Immunobiol. (2001) 203, pp. 725–742
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http://www.urbanfischer.de/journals/immunobiol

Immunobiology

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An Immature Rat Lymphocyte Marker CD157: Striking Differences in the Expression between Mice and Rats

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Received October 4, 2000 · Accepted in revised form March 23, 2001

Abstract

We have established a novel monoclonal antibody that recognises mouse and rat CD157, and uncovered striking differences in both the level and stage of expression of this antigen in the primary lymphoid organs between these two species. Unlike mouse, the majority of rat thymocytes express CD157. SHR and WKY rats were the exception, having unusually low levels (similar to those of the mouse) of these cells. However, in both species, a subset of $CD3^-CD4^-CD8^-$ thymocytes exhibited high levels of CD157. Surprisingly, these $CD157^{high}$ cells temporarily upregulated MHC class I molecules in both species. Furthermore, a third of $CD157^{high}$ rat thymocytes were $CD45RC^+$, a marker found on immature thymocytes with regenerative capacity.

Examination of the bone marrow lymphoid population shows that the expression of rat CD157 is largely observed at the $CD45R^+IgM^-$ pre-B-II cell stage, and unlike mouse, extension of expression into the IgM^+ immature B cell stage was marginal. Similar to $CD157^{high}$ immature thymocytes, these immature B cells also expressed high levels of MHC class I. With the exception of the LEC, SHR and WKY rat strains, which have three- to four-fold less $CD157^+$ bone marrow myeloid cells, percentages of these cells are similar between these two species. Thus, marked differences in the level and stage(s) of CD157 expression on lymphoid cells in mouse and rat indicate that CD157 may not, as previously thought, have a direct role in T or B cell differentiation.

Introduction

Mouse CD157 is a 38–48 kDa glycosylphosphatidylinositol (GPI) anchored cell surface glycoprotein (1, 2). The expression on thymocytes is mainly observed at the $CD44^{low/-}CD25^+$ stage (3, 4) when rearrangement of the T cell receptor (TCR) β-chain

Abbreviations: BCR = B cell receptor; CHO = chinese hamster ovary; DN = double negative; FITC = fluorescein isothiocyanate; GPI = glycosylphosphatidylinositol; int = intermediate; mAb = monoclonal antibody; MHC = major histocompatibility complex; PE = phycoerythrin; PI-PLC = phosphatidylinositol-specific phospholipase C; SAv-Cy-Chrome = streptavidin Cy-Chrome; SP = single positive; TCR = T cell receptor.

0171-2985/01/203/05-725 $ 15.00/0

genes occurs. However, expression is independent of the rearrangement, as RAG-1 deficient mice express CD157 at this stage (3). Bone marrow B cells also express this antigen from the $B220^+IgM^-$ pre-B-I (having completed D_HJ_H gene rearrangement) through to the $B220^+IgM^+$ immature B cell stage (4). CD157 is not restricted to immature T and B cells, it can also be detected on: neutrophils, macrophages, the intestinal epithelial cells and collecting tubules of the kidney. It is also expressed on reticular cells in lymph nodes, Peyer's patches and the splenic white pulp, but not on reticular cells located in the thymus, bone marrow or splenic red pulp (1, 2).

Although the precise function of CD157 is not known, it may belong to the family of ecotoenzymes, as its sequence shows 29% and 33% homology with the ecto-enzymes, mollusc *Aplysia* ADP-ribosyl cyclase and human CD38, respectively (5). Furthermore, like CD38, CD157 has been reported to possess ADP-ribosyl cyclase and cyclic ADP-ribose hydrolase activities (6, 7). The close linkage between mouse *Cd157* and *Cd38* on chromosome 5 has been documented (8). Similarly, in humans, *CD157* is linked to *CD38* located on chromosome 4p15 (9). Therefore, it is thought that these two genes are the result of gene duplication and evolved from a common ancestral gene.

Functional studies by VICARI et al. showed that an antibody specific for CD157 enhanced anti-CD3-induced proliferation of murine pre-T cells, and accelerated differentiation of $\alpha\beta$T cells in foetal thymic organ culture (3). Although these experiments indicate the involvement of CD157 in T cell differentiation/activation, recently established CD157-knockout mice exhibit almost normal lymphocyte development, except for the delayed appearance of peritoneal B1 cells and increased levels of $CD38^{low/-}$ B lineage cells in the bone marrow and spleen (10).

We have established a monoclonal antibody (mAb) that recognises both mouse and rat CD157. For the first time, it is now possible to investigate the expression of rat CD157. Using this antibody, we examined rat CD157 in comparison with mouse CD157 (BP-3/BST-1) as it relates to (i) tissue and strain distributions and (ii) differentiation stages at which expression in lymphocytes occurs.

Materials and Methods

Animals

LEW/Crj rats were purchased from Charles River Japan (Yokohama). ACI/N, BN/SsN, DA/Slc, F344/N, LEC, WKAH/Hkm, SD, SHR/Izm and WKY/Izm rats were obtained from SLC Japan (Hamamatsu) and PVG/Sea from Seac Yoshitomi (Yoshitomi, Japan).

BALB/c, B10.BR, C3H/HeJ and C57BL/6J mice were purchased from SLC Japan (Hamamatsu). CBA/Ca, SWR/J and C57BR/cdJ were obtained from the National Institute for Genetics (Mishima, Japan) and Jackson Laboratory (Bar Harbor, ME, USA), respectively.

Antibodies and reagents

Mouse CD157-specific mAb, BP-3 (1), was a kind gift from Dr. MAX D. COOPER, University of Alabama at Birmingham. Fluorescein isothiocyanate (FITC)-, R-phycoerythrin (R-PE)- or biotin-conjugated mAbs specific for mouse, rat and human cell surface molecules were used for two- and three-colour staining. mAbs specific for mouse: KT3 (anti-CD3), KT120 (anti-K^k), KT6 (anti-CD4) and KT16 (anti-CD90) had been established by us (11); hybridoma cells secreting CD25-specific mAb, 7D4, was obtained from ATCC (Rockville, MD, USA); and CD2, CD11b/Mac-1, CD45R (B220), CD62L and IgM were obtained from Caltag Laboratories (South San Francisco, CA, USA). mAbs

specific for rat cell surface molecules: CD2, CD3, CD4 (OX38), CD11b/Mac-1, CD25, CD45R, CD45RC (OX22), CD62L, CD90, MHC class I (RT1A), IgM and secondary reagent streptavidin Cy-Chrome (SAv-Cy-Chrome) were obtained from PharMingen (San Diego, CA, USA). mAb specific for rat CD8 (OX8), TRI-COLOR coupled antibodies, CD8/TC (anti-mouse CD8α) and OX8/TC (anti-rat CD8 α), and streptavidin-R-PE were obtained from Caltag Laboratories. FITC-coupled goat anti-mouse IgG antibody was purchased from Jackson ImmunoResearch Laboratories Inc. (West Grove, PA, USA) and phosphatidyl-inositol-specific phopholipase C (PI-PLC) from Boehringer Mannheim (Mannheim, Germany). KT605, KT33 (established in our laboratory) and OKT3 (obtained from ATCC) were used as isotype (mouse IgG2a/κ) matched control antibodies for B15.128.

The *mBST-1/pUC19* plasmid was obtained from Riken (Wako, Japan); the pTarget vector was purchased from Promega Corp. (Madison, WI, USA); DH10 electrocompetent *E. coli* and lipofectin from GibcoBRL (Rockville, MD, USA).

