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Analysis of lymphopoietic stem cells with a monoclonal antibody to the rat transferrin receptor

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Summary. A mouse monoclonal IgG2a antibody, designated MRC OX-26, is shown to be specific for the rat transferrin receptor, but does not block transferrin binding. The antibody labelled a myeloma, three leukaemia cell lines and normal dividing cells of various types, but also bound to a number of non-dividing normal tissues. No labelling of lymphopoietic stem cells could be detected, even though approximately 25% of bone marrow and over 95% of fetal liver cells were clearly labelled.

INTRODUCTION

The expression of transferrin receptors at a cell surface has been associated with cell division (Larrick & Cresswell, 1979; Trowbridge & Omary, 1981; Sutherland *et al.*, 1981). Thus, these receptors may be interesting markers to distinguish stages of active division from non-cycling stages in early blood cell differentiation. Transferrin receptors have also been suggested as a target for eliminating tumour cells with antibodies conjugated to toxic substances, or by other depletion methods (Trowbridge & Domingo, 1982). The most likely application for such antibody–toxin conjugates is the removal *ex vivo* of tumour cells from autologous bone marrow which is to be returned to patients after therapy to destroy tumour cells (Trowbridge & Domingo, 1982; Mason, Thorpe & Ross, 1982). This procedure will not be possible if antibodies to transferrin receptors bind to stem cells.

In this paper, we describe the identification of a mouse monoclonal antibody which reacts with rat transferrin receptor. The binding of this antibody to stem cells was examined by reaction with bone marrow or fetal liver cells, and sorting for labelled and unlabelled cells on a fluorescence-activated cell sorter. The stem cell activity was assayed by determining the ability of cells to produce T and B lymphocytes (Hunt & Fowler, 1981) or spleen colonies (CFU-S) in irradiated recipients (Hunt, 1979). The binding of the monoclonal antibody to frozen sections from various normal tissues was also studied to see whether expression of transferrin receptor was restricted solely to dividing cells *in vivo*.

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Abbreviations: BSA, bovine serum albumin; CFU-S, colony forming unit-spleen; Con A, concanavalin A; DAB, Dulbecco's A+B medium; DMEM, Dulbecco's minimal essential medium; FACS, fluorescence-activated cell sorter (Becton–Dickinson, Sunnyvale, CA); PHA, phytohaemagglutinin; PMSF, phenyl methyl sulphonyl fluoride; RAM rabbit $F(ab')_2$ anti-mouse immunoglobulin antibody; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

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MATERIALS AND METHODS

Animals

Inbred 8–12-week-old PVG rats of either sex were from the Specific Pathogen Free Unit at the MRC

Cellular Immunology Unit. The donors in the stem cell assays were PVG-(Pta_{DA} + Igk-1^b) maintained conventionally (Hunt & Fowler, 1981). These rats differ from the PVG strain in their surface immunoglobulin kappa chain allotype, and also in the allotype of the peripheral T-cell antigen Pta.A (Workshop Discussion, 1983). For timed matings, the morning of finding a vaginal plug was taken as day 0. Bone marrow donors were 8-week-old males.

Antibodies

The triple-cloned hybrid cell line, MRC OX-26, was derived from a fusion of spleen cells from a BALB/c mouse immunized with phytohaemagglutinin (PHA)-activated PVG rat lymph node cells with NS1/1.Ag 4.1 myeloma cells (Galfré & Milstein, 1981). Cultures of hybrid cells were screened for antibody with an indirect binding assay (Williams, Galfré & Milstein, 1977) on PHA blasts, and positives were subsequently cloned by dilution cloning. MRC OX-26 was of IgG2a subclass by reaction in agar with rabbit anti-mouse IgG2a from Miles Laboratories Ltd (Slough, Berks). Other mouse monoclonal antibodies used were MRC OX-21 (Hsiung *et al.*, 1982) (IgG1 anti-human C3b inactivator); MRC OX-1 (Sunderland, McMaster & Williams, 1979) (IgG1 anti-rat leucocyte common antigen); and a monoclonal antibody against a squid glycoprotein (A. F. Williams & M. Puklavec unpublished observations). Affinity-purified rabbit anti-mouse IgG antibody was degraded to $F(ab')_2$ and labelled with fluorescein or ^{125}I (Williams *et al.*, 1977) or conjugated with peroxidase (Barclay, 1981).

