 ± 1.3	34.2 ± 14.1	54.2 ± 14.9
15	3.6	5.7 ± 2.9	18.3 ± 5.1	77.1 ± 4.2
50[d]	5.25	0.5 ± 0.1	10.0 ± 5.1	89.5 ± 5.2

[a]Whole blood procedure was used except for kits containing 2 µg tin.
[b]90.1 ± 4.5% of non-membrane associated tin bound to heme; 12.9 ± 4.1% to globin.
[c]Includes wash.
[d]n = 3

TABLE 4 Effect of Sodium Hypochlorite (NaOCl), EDTA, and Plasma On ^{99m}Tc-RBC Labeling[a]

Blood Sample	Percent ^{99m}Tc Activity		
	RBC	Supernatant	2, 2 ml saline washes
Whole blood			
No NaOCl	1.8 ± 0.3	87.1 ± 1.6	11.2 ± 0.5
NaOCl added[b]	93.3 ± 0.4	5.2 ± 0.4	1.5 ± 0.2
NaOCl + EDTA added[c]	98.0 ± 1.2	1.5 ± 0.3	0.5 ± 0.1
RBC (Plasma removed after tinning)			
NaOCl added[b]	99.6 ± 0.3	0.4 ± 0.3	--

[a]Lyophilized kits with the following composition were used: 15 µg Sn^{2+}, 3.67 mg trisodium citrate dihydrate, 5.5 mg dextrose; n = 10.
[b]0.6 ml of a freshly prepared 0.1% NaOCl solution in saline was added to the tinned blood, prior to pertechnetate addition.
[c]In addition to NaOCl as above, 1.0 ml 4.4% Na_2EDTA, pH 7.0, was added.

Of the many ligands studied, citrate and glucoheptonate were most effective in transporting tin into the RBC. The tin uptake depended on the total tin content of the kits, blood sample volume, ligand concentration, and temperature. The limiting factor (using 0.5 - 50 µg tin per ml blood) was not cell saturation. The uptake was rapid initially (5-10 min) and very slow thereafter (Fig. 4). No uptake resulted when the kits contained stannic tin (Sn^{4+}). Removal of plasma Sn^{2+} before $^{99m}TcO_4^-$ addition (either by centrifugation or by adding NaOCl + EDTA

or ACD, to sequester and oxidize Sn^{2+} to Sn^{4+}) gave >95% ^{99m}Tc labeling of RBC. Using 50 µg kits + 1 ml blood, greater than 95% labeling yields were obtained with $^{99m}TcO_4^-$ solutions containing ^{99}Tc from up to 200 mCi of ^{99}Mo decay.

TABLE 5 Percent Distribution of ^{99m}Tc in Blood Components Following In-Vitro Labeling (n = 10)[a]

Tin(2+) Content of kit, µg	Red Cell Bound		Plasma	Wash
	Membrane	Non-Membrane[b]		
2	1.7 ± 0.3	94.8 ± 2.0	2.7 ± 1.7	0.6 ± 0.3
5[c]	1.6 ± 0.4	84.5 ± 4.6	9.3 ± 0.3	1.5 ± 0.8
15	1.3 ± 0.4	93.3 ± 3.2	6.1 ± 3.0	0.7 ± 0.1
50[c]	21.8 ± 8.7	76.0 ± 8.9	1.8 ± 0.7	0.1 ± 0.1

[a]Whole blood (1 ml) procedure was used except for kits containing 2 µg tin.
[b]18.3 ± 9.8% of non-membrane associated ^{99m}Tc bound to heme; 80.5 ± 10.0% to globin.
[c]n = 3

