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MRC OX-52: a rat T-cell antigen

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SUMMARY

A mouse monoclonal antibody MRC OX-52 has been shown to label rat T lymphocytes and thymocytes. The molecule precipitated by this antibody from both thymocytes and T lymphocytes had a two-chain structure of 120,000 MW and 95,000 MW.

INTRODUCTION

In man, several monoclonal antibodies have been described that are specific for human T cells, a number having been characterized that recognize CD3 (Van Wauwe, De Mey & Goossens, 1980; Tax *et al.*, 1983), and, recently, one that binds to the T-cell receptor itself (Spits *et al.*, 1985). However, the situation in experimental animals is less satisfactory. There are few monoclonal antibodies that recognize T cells, and none of these appear strictly T-cell specific. We describe here a monoclonal antibody MRC OX-52 that appears to recognize a molecule largely restricted to cells of the T-lymphocyte lineage and, hence, of use in identifying such cells in tissues or cell suspensions. The molecule recognized has a MW of 95,000–120,000, but has not been associated with a particular function of T cells.

MATERIALS AND METHODS

Animals

BALB/c (H-2^b) mice were obtained from Olac Limited (Bicester, Oxon).

Inbred PVG-RT1^c, PVG-RT1^u, DA-RT1^a rats and F_1 hybrids between these strains were obtained from the Specific Pathogen-Free Unit of the MRC Cellular Immunology Unit, Oxford.

Antibodies

The cloned hybrid cell line secreting MRC OX-52 antibody was derived from a fusion between mouse spleen cells and the mouse myeloma NSO/U (Clark & Milstein, 1981). The spleen cells were from an animal immunized intraperitoneally (i.p.) with 4×10^7 PVG-RT1^c thoracic duct leucocytes (TDL) followed 10 days later by a second i.p. injection of 4×10^7 PVG-RT1^c spleen cells. The animal was boosted with 5×10^7 PVG-RT1^c spleen cells intravenously and the spleen harvested 4 days later for fusion. The fusion was performed following the method of Köhler & Milstein (1976) as modified by McMaster & Williams (1979). After growth of hybrid cell lines, aliquots of supernatant from each well were analysed for antibody activity in an indirect binding assay (Mason & Williams, 1980) using PVG-RT1^c TDL as targets. Selected supernatants were then screened on the fluorescence activated cell sorter (FACS II) using rabbit $F(ab')_2$ anti-mouse IgG conjugated to fluorescein (RAM FITC) (Williams, Galfré & Milstein, 1977). MRC OX-52 antibody was used throughout as tissue culture supernatant. Other monoclonal antibodies used were: mouse anti-squid glycoprotein (from Dr A. F. Williams, MRC Cellular Immunology Unit, Oxford); W3/25 which recognizes thymocytes (Williams *et al.*, 1977), T helper cells (White *et al.*, 1978) and macrophages (Barclay, 1981b); W3/13 which recognizes thymocytes, T cells, polymorphs, brain (Williams *et al.*, 1977), plasma cells (Dallman, Thomas & Green, 1984) and natural killer cells (Cantrell *et al.*, 1982); MRC OX-6 which recognizes a non-polymorphic determinant on rat Ia, MHC Class II antigen (McMaster & Williams, 1979); MRC OX8 which recognizes T cytotoxic cells (Mason *et al.*, 1980) and natural killer cells (Cantrell *et al.*, 1982); MRC OX-12 which recognizes mouse anti-rat kappa chain (Hunt & Fowler, 1981) and MRC OX-19 which recognizes T cells and thymocytes (Dallman *et al.*, 1984).

