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Two distinct antigenic markers for rat thymus and T cells defined by monoclonal antibodies*

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Summary. Rat thymus and T-cell antigens were defined by using two distinct monoclonal antibodies (R-1-3B3 and R-1-12C5). R-1-3B3 antibody, when tested for its reactivity with rat lymphoid cells by immunofluorescence, labelled virtually all thymus and T cells but not B cells and bone marrow cells. The antigen defined by this R-1-3B3 antibody occurred more abundantly on medullary thymocytes and peripheral T cells than on cortical thymocytes. Immunochemical data showed that R-1-3B3 antibody recognized a single glycoprotein with a molecular weight of 67,000, which were able to interact with *Lens culinaris* haemagglutinin. R-1-12C5 antibody, on the other hand, reacted with all of thymus and T cells as well as with a subpopulation (approximately 20%) of bone marrow cells. In contrast to the antigen defined by R-1-3B3, that detected by R-1-12C5 antibody existed largely on cortical thymocytes and to a much lesser extent on medullary thymocytes and peripheral T cells. R-1-12C5 antibody detected a single glycoprotein with a 95,000 molecular weight, which could also interact with *Lens culinaris* haemagglutinin. Based on these data described above and since both antigens defined by R-1-3B3 and R-1-12C5 antibodies were absent from rat brain tissue, we concluded that they were distinct from brain-associated thymic antigens in rats including Thy-1 and W3/13 antigen systems.

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* We are willing to provide antibodies described in this work to other workers who might need these antibodies for their research purposes.

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INTRODUCTION

A variety of cell-surface antigens, which behave as general T-cell markers, have been identified in the murine system (Ledbetter & Herzenberg, 1979). These antigens include glycoproteins bearing determinants of either Thy-1 (Reif & Allen, 1964) or Ly-1 antigen system (Boyse, Miyazawa, Aoki & Old, 1968). Similar antigen molecules to mouse Thy-1 or Ly-1 have also been demonstrated on human thymus and T cells (Ades, Zwerner, Acton & Balch, 1980; Ishii, Fujimoto, Koshiba & Kikuchi, 1981; Ishii, Fujimoto, Kon Ogasawara, Koshiba, Matsuura & Kikuchi, 1982), though there is a debate as to the exact tissue distribution of human Thy-1 antigen (Dalchau & Fabre, 1979; McKenzie & Fabre, 1981).

In the rat, Thy-1 antigen is not restricted to thymus and T cells, but is found on a subpopulation of bone marrow cells such as haemopoietic stem cells and at least some of their lymphoid descendants (Williams, 1976; Hunt, 1979; Crawford & Goldschneider, 1980; Uede, Ishii, Matsuura & Kikuchi, 1982). Moreover, the T-cell antigen comparable to mouse Ly-1 system has not been defined on rat T cells. Thus, only an

antigen, designated W3/13, has been chemically characterized and utilized as a marker for distinguishing T cells from B cells in the rat, though this antigen is expressed not only on thymus and T cells but also on other non-lymphoid cells such as granulocytes and brain (Williams, Galfre & Milstein, 1977).

In our previous paper, we have described the T-cell specificity of a xenoantiserum raised in rabbits against rat T cells (Ishii, Koshiba, Yamaoka & Kikuchi, 1976). In the present study, we have developed two different monoclonal antibodies (MoAB), which can detect two distinct cell-surface glycoproteins with molecular weights (M.W.) of either 67,000 (T67) or 95,000 (T95) on rat thymus and T cells. Based on their tissue distribution and molecular characteristics, the T67 antigen was considered to be homologous to mouse Ly-1 antigens, while the T95 antigen appeared to be newly defined one distinct from W3/13 antigen, although there are some similarities between T95 and W3/13 antigens.

MATERIALS AND METHODS

Animals

Inbred Wistar-King Apteckman (WKA) and ACI rats were used. Buffalo/Mna rats, which spontaneously develop thymoma and associated muscular disfunction (Matsuyama, Amo, Takayanagi & Iida, 1981), were kindly provided by Dr M. Matsuyama, Aichi Cancer Center. BALB/C mice, bred at the Faculty of Science, Hokkaido University, were also used in this study.

Cell suspension

Single-cell suspension of rat thymus (THY), lymph node (LNC), spleen (SPC) or bone marrow cells (BMC) was prepared in RPMI 1640 medium, as previously described (Ishii *et al.*, 1976). Leukaemic B cells (KNL-14), which were induced in WKA rats by oral administration of nitrosobutylurea, were maintained in ascites form by serial intraperitoneal inoculation of syngeneic WKA rats. These leukaemic cells expressed IgM, RT1.B (rat Ia) and Thy-1 antigens on their cell surfaces (Ohhashi, Inomata, Fujimoto, Nakagawa, Natori & Aizawa, 1981), suggesting that they were derived from immature B cells.

