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Characteristics of a Cell Surface Antigen Defined by an Anti-human Osteogenic Sarcoma Monoclonal Antibody*

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Abstract—*The expression of a cell surface antigen defined by an anti-human osteogenic sarcoma monoclonal antibody was analysed by flow cytofluorometry using fluorescein-labelled antibody. Quantitative absorption tests established that the antigen was associated with plasma membranes, whereas cytosol, cellular lipids and nuclei were largely devoid of activity. Single-phase aqueous butanol solutions at non-cytolytic concentrations failed to solubilize the antigen, although treatment of cells with papain virtually abolished antigenic activity. The antigen was shown to be solubilized by the non-ionic detergent Nonidet P-40, and following lactoperoxidase-catalysed radioiodination of viable cells, extraction with detergent, immunoprecipitation of antigen with monoclonal antibody and Sepharose-Protein A, the molecular weight of antigen was determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The findings indicate that this human osteogenic sarcoma antigen is a monomeric integral membrane protein with an apparent molecular weight of 72,000, which is predominantly expressed at the external face of the tumour cell plasma membrane.*

INTRODUCTION

MONOCLONAL antibodies have been prepared against a human osteogenic sarcoma cell line designated 791T [1, 2]. As with a number of xenogenic anti-human tumour monoclonal antibodies already described (e.g. [3–6]), one of the antibodies produced, anti-791T/36, displays strong reactivity with the immunizing tumour cell line and several other cell lines derived from tumours of the same histological type, and weak or no reactivity with cells obtained from normal tissues. In addition, this antibody reacts characteristically with several other established tumour cell lines [1, 2]. There is no formal proof that the anti-791T/36 antibody specifies tumour-associated antigens recognized by cancer patients. However, recent investigations have emphasized its potential usefulness to provide new approaches for the *in vivo* localization of malignant tissue deposits and possibly as a vehicle for targeting anti-tumour agents. Radioiodinated preparations of anti-791T/36 antibody preferentially localize within 791T xenografts in immunodeprived mice [7], and have been employed for external imaging of colon carcinomas and their metastases in patients [8]. In addition, conjugates of monoclonal antibody and vindisine have been shown to exert cytotoxicity for tumour cells *in vitro* [9].

In the present communication, the biochemical characteristics of the cell surface antigen defined by the anti-791T/36 monoclonal antibody have been investigated in order to establish a basis for further detailed chemical analysis and to provide information with regard to its molecular expression which is relevant to projected clinical programmes.

MATERIALS AND METHODS

Cells

The human tumour cell lines employed in the present study are listed in Table 1. Also summarized in this table is the reactivity of each cell line with anti-human osteogenic sarcoma 791T/36 monoclonal antibody in hybridoma supernatants as assessed using the indirect [^{125}I]-Protein A cell-binding assay [1, 2]. Cell lines were

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Table 1. Summary of cell lines employed and their reactivity in the indirect [^{125}I]-Protein A cell-binding assay with anti-791T/36 monoclonal antibody

Cell line	Cell type	Reactivity of anti-791T/36 monoclonal antibody [1, 2]
791T	Osteogenic sarcoma	+++
788T	Osteogenic sarcoma	+++
HC Lo	Colon carcinoma	+++
A549	Lung carcinoma	+
T24	Bladder carcinoma	-
RPMI 1566	Melanoma	-

grown as monolayers in Eagle's minimum essential medium supplemented with 10% (v/v) foetal calf serum, and cells were harvested following brief exposure to trypsin unless otherwise stated.

Monoclonal antibodies

Hybridomas 791T/36 Clone 3 and 791T/48 Clone 15 [1, 2] provided the source of antibody in supernatants from *in vitro* cultures. These antibodies (both mouse IgG2b with κ light chain) were purified by affinity chromatography using Sepharose-linked Protein A (Pharmacia, Uppsala, Sweden) as previously described [10]. Anti-HLA-ABC (shared determinant) Clone W6/32 HL monoclonal antibody was obtained as serum/ascites fluid (Ig content 2 mg/ml) from Sera-Lab (Crawley Down, Sussex). Normal mouse IgG was obtained from Miles Laboratories (Stoke Poges, U.K.).