Establishment of an antibody specific for both mouse and rat CD157

An A/J mouse was hyperimmunized with BALBRV4 tumour cells. Spleen cells from the immunized mouse were fused with NS1 myeloma cells as described in a previous paper (12). The antibodies secreted by the resultant hybridoma cells were screened for their ability to induce cytolysis of BALBRV4 cells in the presence of rabbit complement. We found 48 hybridomas secreting antibodies with the same specificity. One of them was cloned using a limiting dilution method and designated as B15.128 (IgG2a, κ).

Flow cytometry

Cells from thymus, bone marrow, and various cell lines (1, 13–16) were stained with a variety of antibodies and analyzed with an EpicsXL flow cytometer as described in a previous paper (17).

PI-PLC treatment

Six $\times 10^5$ cells were incubated in 100 μl of 2% FCS-containing RPMI 1640 medium supplemented with 0.5 unit/ml of PI-PLC or medium alone at 37 °C for 75 min. After two washes the cells were incubated with either KT33 (isotype matched control) or B15.128 biotinylated antibodies, washed and then treated with streptavidin R-PE prior to examination with a flow cytometer.

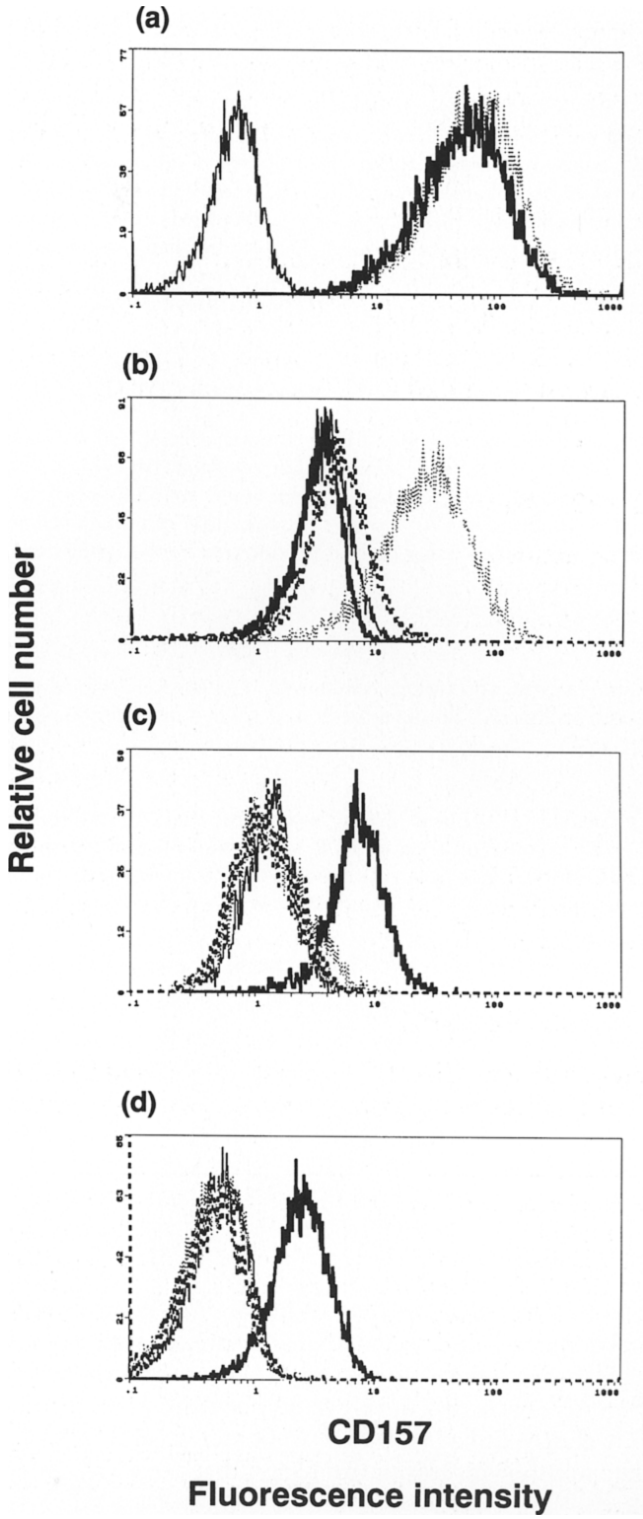
Transfection of the mouse *Cd157* gene into chinese hamster ovary (CHO) cells

The *mBST-1/pUC19* plasmid was electroporated into DH10 *E. coli* and expanded. After isolation and purification of the plasmid, the *Cd157* gene was excised at the *Eco*RI site and ligated into the pTarget vector. After a further round of electroporation, expansion, and purification, the construct was transfected into CHO cells using lipofectin.

Results

Specificity of B15.128 mAb

We examined the strain distribution pattern of the antigen recognized by B15.128 mAb (derived from an A/J mouse) and found that it was expressed on a small percentage of thymocytes in all other mouse strains tested (data not shown). Hence, using 98 {(A/J×Czech II)F1×A/J} backcross mice, we mapped the gene encoding the B15.128 antigen to a region closely linked (3.0 ± 1.7 cM) to *D5Mit80/D5Mit81/D5Mit13* on chromosome 5 (data not shown). It is in this region that the *Cd157* gene (*Bp-3*) is located (8).


(a)
(b)
(c)
(d)
Relative cell number
CD157
Fluorescence intensity

Further examination of cells from primary and secondary lymphoid organs (thymocytes, bone marrow cells, splenocytes, and lymph node cells) revealed that the staining profiles of B15.128 were similar to those reported with mouse CD157-specific mAbs, BP-3 (1) and G12 (4) [data not shown, but see Fig. 2 (right panel), Fig. 3b, Fig. 5b and Fig. 6b].

B15.128 mAb is specific for mouse CD157

To confirm that B15.128 recognizes the same antigen as BP-3 (i.e. CD157), we transfected mouse *Cd157* into CHO cells and examined the cell surface expression of CD157. As shown in Figure 1a, CHO cells transfected with mouse *Cd157* could be stained by both mAbs, BP-3 and B15.128. Furthermore, incubation of the transfected cells with PI-PLC cleaved off cell surface GPI-anchored CD157 as indicated by a 90.7% reduction in fluorescence intensity of B15.128 staining (Fig. 1b).

Unlike BP-3, B15.128 mAb stained rat cells

A panel of lymphoid and non-lymphoid cell lines was examined for their expression of CD157 with mAbs, B15.128 and BP-3. Indirect immunofluorescence analysis of the 23 lines showed that of the murine lines, five (BALBRV4, YAC-1, SCID-thy9, P388D1 and SF/1) were positive for both BP-3 and B15.128 mAbs (Table I). Furthermore, the fluorescence intensity observed with B15.128 was higher than that seen with BP-3. Interestingly, two rat cell lines, the MTHC-1 thymoma (14) (Fig. 1c) and a dendritic-like cell line (SF-DL1) stained for B15.128 but not BP-3 (Table I); indicating that B15.128 recognises both rat and mouse CD157. This reflects the high similarity in CD157 sequence between rat and mouse (5, 18). To confirm that this was the case, MTHC-1 cells were treated with PI-PLC and then stained with B15.128. As shown in Figure 1d, complete abrogation of B15.128 antigen expression was observed.
However, neither antibody recognized CD157$^+$ human cell lines (13, 16, 19) (U937 and Jurkat) nor human peripheral blood cells (data not shown).