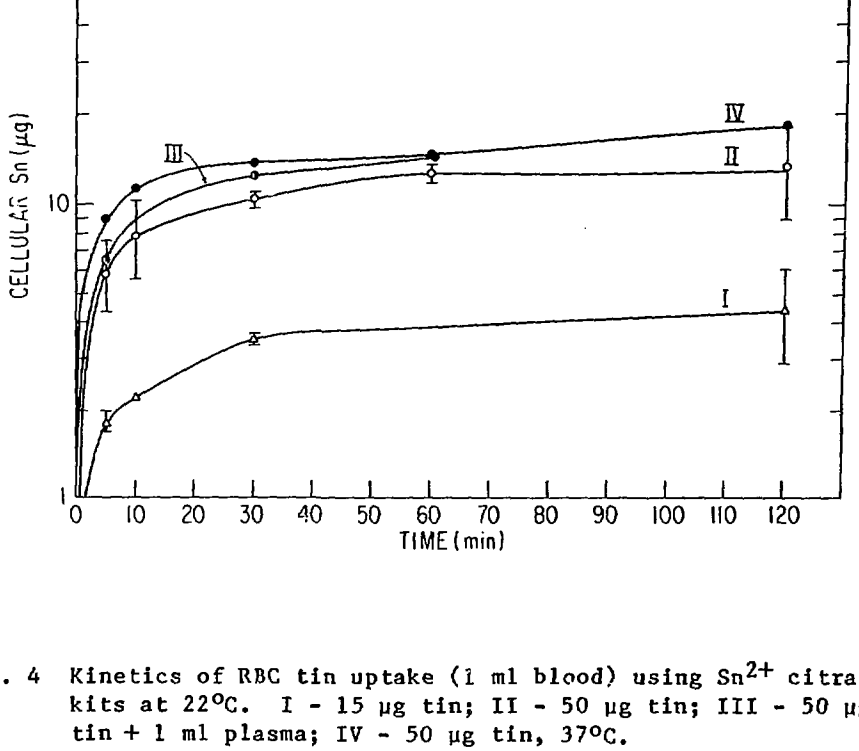


Fig. 4 Kinetics of RBC tin uptake (1 ml blood) using Sn^{2+} citrate kits at 22°C. I - 15 µg tin; II - 50 µg tin; III - 50 µg tin + 1 ml plasma; IV - 50 µg tin, 37°C.

Association of ^{99m}Tc and tin with membrane and non-membrane (hemoglobin) fractions appears to be chemically similar although evidence for or against the presence of a mixed-metal complex is not conclusive. The binding of ^{99m}Tc to hemoglobin seems to have a greater ionic character than most metal-protein complexes. The tag is labile to dilution with water and in low ionic strength solution at neutral pH, and dissociates in an electrical field. In-vivo stability of ^{99m}Tc-RBC is attributable to the cell membrane that maintains a high hemoglobin concentration within the RBC and considerably slows the outward migration of reduced technetium species.

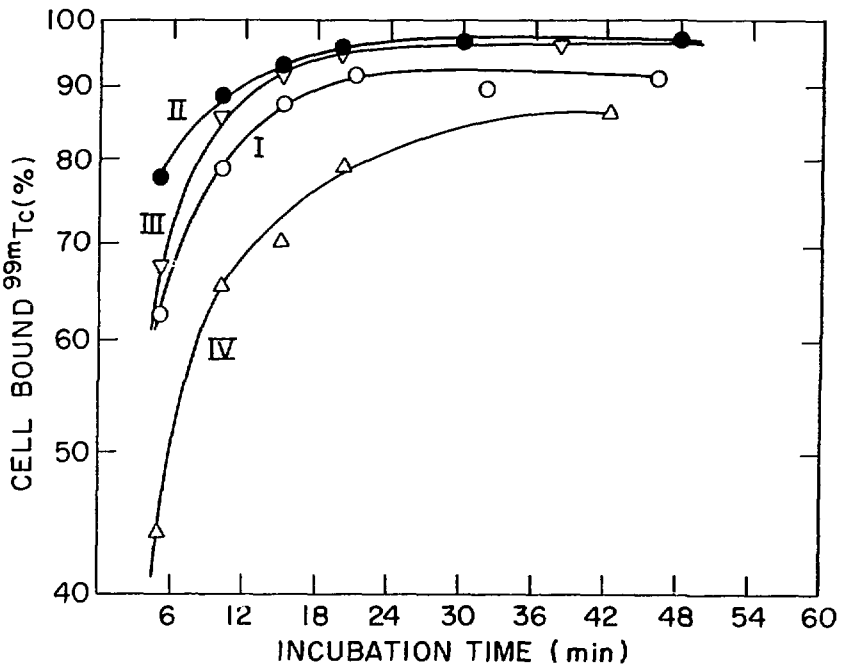


Fig. 5 Kinetics of ^{99m}Tc uptake into RBC (1 ml blood) using Sn^{2+} citrate kits (50 µg tin, whole blood procedure). I - NaOCl only; II - NaOCl + $CaNa_2EDTA$; III - 1 ml saline added; IV - 1 ml plasma added.