FITC-labelled anti-791T/36 antibody cell-binding assay

Fluorescein isothiocyanate (FITC)-labelled anti-791T/36 antibody was prepared by adding antibody preparations (1 mg antibody protein in 1 ml 0.5 M carbonate buffer, pH 9.5) to glass tubes coated with FITC (60–80 μg; FITC was dried onto glass from a 1-mg/ml solution in acetone). Labelled antibody was separated from unconjugated FITC by gel filtraton upon Sephadex G25 using pre-packed PD-10 columns (Pharmacia, Uppsala, Sweden) equilibrated and eluted with phosphate-buffered saline, pH 7.3 (PBS). FITC: IgG molar ratios (from 4.6:1 to 7:1) and protein concentrations were determined by the method of Forni [11].

The reactivity of FITC-labelled anti-791T/36 antibody with tumour cells was analysed by flow cytometry. Target cells (2×10^5) were incubated with various amounts of antibody (as indicated in the text) for 45 min at 4°C with rolling of samples and analysed without washing in a FACS IV Flow Cytofluorometer (Becton Dickinson, Sunnyvale, CA, U.S.A.). Excitation was at 120 mW at 488 nm from an argon ion laser and fluorescence was collected via a 10-nm band pass filter centred at 515 nm. Appropriate forward angle scatter gating was used to confine the fluorescence analysis to viable tumour cells, excluding dead cells and any cell debris. Linear amplification was used to quantitate mean fluorescence intensity, and fluorescence signals were also amplified logarithmically for direct comparison of fluorescence intensity.

In tests using unabsorbed FITC-labelled anti-791T/36 antibody or labelled antibody absorbed with cells, subcellular membranes, cytosol or nuclei, cells and antibody were prepared in Hanks' BSS + 0.1% (w/v) BSA. When labelled antibody was absorbed with detergent extracts of tumour cells, 791T target cells were fixed with 0.01% (v/v) glutaraldehyde [12] and suspended in Hanks' BSS containing 10% (v/v) newborn calf serum and 3% (w/v) BSA. All absorption tests were performed using 2×10^5 791T target cells and 10^2 ng FITC-labelled anti-791T antibody with an absorption period of 60 min at 4°C.

Extra-nuclear membranes (100,000 *g* pellets of 600 *g* supernatant of homogenates), cytosol (100,000 *g* supernatants of homogenates) and nuclei were prepared as previously described [13]. Total lipid extracts of 791T cells were isolated by chloroform/methanol extraction according to Magnani *et al.* [14] and evaporated in glass tubes under a stream of nitrogen. The dried lipid extract was dispersed in 0.1% (v/v) Nonidet P-40 (NP-40) prior to its use in the absorption assay. Detergent extracts were prepared by incubating cells in TNEN buffer (20 mM Tris–HCl, 100 mM NaCl, 1 mM EDTA, 0.5% NP-40, pH 8.0) [15] at 5×10^6/ml for 2 hr at room temperature. Extracts were centrifuged at 100,000 *g* for 60 min and the supernatants were stored at -20°C.

After absorption of FITC-labelled anti-791T/36 antibody with the various fractions, cells and nuclei were removed by centrifugation at 10,000 *g* for 5 min and extra-nuclear membranes were

sedimented at 100,000 ***g*** for 60 min, and the cell-binding activity of supernatants was evaluated. Labelled antibody absorbed with soluble or solubilized fractions was examined directly for 791T cell-binding activity at the end of the absorption period (60 min at 4°C).

Enzyme treatment of 791T cells

791T cells (2×10^5 cells/tube) were treated with 1-ml aliquots of solutions of trypsin, α-chymotrypsin, papain, β-galactosidase, β-glucosidase and neuraminidase (Sigma Chemical Co., London, U.K.) for 30 min at 37°C at concentrations stated in the text. Each enzyme was dissolved in Hanks' BSS, although with papain the solution also contained 10 mM L-cysteine. After incubation the cells were washed twice with cold (0°C) Hanks' BSS + 5% calf serum. Saturating quantities of FITC-labelled antibody were added to the final cell pellets (10^3 ng/ml/2×10^5 cells) prior to their examination by flow cytometry after incubation for 45 min at 4°C.