Monoclonal antibodies

Eight-week-old female BALB/C mice were immunized intraperitoneally with 2×10^8 WKA rat thymocytes at 2-week intervals for 6 weeks. Three days after the last challenge, their spleen cells (1×10^8) were fused with NS-1 myeloma cells (1×10^7), which were the gift by Dr M. Green, Harvard Medical School, according to the method originally described by Köhler & Milstein (1975). After cell fusion with polyethylene glycol (M.W. 4000), the cells were suspended in 300 ml of RPMI 1640 medium with 10% foetal calf serum (FCS) and distributed into 300 wells of Nunc 168357 multi well plates (Nunc, Kamstrup, Denmark) in the presence of feeder cells obtained from BALB/C spleens. After cultivation for 10 days in hypoxanthine-aminopterin-thymidine (HAT) medium, the culture medium was replaced by HT-containing medium (Littlefield, 1964), and the supernatants of growth-positive cultures were screened for antibody activity on a variety of rat target cells by indirect membrane immunofluorescence as described below. Out of 300 wells, 162 wells were growth-positive and 35% of growing clones produced antibodies. Eighteen clones were selected for cloning by limiting dilution technique (Lemke, Hämmerling, Hohmann & Rajewsky, 1978). Among these eighteen clones, three hybridoma clones produced MoAB with T-cell specificity, and two of these clones (R-1-3B3 and R-1-12C5) were subjected to further studies. The immunoglobulin (Ig) heavy chain classes of these two MoAB, determined by Ouchterlony double immunodiffusion with rabbit antisera to various mouse Ig heavy chain classes (Miles Laboratories, Kenkakee, Illinois), were IgG2a for R-1-3B3 and IgG1 for R-1-12C5.

Immunofluorescence

Viable lymphoid cells (5×10^5) were reacted with a saturating amount of MoAB (100 μl) or rabbit anti-rat Ig (100 μl of 1:20 diluted serum) at 4° for 30 min (Ishii, Ueno & Kikuchi, 1974). The cells were then washed twice with phosphate-buffered saline and incubated with fluorescein isothiocyanate (FITC)-conjugated goat antibody to mouse or rabbit Ig at 4° for 30 min. Cross-reactivity of anti-mouse Ig to rat Ig was removed by passing the antibody through a Sepharose 4B column coupled with rat Ig (Porath, Aspherg, Drevin & Axen, 1973). After washing twice with phosphate-buffered saline, the cells mounted on the slides were examined under a Leitz Ortholux II microscope with a vertical UV illuminator, and the percentage of cells with surface fluorescence was counted. Control specimens, which were reacted with NS-1 culture supernatant supplemented with 1% normal mouse (BALB/C) serum (NMS) or with

normal rabbit serum (NRS), were further reacted with the second reagents, always giving less than 1% background staining.

Fractionation of rat T and B cells

T cells were separated from mesenteric lymph nodes of WKA rats by nylon fibre column filtration (Julius, Simpson & Herzenberg, 1973). Splenic B cells, which were adherent to the nylon fibre column were recovered from the nylon fibre by mechanical shaking. Purity of those lymphocytes in each fraction was nearly 100% for T cells and approximately 70% for B cells.

Immunoperoxidase staining

Cryostat sections of rat lymphoid tissues were fixed in periodate-lysine-paraformaldehyde (PLP) fixative for 15 min at 4° (McLean & Nakane, 1974). Then, the sections were washed with phosphate-buffered saline for 30 min, and were reacted with MoAB or control culture supernatant for 45 min at room temperature. The sections were washed three times with phosphate-buffered saline for 30 min and reincubated with peroxidase-conjugated goat anti-mouse Ig (Cappel Laboratories, Cochranville, Pen.) for 45 min at room temperature. The enzyme reaction was developed with 0·05% 3,3′,5,5′-diaminobenzidine tetrahydrochloride and 0·01% hydrogen peroxide in 0·05 M Tris-HCl buffer (pH 7·6).

Cell-surface radioiodination

Freshly prepared lymphocytes were labelled with ^{125}I using lactoperoxidase (Sigma Chemical Co., St Louis, Miss.) according to the method previously described (Ishii *et al.*, 1981). The labelled cells were solubilized with 2% Lubrol-PX (Nakarai Chemicals, Kyoto, Japan) in phosphate-buffered saline at 4° for 1 hr. After centrifugation at 4000 *g* for 15 min, the supernatant of cell lysate was collected, passed through a Millipore 0·45 μm membrane and dialysed against phosphate-buffered saline containing 0·2% Lubrol-PX for 24 hr at 4°. In some experiments, the whole cell lysate was applied to a Sepharose-4B column coupled with *Lens culinaris* haemagglutinin (LcH; Pharmacia Fine Chemicals, Uppsala, Sweden), and cell-surface glycoproteins bound to the column were eluted with 4% methyl-D-mannoside and dialysed against phosphate-buffered saline for 24 hr at 4°. Each sample was counted for radioactivity and subjected to radioimmunoprecipitation (RIP) experiments as described below.

Radioimmunoprecipitation

Aliquots of the labelled cell lysate (2×10^6 c.p.m.) or glycoproteins (2×10^5 c.p.m.) were mixed with 100 μl of MoAB or control culture supernatant and were incubated at 37° for 30 min and at 4° for 20 hr. Since R-1-3B3 MoAB was capable of binding to staphylococcal protein-A, immune complexes formed between this monoclonal and the labelled cell sample were precipitated directly by adding 200 μl of 10% *Staphylococcus aureus* Cowan I (SACI; The Enzyme Center Inc., Boston, Ma.) to the mixture (Cullen & Schwarz, 1976). When R-1-12C5 MoAB, which did not bind to protein-A, was used, rabbit anti-mouse Ig serum (10 μl) was added to the mixture, incubated at 37° for 2 hr, and then resulting immune complexes were precipitated with 200 μl of 10% SACI. We absorbed the labelled cell lysate with SACI prior to RIP, because considerable radioactivity was non-specifically bound to SACI, when the whole cell lysate was used for the RIP experiments.