Rat CD157 expression in the thymus

Three-colour analysis with anti-CD4/FITC, anti-CD8/TC and B15.128/Biotin was performed on LEW rat thymocytes to determine the differentiation stage at which CD157

Figure 1. CD157 expression on CHO cells and a rat thymoma, MTHC-1. (a) CHO cells transfected with mouse *Cd157* gene: B15.128 antibody (thick plain line), BP-3 antibody (thin dotted line) and isotype matched mAb (plain line). Bound antibody was detected by FITC- conjugated goat anti- mouse IgG. (b) Cleavage of CD157 from CD157$^+$ CHO cells with PI-PLC: B15.128 staining with (thick dotted line) and without PI-PLC (thin dotted line) treatment. Thick and thin plain lines represent isotype matched control staining with and without PI-PLC treatment, respectively. (c) Expression of rat CD157 on a rat thymoma, MTHC-1: B15.128 staining (thick plain line), BP-3 staining (thin dotted line), isotype matched mAb (thick dotted lines) and medium alone (thin plain line). Bound antibody was detected by FITC- conjugated goat anti- mouse IgG. (d) Cleavage of rat CD157 from MTHC-1 cells with PI-PLC: B15.128 staining with (thick dotted line) and without (thick plain line) PI-PLC treatment. Thin dotted and thin plain lines represent staining with isotype matched mAb with and without PI-PLC treatment, respectively.

is expressed. C3H/He mouse thymocytes were similarly stained for comparison. As shown in Figure 2 (left panel), rat CD157 is expressed on the majority of thymocytes. However, a subset of $CD4^{-}CD8^{-}$ double negative (DN) cells was negative and appears to belong to the most immature thymocytes. The highest level was observed on a subset (21.0%) within the DN cells. The levels of expression then decreased as the thymocytes differentiated and finally the lowest level was seen on the mature $CD4^{+}CD8^{-}$ single positive (SP) cells. In contrast, and in agreement with previously published BP-3 data (1, 3, 4) expression of mouse CD157 was predominately seen on DN thymocytes; a subset (36.9%) of which, expressed high levels (Fig. 2, right panel).

Table I. Expression of B15.128 and BP-3 antigens on transformed cells

Cell Line	Origin	Cell Type	B15.128	BP-3
Mouse				
BALBRV4	BALB/c	thymoma	5.3	3.4
SCID thy9	C.B-17/lcr-*scid*	thymoma	15.2	11.7
YAC-1	A	lymphoma	17.5	10.4
SF/1	C57BR/cdJMs	thymic stromal cell line	4.7	2.2
P388D1[1]	DBA/2	macrophage-like cell line	1.9	1.5
AKR339	AKR	thymoma	0.0	0.0
AKR344	AKR	thymoma	0.0	0.0
AKR365	AKR	thymoma	0.0	0.0
AKR369	AKR	thymoma	0.1	0.0
AKR389	AKR	thymoma	0.0	0.0
AKR424	AKR	thymoma	0.2	0.1
BWCD4	AKR	thymoma	0.0	0.0
EL4	C57BL/6	thymoma	0.0	0.0
1/29	I/St	B cell lymphoma	0.0	0.0
LBBA	BALB/c/RF/J	B cell lymphoma	0.0	0.2
MMTV Cz	Czech II	mammary tumour	0.0	0.0
MMTV Ms	C57BR/cdJMs	mammary tumour	0.3	0.1
RADA-1	A	T cell leukemia	0.0	0.0
ST2	BC8	bone marrow stromal cell line	0.0	0.0
Human				
U937	Human	histiocytic lymphoma	0.0	0.0
Jurkat	Human	acute T cell leukemia	0.0	0.0
Rat				
MTHC-1	rat	thymoma	4.37	0.0
SF-DL1	rat	dendritic-like cell line	6.1	0.0

All cell lines were stained with B15.128 or BP-3 culture supernatants followed by FITC-conjugated goat anti-mouse IgG. Isotype matched control mAbs were used as negative controls. The data shown are mean fluorescence intensity values after deduction of isotype control.

[1] P388D1 has been reported to be negative for BP-3 (1). Our staining shows that it is weakly positive for BP-3 and B15.128. This may reflect a sub-line difference.