Butanol treatment of 791T cells

791T cells in Eagles-HEPES buffer containing 5% calf serum (1 ml containing 10^7 cells) were labelled with ^{51}Cr by the addition of 50 μCi $Na_2[^{51}Cr]O_4$ (Radiochemical Centre, Amersham, U.K.). Following incubation at 37°C for 60 min, the cells were washed twice with Eagles–HEPES + 5% calf serum and once with PBS and dispensed at 2×10^5 cells/tube. The cells were sedimented and aliquots (1 ml) of butanol solutions in PBS were added. Following incubation for 5 min at room temperature, the cells were washed twice with cold (0°C) PBS and the remaining cell-associated radioactivity was determined. The cells were re-suspended in FITC-labelled antibody (10^3 ng/2×10^5 cells), and following incubation (45 min at 4°C) the binding of labelled antibody to treated cells was measured by flow cytofluorimetry.

Radioimmunoprecipitation of anti-791T/36-defined antigen

791T cells were surface-labelled with ^{125}I according to the procedure of Brown *et al.* [16]. Cells ($1–2 \times 10^7$), which had been detached from culture flasks by trypsinization or using versene, were washed four times with Hanks' BSS and then twice with PBS. The following were added to the final cell pellets: 1 ml 5 mM glucose in PBS, 100 μg glucose oxidase (Sigma Chemical Co.), 20 μg lactoperoxidase (Sigma Chemical Co.) and 1–2 mCi $Na[^{125}I]$. After 10 min incubation at room temperature the cells were washed 4 times with 10 ml PBS and lysed with 0.5–1 ml of TNEN containing 0.5% sodium deoxycholate, and 10 mM NaI (immunoprecipitation, IP buffer). The cells were incubated for 60 min at 0°C and centrifuged at 100,000 ***g*** for 30 min.

To aliquots of radiolabelled membrane proteins (approximately 5×10^6 counts/min ^{125}I) equal volumes of IP buffer containing 4% BSA and 0.2% sodium dodecyl sulphate (SDS) were added, followed by 5–50 μl antibody (5–40 μg). After incubation at 0°C for 2 hr, 100-μl aliquots of a 1/4 suspension of Sepharose-Protein A (i.e., 50 μg Protein A/sample; Pharmacia, Uppsala, Sweden) in IP buffer, 2% BSA and 0.1% SDS were added and incubation was continued for a further 20 min at 0°C. The Sepharose beads were then washed twice with IP buffer containing 2% BSA and 0.1% SDS, once with IP buffer containing 0.1% BSA and 0.1% SDS and twice with a 1/10 dilution of TNEN in water. Each wash volume was 5 ml. The final pellets were suspended in 50–100 μl of sample buffer [17], incubated for 3 min at 100°C and centrifuged for 5 min at 500 ***g***. The supernatants were electrophoresed on an SDS-12% polyacrylamide gel at 50 V [17]. The gel was stained for protein with Coomassie blue, and after destaining the gel was dried and autoradiographed for 5–10 days at −70°C with pre-flashed Fuji X-ray film (Fuji Photo Co. Ltd., Tokyo, Japan) and an intensifying screen (Cawo, F.R.G.). Molecular weight markers were transferrin (85,000), bovine serum albumin (67,000), IgG heavy chain (55,000), ovalbumin (45,000), IgG light chain (25,000), haemoglobin (17,000) and cytochrome *c* (12,400).