Blood clearance data in dogs (Fig. 6) showed that the EDTA treatment of tinned RBC conferred greater stability on the label. The blood clearance half-time was longest for ^{99m}Tc-RBC prepared using the new "whole blood" method.

Evidence was found (cellulose acetate electrophoresis) for a small amount of plasma-bound Sn^{2+} species which resists oxidation by NaOCl. This species is slowly dissociable however, and chelants such as EDTA or citrate (ACD) compete successfully for the plasma bound Sn^{2+} and render it oxidizable. Technetium-99m uptake by tinned RBC was adversely affected in situations of low blood hematocrits (greater plasma content).

Following a single tin-pyrophosphate (Sn-PYP) injection in patients as in the in-vivo labeling method, significant retention of Sn^{2+} was demonstrable even after a period of two months (Srivastava and co-workers, 1982). Blood samples at up to 63 d after the Sn-PYP injection gave significant RBC labeling when incubated in-vitro with ^{99m}Tc (Table 6).

TABLE 6 In-Vitro RBC Labeling with ^{99m}Tc of Blood Samples Obtained at Various Intervals Following Sn-PYP Injection in Normal Human Volunteers (n = 4)

Time after Sn-PYP Injection	Percent Labeling Yield		
	Time (min) of incubation, RBC's + $^{99}TcO_4^-$		
	15	60	300
30 min	98.5 ± 0.7	96.7 ± 1.2	--
24 hr	98.6 ± 0.3	98.4 ± 0.6	94.6 ± 2.3
7 d	55.7 ± 3.7	85.4 ± 5.8	96.5 ± 2.0
21 d	29.5 ± 4.3	47.6 ± 7.1	89.2 ± 5.2
42 d	21.4 ± 3.5	27.3 ± 6.8	61.7 ± 10.8
63 d	20.7 ± 13.7	31.7 ± 28.2	57.1 ± 30.1

The kinetics of labeling with the later blood samples were considerably slower indicating the incremental loss with time of Sn^{2+} from the RBC or its oxidation to Sn^{4+}. When pertechnetate was injected at various times following a single Sn-PYP injection, blood labeling was high at early time periods and decreased later (7 days, 41%; 42 days, 25%). The ratio of RBC to plasma activity of injected ^{99m}Tc also dropped significantly with time (7 days, 2.75; 42 days, 0.20) (Srivastava and co-workers, 1982).

SPECIFIC CELL LABELING USING MONOCLONAL ANTIBODIES

Lipophilic chelates of ^{111}In with oxine, acetylacetone, tropolone, and mercaptopyridine N-oxide have successfully been used for labeling platelets (Mathias and Welch, 1984; Thakur, 1983) as well as leukocytes (Mathias and Welch, 1984; Thakur and co-workers, 1984). These methods require prior cell separation and are generally not very effective in the presence of plasma. Labeling in non-plasma environments often compromises cell viability and function.

Labeling of specific cell populations in whole blood samples would eliminate the possible damage from cell-separation procedures and keep the cells in their native plasma environment throughout the labeling procedure. This is a highly desirable goal. Phagocytic labeling of PMN's with ^{99m}Tc colloids fulfills these criteria,

although it is not clear whether the cells fully retain their chemotactic ability after being labeled using this procedure. A very promising approach is the labeling of cells with monoclonal antibodies directed against specific cell antigens. Various investigators have already started to actively search for cell-specific antibodies and develop methods for in-vitro or in-vivo labeling of leukocytes and platelets in whole blood (Grossman and co-workers, 1985; Khaw and co-workers, 1985; McAfee and co-workers, 1984; Oster and co-workers, 1985; Som and co-workers, 1985; Srivastava and co-workers, 1984; 1985a; 1985b).

Results of a detailed investigation on the use of an antiplatelet monoclonal antibody for specific platelet labeling were reported recently (Oster and co-workers, 1985; Srivastava and co-workers, 1984). The study included developing suitable chemical methods to radiolabel the antibody with various radionuclides including ^{123}I, ^{111}In, and ^{99m}Tc, investigating the binding of the labeled antibody with platelets in whole blood samples, and evaluating antibody-labeled platelets for imaging experimental thrombi and vascular lesions in dogs.