Sodium Dodecyl Sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Immunoprecipitates adsorbed to SACI were dissociated by boiling the bacteria for 4 min in 100 μl of SDS sample buffer containing 50 mM Tris-HCl (pH 7·0), 2% SDS with or without 5% 2-mercaptoethanol. The sample solubilized in SDS sample buffer was applied to SDS-PAGE using 0·1% SDS and 10% polyacrylamide slab gel (Laemmli, 1970). The gel was electrophoresed at 25 mA per plate until a bromophenol blue marker reached the end of the gel. The gel was stained with Coomassie brilliant blue and dehydrated using a gel slab dryer (Bio-Rad Laboratories, Richmond, Ca. U.S.A.). Radioactivity in the slab gel was visualized by autoradiography using a Cornex Intensifying Screen (E.I. Du Pont De Nomours & Co., Wilmington, De., U.S.A.).

Protein-A radioassay

Indirect radiobinding assays using ^{125}I-labelled staphylococcal protein-A (SPA; Pharmacia Fine Chemicals, Uppsala, Sweden) were performed by the method of McCabe, Quaranta, Frugis, Ferrone & Reisfeld (1979). Briefly, 25 μg of SPA were labelled with 0·5 mCi $Na^{125}I$ by the chloramine-T method. For indirect saturation binding assays, $2{\cdot}5 \times 10^6$ of rat thymus or T cells were incubated with 100 μl of R-1-3B3 and R-1-12C5 MoAB, which had been determined to be sufficient for saturating antigenic sites on these cells, at 4° for 2 hr. The cells were washed twice with

phosphate-buffered saline and incubated with 10^6 c.p.m. of ^{125}I-labelled SPA at 37° for 30 min. When MoAB belong to mouse IgG1, the cells sensitized with MoAB were incubated with rabbit anti-mouse Ig serum (20 μl) prior to being reacted with SPA. The amount of radioactivity added to each tube (10^6 c.p.m.) was in large excess to saturate antibodies bound to the cell surfaces of $2{\cdot}5 \times 10^6$ target cells. After washing twice with phosphate-buffered saline, the cells were counted for bound radioactivity in a Packard gamma-counter (Packard, Zurich, Switzerland).

RESULTS

Reactivity of R-1-3B3 and R-1-12C5 MoAB with different types of cells

The reactivity of R-1-3B3 and R-1-12C5 antibodies with various lymphoid cell populations from WKA and ACI rats as defined by immunofluorescence is shown in Table 1. When these MoAB were reacted with thymus and peripheral lymphocytes including T and B cells, almost identical pattern of reactivity was observed with both MoAB. Thus, these two different MoAB stained virtually all of thymus and T cells but not B cells. When either R-1-3B3 or R-1-12C5 monoclonals was admixed with rabbit anti-rat Ig serum and applied for the staining on LNC, we found that nearly 100% of LNC were stained, indicating that these two MoAB reacted with LNC that did not express surface-bound Ig. The intensity of immunofluorescent staining, however, was different between these two MoAB. Whereas R-1-3B3 stained T cells stronger than most of the thymus cells, R-1-12C5 stained thymus cells much brighter than T cells. This finding was confirmed by radioimmunometric antibody-binding assays using ^{125}I-labelled SPA, which clearly demonstrated that R-1-3B3 bound to T cells approximately three times as much as to thymus cells, whereas R-1-12C5 bound to thymus cells six times as much as to T cells (Table 2). Furthermore, R-1-3B3 did not react with BMC (less than 4%), but R-1-12C5 could react with approximately 20% of these BMC.

To determine whether the antigen defined by either R-1-3B3 or R-1-12C5 MoAB is associated with brain tissue, 1:100 dilution of these MoAB was absorbed with packed brain and liver homogenates obtained from WKA rats and was tested for the residual reactivity with rat thymus cells by radioimmunometric antibody-binding assays using ^{125}I-labelled SPA

Table 1. Reactivity of R-1-3B3 and R-1-12C5 MoAB with various types of rat lymphoid cells as defined by immunofluorescence

Cell types	MoAB*		Surface Ig*	Anti-Ig plus 3B3†	Anti-Ig plus 12C5†
	R-1-3B3	R-1-12C5			
WKA rat					
Thymus	98	98	1		
Lymph node	70	67	24	96	95
Spleen	49	45	50		
Bone marrow	2	21	5		
T-cell rich‡	98	96	1		
B-cell rich‡	22	20	73		
ACI rat					
Thymus	98	98	1		
Lymph node	62	59	35	96	94
Spleen	46	41	57		
Bone marrow	3	23	6		

* Cells were stained by indirect immunofluorescence with MoAB or rabbit anti-rat Ig serum. Data were expressed as percentage of fluorescent cells.

† Lymph node cells from WKA and ACI rats were incubated with the mixture of MoAB and rabbit anti-rat Ig at 4° for 30 min, washed twice and further incubated with the mixture of fluoresceinated goat anti-mouse Ig and anti-rabbit Ig sera.