RESULTS

Binding of FITC-labelled anti-791T/36 antibody to 791T cells

The expression of the cell surface antigen defined by anti-791T/36 monoclonal antibody was analysed by flow cytometry using FITC-labelled antibody. With this procedure the distribution of fluorescence on a cell population treated with labelled antibody was determined, and within that distribution the mean fluorescence intensity per cell was calculated. Upon titrating FITC-labelled antibody against a fixed number of 791T cells, it was determined that the maximum uptake of labelled antibody was achieved at approximately 10^3 ng antibody/2×10^5 cells, indicating saturation of anti-791T/36-defined antigen (Fig. 1). The specificity of this binding was established by competitive inhibition in which FITC-labelled anti-791T/36 antibody was mixed with known quantities of unlabelled antibodies. As shown in Fig. 1, when 10^3 ng of labelled antibody was mixed with an equal amount or a 10-fold excess of unlabelled anti-791T/36 antibody, the binding of labelled antibody was reduced to approximately 50 and

10% respectively of the control value. The addition of unlabelled preparations of another anti-791T monoclonal antibody, anti-791T/48, to labelled anti-791T/36 failed to reduce the latter's binding to 791T cells, thus indicating that the 2 antibodies define separate cell surface epitopes. Figure 2 illustrates raw data of the cold antibody competitive inhibition assay. With 10^3 ng of labelled antibody/2×10^5 cells, all cells falling within the viable malignant cell forward angle scatter gate showed considerably increased fluorescence compared with the unstained control. The addition of an equal and 10-fold excess of unlabelled anti-791T antibody quantitively inhibited the binding of labelled antibody (Figs 1 and 2).

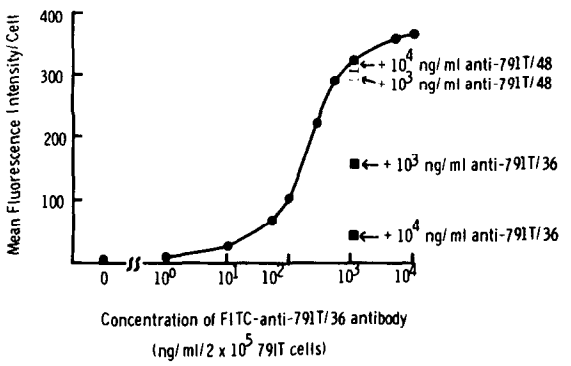

Fig. 1. Titration of the binding of FITC-labelled anti-791T/36 monoclonal antibody against 791T target cells (●——●) as assessed by flow cytometry. In competitive inhibition tests aliquots of FITC-labelled anti-791T/36 antibody (10^3 ng) were admixed with unlabelled anti-791T/36 antibody (■) or unlabelled anti-791T/48 antibody (□) at the concentrations stated, and the cell-binding activity of labelled antibody was determined.

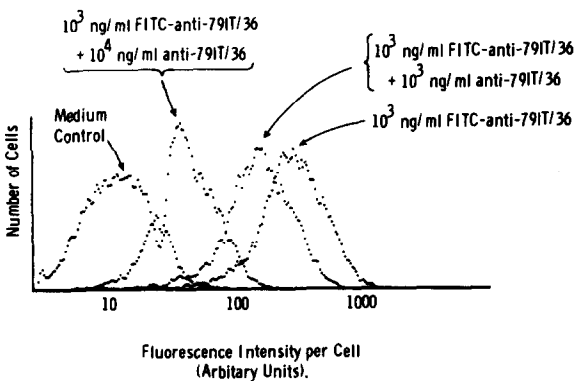

Fig. 2. Competitive inhibition of binding of FITC-labelled anti-791T/36 monoclonal antibody by unlabelled anti-791T/36 antibody at the concentrations stated. The figure illustrates the direct print-out from the FACS IV Cell Sorter with logarithmic amplification of the fluorescence signal measured on single cells in each antibody-treated cell preparation.

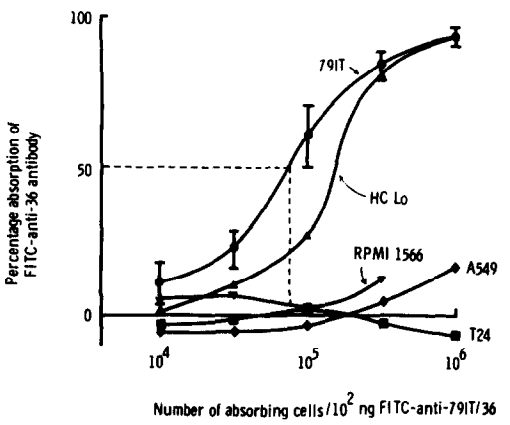

Fig. 3. Absorption of FITC-labelled anti-791T/36 antibody by the cell lines 791T (●——●), HC Lo (△——△) RPMI 1566 (▼——▼) A 549 (◆——◆) and T 24 (■——■) as assessed by flow cytometry.