The antibody, 7E3, has been extensively studied and characterized during the last several years (Coller and co-workers, 1983; Coller, 1985). It belongs to the IgG_1 subclass, and is directed against the fibrinogen receptor on the platelet surface, which is a glycoprotein IIb/IIIa complex. The antibody inhibits ADP-induced platelet aggregation, and the ADP-induced binding of fibrinogen to platelets. There are 50,000 antibody binding sites per human platelet, whereas the dog platelets have 75,000 binding sites each. The antibody was produced by immunizing Balb/c mice with washed human platelets and fusing the splenocytes from the mice with a non-secretory murine myeloma cell line (Coller and co-workers, 1983). The screening assay was based on the inhibition of agglutination of fibrinogen-coated beads by the culture supernatants. The antibody cross reacts with dog platelets and was therefore chosen for further study.

In-vitro binding of the antibody to human and dog platelets demonstrated that: the Scatchard plot is essentially linear indicating only one type of binding site; greater than 90% tracer antibody dose binds to human platelets; ~70% of tracer dose binds to dog platelets; there is no appreciable binding to other blood components; virtually all platelet-bound 7E3 becomes incorporated into thrombin produced clots; 10 µg antibody/ml blood causes total inhibition of platelet aggregation; and 0.5-1 µg antibody/ml blood did not produce any significant change in platelet function (Coller and co-workers, 1983).

The antibody was iodinated with various iodine nuclides including p,5n ^{123}I. A number of procedures were evaluated to obtain a product with maximum retention of binding specificity. The typical optimized procedure consisted of reacting 100 µg of 7E3 with radioiodide at a molar ratio of iodine to antibody of about 0.5, in the presence of 5 µg Chloramine T as the oxidant. TCA precipitation, polyacrylamide gel electrophoresis, and in-vitro binding assay were used to characterize the product. Labeling yields following column purification were between 30-80% depending upon the reaction conditions, and the specific activity ranged between 20-300 µCi/µg in the case of ^{123}I at a substitution level of less than 0.5 atoms of iodine per molecule of the antibody. Labeling with ^{111}In was accomplished after conjugating DTPA to the antibody using the cyclic anhydride method (Hnatowich and co-workers, 1983) (ratio of DTPA anhydride to antibody, 50:1). Labeling yields (using 100 µg antibody) were ~80% and the specific activity ranged between 10-40 µCi/µg at an average substitution level of 0.2 to 0.5 indium atoms per molecule of the antibody. Both iodinated as well as indium labeled 7E3 displayed greater than 90% binding specificity in the fibrinogen-coated bead assay.

The binding of radiolabeled 7E3 to dog platelets at various levels of saturation of the binding sites showed that when 0.5-1 µg antibody was incubated per ml of

blood, resulting in 5-10% saturation, 75 ± 5% of the activity became bound to the cellular fraction, of which >95% was associated with the platelets. Twenty to 30% of the label remained in the plasma, and >90% of this was found associated with the IgG fraction. Repeated washing of the cells did not elute the label from the platelets. Experiments with human blood showed >90% binding of the activity to platelets upon incubation of trace levels of antibody to whole blood samples. When the blood was clotted by adding thrombin, 80-90% of the activity became incorporated into the clot despite repeated washing. Fifty percent of the maximum binding took 6-8 minutes, and the maximum binding was observed in 45-60 minutes. Radiolabeled 7E3 preparations bound to human platelets with higher affinity and to a greater extent than with dog platelets. Under further development is another antibody, 10E5 (Coller and co-workers, 1983), which binds avidly to human platelets but not to dog platelets. Either 7E3 or 10E5, or a combination of the two, are expected to prove effective for eventual human application.

Blood clearance in dogs of the 7E3-labeled platelets is shown in Fig. 7. Initial recovery was about 70%, very similar to indium-oxine-labeled platelets. Approximately 50% of the injected activity remains in the blood at 30 minutes. At about 60 minutes, the blood activity drops to 40-45% and thereafter remains fairly constant up to 4 hr. It is interesting to note that both iodine- and indium-7E3-labeled platelets show a very similar blood clearance. The plasma levels of indium stabilize at about 10% as opposed to ~5% for ^{123}I. Total urinary excretion at 4 hr was less than 2%. These studies will be carried out for longer intervals after injection in order to assess the platelet survival as a function of the degree of substitution of the antibody on platelet binding sites.