Cells

TDL were obtained by thoracic duct cannulation and collected overnight into ice-cold Dulbecco's A+B medium (DAB) containing 20 u/ml heparin. Mesenteric lymphadenectomy (MLNX) and dendritic cell-enriched MLNX TDL were produced by the method of Pugh, MacPherson & Steer (1983). Bone marrow cells were obtained by flushing the marrow cavity of both femurs with phosphate-buffered saline (PBS) containing 20 u ml/heparin (PBS 20). Thymus, spleen or lymph nodes were removed and teased into single cell suspensions in ice-cold DAB

Abbreviations: BSA, bovine serum albumin; DAB, Dulbecco's A+B medium; DMEM, Dulbecco's minimal essential medium; FACS, fluorescence-activated cell sorter (Becton-Dickinson, Sunnyvale, CA); FITC, fluoroscein isothiocyanate; i.p., intraperitoneally; MLNX TDL, TDL obtained from mesenteric lymphadenectomized rats; M_rW, molecular weight; PBS, phosphate-buffered saline; RAM, immunoabsorbent purified rabbit anti-mouse IgG; SDS–PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; TDL, thoracic duct leucocytes.

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containing 0·2% bovine serum albumin (BSA). Resident peritoneal cells were obtained by massaging the abdomen of a freshly killed rat after injection of 10 ml of PBS 20. The peritoneum was then opened and the fluid removed.

Immunoprecipitation

TDL were examined after depletion of B cells using MRC OX-12 and MRC OX-6 in an indirect rosetting technique described by Mason (1981) after Parish & Hayward (1974).

Thymocytes or B-cell depleted TDL were surface-labelled with ^{125}I using the lactoperoxidase procedure described by Trowbridge, Ralph & Bevan (1975) and modified by Jefferies *et al.* (1985). Cell lysis and indirect immunoprecipitation using protein-A Sepharose CL-4B saturated with rabbit IgG anti-mouse IgG (RAM) (Mason & Williams, 1980) were performed as described by Thomas & Green (1983), except that the lysing buffer contained 3% NP40 (BDH Chemicals Ltd, Poole, Dorset) instead of 3% Brij 96. To preclear, monoclonal mouse anti-squid Thy-1 was added to the supernatant. On occasion, in addition this was followed by preclearing with W3/13 or MRC OX-52 antibody. After washing the beads, immunoprecipitates were released from the beads by boiling for 5 min in 0·05 ml sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) sample buffer (Laemmli, 1970) containing 5 mM iodoacetamide. Precipitates were analysed by SDS–PAGE (Laemmli, 1970) and autoradiography. Molecular weight markers (Sigma Chemical Co., Poole, Dorset) were: myosin 200,000 MW, β galactosidase 118,000 MW, phosphorylase B 94,000 MW, BSA 68,000 MW and ovalbumin 43,000 MW.

Miscellaneous methods

Labelling of cells with monoclonal antibodies for analysis of cells on the FACS II was performed as described previously (White *et al.*, 1978). Histology of frozen thin sections and the immunoperoxidase technique were as described by Barclay (1981a) as modified by Dallman, Mason & Webb (1982). Cryostat sections of spleen, thymus, lymph node, Peyer's patch, small intestine, skin, kidney, liver and brain were examined. Smears and cytocentrifuge preparations were made in 10% BSA. Phagocytosis of latex was performed in DMEM 0·2%