Cells

All cell manipulations, except tissue culture, were carried out at 4° in DAB with 0·2% BSA, or 5% fetal calf serum and 10 mM NaN_3. TDL were collected overnight in DAB containing 20 IU/ml heparin. Bone marrow cells were obtained from the femur and tibia. Fetal liver cells were prepared by dissection, and disrupted through 19 and 23 gauge needles, from 17 day-old fetuses. All other cell suspensions were prepared by standard methods (Ford, 1978). Dividing cells (blasts) were obtained by incubating lymphocytes for 3 days with 5 μg/ml Con A (Sigma Chemical Co., Poole, Dorset) or PHA with 2·5% fetal calf serum in DMEM. Y3/Ag 1.2.3 myeloma cells were from Dr C. Milstein, MRC Molecular Biology Laboratory, Cambridge (Galfré & Milstein, 1981).

Cell sorting

Approximately $2–3 \times 10^8$ bone marrow or fetal liver cells were incubated with MRC OX-26 tissue culture supernatant for 20 min, washed twice with DAB/0·2% BSA and incubated with fluorescein-$F(ab')_2$ RAM for 20 min. After one wash, the cells were suspended at 35×10^6 ml^{-1} in 70% Percoll (Pharmacia Ltd., Milton Keynes, Bucks) buffered to isotonicity to help prevent agglutination due to sedimentation. Recoveries after staining were 91% (bone marrow) and 54% (fetal liver). On the FACS, red cells and dead cells were excluded by low-angle light scatter gating, and thresholds for sorting were determined by placing a 20–30 channel gap around the trough in the fluorescence profiles. In some of the fetal liver separations, latex beads (15·1 μm diameter; Coulter Electronics, Luton, Beds) were added as inert non-fluorescent particles to the cell suspension before sorting, so that the number of droplets going into the positive and negative fractions was roughly equal (see Results). Post-sort recoveries were about 30% of input cells.

The FACS was operated at 488 nm; 200 mW, photomultiplier voltage 580 or 680; fluorescence pre-amp gain 4/1.

Chimaera preparation and analysis—CFU-S assay

The chimaera assay of Hunt & Fowler (1981) was as described, except that monoclonal antibodies were used to score Pta allotype-bearing T cells (P4/16 anti-Pta_{PVG}: GY1/12 anti-Pta_{DA}; gifts from Dr G. Butcher, AFRC Animal Physiology Institute, Babraham, Cambridgeshire) and to detect immunoglobulin kappa allotype Igk-1^a (MRC OX-11, Dyer, 1980). Staining by these antibodies was revealed by indirect immunofluorescence with fluorescein $(Fab')_2$ anti-rat IgG2 antibody (Jensenius & Williams, 1974), and 5×10^4 cells were scored on the FACS operated as in cell sorting.

Biochemical procedures

(i) ^{125}I transferrin binding. 1 ml of log phase Y3 cells at 2×10^7 cells ml^{-1} in RPMI 1640 medium were incubated for 30 min at 37° with 1·25 μg ^{125}I-human transferrin (from Sigma Chemicals, then purified on Sephacryl S-200) containing $3·5 \times 10^7$ c.p.m. The cells were then washed four times by centrifugation and solubilized as in (iv) below.

(ii) Surface labelling with ^{125}I. Con A blasts were incubated for 10 min with methyl mannoside at 20 μg/ml to remove Con A, and centrifuged on an isopaque-ficoll gradient to remove dead cells. Labelling with ^{125}I was by the lactoperoxidase/glucose oxidase method, essentially as in Trowbridge, Ralph &

Bevan (1975): 2×10^7 cells were suspended in 1 ml PBS plus 50 mM glucose, lactoperoxidase (Boehringer Ltd, Lewes, Sussex) 20 μg/ml, and glucose oxidase (Sigma-type V, Sigma Chemical Co.) 0·5 μg/ml (0·1 units/ml), 1 mCi Na ^{125}I (Amersham International Ltd., Amersham, Bucks), at 20° incubated for 15 min; the reaction was halted with DMEM, 10 mM NaN_3, 4°, and the cells were washed three times in DMEM.