Arterial and venous clots were produced in dogs either by thrombin injection into vein segments, by transcatheter placement of a copper coil into blood vessels, or by electrocoagulation. A 100-150 ml blood sample was incubated ex-vivo with about 100 µg of ^{111}In-labeled 7E3 and the separated saline-resuspended cells were reinfused slowly into the animals. Fig. 8 shows an anterior view of the chest and abdomen indicating increased uptake of ^{111}In-7E3-platelets, 3 hr post-injection, into clots in the lung. The clots were visualized despite the overlying heart blood pool activity. Image of the excised lung from this dog showed approximately 15 times greater uptake in the clots compared to the surrounding blood pool. The computer generated clot-to-blood ratios in various experiments ranged between 5 and 35. Fig. 9 is an image in another dog with a clot in the right carotid artery, 90 minutes post-injection. Uptake of activity in the clot as well as at the sites of incision is seen clearly. The left carotid artery (with no clot) is visualized only very faintly.

This study has thus demonstrated (Oster and co-workers, 1985) that the antiplatelet monoclonal antibody, 7E3, binds with high selectivity to human or dog platelets in whole blood samples, and that the antibody could be labeled with ^{123}I and ^{111}In with high specific activity without loss of binding efficiency to platelets. In dogs, 7E3-labeled platelets generate early and clear images of experimental arterial and venous thrombi without the need for blood pool subtraction.

Radiolabeled 7E3 and possibly other antibodies such as 10E5 thus hold considerable promise for specific platelet labeling and for localizing in-vivo thrombi and vascular lesions. Cell separation prior to injection may not be necessary with human blood, since the binding is essentially quantitative.

The native behavior of platelets can be manipulated by adjusting the degree of substitution of the antibody or its fragments on the platelet binding sites. To achieve faster blood clearance while maintaining sufficient accretion of activity into the clots is a distinct possibility. Tracer doses of the antibody may enable the study of platelet kinetics and survival.

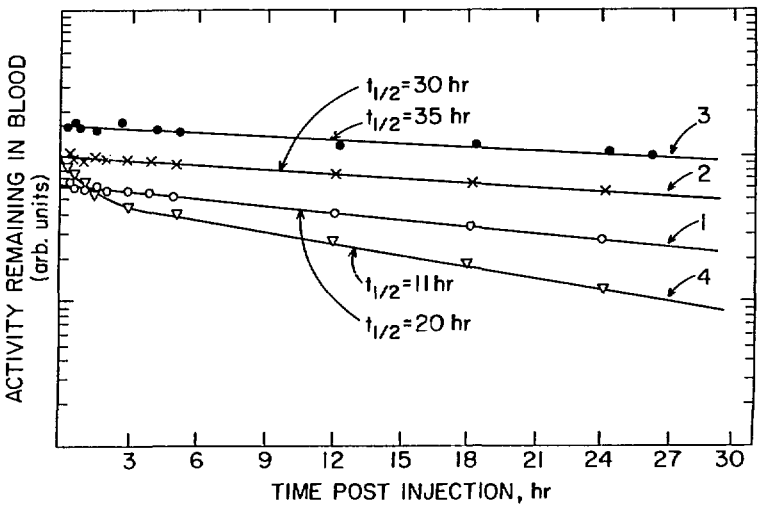


Fig. 6 Blood clearance of in-vitro labeled ^{99m}Tc-RBC in asplenic dog. Curves 1, 2, and 4 obtained using the procedure involving incubation of blood with 2 µg tin (stannous citrate) kit and cell separation prior to addition of pertechnetate. 1, saline dilution after tinning; 2, EDTA addition after tinning; 4, saline dilution after tinning and heat damaged during incubation with $^{99m}TcO_4^-$. Curve 3 obtained using the whole blood procedure with 50 µg tin (stannous citrate) kits.

