MRC OX-22, A MONOCLONAL ANTIBODY THAT LABELS A NEW SUBSET OF T LYMPHOCYTES AND REACTS WITH THE HIGH MOLECULAR WEIGHT FORM OF THE LEUKOCYTE-COMMON ANTIGEN

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Thymus-derived cells of the mouse, rat, and human can be divided, on the basis of differential expression of cell surface antigens, into phenotypically distinct subsets, and this phenotypic heterogeneity is correlated with functional differences (1–3). In the rat, the monoclonal antibody W3/25 (5) has been shown to label the T subset that contains helper cells for B cells (5) and cytotoxic T cells (6, 7) and also cells that mediate graft-versus-host (GvH)[1] reactivity (5). The monoclonal antibody MRC OX-8 labels the subset that contains suppressor and cytotoxic activities (2, 6, 8).

In this paper a new monoclonal antibody MRC OX-22 is described that labels a subset of the W3/25$^+$ cells in the rat. Data are presented demonstrating that this phenotypic division is correlated with a functional one, with cells mediating help for B cells and those mediating GvH reactivity in different T cell subsets. Biochemical studies of the MRC OX-22 antigen demonstrate that it reacts with the high molecular weight form of the rat leukocyte-common antigen (L-CA).

Materials and Methods

Rats

Inbred rats from the specific pathogen-free unit at the MRC Cellular Immunology Unit were used. The strains were PVG/c (RT1^c; Igl^b), PVG-1^a (RT1^c; Igl^a), and (PVG × DA)F$_1$ (RT1^c × RT1^a). PVG/c and PVG-1^a are congenic strains that differ in their immunoglobulin light chain allotypes. Irradiations were performed using a ^{137}Cs source at 94 rad/min (Gammacel; Atomic Energy of Canada).

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; DAB, Dulbecco's A + B medium; DFP, diisopropylfluorophosphate; DNP-BGG, dinitrophenyl bovine gamma globulin; FACS, fluorescence-activated cell sorter; GvH, graft-versus-host; L-CA, leukocyte-common antigen; MHC, major histocompatibility complex; NCS, newborn calf serum; NP-40, Nonidet P-40; PMSF, phenylmethyl-sulphonyl fluoride; RAM, rabbit F(ab')$_2$ anti-mouse Ig antibody; RAM-FITC, fluorescein isothio-cyanate-conjugated RAM; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; TDL, thoracic duct lymphocytes.

Antibodies

MRC OX-22 monoclonal antibody was derived by a fusion of spleen cells from a BALB/c mouse immunized with phytohemagglutinin-activated rat lymph node cells with NS1 myeloma cells. Rat lymph node cells were cultured in 10 μg/ml phytohemagglutinin type V from *Phaseolus vulgaris* (Sigma Chemical Co., Detroit, MI) in RPMI 1640 medium plus 5% DA rat serum, 2.5×10^{-5} M mercaptoethanol and 2 mM glutamine for 3 d. BALB/c mice were immunized twice with 10^8 blast cells per mouse intraperitoneally at an interval of 10 d, and 6 wk later were immunized again with blast cells intravenously. 3 d later the spleen cells were fused with the NS1/1.Ag4.1 cell line and hybrids selected according to the procedures of Galfre and Milstein (9). Cultures with hybrid cells were screened for antibody with the indirect binding assay on blast cells, and clones were obtained from positive cultures by dilution cloning with rat thymocytes as feeders. The positive cultures were recloned at intervals and after growing in mice as ascites. MRC OX-22 was found to be of IgG_1 class by formation of precipitin in agar using rabbit anti-mouse IgG_1 antiserum, kindly provided by M. Parkhouse and rabbit anti-mouse IgG_{2a} and IgG_{2b} serum from Miles Laboratories Ltd. Other mouse monoclonal anti-rat cell antibodies used were: W3/13 and W3/25 (4); MRC OX-7 (10); MRC OX-8 (2); MRC OX-12 (11), and MRC OX-19 (6). Rabbit anti-mouse (RAM) $F(ab')_2$ and its fluorescein isothiocyanate (FITC) conjugate (RAM-FITC) were prepared as described previously (4). Rat anti-rat 1^a allotype was as described in (11) and rabbit anti-rat $F(ab')_2$ as in (12). Antibodies were labeled with ^{125}I by the chloramine T method to a specific activity of ~25 μCi/μg (10).

Media

Cells were suspended in Dulbecco's A + B medium (DAB), with either 2% newborn calf serum (NCS) or 0.2% bovine serum albumin (BSA). 10 mM sodium azide was added to cells for fluorescence-activated cell sorter (FACS) (B-D FACS Systems, Sunnyvale, CA) analysis and sorting.

Cells

Thoracic duct lymphocytes (TDL) were collected overnight into DAB containing 20 IU/ml heparin at 4°C. Spleen cells were obtained by pressing spleens through a wire gauze into ice-cold DAB/NCS, and after washing, erythrocytes and dead cells were removed by suspending the cells in 5 ml distilled water for 5 s after which isotonicity was restored by the addition of 5 ml 1.8% NaCl. The dead cells were clumped and filtered on a small cotton wool plug and red cell ghosts were removed by washing cells twice by centrifugation.

Cell Separation by Rosetting

T cells or B cells were isolated from rat TDL or splenocytes by depletion of cells that rosetted with sheep erythrocytes coated with anti-rat Ig or W3/13 antibody, as described by Mason (13).