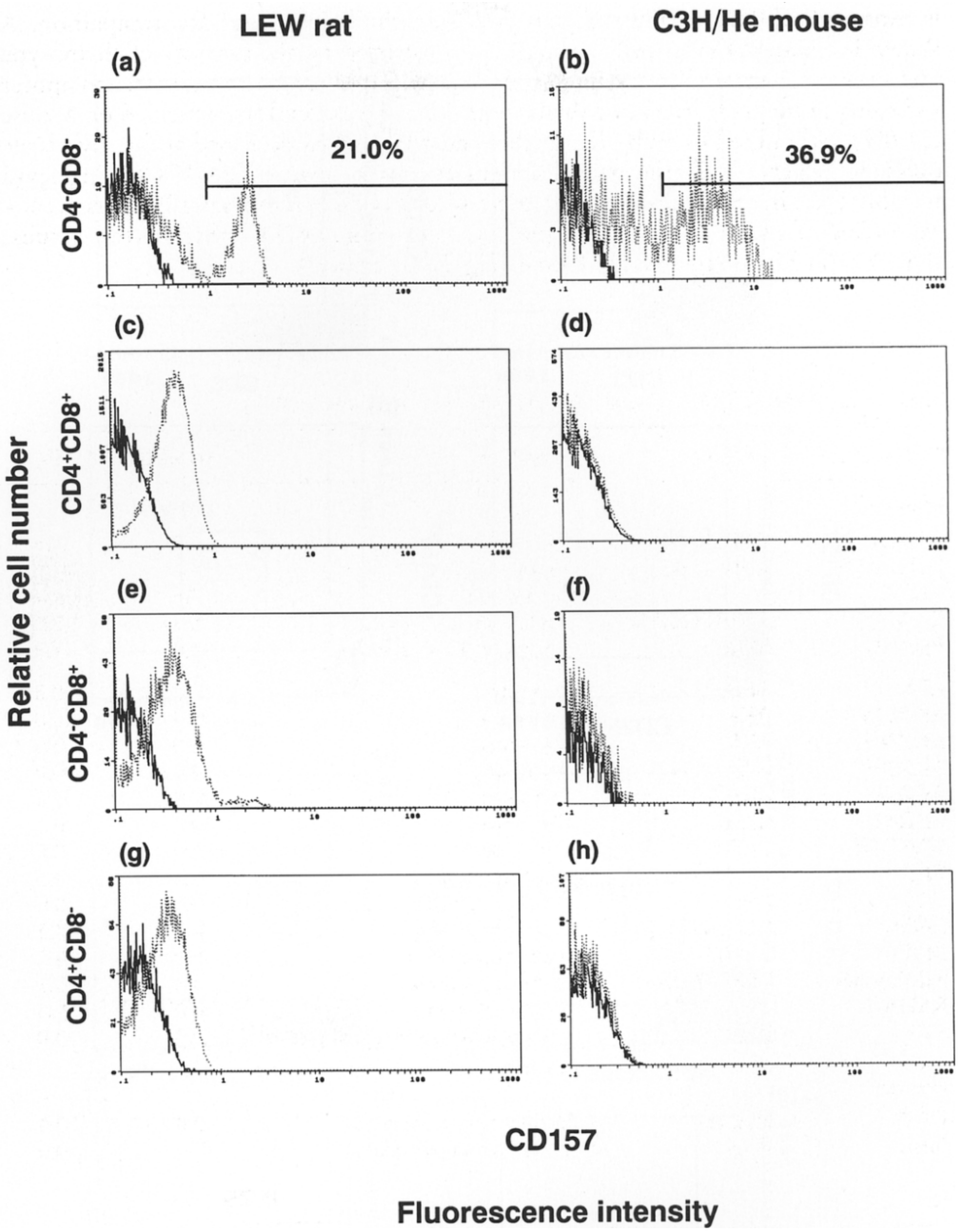
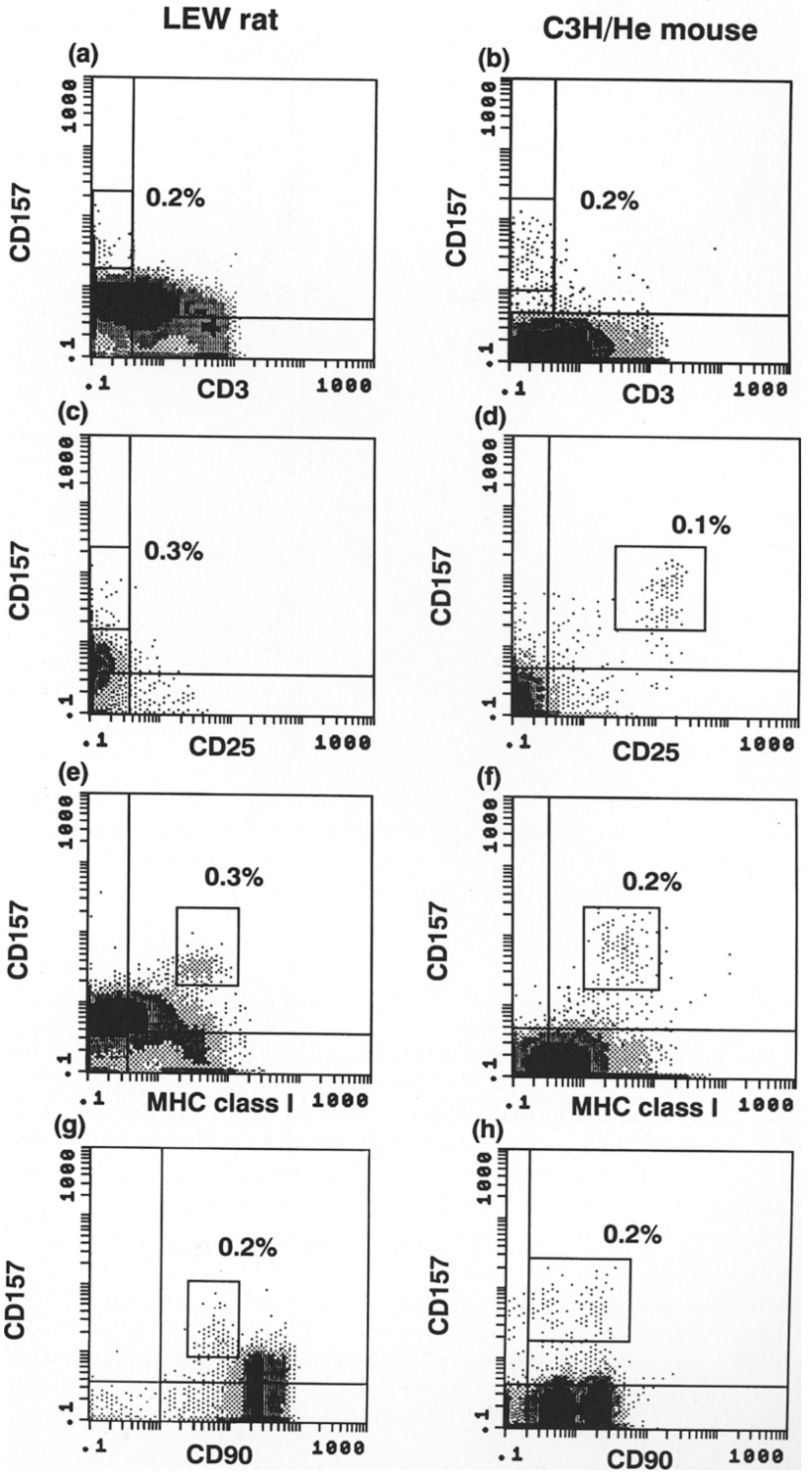


Figure. 2. Three-colour analysis of CD157 on rat and mouse thymocyte subsets. LEW rat (left panel) and C3H/He mouse (right panel) thymocytes were stained with biotinylated B15.128, anti-CD4/FITC and anti-CD8/TC and then with streptavidin R-PE. Subsets of thymocytes were analysed for expression of CD157 (B15.128). Dotted lines represent CD157 staining, solid lines correspond to isotype matched control mAb. The percentage of cells expressing CD157 at high levels within the $CD4^{-}CD8^{-}$ DN population is shown.


LEW rat
C3H/He mouse
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
CD157
CD3
CD25
MHC class I
CD90
0.2%
0.2%
0.3%
0.1%
0.3%
0.2%
0.2%
0.2%
.1
1000

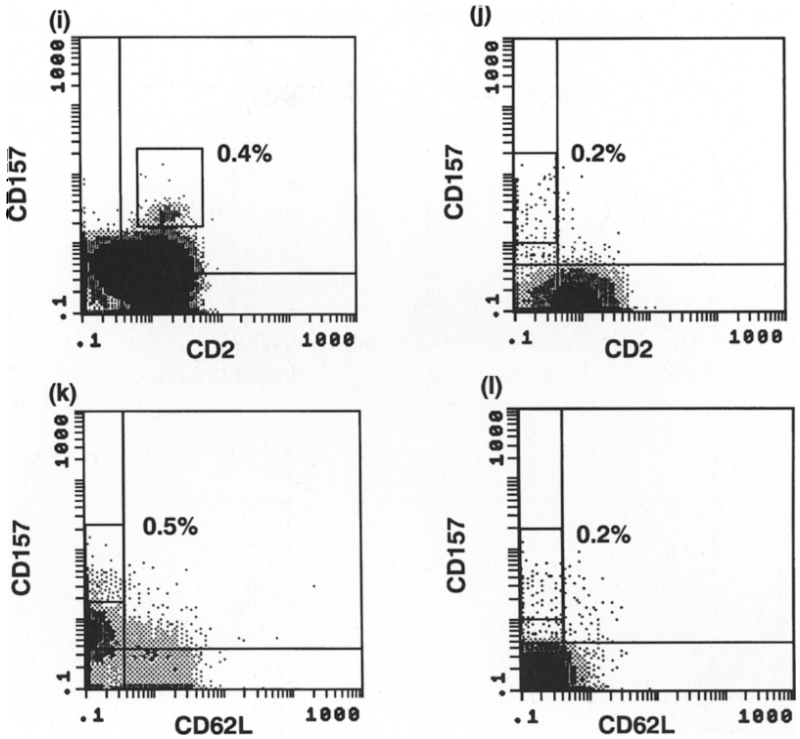


Figure 3. Two-colour analysis of CD157^{high} thymocytes in rat (left panel) compared with mouse (right panel). Thymocytes were stained with biotinylated anti-CD157 (B15.128) in combination with the following FITC conjugated mAbs: (a) and (b), anti-CD3; (c) and (d), anti-CD25; (e) and (f), anti-MHC class I; (g) and (h), anti-CD90; (i) and (j), anti-CD2; (k) and (l), anti-CD62L. Biotinylated antibodies were detected using streptavidin-R-PE. Quadrants were set according to the profile of the negative control (isotype matched mAb). The CD157 high expressing populations are indicated by boxes.