(iii) Metabolic labelling. Con A blasts were incubated for 12 hr with ^{35}S methionine at 1 mCi per $6{\cdot}25 \times 10^7$ cells, as in Thomas & Green (1983).

(iv) Solubilization and immunoprecipitation. Washed cells from the above procedures were lysed in 1 ml 1% NP40, 10 mM Tris pH 8·0, with 1 mM PMSF, 5 mM iodoacetamide and 1% rat serum (to exchange with any labelled transferrin) at 4°. Nuclei and cell debris were removed with a 5-min microfuge spin, followed by 30 min centrifugation at 100,000 *g*. Immunoprecipitation from the supernatant was done, either by adding mouse monoclonal antibody followed by protein A-Sepharose 4B beads with RAM bound, as in Thomas & Green (1983), or by using monoclonal antibodies conjugated directly to Sepharose 4B at 10 mg/ml (Brown & Williams, 1982). In the latter case, 15 μl beads were added to 300 μl supernatant, and the beads were washed as in Thomas & Green (1983). Bound material was released by suspending beads for 5 min at room temperature in 5% SDS, 20% glycerol, 0·5 M Tris; NaOH, pH 11·5, 0·005% bromophenol blue. Released material was neutralized to pH 8 with 1 M Tris HCl, pH 6·8, prior to SDS-PAGE (7·5% gel) analysis. Samples were run non-reduced or reduced with 5% 2-mercaptoethanol. Radiolabelled material was detected by fluorography and radiography, as in Brown & Williams (1982).

Localization of antigens in cryostat sections

Cryostat sections (5 μm) were cut from brain, spleen, kidney, thymus, lymph node, liver, heart, pancreas, testis, small intestine, skin, tongue and hind limb skeletal muscle of PVG rats. The sections were fixed with ice-cold ethanol, and then stained with undiluted tissue culture supernatant containing MRC OX-26 or MRC OX-21 antibody, followed by peroxidase conjugated $F(ab')_2$ RAM, as in Barclay (1981). Labelling was also assessed *in vivo* by injecting 2·5 mg OX-26 or control antibodies i.v. into 5-week-old PVG rats. The animals were killed 20–60 min after injection, and sections were prepared from their tissues and stained with RAM peroxidase without any prior incubation with monoclonal antibody. Phagocytic cells in the peritoneum or liver were identified by uptake of carbon particles injected i.p. or i.v., respectively, as 0·5 ml of 1/100 dilution of Pelikan ink.

RESULTS

MRC OX-26 antibody recognizes rat transferrin receptor

Rat myeloma Y3 cells specifically bound ^{125}I-labelled human transferrin, but this binding was not inhibited by MRC OX-26 antibody (data not shown). In order to show that the antibody binds to transferrin receptor, Y3 cells were incubated with ^{125}I transferrin, and then washed and extracted with NP-40 detergent. Antibodies were added to the detergent extracts and precipitated with rabbit anti-mouse IgG antibody attached to protein A Sepharose 4B. When MRC OX-26 was added to the extract, 9600 c.p.m. were specifically precipitated, while with the control antibody MRC OX-21 (which does not react with rat proteins) or MRC OX-1 (which reacts with the rat leucocyte-common antigen), fewer than 650 c.p.m. were precipitated. This experiment was repeated three times with similar results. The radioactivity in the MRC OX-26 precipitate co-migrated with authentic human transferrin on SDS-PAGE. MRC OX-26 does not react with human transferrin itself, and thus, presumably, precipitates the ^{125}I transferrin by reacting with transferrin receptor plus bound ligand.