Cell Sorting Using a FACS II

2.5×10^8 lymphocytes were incubated with the appropriate monoclonal antibody for 1 h at 4°C, washed twice in DAB/NCS, and then incubated with RAM-FITC for 1 h also at 4°C. After a final wash they were suspended in 15 ml DAB/NCS/azide. Red and dead cells were excluded by the appropriate scatter gating.

Assays

GvH Assays. The popliteal lymph node assay was used exactly as described by Ford et al. (14). Lethal GvH disease was induced by the protocol described in (13). Details of cell doses for these assays are given in the main text.

Helper Cell Assay. An adoptive transfer method was used in which sorted lymphocytes from animals primed with dinitrophenyl-bovine gamma globulin (DNP-BGG) were injected intravenously together with 1 mg of DNP-BGG into irradiated syngeneic recipients

(700 rad) (5). Serum was collected 7 d later and assayed for anti-DNP antibody.

Binding Assay for Anti-DNP Antibody. Rat sera were diluted in DAB/2% NCS and 50-μl aliquots were assayed in triplicate at 4°C for binding to DNP-BGG adsorbed to wells of 96-well polyvinyl chloride Linbro plates (Linbro Chemical Co., Hamden, CT). Bound antibody was detected by a second incubation with 50 μl of ^{125}I-rabbit anti-rat F(ab')$_2$ antibody with sera from PVG/c donors or ^{125}I-rat anti-rat 1^a allotype antibody for sera from PVG-1^a donors. To prepare the plates 50 μl per well of DNP$_{29}$-BGG at 200 μg/ml in DAB/0.2% NCS was left in the wells overnight followed by washing with water, incubation for 1 h with 10% rabbit or human serum in DAB/10 mM NaN$_3$, and washing again with water. In the assays both incubations were for 1 h with 3 washes with DAB/0.2% NCS after each step. Finally, the plates were air-dried, the wells were cut off with a hot wire, and ^{125}I was counted with an LKB Rackgamma counter (LKB Instruments, Inc., Rockville, MD).

Quantitative Absorption Assays with Cells and Tissue Homogenates (15). MRC OX-22 ascites fluid was diluted 1:25,000 and aliquots were incubated at 4°C for 2 h with various dilutions of cell suspensions of spleen, thymus, and erythrocytes, or homogenates of kidney and liver. The erythrocytes and kidneys were derived from animals irradiated 3 d before with 950 rad to remove lymphocytes. Then the mixtures were centrifuged and the supernatants assayed for residual antibody by the indirect binding assay using trace conditions (15, 16).

Localization of Antigens in Cryostat Sections

Cryostat sections of rat brain, spleen, kidney, skin, and thymus were stained for cells reactive with monoclonal antibodies MRC OX-22, MRC OX-18 (mouse anti-rat class 1 major histocompatibility complex (MHC) antibody [17]) and MRC OX-21 (mouse anti-human C$_3$b inactivator antibody [18]) using the immunoperoxidase method described by Barclay (19). The peroxidase-conjugated rabbit Fab anti-mouse Ig reagent was the kind gift of Dr. A. N. Barclay (19).

Biochemical Analysis

For analysis of surface-labeled glycoproteins, TDL were labeled with [^{3}H]borohydride after oxidation with periodic acid (20, 21), solubilized, and subjected to affinity chromatography exactly as in (22) except that 1 mM phenylmethylsulfonyl fluoride (PMSF) was used as a proteolytic inhibitor rather than diisopropylfluorophosphate (DFP). Samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with radiolabeled material visualized by fluorography, as described in (22).

Results

Tissue Distribution of Cells Binding MRC OX-22 Monoclonal Antibody

Flow Cytofluorograph Analysis. Cells were incubated with MRC OX-22 monoclonal antibody followed by fluorescein-conjugated rabbit F(ab')$_2$ anti-mouse Ig antibodies, and binding was analyzed by flow cytofluorography. As shown in Table I and Fig. 1, a total of 84% of cells were labeled and the fluorescence histogram showed three populations, one unlabeled, one weakly positive, and one strongly so. TDL were incubated with MRC OX-22 antibody along with other mouse anti-rat monoclonal antibodies to T subsets and the results are presented in Table I and were used to construct the subpopulation diagram in Fig. 2. MRC OX-22 antibody labels virtually all B cells and cytotoxic/suppressor cells but only $^{2}/_{3}$ of the W3/25$^+$ T cell subset. The heavily labeled MRC OX-22$^+$ cells are mainly B cells since labeling of cells with MRC OX-22 and MRC OX-12 (anti-kappa) together increased the fluorescence of these bright cells but not the weakly labeled ones. Co-labeling with MRC OX-19 (pan T) had the opposite

TABLE 1

Percentages of Rat TDL and Bone Marrow Cells Labeled by Mixtures of MRC OX-22 and Other Mouse Anti-Rat Monoclonal Antibodies

Antibodies (subset labeled)	Percentage of cells labeled	
	TDL	Bone marrow
MRC OX-22 only	84	46.4
MRC OX-12 (anti-kappa chain of rat Ig)	50.4	12.4
MRC OX-19 (pan T cell)	46.1	2.7
MRC OX-8 (T$_{c/s}$)	6.9	ND
W3/25 (T$_h$)	37.1	ND
W3/13 (pan T cell, granulocytes)	ND	40.3
MRC OX-7 (anti-rat Thy-1.1)	ND	52.8
MRC OX-22 + MRC OX-12	84.8	49.6*
MRC OX-22 + MRC OX-19	93	44.0
MRC OX-22 + MRC OX-8 + MRC OX-12	85	ND
MRC OX-22 + W3/25	93.6	ND
MRC OX-22 + W3/13	ND	73.8
MRC OX-22 + MRC OX-7	ND	53.0