Phenotypic characterisation of rat CD157high cells in the thymus

As shown in Figure 3, two-colour analysis of rat and mouse thymocytes confirmed that cells expressing high levels of CD157 represented a minor population (approximately 0.3% in the LEW rat and approximately 0.2% in the C3H/He mouse) of thymocytes. These cells were confirmed to be immature thymocytes as shown by the expression of CD25 (in mouse) and the absence of cell surface CD3. Although CD25 is not a marker for rat DN thymocytes (20) (Fig. 3c), in both species these CD157^{high} CD3^{-} cells were also CD90^{+}(from intermediate to high levels), MHC class I^{high}, and CD62L^{-}. Similarly, in both species, these CD157^{high} cells did not stain for TCRαβ, TCRγδ, NKRP1c, CD45R and MHC class II cell surface molecules (data not shown). However, it is of note that rat but not mouse, CD157^{high} thymocytes were also CD2^{high}.

The CD45RC antigen (OX22) has been clearly shown to be expressed on a regenerative subset of rat DN thymocytes (21). As the PVG rat expresses high levels of CD45RC^{+} DN thymocytes (21), we decided to use this rat strain to determine whether CD157^{high} cells belonged to this regenerative subset of DN thymocytes. Three-colour analysis with

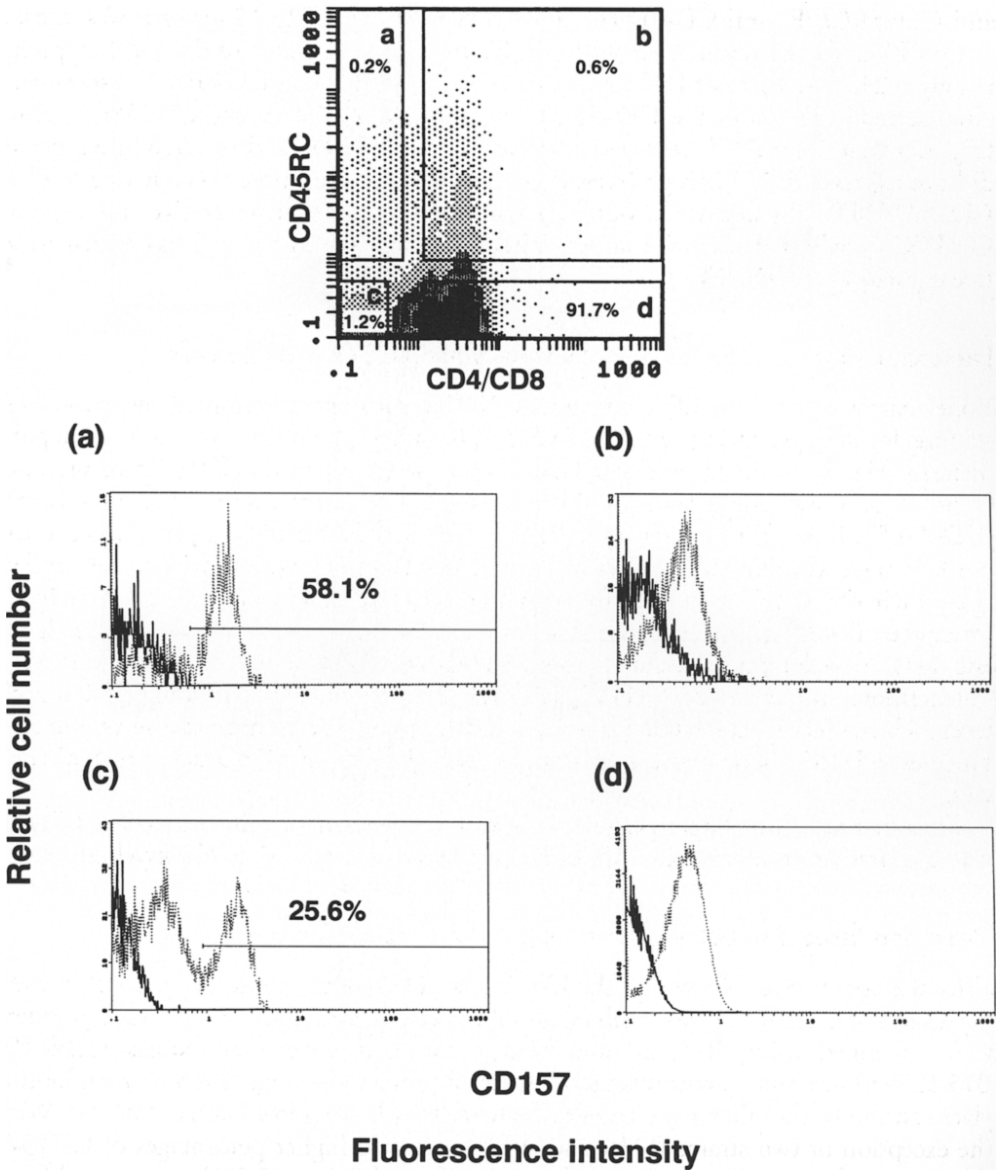


Figure 4. Three-colour analysis of CD157^{high} cells within rat CD45RC^{+} and CD45RC^{-} thymocyte subsets. PVG rat thymocytes were stained with biotinylated anti-CD157(B15.128), anti-CD4/FITC, anti-CD8/FITC, anti-CD45RC/PE and then with SAv-Cy-Chrome. The top figure shows the distribution of CD45RC staining in relationship to CD4 and/or CD8 expression. Each subset is defined by a gating region (a,b,c and d). Expression of CD157 in the regions is presented in histograms below. Dotted lines represent CD157 staining, solid lines correspond to isotype matched control mAb. The percentage of cells expressing CD157 at high levels within the CD45R^{+}CD4^{-}CD8^{-} and CD45R^{-}CD4^{-}CD8^{-} DN populations are shown.

anti-CD45RC/PE, anti-CD4/FITC, anti-CD8/FITC and B15.128/Biotin was carried out on PVG rat thymocytes. As shown in Figure 4 (gated regions of dot plot), approximately 0.2% and 1.2% of DN thymocytes are CD45RC$^+$ and CD45RC$^-$, respectively. Unexpectedly, 58.1% of the CD45RC$^+$ (Fig. 4a) and 25.6% of the CD45RC$^-$ subset (Fig. 4c) were CD157^{high} cells. However, as expected, CD4$^+$ and/or CD8$^+$ thymocytes did not express CD157 at high levels (Fig. 4b, d). Therefore, approximately one third of CD157^{high} DN thymocytes belong to a sub-population with regenerative capacity (i.e. CD45RC$^+$), whilst the remaining two thirds belong to a subset which has no regenerative capacity (i.e. CD45RC$^-$).