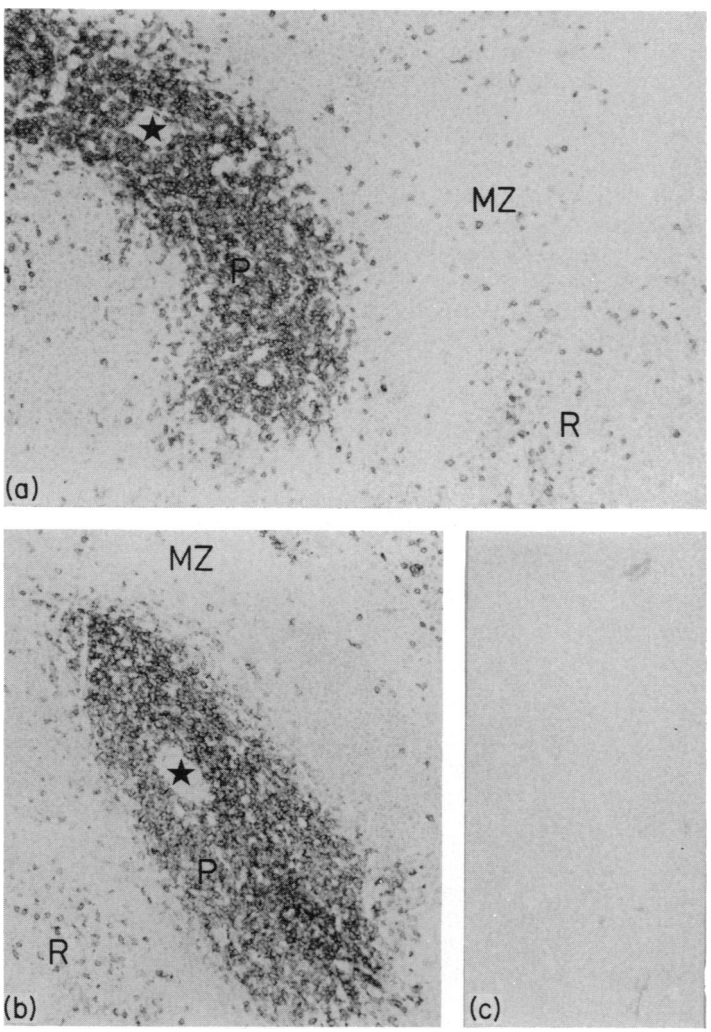

Figure 1. Cryostat sections of spleen labelled by the immunoperoxidase technique using: (a) MRC OX-52 antibody, (b) MRC OX-19 antibody, (c) MRC OX-21 antibody, and counterstained with haematoxylin. (*) Central artery of the periarteriolar lymphoid sheath; P, periarteriolar lymphoid sheath; MZ, marginal zone; R, red pulp. Magnification × 55.

BSA using ~ 10^6 cells/ml. Cells were incubated at 37° for 30 min in Eppendorf tubes to which there was no significant cell adherence.

The mixed leucocyte allogeneic response was performed according to the method previously described (Mason, Pugh & Webb, 1981). Monoclonal antibodies were added to the assay at a final concentration of 1/10, 1/30, 1/90 or 1/270 of tissue culture supernatant.

The generation of cytotoxic T cells and the ^{51}Cr-release assay were performed as follows. PVG-RT1^c spleen cells (5×10^5) were cultured with 2×10^5 (PVG-RT1^u × PVG-RT1^c) F_1 irradiated spleen cells in RPMI containing porcine serum. The F_1 stimulator cells were irradiated with 1000 rads using a ^{137}Cs source (Gamma cell, Atomic Energy of Canada). After 5 days, cytotoxic T cells in the culture were detected in a ^{51}Cr-release assay (Dallman *et al.*, 1982; Pearson, Hodes & Friberg, 1969) using Y3, a rat myeloma line carrying the RT1^u major histocompatibility antigens (Cotton & Milstein, 1973), as specific target cells, and NSI, a mouse myeloma line (Williams *et al.*, 1977), to check the specificity of killing. After a 6-hr assay, the percentage specific release was calculated by the formula:

$$\% \text{ specific release} = \frac{(\text{experimental release} - \text{spontaneous release})}{(\text{maximum release} - \text{spontaneous release})} \times 100.$$

RESULTS

Using the immunoperoxidase technique, MRC OX-52 antibody staining was found mainly confined to the T-cell areas of the spleen (Fig. 1), lymph node and Peyer's patch, where labelled cells were small with a rounded morphology. Occasional positive cells were seen in the B-cell areas of these organs. In the thymus, MRC OX-52 antibody labelled all thymocytes, but the medullary cells were more strongly positive than the cortical cells. In all these organs the staining pattern resembled that seen with MRC OX-19 (Fig. 1) (Dallman *et al.*, 1984). Non-lymphoid tissues examined, including brain, kidney, liver and skin, did not label with MRC OX-52 antibody.