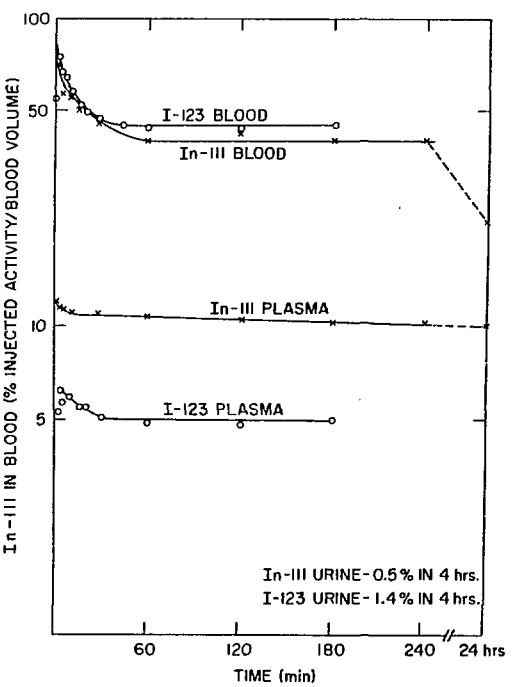


Fig. 7. Blood and plasma clearance of ^{123}I-7E3- and ^{111}In-7E3-labeled platelets in dogs.

Fig. 8. Anterior image of the chest in dog, 3 hr following injection of ^{111}In-7E3-platelets. The thrombi in segmental pulmonary artery are visualized despite the overlying heart blood pool.

Fig. 9. Scintiphotograph of the neck area in a dog, 90 min following injection of ^{111}In-7E3-platelets. The clot in the right carotid artery is clearly seen. There is no uptake of activity in the normal left carotid area.

A study was carried out recently to further investigate the various chemical factors involved in the radioiodination and chelation labeling of 7E3 (Srivastava and co-workers, 1985a; Srivastava, 1985). The effectiveness of ^{131}I-7E3-platelets for thrombus localization was compared with the ^{111}In-oxine technique in the same animals in another study (Srivastava and co-workers, 1985b). The venous thrombi (DVT) were clearly imaged within 5-30 min with ^{131}I-7E3-platelets and in ~60 min with ^{111}In-oxine-platelets. The clot to blood ratios were approximately twice as high as with ^{111}In-oxine-platelets. Coronary thrombi were visualized one-third of the time with the antibody at 3-4 hr after injection. Indium-111-oxine images at this time period were negative. More rapid blood clearance of 7E3-platelets will be required to allow for prompt and reproducible imaging of coronary thrombi. This may become possible through a manipulation of the degree of substitution of the antibody on the platelets, or by using antibody fragments.

Several advantages of the antibody technique over the conventional indium-oxine method are immediately obvious. Since the antibody can be iodinated or labeled with metallic nuclides, and since imaging is possible quite early after injection, a variety of nuclides can be employed, e.g. ^{123}I, ^{99m}Tc, ^{97}Ru, etc. Furthermore, an antibody-DTPA kit could be developed to make the procedure even more convenient by allowing instant labeling of 7E3, 10E5, or their fragments with various nuclides.

The clinical effectiveness of this procedure remains yet to be established.

LEUKOCYTE LABELING WITH ^{99m}Tc

While various investigators have attempted to label leukocytes with ^{99m}Tc, reported procedures neither provide sufficiently high labeling yields, nor satisfactory stability of the label. The described methods generally require large blood samples and utilize either the pretinning approach or phagocytic mechanisms (in the case of polymorphonuclear leukocytes) (McAfee and Thakur, 1976b), or lipophilic complexes (McAfee and Thakur, 1976a; Spitznagle, Marino, and Kasina, 1981; Subramanian and co-workers, 1981). For pretinning the cells, a variety of Sn^{2+} complexes have been used, e.g., chloride (Glenn and co-workers, 1976), pyrophosphate (Farid and co-workers, 1983; Kelbaek and Fogh, 1985; Linhart and co-workers, 1983; Srivastava and Straub, 1985; Uchida and co-workers, 1979), MDP (Kelbaek and Fogh, 1985), citrate (Srivastava and Straub, 1985), and others. In some methods, mixed white-cell populations were used, while in others the labeling of separated polymorphonuclears was carried out. Phagocytic labeling using ^{99m}Tc-colloids has been reported using a tin-technetium colloid (Schroth, Oberhausen, and Berberich, 1981; Hanna and co-workers, 1984), and ^{99m}Tc-sulfur colloid (English and Andersen, 1975).