In order to examine the size of the antigen recognized by MRC OX-26 antibody, Con A blasts were biosynthetically labelled with ^{35}S methionine, or labelled with ^{125}I at the cell surface using the lactoperoxidase method, and labelled material was then immunoprecipitated from detergent extracts of the cells. The material precipitated was examined by SDS-PAGE, and it can be seen (Fig. 1), that, with ^{125}I-labelled material, MRC OX-26 antibody yielded a band which electrophoresed with an apparent MW of 195,000 without reduction, and 95,000 after reduction. The ^{35}S-labelled material electrophoresed without reduction as a doublet at around 195,000 MW. The reason for the doublet is unknown, but it could be due to differences in glycosylation or the presence of a precursor of the antigen. Upon reduction, the ^{35}S-labelled material ran as a single band at 95,000 MW. These results were twice reproduced, as shown in Fig. 1. In a number of other experiments where bound

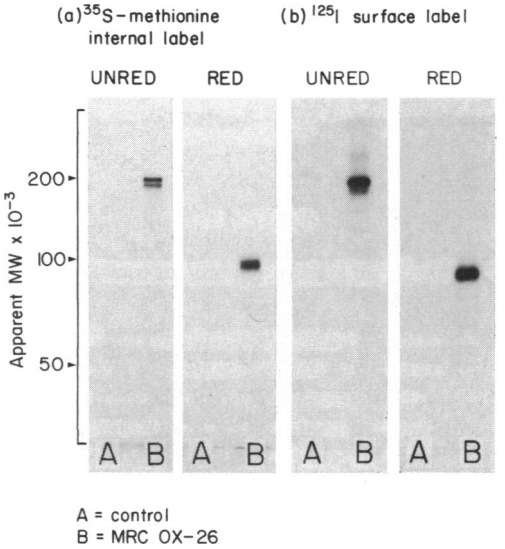

Figure 1. Analysis by immunoprecipitation of the molecule recognized by the monoclonal antibody MRC OX-26. Con A blasts labelled with ^{35}S-methionine internally, or with ^{125}I at the cell surface, were solubilized in detergent and reacted with antibodies coupled to Sepharose 4B beads. (A) Control antibody against a squid glycoprotein; (B) MRC OX-26 antibody. The bound material was eluted with pH 11·5 buffer plus SDS, and electrophoresed on 7·5% SDS-PAGE with (RED) and without (UNRED) reduction.

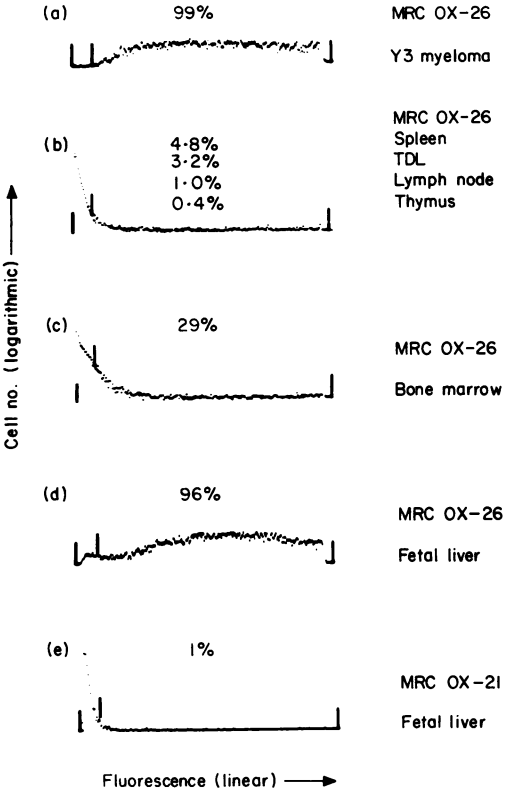

Figure 2. Labelling of cells analysed with the FACS. Lymphoid cells were washed three times in DAB + 0·5% BSA + 10 mM NaN_3, and were then incubated at 4° for 1 hr with a saturating concentration of MRC OX-26 antibody tissue culture supernatant, or MRC OX-21 antibody as negative control, and then reincubated with fluorescein-conjugated rabbit anti-mouse IgG antibody. 10^4 cells, gated to exclude erythrocytes and dead cells, were analysed for their fluorescence intensity. Negative controls with MRC OX-21 were similar to the representative profile shown at the bottom for fetal liver. TDL = thoracic duct lymphocytes. The profile depicted in (b) is of MRC OX-26 antibody binding to spleen cells.

material was released from the beads by boiling rather than by elution with high pH and SDS at room temperature, the same result was obtained but much more non-specific material was also seen.