The monoclonal antibodies in the table were used either singly or in mixtures to label rat TDL and bone marrow cells. After 1 h incubation the cells were washed, incubated for a further 1 h with RAM-FITC, and examined on the FACS. The percentage of labeled cells was obtained from histograms of 5×10^4 cells. Using W6/32 monoclonal antibody, which does not react with normal rat tissues, as a negative control, <1% of TDL or bone marrow cells were labeled. ND, not determined.
* In two other experiments, MRC OX-22 plus MRC OX-12 was exactly the same as MRC OX-22 alone, indicating that all surface Ig$^+$ cells were also MRC OX-22$^+$.
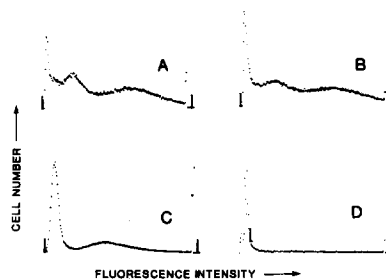

FIGURE 1.   Fluorescence histograms of TDL, bone marrow, spleen, and thymus cells labeled with monoclonal antibody MRC OX-22. Single-cell suspensions were incubated with MRC OX-22 monoclonal antibody, washed, and then incubated with RAM-FITC. Fluorescence histograms were obtained on the FACS using 10^5 cells. Both scales are linear. (A) TDL, (B) spleen, (C) bone marrow, (D) thymus.

effect (data not shown). The pattern of labeling on spleen cells was essentially identical to that of TDL but only 2.1% of thymocytes were labeled by MRC OX-22 antibody (Fig. 1).

Analysis of bone marrow revealed a bimodal profile with 46% of cells labeled (Fig. 1, Table I). ¾ of these cells were unlabeled by W3/13 monoclonal antibody, which labels marrow polymorphs (4) and hemopoietic stem cells (23) but not
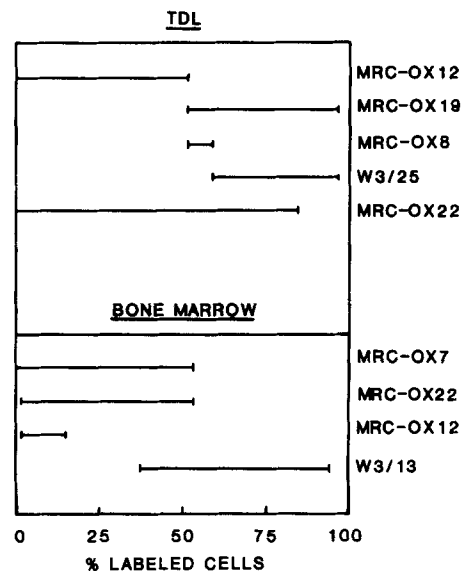
TDL

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            ┌────────────────────────┐
            │ ├──────────────┤        │ MRC-OX12
            │     ├────────────────┤  │ MRC-OX19
            │        ├─┤              │ MRC-OX8
            │      ├─────────────┤    │ W3/25
            │ ├──────────────────────┤│ MRC-OX22
            │                        │
            │  BONE MARROW           │
            │                        │
            │ ├────────────────┤     │ MRC-OX7
            │ ├───────────────┤      │ MRC-OX22
            │ ├───┤                  │ MRC-OX12
            │        ├──────────────┤│ W3/13
            └────────────────────────┘
            0    25    50    75    100
                 % LABELED CELLS
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FIGURE 2. Relationship of subsets of lymphocytes and bone marrow cells, defined by MRC OX-22 antibody, to previously defined lymphocyte subsets.

most marrow lymphocytes. In contrast, all of the MRC OX-22$^+$ cells were also labeled by MRC OX-7 antibody (Thy-1$^+$), which labels stem cells and the marrow lymphocytes that are thought to be immature B cells (24). It thus seems likely that MRC OX-22 antibody labels most cells of the B lineage in the marrow as well as in the periphery.

Detection of MRC OX-22$^+$ Cells on Cryostat Sections. Immunoperoxidase staining of cryostat sections of spleen revealed a reaction pattern that was consistent with that obtained by flow cytofluorography in that, although both B and T cells were labeled, the staining of the B areas was more intense than that of the T areas. The staining pattern in the thymus was unique in that, though few cells were stained, the majority of these positive cells were found at the edge of the medulla, which was defined by staining with MRC OX-18 antibody (Fig. 3). Additionally, there were labeled cells scattered through the cortex with an apparently random distribution. Staining of sections of liver, brain, and skin failed to detect any antigen present, while in the kidney very faint staining was seen only in the glomeruli (the significance of this latter finding is doubtful).