‡ T and B cells were fractionated from WKA rat lymph node and spleen cells by nylon fibre column filtration.

Table 2. Binding of ^{125}I-labelled SPA to WKA rat thymus and T cells sensitized with R-1-3B3 or R-1-12C5 monoclonal antibody

Treatment	Binding to (c.p.m.)*	
	Thymocyte	T cell†
R-1-3B3	91935 (44713)¶	167434 (121572)¶
Control‡	47222 (0)	45862 (0)
R-1-12C5	529837 (439771)¶	162762 (77078)¶
Control§	90066 (0)	85684 (0)

* $2{\cdot}5 \times 10^6$ thymus and T cells were sensitized with R-1-3B3 or R-1-12C5 antibody. The cells sensitized with R-1-12C5 antibody were further treated with rabbit anti-mouse Ig serum. The cells were washed twice and incubated with ^{125}I-labelled SPA (1×10^6 c.p.m).

† T cells were purified from lymph node cells by nylon fibre column filtration.

‡ NS-1 culture supernatant supplemented with 1% normal mouse serum was used as a control reagent.

§ Control sample was prepared by treating target cells with NS-1 culture supernatant supplemented with 1% normal mouse serum and further with rabbit anti-mouse Ig serum.

¶ Data were expressed as c.p.m. bound to cells sensitized with monoclonal antibodies or with control reagents. Specific binding, which was calculated by subtracting c.p.m. bound to cells treated with control reagents from that bound to antibody-treated cells, is in parenthesis.

(Table 3). Whereas both brain and liver tissues were ineffective to absorb antibody activity in both R-1-3B3 and R-1-12C5 MoAB, brain but not liver tissue could indeed absorb anti-rat Thy-1 activity found in an anti-rat Thy-1 MoAB (R-1-6C1).

Since the reactivity of R-1-12C5 MoAB with rat lymphoid cells appeared to resemble that reported for W3/13 antibody (Williams *et al.*, 1977; Barclay, 1981), we compared the pattern of reactivity with THY, SPC, LNC and BMC from WKA rats between these two monoclonals (Table 4). Both R-1-12C5 and W3/13 antibodies, as expected, reacted similarly with THY, SPC and LNC, but, when BMC were stained with these antibodies, we found that W3/13 antibody stained many more BMC than did R-1-12C5 antibody.

The reactivity of R-1-3B3, R-1-12C5 and W3/13 monoclonals with neoplastic T and B cells was also tested by immunofluorescence (Table 5). All of these monoclonals labelled more than 90% of thymoma cells from Buffalo/Mna rats. Whereas R-1-3B3 did not stain leukaemic B cells (KNL-14) expressing both Thy-1 and IgM on their cell surfaces, R-1-12C5 and W3/13 monoclonals clearly labelled these KNL-14 leukaemic B cells. Again, R-1-12C5 stained thymoma cells much brighter than did R-1-3B3 antibody.

Tissue localization of lymphocytes reactive with R-1-3B3 and R-1-12C5 MoAB

Cryostat sections prepared from thymuses, spleens and lymph nodes of WKA rats (8–10 weeks old) were stained by immunoperoxidase technique with R-1-3B3 and R-1-12C5 MoAB. When the thymus was stained with R-1-3B3, medullary cells were more intensely stained than were cortical cells (Fig. 1a). R-1-12C5, in contrast, more strongly labelled cortical than medullary cells (Fig. 1b).

Both of these MoAB gave positive staining on T cells located in thymus-dependent areas, i.e., the paracortex of lymph node and periarteriolar lymphatic sheath of spleen, except for a few cells stained with these antibodies that were occasionally seen in lymphoid follicles (Fig. 2). A number of cells stained by either R-1-3B3 or R-1-12C5 MoAB were scattered in the medulla of lymph node and red pulp of spleen, but the marginal zone of splenic white pulp, where maturating B cells exist (Kumararatne, Bazin & MacLennan, 1981), was not stained with both MoAB.

Chemical characterization of antigens defined by R-1-3B3 and R-1-12C5 MoAB

For the immunochemical characterization of cell-surface antigens defined by R-1-3B3 and R-1-12C5 MoAB, we used indirect RIP and reacted these MoAB with rat thymus cell lysate that had been labelled with ^{125}I by lactoperoxidase and absorbed with equal volume of packed SACI at 4° for 1 hr. SDS-PAGE analysis performed on immunoprecipitates made with R-1-3B3 clearly demonstrated a single component with a M.W. of 67,000 (T67). This component appeared to consist of a single polypeptide chain containing no intrachain disulfide linkages, as suggested by the fact that it ran identically in gels under both reducing and non-reducing conditions (Fig. 3). The T67 antigen was considered to have carbohydrate moieties, because it could bind to LcH-coupled Sepharose 4B column (Fig. 5).

SDS-PAGE profiles of immunoprecipitates formed between R-1-12C5 and ^{125}I-labelled thymus cell lysate

Table 3. Absorptive capacities of WKA rat brain and liver tissues for reactivity of various MoAB on rat thymus cells

MoAB (Ig classes)*	Unabsorbed	Absorbed with brain† 1:1	1:1/2	1:1/4	Absorbed with liver (1:1)†
R-1-3B3 (IgG2a)	11201‡	10275	10802	11206	10853
R-1-12C5 (IgG1)	21321	20602	20608	22402	19571
R-1-6C1 (IgG3)§	14327	1414	1873	2130	13160

* 5×10^6 of WKA rat thymocytes were treated with 40 μl of each MoAB (1:100 dilution). The cells sensitized with R-1-12C5 antibody were further treated with rabbit anti-mouse Ig serum. The cells were washed twice and incubated with ^{125}I-labelled SPA (3×10^5 c.p.m.).

