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Haematopoietic cell lines capable of colonizing the thymus following *in vivo* transfer expressed T-cell receptor δ-gene immature mRNA

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SUMMARY

To clarify the mechanism by which progenitor T (pro-T) cells recognize and enter the thymus, an attempt was made to produce haematopoietic cell lines by the fusion of BALB/c nude mouse bone marrow or foetal liver cells (gestation 14 and 15 days) with AKR thymoma BW5147, thereby immortalizing cells with potency to colonize the thymus, a characteristic of pro-T cells rarely found in adult bone marrow or foetal liver. The hybridomas thus produced were classified according to the phenotype of surface markers, T-cell receptor (TcR) gene configuration and expression. All hybridomas were negative in the surface expression of T-cell markers such as TcR $\alpha\beta$, TcR $\gamma\delta$, CD3, CD4 and CD8. They had TcR β-, γ- and δ-genes, each with a different status with respect to configuration and transcription. Some possessed partially rearranged TcR genes and others expressed immature TcR mRNA. The cell lines were examined for their capacity to colonize the thymus following intravenous injection into recipient mice. It was found that the cells with capacity of colonizing the thymus expressed immature TcR δ mRNA, while the cell lines lacking TcR δ-genes did not home to the thymus. These findings imply that the potency for migrating to thymus is closely associated with the particular stage of prethymic cell differentiation which could be estimated by the analysis of TcR genes, and that some cell lines with the expression of TcR δ-gene mRNA and the ability to colonize the thymus are derived from pro-T cells.

INTRODUCTION

T cells are derived from multipotent haematopoietic stem cells in bone marrow or foetal liver. Progenitor T (pro-T) cells migrate to the thymus to differentiate into mature T lymphocytes. However, the exact time at which multipotent stem cells become committed to the T-cell lineage, before or following entrance into the thymus, and the means by which they recognize and enter the thymus, are matters yet to be elucidated. The frequency of pro-T cells in mouse bone marrow capable of repopulating the thymus was determined to be only one in 5000 by limiting dilution analysis of colony-forming units in the thymus.[1] Due to the limited number of pro-T cells and absence of specific surface markers for them, it has not been feasible to prepare homogeneous pro-T cells from bone marrow or foetal liver.

In order to characterize pro-T cells, it is preferable to establish immortalized clones possessing the features of pro-T cells. The immortalization of T-cell progenitors has been attempted so far by establishing pro-T-cell clones from mouse bone marrow with the supplement of lymphokines,[2] from foetal thymocytes with Abelson Leukaemia virus infection,[3] and by establishing hybridomas produced by fusion between foetal liver cells, thymocytes or nude mouse bone marrow cells and thymoma, BW5147.[4] In the last case, pro-T-cell hybridomas were selected from others based on the expression of the Thy-1 antigen and the ability to secrete interleukin-2 (IL-2) by mixed lymphocyte reaction, but there was no further characterization beyond this. Using the pro-T-cell clones[5,6] or hybridomas,[7] possible pro-T cell or haematopoietic stem cell-specific surface markers have been proposed.

In the present study, an attempt was made to produce pro-T-cell lines by the cell fusion method from cells of mouse haematopoetic organs since pro-T cells freshly prepared from haematopoietic organs could be immortalized as hybridomas, each representing distinct stage in the differentiation of pro-T cells. The hybridomas thus obtained were analysed for surface antigen expression, T-cell receptor (TcR) gene status and ability to colonize the thymus following their intravenous injection into recipient mice. The cell lines with potential to colonize the thymus expressed immature TcR δ mRNA, while the cells lacking TcR δ-genes did not colonize the thymus. These findings may be an implication that the stage of prethymic T-cell differentiation is correlated with the ability to migrate to the thymus in these cell lines.

Abbreviation: pro-T cell, progenitor T cell.

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MATERIALS AND METHODS

Mice

All except AKR mice were purchased from Sankyo Labo Service Co. (Shizuoka, Japan). AKR mice were obtained from Seiwa Experimental Animals Ltd (Oita, Japan).