Quantitative Absorption Analysis. Rat erythrocytes, splenocytes, thymocytes, and homogenates of kidney and liver were used to absorb MRC OX-22 antibody, and the completeness of absorption was determined by assaying the residual antibody on TDL using a cellular radioimmunoassay. Thymocytes had 2.3% of the absorptive capacity of spleen cells, but erythrocytes, kidney, and liver failed to produce any absorption even when weights of wet tissue were used that were 100 times that of the amount of spleen needed to give complete absorption of the antibody. Thus the absorption data is fully consistent with the results for antigen distribution by FACS analysis and from the staining of cryostat sections.
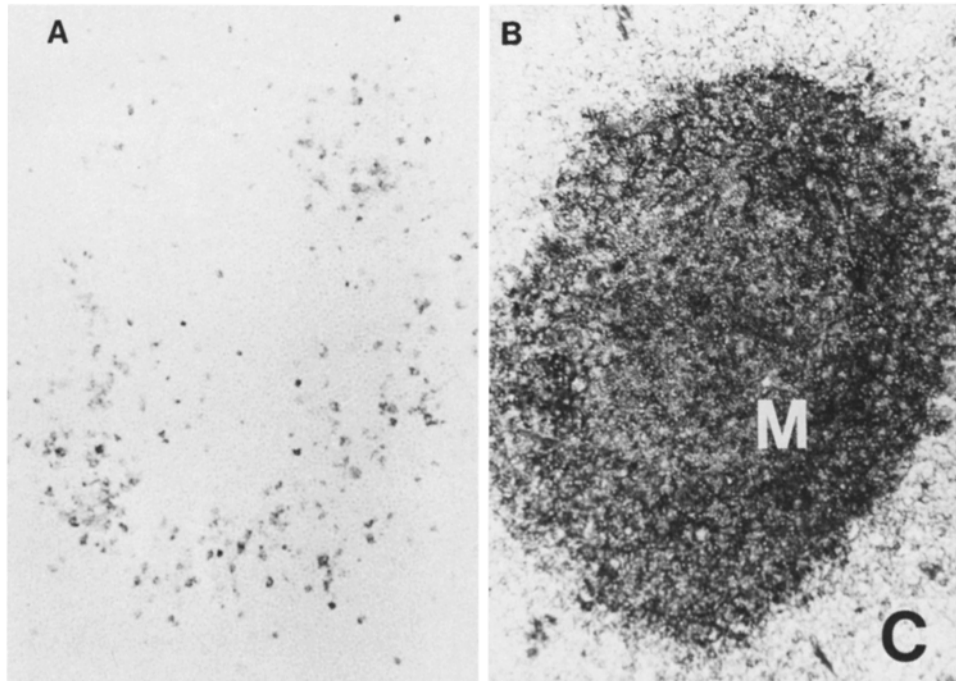
FIGURE 3. Serial cryostat sections of rat thymus stained with MRC OX-22 and MRC OX-18 (anti-MHC class I antigen) monoclonal antibodies. Ethanol-fixed cryostat sections of thymus were incubated with MRC OX-22 or MRC OX-18 antibodies, washed, and incubated with peroxidase-conjugated rabbit Fab anti-mouse IgG. After a further wash the presence of bound enzyme was revealed by incubating the section in diaminobenzidine in the presence of hydrogen peroxide (19). (B) The MRC OX-18 antibody stains the medulla (M) more heavily than the cortex (C). The MRC OX-22$^+$ cells are found predominantly as a ring of cells lying just within the medulla (A). MRC OX-21 antibody (anti-human C_3b inactivator) was used as a negative control and gave no staining (data not shown).

Biochemical Characterization of the MRC OX-22 Antigen

The sialic acid of glycoproteins of TDL was labeled with ^3H by reduction with [^3H]Na borohydride after oxidation with periodic acid (20). Membrane molecules were solubilized in 1% Nonidet P-40 (NP-40) detergent and the extract was passed through an MRC OX-22 antibody affinity column. A glycoprotein of 220,000 apparent mol wt was eluted from the column (Fig. 4). The binding of this glycoprotein was specific since this band was not retained on control columns (data not shown). The 220,000 mol wt glycoprotein also binds to an affinity column containing MRC OX-1 antibody specific for L-CA (Fig. 4). Bands at 200,000, 190,000, and 180,000 mol wt also bind to the MRC OX-1 column. These lower mol wt bands are thought to be derived from T cells (25), and in Fig. 4 it is clear that the 190,000 and 180,000 mol wt bands do not bind to the MRC OX-22 column. However, the 200,000 mol wt band does appear to bind at least in part to MRC OX-22 antibody; whether this might be the T cell form of the MRC OX-22 antigen remains to be established. Also there appeared to be some bands of ~220,000 mol wt that were bound by the MRC OX-1 but not the MRC OX-22 column, and the origin of these also requires further investi-
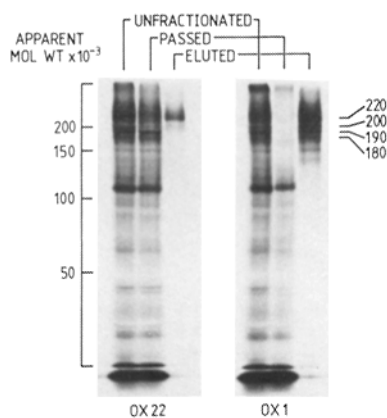
FIGURE 4. SDS-PAGE of ^{3}H-labeled glycoproteins purified on MRC OX-22 or MRC OX-1 affinity columns. 9 × 10^8 TDL were oxidized with periodate and reduced with 10 mCi NaB[^{3}H]$_4$. The membrane proteins were solubilized in 6 ml 1% NP-40 and after centrifugation aliquots of the extract were passed through MRC OX-22 and MRC OX-1 affinity columns (5 ml with 10 mg IgG/ml Sepharose 4B). The columns were washed with 0.5% deoxycholate in 10 mM Tris HCl, pH 8, and bound material was then eluted with the same buffer plus 0.05 M diethylamine HCl, pH 11.5. The gels show electrophoresis of trichloroacetic acid-precipitable material from starting extract (unfractionated), extract passing through the column (passed), and eluted materials (eluted). The unfractionated and passed tracks used material derived from 75 μl of starting extract, whereas the eluted tracks used material from 150 μl of starting extract.

gation.