† One millilitre of MoAB (1:100 dilution) was absorbed with 1 to 1/8 ml of packed tissue homogenates prepared from WKA rat brain and liver at 4° for 2 hr. After centrifugation at 4000 *g* for 20 min, the supernatant was collected and tested for the residual antibody activity on rat thymus cells.

‡ C.p.m. bound to antibody-sensitized cells were subtracted by that bound to cells treated with control reagents. Control cells treated with NS-1 culture supernatant plus 1% NMS or further with rabbit anti-mouse Ig serum give background c.p.m. of 4192 and 9281, respectively.

§ This R-1-6C1 MoAB, produced also in our laboratory, has been determined to react with rat Thy-1 antigen.

Table 4. Comparison of reactivity between R-1-12C5 and W3/13 monoclonal antibodies with WKA rat lymphoid cells

Cell types	Monoclonals* R-1-12C5	W3/13†
Thymus	98‡	98
Lymph node	62	65
Spleen	48	50
Bone marrow	23	52

* Cells were stained by indirect immunofluorescence.

† W3/13 antibody was obtained from Sera-Lab (Sussex, England).

‡ Data were expressed as percentage of fluorescent cells.

are shown in Fig. 4. This R-1-12C5 MoAB precipitated a 95K M.W. component (T95) from rat thymus cells. No difference in its electrophoretic mobility was seen when the T95 protein was electrophoresed under both reducing and non-reducing conditions. The T95 antigen also seemed to be a glycoprotein, as suggested by its binding to LcH (Fig. 5). When the parallel immunoprecipitation experiments using ^{125}I-labelled rat thymus cell glycoproteins purified by a LcH-coupled Sepharose 4B column and R-1-12C5 or W3/13 antibody were performed, it was found that R-1-12C5 precipitated a 95K M.W. component from the labelled thymus cell glycoproteins, but none was precipitated by W3/13 antibody from the same glycoprotein preparation (Fig. 6).

DISCUSSION

Murine T cells express cell-surface antigens, which enable us to distinguish those T cells from other lymphoid elements including B cells (Ledbetter & Herzenberg, 1979). These antigens with specificity for murine T cells include Thy-1 and Ly-1 alloantigen systems. Human T cells also bear a T-cell antigen designated Leu-1 or OKT1 (Ledbetter, Evans, Lipinski, Cunningham-Rundels, Good & Herzenberg, 1981; Reinherz, Kung, Goldstein, Levey & Schlossman, 1980), which shows similarities in tissue distribution and molecular characteristics to mouse Ly-1 antigens (Ishii *et al.*, 1981). In addition, human T cells possess other T-cell antigens such as Leu-4 (OKT3) or Leu-5 (OKT11) (Howard, Ledbetter, Wong, Bieber, Stinson & Herzenberg, 1981; Verbi, Greaves, Schneider, Koubek, Janossy, Stein, Kung & Goldstein, 1982).

Table 5. Reactivity of R-1-3B3, R-1-12C5 and W3/13 monoclonal antibodies with neoplastic T and B cells in rats

Cells	Monoclonal antibodies*			
	R-1-3B3	R-1-12C5	W3/13	Surface Ig*
Thymoma (Buffalo/Mna)	96	97	98	< 1
B-cell leukaemia (KNL-14)	0	80	90	93

* Cells were stained with monoclonal antibodies or rabbit anti-rat Ig serum by indirect membrane immunofluorescence. Data were expressed as percentage of fluorescent cells.