Expression of rat CD157 on bone marrow lymphoid and myeloid cells

Bone marrow cells from LEW rats and C3H/He mice were examined by two-colour staining for their expression of CD157 (B15.128) on lymphoid (Fig. 5) and myeloid populations (Fig. 6). In the former population, both species expressed CD157 predominately on B cells with high levels of MHC class I and intermediate levels of CD45R (CD45R^{int}). In rat, the majority of these CD157$^+$CD45R^{int} B cells appeared to be at the pre-B-II stage as indicated by their levels of CD45R expression and lack of cell surface IgM: 9.8% of CD157$^+$ bone marrow lymphoid cells were IgM$^+$, while 90.2% were IgM$^-$ (calculated from Fig. 5c). In contrast, in mouse, distributions of CD157$^+$ cells in IgM$^+$ and IgM$^-$ populations (calculated from Fig. 5d) were 27.8% and 72.2%, respectively. Furthermore, unlike mouse, CD157 was barely detected on cells expressing IgM at high levels. Thus, CD157 expression terminates earlier in rats than in mice and similar to thymocytes, CD157$^+$ bone marrow cells expressed high levels of MHC class I in both species.

Examination of the larger granulated Mac-1$^+$ bone marrow cells shows that CD157 was also present on more than 50% of rat (Fig. 6a) and mouse (Fig. 6b) myeloid cells.

Strain and tissue distribution pattern of CD157 in rat and mouse

Since the above results show that the LEW rat and C3H/He mouse strain differ in their expression of CD157, 11 rat (9 inbred and 2 closed colonies) and 7 inbred mouse strains were examined using B15.128 and isotype matched control antibodies (Table II). B15.128 culture supernatant was used because it produces stronger staining than biotinylated antibody, thus making it easier to detect cells expressing low levels of CD157. With the exception of two strains (SHR and WKY), rats had higher percentages of CD157$^+$ thymocytes (from 88.3% to 92.8%) than mice (from 4.5% to 13.3%), as expected from Figure 2. Furthermore, a subset of thymocytes expressing CD157 at high levels were also observed (from 0.3% to 1.9% in rat and from 0.3% to 0.9% in mouse). This subset corresponds to the minor population detected within the DN population in the three-colour analysis of rat and mouse thymocytes (Fig. 2a, b).

Comparisons between bone marrow lymphoid cells showed that the percentage of CD157$^+$ lymphoid cells is generally lower in rat (from 5.9% to 22.0%) than in mouse (from 13.0% to 42.0%). Exceptionally, these cells were detected at negligible levels in SHR and WKY rats. However, examination of the bone marrow myeloid population revealed that 63.3% to 85.1% of rat cells expressed CD157 compared with 51.1% to 72.0% in the mouse. The SHR, WKY and also the LEC strain differed, having only

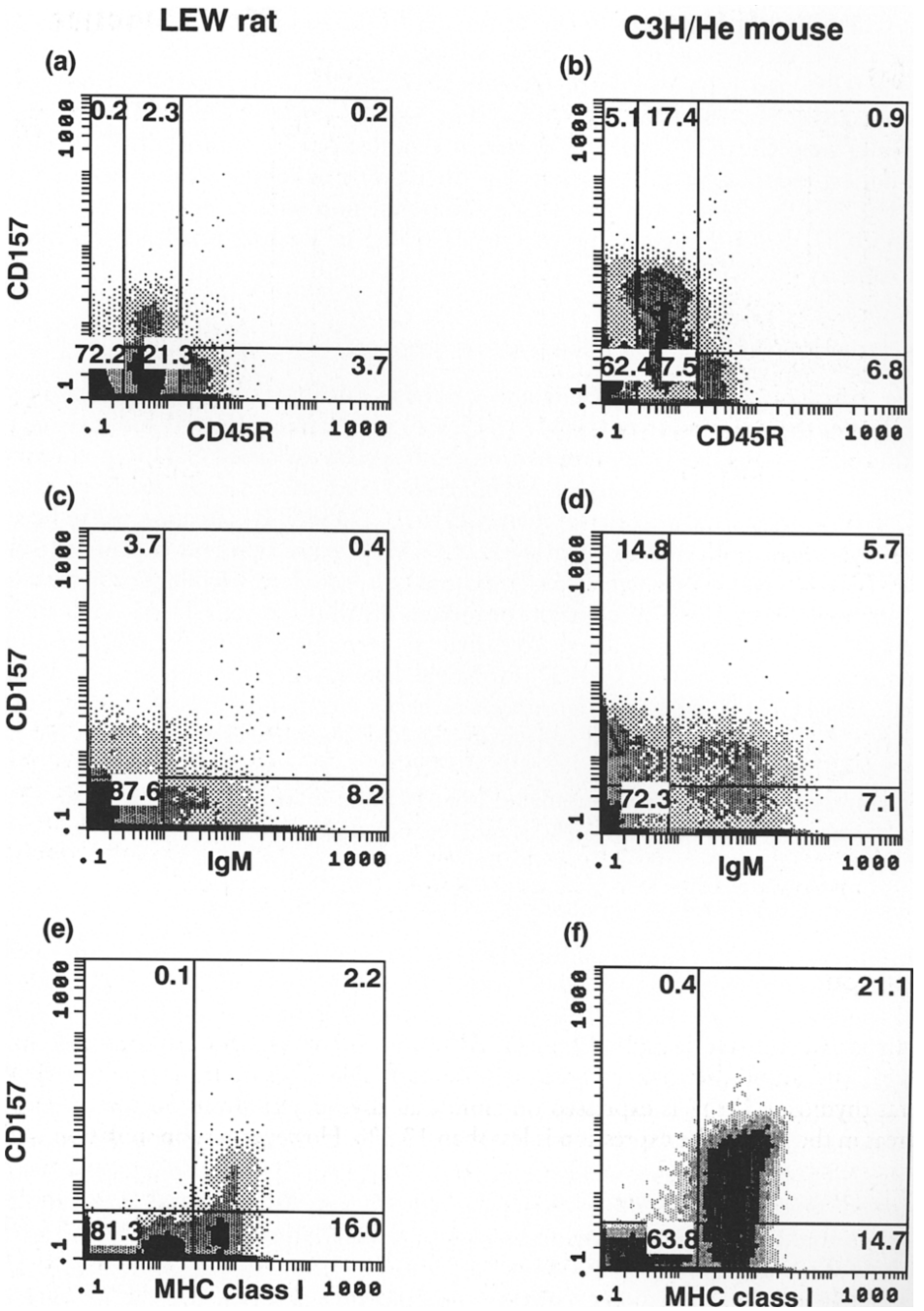


Figure 5. CD157 expression on rat and mouse bone marrow lymphoid cells. Two-colour analysis was carried out on bone marrow cells using biotinylated anti-CD157 (B15.128) with either of the following FITC-coupled mAb: (a) and (b), anti-CD45R; (c) and (d), anti-IgM; (e) and (f), anti-MHC class I. Biotinylated antibodies were detected using streptavidin-R-PE. Quadrants were set according to the profile of the negative control (isotype matched mAb).