We have carried out a detailed investigation in order to develop better ^{99m}Tc agents for labeling lymphocytes, PMN's and platelets (Gil and co-workers, 1985). It was demonstrated earlier that among the various commonly used Sn^{2+} chelates, stannous-glucoheptonate (Sn-GH) is transported more effectively into cells and that the uptake appears irreversible (Srivastava and co-workers, 1984a; 1984b; Srivastava and Straub, 1985). Preliminary data (Gil and co-workers, 1985) show that lymphocytes, PMN's, and platelets, pretinned with Sn-GH, provide consistently reproducible and much higher labeling yields (80 $\pm$ 5%) compared to other reported methods. Stability of the label is quite satisfactory (10 $\pm$ 5% washout in 24 hr), and blood samples as small as 3 ml can be used. Lymphocytes, PMN's, and platelets isolated from 3-10 ml rabbit, rat, and human blood repeatedly gave ~80% labeling with up to 50 mCi ^{99m}Tc. The labeling yields are not affected significantly by cell concentration when 2-20 µg total Sn^{2+} is utilized in the procedure (Gil and co-workers, 1985).

Fig. 10 shows a typical image in rabbits obtained with ^{99m}Tc-PMN's labeled using this procedure. At 2 hr after injection, intense localization of the activity was seen in the turpentine-induced abscess in the right leg, compared to the normal left leg area (Fig. 10B). The PMN's localized initially in the lungs and the liver and then cleared slowly from these organs and the blood (Fig. 10A). Kidney and bladder activity was high due to a significant, initial excretion of ^{99m}Tc.

Further optimization of this method, mechanistic studies, and a detailed evaluation using various animal models, are in progress (Gil and co-workers, 1985).

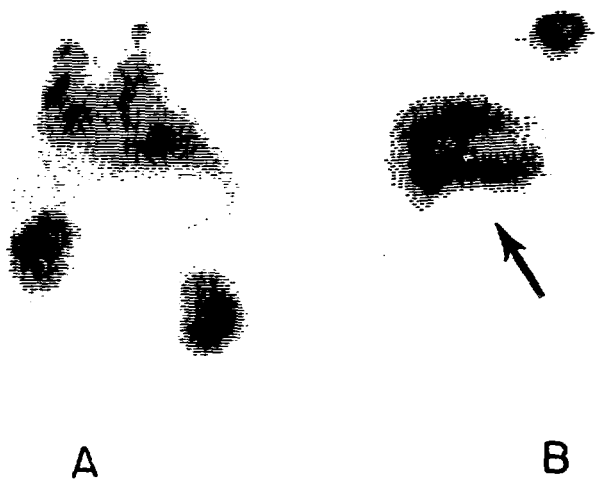


Fig. 10. Images in a rabbit 2 hr following injection of ^{99m}Tc-labeled polymorphonuclear leukocytes. A, thorax and upper abdomen area; B, area of turpentine-induced abscess in the right leg (arrow).

CONCLUSIONS

A number of newer blood cell labeling methods, presently in an experimental stage, hold considerable future promise. Although great advances have been made in cell labeling research during the past ten years, a number of challenges remain to be met. The ultimate goal is the labeling of specific cell populations in whole blood with a variety of radionuclides tailored for particular applications.

Monoclonal antibodies and their fragments, directed against specific cell surface receptors/antigens, appear particularly promising in meeting this challenge. Further research should lead to selective labeling of individual cell types as more and more specific antigens are identified, isolated, and become available. Some progress has already been made in the area of platelet and leukocyte labeling using the antibody approach.

In the meantime, several improvements in the current techniques are expected to provide better labeling yields, greater methodological convenience, and higher

cell viability. Examples are the use of ^{111}In-tropolone and ^{111}In-MERC for labeling leukocytes and platelets in plasma, a new in-vitro kit method for labeling red cells in small (~1 ml) whole blood samples with ^{99m}Tc, and the tin-glucoheptonate method for labeling lymphocytes, polymorphonuclear leukocytes, and platelets with ^{99m}Tc.

ACKNOWLEDGMENTS

Thanks are due to H.L. Atkins, B. Coller, Z.H. Oster (SUNY, Stony Brook), P. Som (Brookhaven), and M. Ezekowitz (Yale) for their valuable help and collaboration during various stages of the research described in this article. Work on leukocyte labeling was carried out at the Chilean Nuclear Energy Commission under Project CHI/2/008, supported by the International Atomic Energy Agency. Research at Brookhaven National Laboratory was performed under the auspices of the Office of Health and Environmental Research, the U.S. Department of Energy, Contract #DE-AC02-76CH00016.

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