Cell suspensions analysed using the immunoperoxidase technique or by FACS II analysis of immunofluorescent cells showed that MRC OX-52 antibody labelled 1% of bone marrow cells, 56% of TDL, all thymocytes and 25% of spleen cells (Table 1). In TDL W3/25 positive cells and MRC OX-8 positive cells were found within the MRC OX-52 positive population and accounted for virtually all of these cells. Similarly, as expected from this result, MRC OX-19 and MRC OX-52 antibodies labelled the same population. All cells expressing MRC OX-52 were W3/13 positive. Examination of the fluorescence histograms obtained from the FACS (Fig. 2) showed that the expression of the MRC OX-52 antigen on TDL was quite heterogeneous, but there was a sharp distinction between the antigen-positive T cells and the antigen-negative B cells. The mean fluorescence intensity was less than that obtained with W3/25 antibody (Fig. 2), indicating that the MRC OX-52 antigen is expressed in rather low amounts on peripheral T cells, i.e. on average less than 20,000 molecules/cell.

In spleen cell suspensions, interpretation of the data was complicated by the presence of non-lymphoid cells expressing antigens also found on lymphocytes, such as MRC OX-8 on natural killer cells and W3/25 on macrophages. However, the great majority of cells labelled with MRC OX-19 antibody labelled with MRC OX-52 antibody indicating MRC OX-52 antigen is present only on T lymphocytes (Table 1).

MRC OX-52 antibody labelling was not seen on mononuclear phagocytes or granulocytes from the peritoneal cavity, but appeared weakly on approximately 50% of dendritic cells from MLNX TDL.

Table 1. Percentage of cells in rat tissues labelled with MRC OX-52 and other antibodies

Antibodies	Percentage of cells labelled		
	TDL	Lymph node cells	Spleen cells
MRC OX-52	56·2	55·0	30·0
MRC OX-19	57·5	63·4	30·0
MRC OX-8	10·4	28·0	17·9
W3/25	48·5	38·0	26·7
MRC OX-12	41·7	36·4	58·0
W3/13	58·6	ND*	43·0
MRC OX-52 and MRC OX-19	61·6	62·5	33·4
MRC OX-52 and MRC OX-8	57·4	60·8	32·5
MRC OX-52 and W3/25	57·8	60·1	35·3
MRC OX-52 and MRC OX-12	94·9	88·9	82·6
MRC OX-52 and W3/13	56·1	ND	42·3

MRC OX-21, which does not react with rat tissues, was used as a negative control, and labelled less than 1% of TDL and lymph node cells and 5% of spleen cells. These experiments were all repeated with similar results.

* ND, not done.

The nature of the molecule recognized by MRC OX-52

Suspensions of thymocytes or TDL were radio-iodinated at the

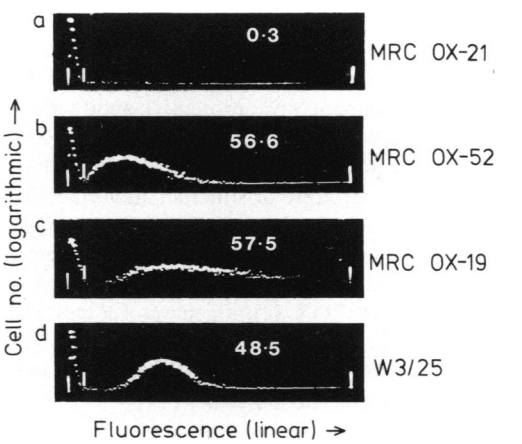

Figure 2. Fluorescence histograms of TDL labelled with (a) MRC OX-21, (b) MRC OX-52, (c) MRC OX-19, and (d) W3/25 antibodies. Single cell suspensions were incubated with a single monoclonal antibody, washed, and then incubated with RAM FITC. Fluorescence histograms were obtained using 5×10^4 cells. The numbers given on each profile indicate the percentage of cells that fell into fluorescence channels above the lower marker.