The clear point from Fig. 4 is that MRC OX-22 antibody reacts mainly with the high mol wt form of the L-CA. Proof that the MRC OX-22 glycoprotein is part of the L-CA complex is given in Table II, which shows the counts per minute of ^{3}H-labeled glycoproteins eluted from MRC OX-1 and MRC OX-22 columns run in series. When the MRC OX-1 column preceded the MRC OX-22 column, almost no cpm were eluted from the second column, whereas when the MRC OX-22 column was first, it bound ~20% of the radioactive material that can be bound by the MRC OX-1 column.

Functional Studies

Previous studies have shown that cells which mediate helper functions for B cell responses and the GvH reaction as measured by the popliteal lymph node assay are found exclusively in a T cell subset that is uniformly labeled by W3/25 antibody. Labeling with MRC OX-22 antibody subdivides the W3/25$^+$ cells (Fig. 1) and functional studies were carried out to see whether this subdivision correlated with different functional activities.

GvH Reactivity and GvH Disease. The results in Fig. 5 shows that the cells which mediate popliteal lymph node enlargement are all labeled by MRC OX-22 antibody and that the unlabeled cells do not show this activity. This is true whether the sorted cells were injected in doses proportional to the frequency of MRC OX-22$^-$ and MRC OX-22$^+$ cells in the original parental TDL (physiological doses) or if cell doses were adjusted so as to give all recipients equal numbers of T cells (equal T cell doses).

In contrast to GvH reactivity as demonstrated by the popliteal lymph node

TABLE II

Binding of ^3H-labeled Glycoproteins to MRC OX-1 (anti-L-CA) and MRC OX-22 Affinity Columns

	^{3}H-glycoprotein eluted	
	Order of columns in series	
	MRC OX-1 MRC OX-22	MRC OX-22 MRC OX-1
	cpm	
First column	1.6×10^6	2.8×10^5
Second column	2.7×10^4	1.4×10^6

7×10^8 TDL were oxidized with periodic acid and labeled with 10 mCi of $NaB[^3H]_4$. Membrane proteins were solubilized in 6 ml 1% NP-40 and 2.4-ml aliquots of this extract were passed through two sets of affinity columns containing MRC OX-1 and MRC OX-22 antibody in series as shown above (each column was 5 ml with 10 mg IgG/ml Sepharose 4B). On each set of columns was loaded 8.8×10^6 cpm of trichloroacetic acid-precipitable radioactivity, and the amount unbound by the columns was 6.7×10^6 cpm for the MRC OX-22/MRC OX-1 experiment and 7.1×10^6 cpm for the MRC OX-1/MRC OX-22 experiment. The columns were washed with 0.5% deoxycholate, 10 mM Tris HCl, pH 8, buffer and then eluted with the same buffer plus 50 mM diethylamine HCl, pH 11.5. The trichloroacetic acid-precipitable counts were determined and are shown in the table. In each case they were ~60% of the total counts per minute eluted.
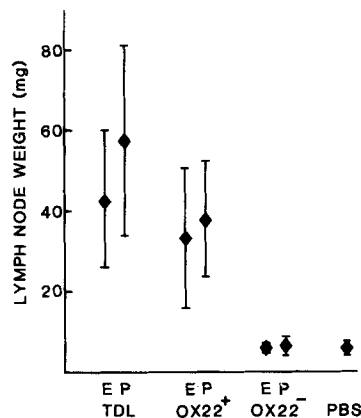

FIGURE 5.   Ability of MRC OX-22$^+$ and MRC OX-22$^-$ subsets of parental strain rat TDL to mediate popliteal lymph node enlargement in F_1 recipients. Parental strain TDL were separated on the FACS into MRC OX-22$^-$ and MRC OX-22$^+$ subsets. These cells were then injected into the footpads of F_1 hybrid rats and the popliteal lymph nodes removed for weighing 7 d later (14). E, experiments in which the T cell dose for all groups was identical; P, experiments in which the subpopulations were used in their physiological ratios (see text) based on a TDL dose of 10^7 cells. Purities were >99% for both fractions. Results are given as means ± standard deviation.

assay, lethal GvH disease can be mediated by both the W3/25$^+$ and MRC OX-8$^+$ T cell subsets (13). Parental TDL were fractionated after labeling with MRC OX-22 antibody and injected into sublethally irradiated F_1 hybrids. Only the recipients of MRC OX-22$^+$ cells developed the erythema, ruffled fur, and weight

loss characteristics of GvH disease (Fig. 6). Rats given MRC OX-22$^-$ cells gained weight and remained as healthy as those given only saline. These results demonstrate that W3/25$^+$, MRC OX-22$^-$ cells are unable to mediate GvH disease but do not exclude the possibility that the GvH disease observed when unfractionated W3/25$^+$ cells are used (13) arises as a consequence of a cell-cell interaction between the MRC OX-22$^-$ and MRC OX-22$^+$ components of the W3/25$^+$ population. However, when W3/25$^+$, MRC OX-22$^+$ were assayed alone for their ability to cause lethal GvH disease, recipients did develop disease, indicating that synergy with W3/25$^+$, MRC OX-22$^-$ cells was not required (data not shown).

T Help for Anti-hapten Antibody Responses. Table III (experiment 1) shows the results of an experiment designed to assay the ability of MRC OX-22$^-$ cells, MRC OX-22$^+$ cells, or a mixture of the two to adoptively transfer a secondary antihapten response to sublethally irradiated recipients. As the table shows, neither fraction alone produced a response comparable to that of unfractionated TDL, but a mixture, in physiological proportions, of the two fractions was as active as unfractionated cells.