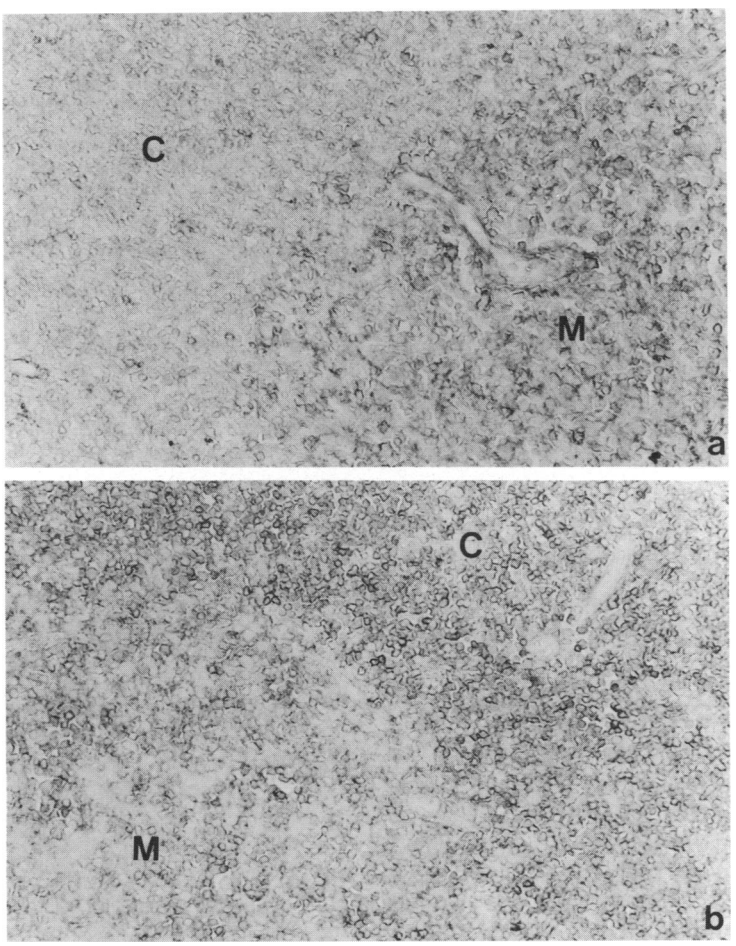

Figure 1. Thymus tissue sections of WKA rat stained by immunoperoxidase technique with (a) R-1-3B3 and (b) R-1-12C5 antibodies. Whereas R-1-3B3 more intensely stained medullary than cortical cells, R-1-12C5 reacted strongly with cortical cells and weakly with medullary cells. (Magnification × 140)

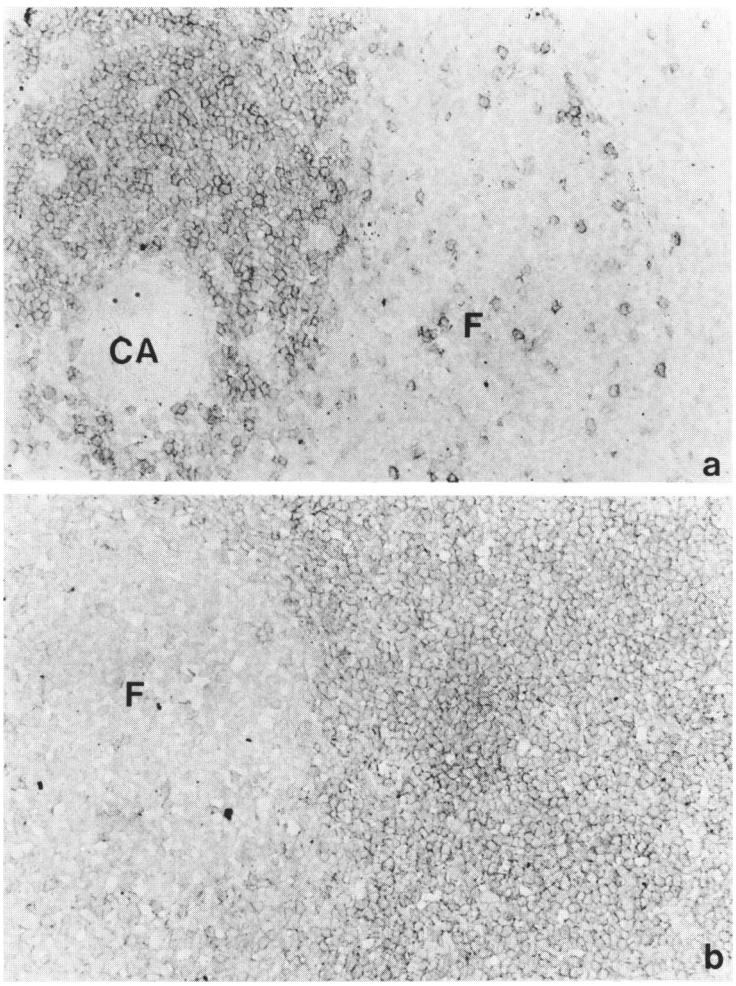

Figure 2. (a) Spleen and (b) lymph node tissue sections of WKA rat stained by immunperoxidase technique with R-1-3B3 and R-1-12C5 antibodies, respectively. R-1-3B3 stained T cells located around central arteriole (CA) of splenic white pulp but not cells in lymphoid follicle (F) and marginal zone of the spleen (a). R-1-12C5 also stained T cells located in paracortical area of the lymph node (b). (Magnification × 140)

Definite general markers for rat T cells, however, have not been well documented, though several xenoantisera to rat T cells have been shown to exhibit operational specificity for rat T cells (Goldschneider & McGregor, 1972; Ishii *et al.*, 1976). Recently, Williams *et al.* (1977) produced a MoAB (W3/13) that preferentially labelled thymus and T cells but not B cells. This antigen defined by W3/13 antibody, however, is not restricted to rat thymus and T cells but is found on other cell types including myelogenous cell series and brain tissue. The chemical nature of this W3/13 antigen was also examined, and it appears that it is a sialoglycoprotein with a 95K M.W. as estimated by SDS-PAGE (Standring, McMaster, Sunderland & Williams, 1978).