Absorption of FITC-labelled anti-791T/36 antibody by cells

In tests evaluating the capacity of cells, subcellular preparations and soluble and solubilized cell fractions to absorb or neutralize antibody cell-binding activity, the input of FITC-anti-791T/36 antibody was decreased below saturation levels to 10^2 ng antibody/2×10^5 target cells in order to increase the sensitivity of the assay for antigen detection. Figure 3 illustrates the absorption of FITC-labelled anti-791T/36 antibody by five cell lines: 791T and the cross-reactive line HC Lo, and the three lines RPMI 1566, T24 and A549, which are non- or only weakly reactive with anti-791T/36 antibody (Table 1). Absorption of labelled antibody with the latter three lines at up to 10^6 cells/10^2 ng of antibody failed to effect significant reduction in antibody binding to 791T cells, whereas both 791T and HC Lo cell lines produced approximately 95% absorption of labelled antibody binding activity at 10^6 cells/10^2 ng antibody. In Fig. 3 the absorption curve for 791T cells was constructed from data from three individual absorption experiments performed using different batches of target and absorbing cells, and the reproducibility of the assay is reflected in the small standard deviations from the means derived from separate tests (Fig. 3).

Absorption or neutralization of FITC-labelled anti-791T antibody by treated cells, subcellular fractions and soluble preparations

For comparative purposes, the number of 791T cells required to effect 50% absorption of 10^2 ng of FITC-labelled anti-791T/36 antibody activity was determined. As shown in Fig. 3, 8×10^4 viable 791T cells produced 50% absorption. When cells were pre-treated with 0.01% glutaraldehyde, 1×10^5 cells were required for 50% absorption (Table 2). Thus cells subjected to this degree of

Table 2. Absorption or neutralization of FITC-labelled anti-791T/36 cell-binding activity

Absorbing material	Absorbing fraction	No. of cells or cell equivalents required to effect 50% reduction of FITC-anti-791T/36 cell-binding activity*
Particulate	791T Cells	8×10^4
	791T Cells/0.01% glutaraldehyde	1×10^5
	791T Extra-nuclear membranes	9×10^4
	791T Nuclei	5×10^6
Soluble	791T Cytosol	$\gg 1.6 \times 10^7$
	791T Chloroform/methanol extract	$\gg 1 \times 10^7$
	791T NP-40 extract	7×10^5
	788T NP-40 extract	5×10^5
	T24 NP-40 extract	$\gg 2.5 \times 10^6$
	RPMI 1566 NP-40 extract	$\gg 2.5 \times 10^6$

*Absorbing materials were evaluated for their capacity to absorb or neutralize the cell-binding activity of 10^2 ng FITC-anti-791T/36 tested against 2×10^5 791T cells.

fixation retained anti-791T/36 binding activity. Comparably, the recovery of antigenic activity in the 'extra-nuclear' membrane fraction prepared from 791T cells approximated to 100% of that expressed upon intact cells, whereas in excess of 5×10^6 791T nuclei/10^2 ng antibody were required for 50% absorption of antibody binding activity (Table 2).

A number of soluble of solubilized cell extracts were evaluated for their capacity to neutralize FITC-labelled anti-791T/36 antibody cell-binding activity. With extracts containing detergent, glutaraldehyde-treated 791T cells were employed as target cells since they are resistant to detergent and antigen expression is preserved. As shown in Table 2, 791T cytosol failed to neutralize labelled antibody binding activity when tested at up to 1.6×10^7 cell equivalents/10^2 ng labelled antibody, indicating that antigenic activity is not present in the soluble cytoplasmic protein fractions of cell homogenates. Similarly, chloroform/methanol extracts of 791T containing the bulk of cellular lipids were inactive when tested at concentrations of up to 10^7 cell equivalents/10^2 ng labelled antibody. Conversely, NP-40 extracts of the osteogenic sarcomas 791T and 788T neutralized antibody binding, whereas equivalent extracts from the non-cross-reactive cell lines T24 and RPMI 1566 lacked this neutralization capacity (Table 2).