Because the MRC OX-22 antibody was known to label most, if not all, B cells (Fig. 2) the synergy observed in the experiment described in Table III suggested that the B memory cells were in the MRC OX-22$^+$ fraction and helper T cells for B cell responses in the MRC OX-22$^-$ one. To test this conclusion directly MRC OX-22$^-$ and MRC OX-22$^+$ cells, from primed donors, were supplemented with primed B cells, and the ability of these mixtures to adoptively transfer secondary antihapten responses was assayed. The results, presented in Table III (experiment 2), showed that the MRC OX-22$^-$ cells were much more active in providing B cell help than were the MRC OX-22$^+$ cells. However, the MRC OX-22$^+$ cells appeared to provide some help and the reconstitution with MRC OX-22$^-$ cells was not complete compared with the response obtained from unfractionated TDL. This result was obtained from three similar experiments. Further comments on this result are deferred to the Discussion.

Cells Mediating Allogeneic Suppression of Antibody Synthesis are MRC OX-22$^+$. Under appropriate conditions the induction of GvH disease in F_1 rats is accompanied by a profound suppression of antibody synthesis (2, 5). This allogeneic suppression is mediated by MRC OX-8$^+$ cells (2), which form $\sim$10% of all cells in TDL. Because the percentage of these cells is so small, it is not
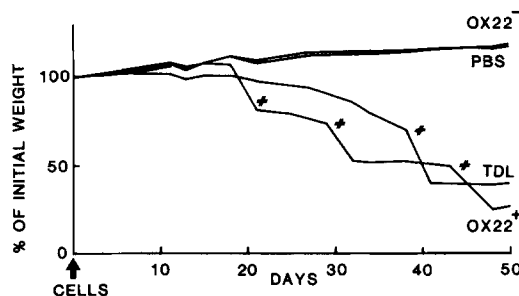

FIGURE 6. Inability of MRC OX-22$^-$ cells to mediate GvH disease. MRC OX-22$^-$ and MRC OX-22$^+$ subpopulations of parental strain rat TDL, isolated on the FACS, were injected in physiological ratios into sublethally irradiated F_1 rats. Rats were weighed on alternate days thereafter. #, death of a rat from GvH disease.

TABLE III

Synergy Between MRC OX-22⁻ and MRC OX-22⁺ Cells in Antihapten
Antibody Responses

Experi-ment	Cells transferred	Percent relative antibody titer*
1	10^6 OX-22⁻	8.1
	8×10^6 OX-22⁺	11.3
	10^6 OX-22⁻ + 8×10^6 OX-22⁺	138
	10^7 TDL	100
2	5×10^6 B cells	0.6
	1×10^7 B + 10^6 OX-22⁻	71
	5×10^6 B + 8×10^6 OX-22⁺‡	14
	10^7 TDL	100

Sublethally irradiated syngeneic recipients were injected with TDL or
subpopulations thereof from DNP-BGG-primed donors and were chal-
lenged with the priming antigen at the time of cell transfer. Anti-DNP
antibody titers were determined 7 d later. There were two animals in
each group for experiment 1 and three per group in experiment 2. In
experiment 1 MRC OX-22⁻ cells were 99.8% pure and MRC OX-22⁺,
99.2% pure. In experiment 2 the corresponding results were 99.9% and
99.3%, respectively. The B cells were 96% pure as judged by percent of
surface Ig cells. The MRC OX-22⁺ cells were isolated on the FACS and
the B cells for experiment 2 by indirect rosette depletion of primed TDL
using W3/13 monoclonal antibody.
* Anti-hapten antibody titers are expressed as a percentage of the control
response produced by unfractionated TDL.
‡ The 8×10^6 MRC OX-22⁺ cells contained ~5×10^6 B cells.

possible to prove by the type of population analysis in Table I that all MRC OX-
8⁺ cells are also MRC OX-22⁺. To verify that the cells responsible for allogeneic
suppression were MRC OX-22⁺, sublethally irradiated F_1 rats were given hapten-
carrier–primed F_1 spleen cells and either unfractionated, primed parental TDL
or parental TDL that had been fractionated on the FACS into MRC OX-22⁻
and MRC OX-22⁺ subsets. The allogeneic suppressive activity of the parental
spleen cells was wholly contained within the MRC OX-22⁺ population (Table
IV). This experiment was repeated with the same result.

Phenotype of the Memory B Cell.   Memory B cells in rat TDL constitute only a
few percent of all the cells present (26), so although the population analysis
shown in Table I indicated that the great majority of all B cells in TDL were
MRC OX-22⁺, this did not conclusively demonstrate that the memory B cell also
expressed this antigen. Further, the small but significant antibody response
observed (Table III) when MRC OX-22⁻ cells were transferred into sublethally
irradiated hosts was of uncertain origin, since, in principle, the T helper cells in
the population could have been collaborating with any donor B cells present in
the MRC OX-22⁻ inoculum or with radio-resistant B cells in the host (27). To
determine directly whether any B memory cells were MRC OX-22⁻, an adoptive
transfer experiment was carried out using hapten-carrier–primed TDL from 1ᵃ
light chain allotype donors and sublethally irradiated congenic recipients of the
1ᵇ allotype (11). To ensure that T helper cells were not limiting in the assay, all
recipients of MRC OX-22⁻ cells and MRC OX-22⁺ cells also received hapten-

TABLE IV

Phenotype of the Cell Responsible for Suppression of Antibody Synthesis in GvH Disease