These observations, that the precipitated antigen binds transferrin and consists of two disulphide-linked 95,000 MW chains, provide strong evidence that MRC OX-26 antibody recognizes the rat transferrin receptor, and that the rat molecule is similar in size and electrophoretic behaviour to human and mouse transferrin receptor (Schneider *et al.*, 1982; Trowbridge, Lesley & Shulte, 1982).

Reaction of MRC OX-26 antibody with haemopoietic cells and cell lines

Analysis on a fluorescence-activated cell sorter showed that MRC OX-26 antibody reacted specifically with most cells from the rat Y3 myeloma cell line grown in culture (Fig. 2a), and also with the rat leukaemia W/Fu (C58NT) D (Conzelmann *et al.*, 1982), the Roser leukaemia (Dibley, Dorsch & Roser, 1975) and Pugh leukaemia (W. A. Jefferies, G. MacPherson and C. W. Pugh, unpublished observations) (data not shown). Amongst rat lymphocytes transformed with Con A, more than 95% were clearly labelled (not shown) with a profile similar to that seen with Y3 (Fig. 2a). In contrast, labelled cells constituted less than 5% of cells prepared from any normal lymphoid tissue (Fig. 2b), most of which consists of non-dividing cells. In adult rat bone marrow, about 29% of nucleated cells were labelled (Fig. 2c), while 96% of fetal liver cells at day 17 of gestation were

labelled (Fig. 2d). At this stage, most fetal liver cells are of the erythroid lineage. Mature erythrocytes from blood were not labelled by MRC OX-26 antibody.

Labelling of haemopoietic stem cells

After reaction with MRC OX-26 antibody as above, bone marrow or fetal liver cells were separated into labelled and unlabelled fractions on the FACS, and were assayed for their ability to reconstitute permanently both the B and T lymphoid compartment of rats (Hunt & Fowler, 1981; Hunt, 1979). In this assay, cells from a congenic rat strain expressing alloantigenic markers of both B cells and T cells are transferred to irradiated (9·5 Gy) recipients of the congenic partner, along with 10^7 bone marrow cells of host-type given to ensure the survival of the animals and to act as a reference for the genetically marked test cells. After a minimum of 5 weeks, lymph node biopsies were taken and assayed for the percentage of B and T cells derived from the marked donor cells. With bone marrow, the chimaerism remains essentially constant, regardless of the delay before analysis, and is linearly related to the percentage of marked cells injected, but for fetal liver, the marked cells gradually overgrow the reference marrow, and the chimaerism generated does not show a straight-line relationship to the percentage injected (Hunt & Fowler, 1981). The results are shown in Table 1 for one of three similar bone marrow experiments, and for two of four fetal liver experiments.

Before considering the separated cells, it is important to discover whether antibody labelling itself affected stem cell activity. With bone marrow cells, no substantial loss occurred with labelled, compared with control cells (Experiment A, Table 1). With fetal liver cells, Experiments B and C had a total of three animals given labelled unfractionated cells for analysis and, in two other experiments (not shown), seven further similar animals were analysed. Taken together, the 10 animals gave chimaerism not significantly different from the chimaerism given by unlabelled cells. Thus, either the binding of antibody has no effect on the function of the cells *in vivo* or, if it does, then only a small fraction of the stem cells bind antibody.

With sorted bone marrow, no significant activity could be detected among the bright cells, while the dull fraction showed activity which, within the limits of the assay, accounted for all the activity of unfractionated marrow. In setting the threshold for sorting cells (Fig. 2c) we included all cells which might show positive fluorescence in the labelled cell fraction, and the lack of activity in this fraction shows that the amount of transferrin receptor on stem cells must be markedly lower than that of any of the other labelled populations shown in Fig. 2.