Stability of anti-791T/36-defined antigen to enzymes

791T cells were exposed to various proteolytic and glycolytic enzymes and then assayed for their capacity to bind FITC-labelled anti-791T/36 antibody. Of the enzymes tested only papain produced any significant reduction in labelled antibody binding (Table 3), and in a second experiment the enzyme concentration dependence was investigated. With increasing enzyme concentrations from 0.005 to 0.5 mg papain/2×10^5 cells

Table 3. Modification of 791T cell-binding activity of FITC-labelled anti-791T/36 antibody by pretreatment of cells with enzymes

Experiment No.	Pre-treatment of 791T Cells (incubation: 30 min at 37°C)	Concentration of enzyme (mg/2×10^5 cells)	Percentage reduction in the cell-binding activity of FITC-labelled anti-791T/36 antibody*
1	Trypsin	0.5	22
	α Chymotrypsin	0.5	-21
	Papain	0.5	70
	β-Galactosidase	0.5	-2
	β-Glucosidase	0.5	-7
	Neuraminidase	0.5	-13
2	Papain	0.005	-7
	Papain	0.015	8
	Papain	0.05	28
	Papain	0.15	55
	Papain	0.5	83

*After treatment of cells with enzymes and removal of enzyme by washing, the binding of 10^3-ng aliquots of FITC-anti-791T/36 antibody to 2×10^5 cells in each sample was assessed.

there was a progressive loss in antibody binding activity, and in separate tests it was determined that cell viability was maintained at 85% or greater with each of the enzyme concentrations. In control tests the uptake of labelled antibody to papain-treated cells was inhibited by 90% or greater when fluoresceinated anti-791T/36 was mixed first with a 10-fold excess of unlabelled monoclonal antibody.

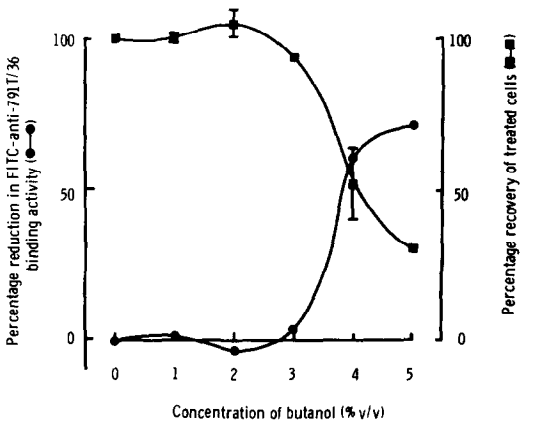

Fig. 4. Effect of n-*butanol treatment of* 791*T cells on the expression of anti-*791*T/*36*-defined antigen. Modification of FITC-labelled anti-*791*T/*36 *cell-binding activity* (●——●) *on butanol-treated cells was assessed by flow cytometry and the viability of treated cells* (■——■) *was determined by the release of intra-cellular* ^{51}Cr.

*Stability of anti-*791*T/*36 *antibody-defined antigen to* n-*butanol*

Single-phase solutions of *n*-butanol at low concentrations (2.5% v/v) have been employed to solubilize tumour-associated surface antigens from cells without loss in cell viability and consequent contamination of the extract with soluble cytoplasmic proteins [18]. The extent of perturbation of membrane components by this butanol extraction is unknown, although 'peripheral' rather than 'integral' membrane proteins are preferentially solubilized [19]. When 791T cells (labelled with ^{51}Cr to assess viability) were treated with increasing concentrations of butanol at pH 7.3 for a period of 5 min, there was no loss in FITC-labelled anti-791T/36 antibody binding activity until butanol concentrations were at 4 and 5% (v/v) (Fig. 4). However, at such concentrations cell viability was markedly reduced as assessed by the release of intracellular ^{51}Cr. Thus the release or inactivation of anti-791T/36-defined antigen was only achieved with the gross disassembly of cells, indicating that the antigen belongs to the class of 'integral' rather than 'peripheral' membrane components.