One of the rat T-cell antigens defined by our monoclonals seems to have some similarities to W3/13 antigen described by Williams *et al.* (1977). Thus, the antigen recognized by R-1-12C5 MoAB was present on virtually all of rat thymus and T cells as well as on thymoma cells, but absent from normal B cells. R-1-12C5, as well as W3/13 antibody, cross-reacted with rat BMC and KNL-14 leukaemic B cells, and could recognize a 95K M.W. glycoprotein on rat

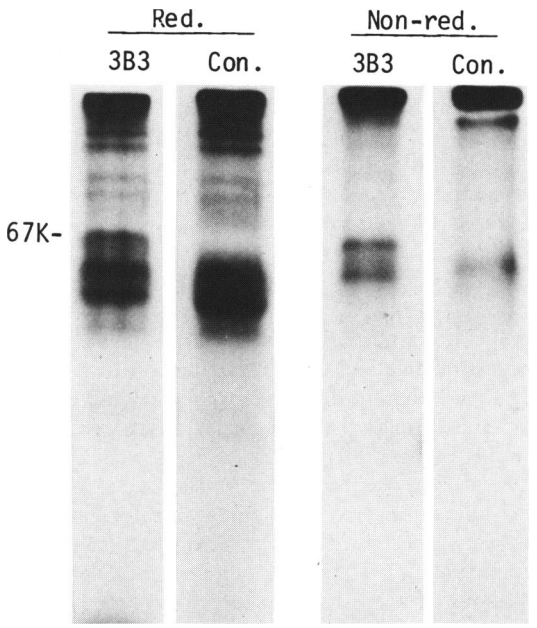

Figure 3. SDS-PAGE analysis of immunoprecipitates of R-1-3B3 antibody (3B3) made with ^{125}I-labelled thymus cell lysate from WKA rat. Note a single component with a M.W. of 67K precipitable with R-1-3B3 antibody but not with control culture supernatant (Con.). The 67K M.W. component (T67) ran identically in gels under both reducing (Red.) and non-reducing (Non-red.) conditions.

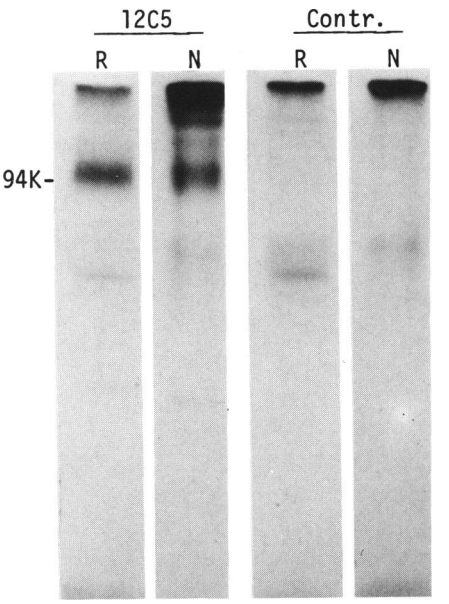

Figure 4. SDS-PAGE analysis of immunoprecipitates of R-1-12C5 (12C5) made with ^{125}I-labelled thymus cell lysate of WKA rat. This R-1-12C5 antibody precipitated a single component with a M.W. of 95K (T95), which ran identically in gels both under reducing (R) and non-reducing (N) conditions. Control immunoprecipitate (Contr.) was prepared by reacting the same thymus cell lysate with NS-1 control culture supernatant.

thymus cells. Despite of these similarities, we concluded that the T95 antigen defined by our R-1-12C5 MoAB was distinct from that detected by W3/13 antibody, because (i) R-1-12C5 stained much less number of rat BMC than that reacting with W3/13, (ii) T95 defined by our R-1-12C5 could not be found in brain tissue, but W3/13 antigen has been reported to exist in rat brain (Williams *et al.*, 1977), (iii) T95 detected by R-1-12C5 could interact with LcH, whereas W3/13 could not (Standring *et al.*, 1978). Parallel immunoprecipitation experiments clearly demonstrated that R-1-12C5 precipitated a 95K M.W. component from labelled thymus-cell glycoproteins purified by an LcH-coupled Sepharose 4B column, but none was precipitated by W3/13 antibody from the same glycoprotein preparation. The amount (100 μl) of W3/13 antibody used for the immunoprecipitation experiments seems to be sufficient, because the same amount of W3/13 antibody could precipitate a 95K M.W. component from LcH-unbound thymus cell proteins (Matsuura & Ishii, 1982). All of these data

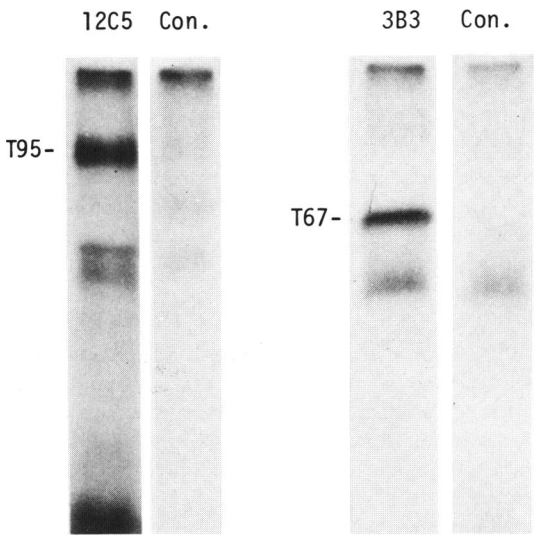

Figure 5. SDS-PAGE analysis of immunoprecipitates of R-1-3B3 (3B3) and R-1-12C5 (12C5) antibodies made with ^{125}I-labelled rat thymus cell glycoproteins, which were purified by LcH-coupled Sepharose 4B column. Both antigens precipitated by R-1-3B3 (T67) and R-1-12C5 (T95) were found in LcH-bound material of the thymus cell lysate.