With sorted fetal liver cells, the result of an initial experiment showed that no activity at all could be detected in the cells labelled with MRC OX-26, while stem cell activity was detected in the MRC OX-26 negative fraction (Table 1, Experiment B). However, this activity was only about one-tenth of that expected from the unfractionated cell controls. A second experiment (not shown) gave a similar result. With our FACS machine, sorted droplets collect on an electrode that monitors the sort, and it is possible that, with very few cells being sorted into the negative fraction, evaporation caused hypertonicity and cell death on the electrode. Thus, in a third experiment, latex particles were added to the cells to give a greater deflection of drops into the negative fractions (Table 1, Experiment C). Also, the lower cut-off for positive cells was pushed to the minimum level so that, even if cells were only weakly labelled with MRC OX-26 antibody, they would register in the positive fraction. As expected, the MRC OX-26 negative cell fraction gave greater potency than in the first experiment and, cell for cell, was about five times more active than the MRC OX-26^+ fraction. The MRC OX-26^+ fraction in this case gave activity at the level of about 10% of unfractionated cells. This could be caused by contamination due to the low threshold set for positive cells. Also, in this experiment, the total activity recovered in the MRC OX-26^+ and MRC OX-26^- fractions approached that expected from the potency of the labelled but unsorted control cells.

In a conventional 9-day CFU-S assay (Hunt, 1979) of bone marrow cells sorted after MRC OX-26 staining, virtually all the stem cell activity was found in the dull fraction (Table 2). The few colonies resulting from the injection of bright cells could have derived from contaminating dull cells. Again, no loss of activity due simply to the binding of antibody could be detected.

Reaction of MRC OX-26 antibody with antigen in tissue sections

Cryostat sections from various rat tissues were labelled using an indirect immunoperoxidase technique to determine histologically the expression of transferrin receptor in a variety of lymphoid and

Table 1. Stem cell assay of MRC OX-26 sorted bone marrow and fetal liver

Cells	Dose $\times 10^{-6}$ (viable)	OX-26^{+}* (%)	OX-26^{-}* (%)	Percentage of B or T cells derived from marked donor cells							
				B	Mean	T	Mean	B	Mean	T	Mean
Bone marrow						*Experiment A*					
Unlabelled, unsorted	10			69, 70	69	72, 59	66				
	4·3			45, 50, 52	49	ND†, 51, 56	53				
	1·1			19, 19, 29	22	ND, 22, 37	29				
Labelled, unsorted	10	17·7‡	80·9	68, 66, 69	68	ND, 71, 72	71				
Sorted, OX-26 bright	0·8	93·0	–	0, 1, 2	1	ND, 8, 18	13				
Sorted, OX-26 dull	9·0	–	99·5	58, 62, 64	62	ND, 59, 61	59				
Fetal liver						*Experiment B*				*Experiment C*§	
Unlabelled, unsorted	4·3			97, 99	98	88, 90	89				
	3·0							72, 88	80	64, 72	68
	1·0			79, 83	81	70, 73	72	59, 65, 71	65	50, 52, 72	58
	0·3			50, 59	55	47, 60	53	25, 27, 40	31	16, 22, 40	26
Labelled, unsorted	1·1	94·0	3·1¶	70, 73	71	30, 52	41				
	0·3	93·4	4·3¶					49		31	
Sorted, OX-26 bright	4·3	98·9	–	0, 4	2	0, 0	0				
	3·0	95·8	–					23, 35, 43	34	7, 25, 37	23
Sorted, OX-26 dull	0·2	–	98·4					64, 67, 68	66	60, 61, 64	62
	0·09	–	96·5	39, 41, 45	42	21, 32, ND	26				

PVG-(Pta^{a}_{A} + Igk-1^{b}) marrow or fetal liver cell suspensions were separated by a FACS according to their MRC OX-26 staining, and injected with 10^{7} PVG bone marrow cells into irradiated PVG recipients. Lymph node biopsies (Experiments A and C, 15 weeks; Experiment B, 33 weeks) were analysed for chimaerism in the B (sIg^{+}) and T (Pta^{+}) compartments by staining with alloantibodies and FACS analysis.

* Percentages above the upper threshold (MRC OX-26^{+}) or below the lower threshold (MRC OX-26^{-}) used for sorting. A small fraction of cells fell in the gap between. The values for the sorted fractions indicate the purity after sorting.

† ND = not determined.

‡ In other experiments, values of 17·0, 30·4, 23·9 were obtained (overall mean = 25%). Negative control < 1%.