*Radioimmunoprecipitation of anti-*791*T/*36*-defined antigen*

From the above it is evident that the anti-791T/36-defined antigen is a tumour cell surface membrane-associated component which is susceptible to release or inactivation by the proteolytic enzyme papain. The antigen belongs to that class of membrane components not affected by extraction with butanol at non-cytolytic concentrations but amenable to solubilization with the detergent NP-40. The findings are therefore compatible with the proposal that the anti-791T/36-defined antigen is an integral plasma membrane-associated protein or glycoprotein. In order to analyse the characteristics of this antigen further, 791T cells were surface-labelled with ^{125}I by lactoperoxidase-catalysed radioiodination. Labelled cells were extracted with 0.5% NP-40 and aliquots of the extract were incubated with anti-791T/36 antibody. The immune complexes were isolated by adsorption to Sepharose-Protein A and analysed by SDS-polyacrylamide gel electrophoresis and autoradiography. As shown in Fig. 5, anti-791T/36 antibody precipitated an antigen from trypsin-harvested cells with an apparent molecular weight of 72,000. With cells harvested using versene, again a single 72,000 molecular weight band was obtained, indicating that trypsin did not generate an antigenic fragment during the preparation of the single-cell suspension from the monolayer culture. Similarly, equivalent precipitation patterns were obtained when samples were prepared under non-reducing conditions, indicating that the 791T antigen is a single-chain polypeptide. As shown in Fig. 5, no bands were detectable using normal mouse IgG instead of anti-791T/36 antibody, and as a positive control the W6/32 monoclonal antibody precipitated bands at 44,000 and 12,000 molecular weight corresponding to HLA heavy chain and β-2-microglobulin respectively.

DISCUSSION

Considerable efforts have been directed to the identification of antigens associated with human tumours and defined by monoclonal antibodies. Without doubt, most success has been achieved with melanoma, and the molecular characteristics of a number of antigens have been described [3, 5, 6, 15, 16, 20, 21]. Of these, the glycoprotein antigen p97 is the most completely characterized, both in terms of chemical structure, including its *N*-terminal sequence, and with respect to its functional relationship to transferrin and lactotransferrin [16, 20]. With osteogenic sarcomas there are many reports suggesting that these tumours possess antigens against which patients