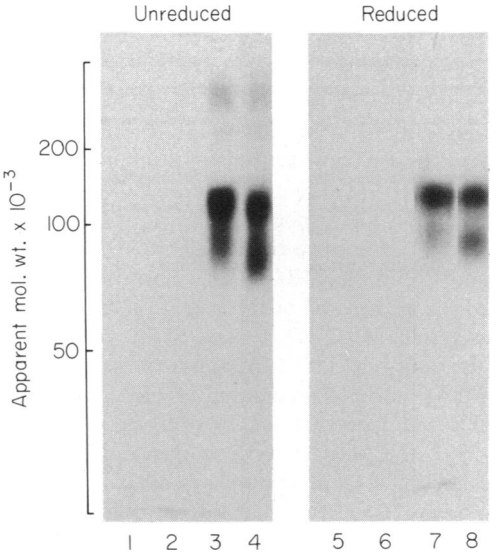

Figure 3. Analysis by immunoprecipitation of the antigen recognized by MRC OX-52 antibody. Thymocytes or B-cell depleted TDL were labelled with ^{125}I at the cell surface, solublized in detergent and immunoprecipitated indirectly with antibodies coupled to Sepharose CL 4B. The control antibody recognized a squid glycoprotein. The cell lysates of thymocytes are shown in Tracks 1, 3, 5 and 7, and of B-cell depleted TDL in Tracks 2, 4, 6 and 8. The immunoprecipitate with MRC OX-52 is shown in Tracks 3, 4, 7 and 8, and with the control antibody in Tracks 1, 2, 5 and 6.

cell surface and the molecule recognized by MRC OX-52 antibody analysed using immunoprecipitation, SDS–PAGE and autoradiography. The molecule precipitated from thymocytes electrophoresed as a broad band extending from 95,000 to 120,000 MW (Fig. 3), with the greatest activity occurring at 110,000–120,000 MW. There was no significant change in the MW or quality of the band under reducing conditions. From TDL, the molecule precipitated with MRC OX-52 antibody electrophoresed as two distinct broad bands of 110,000–120,000 MW and 90,000–105,000 MW (Fig. 3). Again, the bands were unchanged on reduction. The MW of the smaller polypeptide on thymocytes is similar to that of the antigen recognized by W3/13 which appears as a discrete band at 95,000 MW (Standring *et al.*, 1978). The predominant glycoprotein(s) on thymocytes also appears at this MW and some, but not all, are recognized by W3/13 antibody. In order to determine whether MRC OX-52 antibody labelled the same or different molecules on thymocytes, experiments were performed using W3/13 or MRC OX-52 antibodies to preclear cell lysates prior to immunoprecipitation with MRC OX-52 or W3/13 antibodies, respectively. This revealed that the MRC OX-52 antigen was not removed by W3/13 antibody and the W3/13 molecule was not removed by MRC OX-52 antibody (data not shown). Hence, the two antigens recognized are on separate molecules.

Effect of MRC OX-52 on T-cell functions *in vitro*

The presence of MRC OX-52 antibody used as tissue culture supernatant diluted 1/10, 1/30, 1/90 or 1/270 did not inhibit lymphocyte proliferation in the allogeneic mixed leucocyte response. The presence of the antibody did not prevent the generation of T cytotoxic cells in the allogeneic mixed leucocyte responses or inhibit T cytotoxic effector cell function as measured by ^{51}Cr release from specific targets (data not shown).

DISCUSSION

The antigen recognized by MRC OX-52 antibody appears to label only T cells in cell suspensions prepared from lymph node, TDL, thymus and spleen. Labelling of cryostat tissue sections with MRC OX-52 antibody is restricted to the T-cell areas of lymphoid organs, including all thymocytes. However, there was weak staining of some dendritic cells prepared from MLNX TDL, although no labelling of dendritic cells in other tissues was seen. Granulocytes and macrophages did not express MRC OX-52 antigen. Hence, MRC OX-52 antibody is a useful marker in conjunction with other markers for identifying T cells generally in tissues and cell suspensions, and in fact has been used to augment the staining by MRC OX-19 antibody of T cells in cryostat sections.

A molecule, CD6, of similar MW to the MRC OX-52 antigen has been described in man. This antigen, as defined by antibody 12·1 (Kamoum *et al.*, 1981), is present on all mature T cells and thymocytes. The antibody immunoprecipitates a 120,000 MW molecule from T cells (Horibe *et al.*, 1984). Amino acid sequence data are required to decide if these molecules are homologous.

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