§ Latex beads added to increase the frequency of dull particles sorted to about 20%, so as to improve viability of recovered dull cells.

¶ In other experiments, values of 3·1, 4·9 and 4·3 were obtained (overall mean 4·2). Control was 99·8%.

Table 2. Bone marrow CFU-S assay

Bone marrow cells	Dose ($\times 10^{-6}$)	OX-26$^+$ (%)	OX-26$^-$ (%)	Colonies (day 9)
Unlabelled, unsorted	1·0			25, >35
	0·3			9, 11, 13
Labelled, unsorted	1·0	13·4	84·7	25, 25, 27
MRC OX-26 bright	0·21	89		3, 5, 7
MRC OX-26 dull	1·0		99·5	25, 27, >35, >35
Mixed bright & dull	1·0			34, >35
Nil (saline)	0			0, 1, 1

non-lymphoid tissues. Some labelled sections are shown in Fig. 3.

In the thymus, small numbers of heavily labelled cells, along with a greater number of weakly labelled cells, were seen in the medulla. In the cortex, weakly labelled cells were seen scattered amongst unlabelled cells. No evidence was detected for heavily labelled cells in the outer cortex where cells are first believed to divide after entering the thymus. In the spleen, cells in germinal centres were labelled, and such labelling was also seen in lymph nodes and Peyer's patches (not shown). Many heavily labelled cells were seen in the spleen red pulp, and clearly labelled cells were also found immediately beneath the subcapsular sinus in lymph nodes (not shown). Thymic and spleen labelling was also assayed by injecting MRC OX-26 antibody *in vivo* and detecting mouse IgG in sections using peroxidase RAM. Heavily labelled cells were specifically detected as in Fig. 3. It seemed possible that some of the heavily labelled cells were macrophages, and it was established that macrophages could be labelled by the finding that, amongst phagocytic cells from the peritoneum, 50% were labelled with MRC OX-26 antibody.

Many cells were also labelled in non-lymphoid tissues. In the small intestine, dividing epithelial cells in the crypts were heavily labelled, as were non-dividing epithelial cells along the villus. In some cases, intestinal smooth muscle was also labelled (not shown) but the intensity of this varied in different experiments. With *in vivo* administration of antibody, the intestinal epithelial cells were specifically labelled but unequivocal labelling of smooth muscle was not seen. Epithelial cells of skin and tongue were also labelled in tissue sections (not shown). In the testis, cells in the seminiferous tubules were heavily labelled. In pancreas, smooth muscle around arteries reacted strongly, and this was confirmed by *in vivo* labelling. Most other pancreatic cell types showed weak labelling, but no prominent labelling of the islets of Langerhans was seen, as had been described with one antibody but not three others in studies on human transferrin receptor (Gatter *et al.*, 1983). Muscle fibres in skeletal muscle showed weak, but clearly positive, labelling and this was also seen in cardiac muscle and was confirmed by *in vivo* labelling (not shown). In kidney, the tubules of the outer medulla showed uniform labelling, while the collecting ducts and other cells in the inner medulla were unlabelled. Tubules in the cortex were also labelled, but more variably than those in the medulla (not shown). The labelling of kidney tubules could not be confirmed by the *in vivo* method because considerable deposition of IgG was seen in kidney of animals given control antibodies *in vivo*. This was not seen in other tissues. In liver (not shown), parenchymal cells were weakly labelled, and Kupffer cells identified by their phagocytic activity were more strongly positive.

DISCUSSION

We show here that, in both fetal liver and bone marrow of the rat, the predominant detectable stem cell activity is mediated by cells that do not express transferrin receptors reactive with MRC OX-26 antibody. This will have an obvious technical value in enrichment of stem cells from fetal liver. Our results fit well with data recently reported for the mouse which showed that CFU-S are insensitive to killing by anti-transferrin receptor antibody conjugated with ricin (Lesley *et al.*, 1984). Therefore, we conclude that most haemopoietic stem cells are non-dividing, or otherwise that they have an unusual mechanism for obtaining the iron that is presumably needed for cell proliferation. A non-dividing state for many stem cells is consistent with the failure of hydroxyurea or