Cells transferred	Percent relative antibody titer
2.3×10^7 F$_1$ spleen cells + 1×10^6 P OX-22$^-$ TDL	95
2.3×10^7 F$_1$ spleen cells + 8×10^6 P OX-22$^+$ TDL	16.0
2.3×10^7 F$_1$ spleen cells + 10^7 P TDL	6.5
2.3×10^7 F$_1$ spleen cells only	100

DNP-BGG–primed F$_1$ spleen cells were transferred either alone or with various populations of primed parental (P) TDL isolated on the FACS, into sublethally irradiated F$_1$ recipients. All cell recipients were challenged with the primary antigen at the time of cell transfer and the 1^a allotype component (i.e., that derived from the F$_1$ cells) of the anti-DNP response assayed 7 d later. Purities of MRC OX-22$^-$ and MRC-22$^+$ cells were >98%. There were two to three animals in each group.
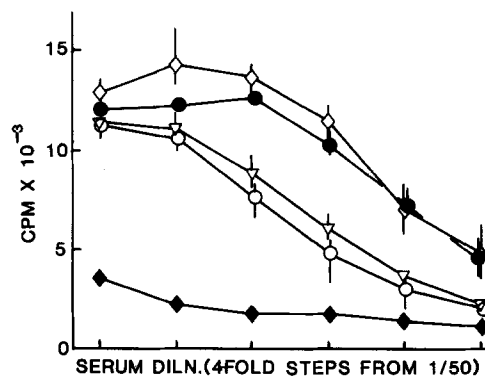

FIGURE 7. The phenotype of the B memory cell. MRC OX-22$^-$ and MRC OX-22$^+$ fractions of TDL from 1^a light chain allotype donors primed to DNP-BGG were transferred, together with primed T cells from donors of the same allotype into sublethally irradiated 1^b allotype recipients. Control rats received primed TDL or primed T cells and all recipients were challenged at the time of cell transfer with DNP-BGG. The 1^a allotype component of the anti-DNP response was determined 7 d later using an allotype-specific solid phase radioimmunoassay. ($\Diamond$) 10^7 primed TDL; (O) 10^6 MRC OX-22$^-$ plus 5 × 10^6 T cells; ($\bullet$) 8.5 × 10^6 MRC OX-22$^+$, 5 × 10^6 T cells; (∇) 5 × 10^6 T cells alone; ($\blacklozenge$) negative serum control. Purities for MRC OX-22$^-$ and MRC OX-22$^+$ were >99%, for T cells (purified by indirect rosette depletion using MRC OX-12 antibody (11, 13), 93.4%. The low antibody titer observed with the purified T cells is accurately accounted for by the residual B cells in this preparation. Results are presented as means with ranges for two rats per group for TDL and T alone; three rats per group for MRC OX-22$^-$ and MRC OX-22$^+$ groups.

carrier–primed T helper cells from 1^a allotype donors. The solid phase radioimmunoassay was made specific for antihapten antibody of donor allotype by using ^{125}I-labeled anti-1^a allotype antibody. As Fig. 7 shows, the MRC OX-22$^+$ cells, when provided with T cell help, provided a response equal to that of unfractionated TDL, while the MRC OX-22$^-$ population was completely inactive. A second

experiment gave the same result.

Discussion

The principal findings of this work are that the MRC OX-22 monoclonal antibody recognizes the high molecular weight form of the rat L-CA and defines a new functional subset of T cells. In rat (25), mouse (28), and man (29), the molecular weight of L-CA varies in a similar manner according to the cell type from which it is extracted. In the rat, the predominant forms of the L-CA from thymocytes, T cells, and B cells run on SDS-PAGE with apparent molecular weight of 170,000, 190,000 (multiple bands), and 220,000 respectively (25). These different forms have different carbohydrate structures, as shown by the differences in binding of peanut and soybean lectin but it is possible that the protein is identical for the different types. In the present analysis on glycoproteins from mixtures of T and B cells, MRC OX-22 antibody was seen to bind mainly to the high molecular weight form of L-CA, which was previously thought to be characteristic of B cells. However, MRC OX-22 antibody labels $\frac{2}{3}$ of T cells at low levels in addition to its heavy labeling of B cells, and this suggests that either T cells express the high molecular weight form of L-CA as well as the dominant bands around 190,000 mol wt, or that a minor band at 200,000 mol wt, which also appeared to bind to MRC OX-22 antibody, is the T cell product. Obviously more work on the molecular weight heterogeneity of T cell L-CA is needed as is analysis of the protein or carbohydrate nature of the antigenic variation.

Comparison of the MRC OX-22 data with the results of Dalchau and Fabre (30, 31) show that rat and human L-CA have similar patterns of antigenic heterogeneity. Dalchau and Fabre described a monoclonal antibody that reacts with the high molecular weight form of L-CA and labels all B cells, all T cells of the cytotoxic/suppressor phenotype (OKT8$^+$ cells), $\frac{1}{3}$ of T helper cells (OKT4$^+$ cells), and a small percentage of thymocytes (30, 31). Apart from the fact that this antibody labels only $\frac{1}{3}$ of the OKT4$^+$ cells in man, whereas MRC OX-22 labels $\frac{2}{3}$ of W3/25$^+$ cells in the rat, these tissue distributions are identical. In the mouse, the 2C2, 14D10, and 14.8 monoclonal antibodies all recognize the high molecular weight form of the L-CA and are expressed on Lyt-2$^+$ cells (32–34). However, except in the *lpr* mouse, the antigen recognized by those antibodies is not expressed on Lyt-2$^-$ cells that seem to be the homologue of rat W3/25$^+$ cells.