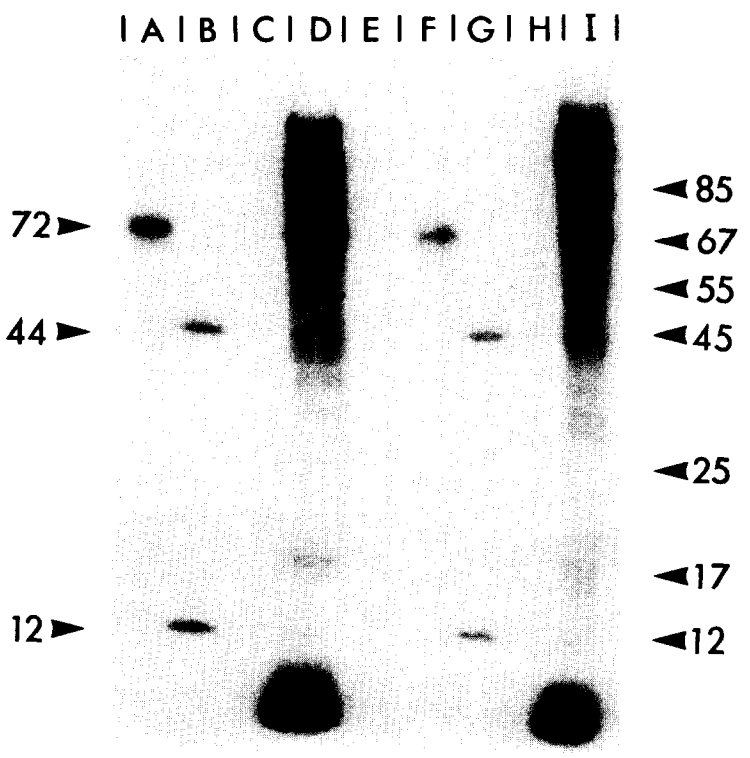

Fig. 5. Radioimmunoprecipitation of anti-791T/36-defined antigen using anti-791T/36 antibody and Sepharose-Protein A as the precipitating agent. In lanes A–D 791T cells were harvested from culture flasks using trypsin, and in lanes F–I cells were harvested using versene. The positions of the molecular weight marker proteins are indicated on the right (molecular weight values are $\times 10^{-3}$*). The 72,000-molecular weight antigen precipitated by anti-791T/36 monoclonal antibody is shown in lanes A and F. Precipitates obtained using the anti-HLA antibody W6/32 are in lanes B and G, and in lanes C and H normal mouse IgG was employed instead of monoclonal antibody as a negative control. The electrophoretic separation of total labelled 791T cell lysates are shown in lanes D and I. Lane E is blank.*

respond with the production of antibodies, although few of these antigens have been biochemically defined [22–26]. In addition, xenogeneic antisera against human osteogenic sarcoma cells and tumour extracts, after absorption, display varying degrees of specificity for sarcoma cells [27, 28]. Also, Hosoi *et al.* [29] have reported the production of three anti-human osteogenic sarcoma monoclonal antibodies which react with osteosarcoma tissues and with one chondrosarcoma specimen but not with other malignant or benign tumours.

Since information concerning the characteristics of osteogenic sarcoma-associated antigens is generally lacking, it is not possible to determine whether the same or different antigens are being detected by the various groups. However, recent tests with the rabbit antisera RM1 and RM3 prepared by Grófová *et al.* [28] have established that they react with antigenic targets other than that defined by the anti-791T/36 monoclonal antibody employed in the present study. Both RM1 and RM3 antisera react with 791T cells, but in competition assays with FITC-labelled anti-791T/36 antibody neither antiserum inhibits the binding of labelled antibody to 791T cells (Price and Grófová, unpublished findings). The two antisera predominantly react with a protein of 115,000 molecular weight but also show weaker reactivity with several cell surface antigens [28].

The results of the present investigation demonstrate that the antigen defined by the monoclonal antibody anti-791T/36 is a monomeric protein or glycoprotein of molecular weight 72,000 which is located at the external surface of the cell plasma membrane. The limited subcellular fractionation studies indicate that the antigen is not present in cytosol, and the finding that the recovery of antigenic activity in 'extranuclear' membranes was similar to that associated with the intact cell (Table 2) suggests that the expression of antigen on intracellular membranes is minimal. The fact that nuclei were able to absorb anti-791T/36 antibody at concentrations greater than 5×10^6 nuclei/10^2 ng antibody (Table 2) has two interpretations: firstly, anti-791T/36-defined antigen may be weakly expressed upon nuclear membranes or, alternatively, the degree of absorption obtained could be attributable to a 2% contamination with intact cells.

The antigen, when expressed upon viable cells, is sensitive to papain (Table 3), although it remains to be established whether this enzyme liberates an antigenically active polypeptide, as was found to be the case with the melanoma antigen p97, protease digestion of which led to the production of a 40,000-molecular weight polypeptide that retained epitopes for several anti-melanoma monoclonal antibodies [16].

Finally, the results of the present study and others [1, 2, 7, 10] have indicated that the anti-791T/36-defined antigen is a major cell surface component, and as such should prove amenable to detailed chemical characterization. Its level of expression makes this antigen a suitable target for the localization of radioiodinated antibody in tumour xenografts in immuno-deprived mice [7]. Radiolabelled anti-791T/36 antibody also localizes to cross-reactive colon carcinomas in patients as assessed by γ-scintigraphy [8] and it is thus important to determine the molecular relationship between these cross-reactive antigens and the 72,000-molecular weight component expressed on 791T cells. This has further relevance since these antigens may represent appropriate determinants for the selective anti-tumour cytotoxicity of drug- or toxin-antibody conjugates [9, 30].

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