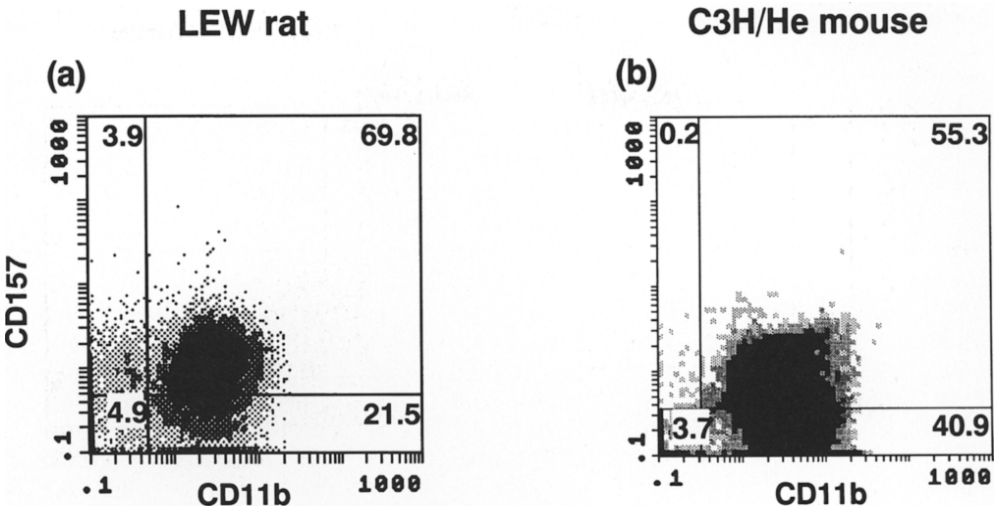


Figure 6. Two-colour analysis of CD157 and CD11b (Mac-1) antigen expression on rat (left) and mouse (right) bone marrow myeloid cells. Bone marrow cells were stained with biotinylated anti-CD157 (B15.128) and FITC-coupled anti-CD11b and then with streptavidin-R-PE. Quadrants were set according to the profile of the isotype matched control antibody.

20.5% to 38.2% of CD157$^+$ cells. Overlapping profiles in Figure 7, show the extremely low levels of CD157 expression on SHR thymocytes (Fig. 7a), bone marrow lymphoid (Fig. 7b) and myeloid populations (Fig. 7c) as compared with the WKAH rat strain.

Thus, with the exception of three rat strains, the above results are consistent with those presented in Figures 3, 5 and 6 which show that CD157 expression within the lymphoid, but not myeloid population in the rat differs from that in the mouse.

Discussion

In this study, we have described a mAb, B15.128, that recognizes both rat and mouse CD157, and found differences in the expression of CD157 in these two species. First, in the rat thymus, CD157 is expressed on almost all thymocytes (from 88.3% to 92.8%), whereas in the mouse the expression is less than 13.3%. However, a subpopulation of thymocytes expressed CD157 at a high level in both species. These cells appeared to be at the late DN stage as had been demonstrated in mice (3, 4), although we have yet to determine whether rat CD157 expression also coincides with rearrangement of the TCRβ-chain genes. Second, in bone marrow, levels of CD157 expression in the lymphoid population were lower in the rat than those in the mouse. Furthermore, the expression terminated earlier in the rat, as (i) there were considerably less rat CD157$^+$ cells (9.8%) than mouse CD157$^+$ cells (27.8%) at the IgM$^+$ immature B cell stage and (ii) rat CD157 was not observed on cells expressing IgM at high levels (Fig. 5c). The above differences may indicate that CD157 does not have an important and direct role in the differentiation of T and B cells (as also indicated by knockout mice (10)).

Table II. Percentage of CD157$^+$ cells in the bone marrow and thymus of various strains of rats and mice

	Bone Marrow		Thymus	
	CD157$^+$ lymphoid cells (%)	CD157$^+$ myeloid cells (%)	CD157$^+$ cells (%)	CD157^{high} cells (%)
Rat strains				
ACI/N	7.2 ± 0.4	70.7 ± 5.9	88.3 ± 1.2	1.2 ± 0.3
BN/SsN	13.2 ± 1.7	63.3 ± 1.3	91.5 ± 1.1	0.5 ± 0.1
DA/Slc	14.2 ± 1.3	85.1 ± 2.6	89.2 ± 4.4	0.5 ± 0.1
F344/N	5.9 ± 1.1	69.4 ± 5.0	92.8 ± 0.9	0.6 ± 0.1
LEC	13.5 ± 1.6	20.5 ± 3.4	91.9 ± 1.9	1.2 ± 0.4
LEW/Crj	10.6 ± 3.3	73.0 ± 0.6	89.9 ± 0.2	0.3 ± 0.0
PVG/Sea	16.0 ± 3.7	77.7 ± 1.6	90.3 ± 0.4	0.8 ± 0.3
SD	7.4 ± 0.3	81.7 ± 1.3	89.2 ± 0.6	0.7 ± 0.3
WKAH/Hkm[1]	22.0, 19.3	81.8, 84.3	89.8, 90.5	2.0, 1.8
SHR/Izm	2.6 ± 0.7	38.2 ± 3.8	9.9 ± 0.3	0.9 ± 0.1
WKY/Izm	3.3 ± 1.9	28.2 ± 0.8	10.2 ± 0.9	0.8 ± 0.2
Mouse strains				
BALB/c	42.0 ± 3.7	51.1 ± 3.5	8.7 ± 5.3	0.3 ± 0.0
B10.BR	22.6 ± 5.8	71.3 ± 3.4	13.3 ± 0.2	0.9 ± 0.1
C57BL/6J	17.0 ± 1.0	67.7 ± 4.0	8.8 ± 2.8	0.6 ± 0.1
C57BR/cdJ	13.0 ± 0.7	62.8 ± 2.2	7.1 ± 1.2	0.6 ± 0.2
CBA/Ca	32.6 ± 3.2	62.3 ± 3.5	9.3 ± 2.2	0.3 ± 0.1
C3H/HeJ	39.3 ± 11.4	72.0 ± 9.1	4.5 ± 0.8	0.4 ± 0.1
SWR/J	27.6 ± 1.9	68.0 ± 2.9	5.5 ± 0.5	0.3 ± 0.0

Cells from thymus and bone marrow were incubated with either B15.128 or isotype matched control culture supernatant. FITC-coupled goat anti-mouse IgG antibody was used as a second reagent. Data are expressed as mean percentage of three animals ± SD (after deduction of isotype matched antibody values).

[1] Percentage values from two rats.

Two exceptional rat strains, SHR and WKY, were found to have not only a lower percentage of CD157$^+$ cells (Table II), but also a lower cell surface expression of the antigen (Fig. 7, and data not shown for WKY). As the *Cd157* genes from the SHR and LEW rats were identical (OA Rosenwasser, unpublished) to the published sequence (18), it is possible that (i) CD157 is glycosylated differently in SHR and WKY rats, and results in a reduction of affinity for the CD157-specific B15.128 mAb, and/or (ii) a mutation in the promoter region of the *Cd157* gene affects the expression in these rats. Work is in progress to determine this issue. SHR is an immunodeficient strain that is also known to develop hypertension (22, 23) and type II diabetes (24), whereas WKY, derived from the same colony as SHR, develops none of these ailments. Therefore, the abnormally low levels of CD157$^+$ cells and CD157 expression appear not to be directly involved in these diseases.