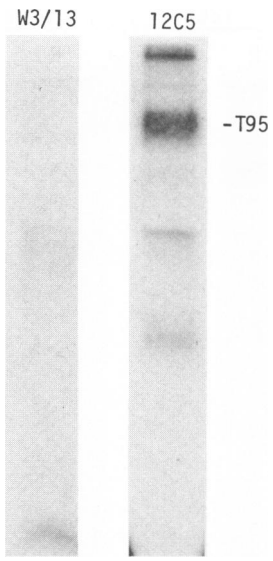

Figure 6. SDS-PAGE analysis of immunoprecipitates of W3/13 and R-1-12C5 (12C5) antibodies made with ^{125}I-labelled rat thymus cell glycoproteins, which were purified by LcH-coupled Sepharose 4B column. Whereas 12C5 precipitated a 95K component (T95) from thymus cell glycoproteins, none was brought down with W3/13 antibody from the same glycoprotein preparation.

mentioned above seem to indicate that R-1-12C5 and W3/13 define different antigen systems on rat thymus and T cells.

In addition to the antigen described above, we found a different type of rat T-cell antigen, which could be recognized by R-1-3B3 MoAB. This antigen appeared to be more restricted to rat thymus and T cells as compared to that defined by R-1-12C5 MoAB, because the former R-1-3B3 antibody reacted with all rat thymus and T cells as well as with thymoma cells but neither with normal and leukaemic (KNL-14) B cells nor with bone marrow cells. Immunochemical studies have shown that R-1-3B3 MoAB precipitated a single polypeptide with a 67K M.W. (T67), containing no intrachain disulphide linkages as judged by SDS-PAGE under reducing and non-reducing conditions. This T67 antigen seems to be a glycoprotein capable of binding to LcH, which shows electric charge heterogeneity as determined by O'Farrell's two-dimensional (2-D) PAGE (Matsuura & Ishii, 1982).

It is of interest to compare the T67 antigen detected by R-1-3B3 MoAB to mouse Ly-1 antigens. General characteristics of Ly-1 antigens can be summarized as follows: (i) Ly-1 are expressed on all of mouse thymus and T cells (Ledbetter, Rouse, Micklem & Herzenberg, 1980); (ii) there are two different phenotypes of Ly-1, e.g. Ly-1,1 and Ly-1,2 (Boyse *et al.*, 1968); (iii) Ly-1 locus resides on chromosome 19 (Itakura, Hutton, Boyse & Old, 1972); (iv) Ly-1 are glycoproteins with M.W. of 67K, as estimated by SDS-PAGE, which are composed of a single polypeptide chain rather than sulphydryl-linked subunits (Durda, Shapiro & Gottlieb, 1978; Ledbetter & Herzenberg, 1979). Among these characteristics, two points can be compared at present. Thus, T67 defined by R-1-3B3 MoAB is similar to mouse Ly-1 at least in regard to its expression on all of thymus and T cells. Furthermore, we found that medullary thymocytes reacted with R-1-3B3 stronger than did cortical thymocytes, comparable to the distribution of Ly-1 within mouse thymus tissue (Ledbetter *et al.*, 1980). The M.W. of rat T67 and mouse Ly-1 are almost identical, as both antigens, labelled with ^{125}I by lactoperoxidase, can be separated as a 67K M.W. component by SDS-PAGE. In addition, it has been demonstrated that Ly-1 are glycoproteins capable of interacting with LcH, and show electric charge heterogeneity on 2-D PAGE (Ledbetter & Herzenberg, 1979), just like T67 shown in this and our previous studies using the similar 2-D PAGE (Matsuura & Ishii, 1982). All of these data are also consistent with those obtained with human Leu-1 or Tgp72 antigen (Ledbetter *et al.*, 1981; Ishii *et al.*, 1981), suggesting that Ly-1 is one of the representative antigens well conserved through the evolutionary process.

Our previous immunohistochemical studies have shown that the tissue distribution of cells reactive with xenoantiserum to rat T cells coincides with thymus-dependent areas, i.e., the paracortex of lymph node and periarteriolar region of splenic white pulp (Ishii *et al.*, 1976; Uede, Ishii, Matsuura & Kikuchi, 1981; Yamanaka, Ishii, Koshiba & Kikuchi, 1981). In the thymus, the antiserum reacted more intensely with the cells located in the thymic medulla than in the cortex. These data are clearly consonant to those obtained in this study with R-1-3B3 MoAB, which also labelled medullary thymocytes and peripheral T cells stronger than cortical thymocytes. In contrast, R-1-12C5 antibody appears to react strongly with cortical thymocytes and weakly with medullary thymocytes and peripheral T cells. Thus, there seems to exist an inverse relationship in distribution of T67 and T95 antigens defined on rat T lineage cells by two distinct MoAB. It is of interest in this regard to assume a possible differentiation pathway proceeding from cortical to

medullary thymocytes and further to peripheral T cells. If this is so, then T-cell development in rats might be accompanied by a gain of T67 and by a corresponding loss of T95. The data obtained from radioimmunometric antibody-binding assays using ^{125}I-labelled SPA seem to support this possibility.

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