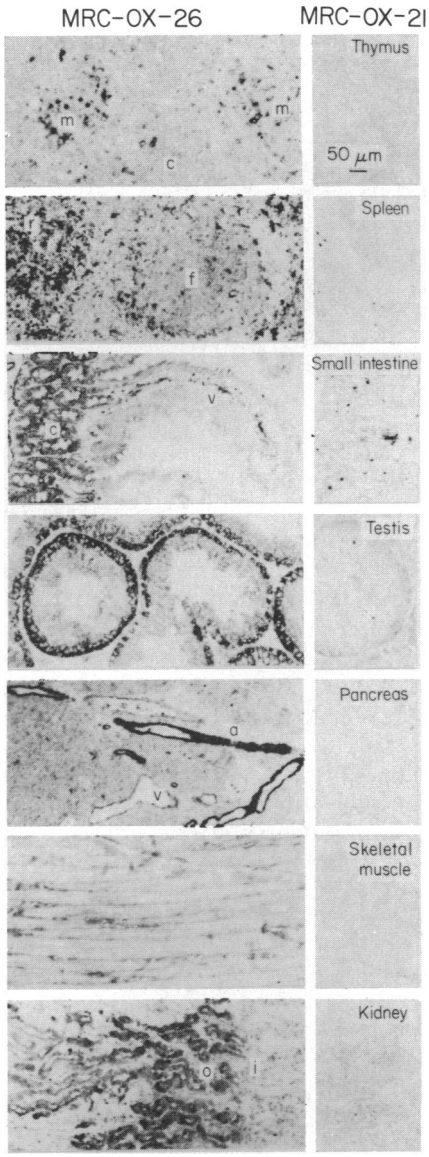

Figure 3. Normal tissues labelled by the immunoperoxidase method with MRC OX-26 antibody. For each tissue, a section that was incubated with MRC OX-26 antibody is shown to the left of a smaller control section reacted with MRC OX-21 antibody. The bar in the thymus control shows 50 μm. Thymus: c, cortex; m, medulla; Spleen: f, follicle; r, red pulp; Small intestine: v, villus; c, crypts; Pancreas: a, artery; v, vein; Kidney: i, inner medulla; o, outer medulla.

tritiated thymidine to kill spleen colony-forming cells with high self-renewal capacity (Vassort *et al.*, 1973; Becker *et al.*, 1965; Ross, Anderson & Micklem, 1982). It might be expected that, under conditions of haemopoietic stress, more stem cells would be dividing and, thus, activity in the MRC OX-26 positive fraction might be detected. This was tested in two experiments (data not shown) where stem cell activity was measured in the bone marrow of irradiated rats (9·5 Gy) which had been reconstituted with fetal liver 7 days earlier. Even in this case, all detectable stem cell activity was MRC OX-26 negative. Thus, it seems clear that transferrin receptor cannot be detected on most mouse and rat stem cells which are assayed in chimaeric tests and, if human stem cells follow this pattern, then anti-transferrin receptor antibodies could be used for removing tumour cells from bone marrow (Trowbridge & Domingo, 1982) for autologous transplantation.

In a partial survey of tissues staining with MRC OX-26 antibody, a variety of cell types were positive, including both dividing and non-dividing cells. Strong reactions with non-dividing cells were most clearly established by the labelling of brain capillaries described elsewhere (Jefferies *et al.*, 1984), but other examples were seen, including reaction with kidney tubules, hepatocytes, pancreatic cells and muscle cells. Positive reactions could be due to irrelevant cross-reactions, but this possibility was rendered less likely by the fact that most reactions were equally seen when MRC OX-26 antibody was given *in vivo*, which presumably restricted the labelling to cell-surface determinants. Indeed, most of the reactions seen in the rat were also seen in a study of human transferrin receptor (Gatter *et al.*, 1983). The only outstanding discrepancy is the labelling of some smooth muscle seen in the present study, which was not identified in the human work (K. Gatter, personal communication). The reason for this difference is, at present, unclear. A possible conclusion from the staining of tissue sections is that transferrin receptor expression is correlated with cells showing high levels of metabolic activity, rather than a tight correlation with dividing cells.

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