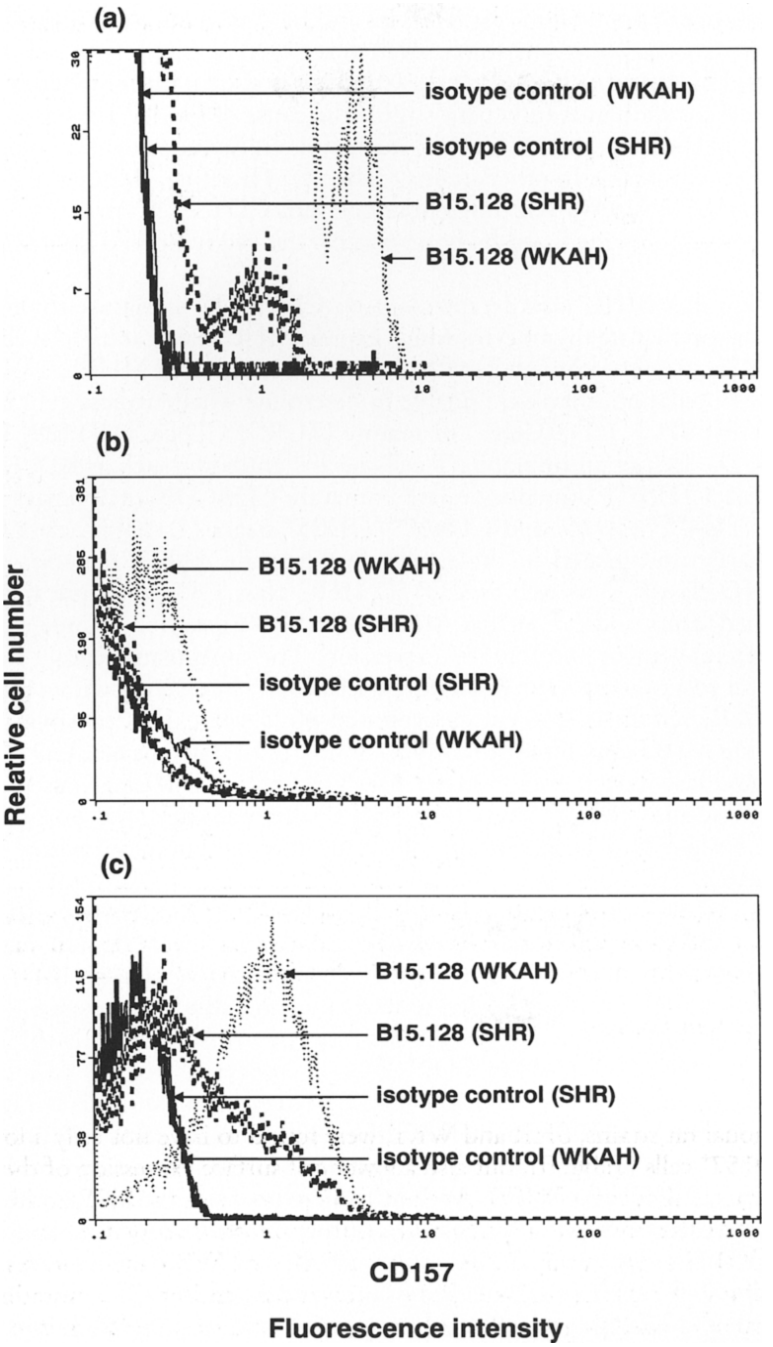


Figure 7. Immunofluorescence analysis of the tissue distribution of CD157 in SHR (thick dotted and thin plain lines) and WKAH (thin dotted and thick plain lines) rats. Thymocytes and bone marrow cells were incubated with B15.128 or isotype matched control (KT33) mAb. Bound antibody was detected by FITC-conjugated goat anti-mouse IgG. (a) represents thymocytes, (b) bone marrow lymphoid and (c) bone marrow myeloid cells.

In contrast, myeloid populations from mouse and rat bone marrow expressed CD157 at similar levels. An exception (i.e. lower level) was observed in LEC as well as in the above mentioned SHR and WKY rats. In LEC (which is also an immunodeficient strain (25) and a model for spontaneous hepatitis (26)) the cause of low level expression is different from that in the latter two, as CD157 expression on thymocytes and bone marrow lymphoid cells is comparable to other strains (Table II). Therefore, this defect is unlikely to be related to the arrest of T cell differentiation from $CD4^+CD8^+$ to $CD4^+CD8^-$ SP thymocytes reported to be mediated by bone marrow derived lymphoid cells in the LEC rat (27).

It is surprising that MHC class I expression on $CD157^{high}$ immature thymocytes is equivalent to that on mature thymocytes which express MHC class I at high levels in both mouse and rat (Fig. 3e, f). Although TSUCHIDA et al. (28) reported MHC class I^{high} cells within the DN population, they were unable to determine whether these cells belonged to either immature ($TCR^-CD4^-CD8^-$) or mature ($TCR^{high}CD4^-CD8^-$) (29) DN thymocytes due to the lack of an immature T cell-specific antibody, such as B15.128. As it is clear that these $CD157^{high}$ thymocytes are immature $CD3^-$ DN thymocytes (Fig. 3a, b), mainly at $CD44^{low/-}CD25^+$ and $CD44^{low/-}CD25^-$ stages (4), MHC class I expression is temporarily upregulated in these cells. Furthermore, C.B-17/lcr-*scid* mice had $CD157^{high}$MHC class I^{high} as well as $CD157^-$MHC class I^{high} immature thymocytes (our unpublished data), indicating that MHC class I^{high} expression is independent of TCRβ-chain rearrangement and CD157 expression. The upregulated class I molecules may have a direct role as a ligand in T cell differentiation by interacting with a receptor(s) (e.g. the pre-TCR, certain NK receptors expressed on T cells). This possibility can be investigated using MHC class I knockout mice. Similarly, in both species, $CD157^+$ bone marrow lymphoid cells which appear to be mainly at the pre-B-II stage, also expressed MHC class I molecules at high levels (Fig. 5e, f). This reinforces the possibility that MHC class I molecules have an important role in the development of immature TCR^- T and BCR^- B cells.

Utilizing a CD45RC-specific antibody, OX22, $CD157^{high}$ DN thymocytes separated into $CD45RC^+$ and $CD45RC^-$ subsets (Fig. 4). As it has been demonstrated that $CD45RC^+$, but not $CD45RC^-$, DN thymocytes have regenerative capacity (21), the conclusion is that $CD45RC^+CD157^{high}$ cells are immature and able to regenerate. It is likely that $CD45RC^-CD157^{high}$ thymocytes are the progeny of $CD45RC^+CD157^{high}$ cells, as it is thought that (i) $CD45RC^+CD2^+$ DN thymocytes are the precursors of $CD45RC^-CD2^+$ thymocytes (21), and (ii) $CD157^{high}$ cells express CD2 at high levels (Fig. 3i). Thus, CD157 and MHC class I, together with CD45RC and CD2 molecules, are useful markers to further delineate the differentiation stages of immature rat DN thymocytes. In mice, CD157 and MHC class I in conjunction with various differentiation markers (e.g. CD25 and CD44) can be used to further define DN thymocyte subsets.

The fact that both *Cd38* and *Cd157* genes are closely linked and their products share similar properties (7–9) and that CD38 expression was delayed in CD157 deficient mice (10) suggest a close association between these two molecules. Therefore, as the stage of expression of CD157 in rat bone marrow lymphoid cells is shorter, it would be worth examining whether the stage of rat CD38 expression is also different from mouse. We are currently trying to establish a rat CD38-specific antibody using mouse cells expressing the rat *Cd38* transgene in order to address this question.

Acknowledgements

The authors thank Dr. MAX D. COOPER (Howard Hughes Medical Institute, University of Alabama at Birmingham, Alabama, USA) for the BP-3 antibody and Drs. NAIKI and NAKAKUKI (Fukui Medical School) for the use of their flow cytometer.

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