The experiments on the fractions of the MRC OX-22$^-$ and MRC OX-22$^+$ subsets of rat TDL revealed a hitherto unknown heterogeneity in the T cell subset that expresses the W3/25 antigen. This is seen in the correlation between rat T cell subsets and functions summarized in Table V. As the table shows, labeling with MRC OX-22 antibody clearly distinguishes W3/25$^+$ cells with helper activity for B cells (W3/25$^+$, MRC OX-22$^-$) from those showing alloreactivity in the popliteal lymph node assay (W3/25$^+$, MRC OX-22$^+$). The question remains as to whether the W3/25$^+$, MRC OX-22$^+$ cells play any role in the helper effect. Although the MRC OX-22$^+$ cells, isolated on the FACS, were ~99% pure, a 1% contamination with MRC OX-22$^-$ cells represents ~10% of all the cells of this phenotype in the unseparated population, so the apparent helper activity of the MRC OX-22$^+$ cells may be artifactual. However, in vitro studies have produced evidence for the existence of two types of T helper cell:

TABLE V

Functions of MRC OX-22-positive and -negative T Cell Subsets

	W3/25⁺, OX-22⁻	W3/25⁺, OX-22⁺	OX-8⁺, OX-22⁺	Surface Ig⁺, OX-22⁺
T helper for B cells	+	±	−	−
GvHR	−	+	−	−
GvH disease	−	+	ND*	−
T suppressor	−	−	+	−
B cell (including B memory cell)	−	−	−	+

* Lethal GvH disease can also be caused by MRC OX-8⁺ cells (13) but it remains to be formally demonstrated that these cells express the MRC OX-22 antigen.

one helping B cells in a one-to-one interaction (monogamous help) and the other providing nondiscriminatory (polyclonal) help via a soluble mediator (35), and it cannot be excluded that the MRC OX-22 antigen distinguishes these two types of helper cells. This possibility is supported by the observation that full immune responsiveness was obtained only when both MRC OX-22⁺ and MRC OX-22⁻ fractions of the W3/25⁺ population were transferred together with B cells into irradiated recipients (Table III). In any event it is clear that if W3/25⁺, MRC OX-22⁺ cells do provide B cell help, they are <10% as potent on a per cell basis as W3/25⁺, MRC OX-22⁻ cells (Table III).

In man and mouse, monoclonal antibodies have been described that label B cells and subsets of T cells (31–34, 36). With the exception of the monoclonal antibody TQ1, described by Reinherz et al. (36), and the monoclonal antibody of Dalchau and Fabre (31), none of these antibodies appears to divide the T helper subset and functional studies have been made only with TQ1. The T4⁺TQ1⁻ T cell subset in man shows similarity with the W3/25⁺, MRC OX-22⁻ population in the rat in that both provide help for B cells but, as with W3/25⁺, MRC OX-22⁺ cells, the T4⁺TQ1⁺ subset showed some weak helper activity. No biochemical characterization of the TQ1 antigen has been made and the homology between the subsets of T helper cells in the two species is not yet firmly established.

The observation that although most peripheral T cells were MRC OX-22⁺, most thymocytes were not labeled by this antibody suggests that the few MRC OX-22⁺ cells in the thymus were mature cells. ~50% of these cells were found at the edge of the medulla where thymocytes are believed to leave the thymus (37), but because small numbers of peripheral T cells migrate back to the thymus medulla (38), it cannot be ruled out that these MRC OX-22⁺ cells are T cells entering the thymus rather than mature thymocytes leaving it. The MRC OX-22⁺ cells in the thymic cortex made up the remaining 50% of cells with this phenotype. These cells are unlikely to be of peripheral T cell origin (38) and their presence raises the possibility that, rather than involving an intramedullary phase, thymocyte maturation may go on to completion within the thymic cortex. Clearly it would be of value to know if these cells express both the W3/25 and MRC OX-8 antigens, as do the great majority of rat cortical thymocytes (17), or whether they express only one or another of these markers, as do most mature T cells.

The MRC OX-22 antibody is potentially of value in the isolation of the rat bone marrow stem cell. As Table I shows, almost all Thy-1⁺ bone marrow cells

also express the MRC OX-22 antigen and it is known that rat stem cells are to be found amongst the Thy-1$^+$ population (24). If it turns out that stem cells are MRC OX-22$^-$, then the isolation of Thy-1$^+$, MRC OX-22$^-$ cells will provide a highly enriched source of these cells.

Summary

A mouse monoclonal antibody (MRC OX-22) is described that labels rat T cells which mediate graft-versus-host reactions and those responsible for the suppression of antibody synthesis in hosts undergoing these reactions. In contrast, most of the T cells that provide help for B cells are MRC OX-22 negative. These results, taken together with those published previously, demonstrate that the rat contains at least three phenotypically and functionally distinct subsets of T cells. The MRC OX-22 antibody also labels all B cells, 50% of bone marrow cells, but only 2% of thymocytes. Of these latter cells about half are found at the edge of the medulla and the remainder are randomly distributed throughout the cortex and medulla. These findings lend support to the view that mature thymocytes leave the thymus at the cortico-medullary junction, and also suggest that both cortex and medulla may be sites where thymocytes mature. Biochemical studies showed that the MRC OX-22 antibody reacts with the high molecular weight form of the leukocyte-common antigen (L-CA). Comparison with data on human L-CA suggests that the molecular and antigenic heterogeneity of this set of glycoproteins has been conserved between rat and man.

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