Cell lines

AKR thymoma, BW5147[8] was purchased from ATCC (Rockville, MD). Cytotoxic T-cell clone OE-4, established by Dr O. Kanagawa,[9] was obtained from Dr H. Takayama (Mitsubishi Kasei Institute of Life Sciences, Tokyo, Japan). TcR $\gamma\delta$-bearing hybridoma NB21[10] was obtained from Dr Y. Takagaki (Mitsubishi Kasei Institute of Life Sciences, Tokyo, Japan).

Antibodies

Rat monoclonal antibody (mAb) GK1.5 (anti-CD4),[11] mouse mAb 83-12-5 (anti-CD8),[12] hamster mAb 2C11 (anti-CD3),[12] and 3A10 (anti-TcR $\gamma\delta$)[13] were obtained from Dr N. Shinohara (Mitsubishi Kasei Institute of Life Sciences, Tokyo, Japan). Hamster mAb H57–597 (anti-TcR $\alpha\beta$-chain)[14] was a gift from Dr R. T. Kubo (National Jewish Center for Immunology and Respiratory Medicine, Denver, Co). Mouse mAb F7D5 (anti-Thy-1.2) and T11D7e (anti-Thy-1.1) were purchased from Serotec (Oxford, U.K.). Rat mAb MEL-14,[15] KM201 (anti-Pgp-1)[16] and PS/2 (anti-VLA-4)[17] were presented by Dr M. Miyasaka (Tokyo Metropolitan Institute of Medical Sciences, Tokyo, Japan).

Production of hybridomas

Hybridomas were obtained by the method of Aihara *et al.*[4] Briefly, bone marrow cells from 8-week-old BALB/c *nu/nu* mice or foetal liver cells from BALB/c embryos on days 14 or 15 of gestation were fused with 8-azaguanine-resistant AKR thymoma, BW5147[8] in the presence of polyethylene glycol (PEG1500, Boehringer Mannheim, Mannheim, Germany). The hybridomas thus obtained were selected in hypoxanthine-aminopterin-thymidine (HAT) containing medium, cloned twice by limiting dilution method and cultured in RPMI-1640 medium supplemented with 15% foetal calf serum (FCS), 50 U/ml penicillin, 50 μg/ml streptomycin and 5×10^{-5} M 2-mercaptoethanol.

Immunofluorescence staining and flow cytometric analysis

Cells were prepared and stained on ice in Hanks' balanced salt solution (HBSS) supplemented with 0·1% bovine serum albumin (BSA) and 0·025% sodium azide. Propidium iodide (1 μg/ml) was added to the immunostained cells to exclude dead cells from the analysis. Flow cytometric analysis was conducted using a FACScan (Becton Dickinson, Lincoln Park, NJ).

Isolation and analysis of nucleic acids

DNA and RNA were prepared and analysed by Southern and Northern blot analyses, respectively, as described by Maniatis *et al*,[18] Pelkonen *et al.*[19] and Takagaki *et al.*[20] A TcR β-gene complex probe (86T5 cDNA)[21] and TcR γ-gene complex probe (8/10-2γ1.1 cDNA)[22] were kindly provided by Dr H. Yagita (Juntendo University, Tokyo, Japan). Jγ1 and Jδ1 probes prepared from KN6 genomic DNA,[20] a Cδ probe from KN12 genomic DNA[20] and a Cγ4 probe from the genomic clone V10.8[23] were obtained from Dr Y. Takagaki (Mitsubishi Kasei Institute of Life Sciences, Tokyo, Japan).

Thymus colonization assay

The cells of each line (1×10^7) were prepared in phosphate-buffered saline and injected into the tail vein of recipient mice [C3H/He(♀) × BALB/c(♂)F_1]. The recipients were killed 3 weeks after the transfer. Single-cell suspension of the thymus of each recipient was prepared and immunostained by biotin-labelled anti-Thy-1.1 mAb followed by streptavidin fluorescein, isothiocyanate (FITC). Donor-derived Thy-1.1$^+$ cells were determined by flow cytometry.

RESULTS

Production and phenotype of hybridomas

BALB/c (Thy-1.2, H-2^d) *nu/nu* mouse bone marrow or BALB/c +/+ mouse foetal liver cells at 14 and 15 days' gestation were fused with AKR (Thy-1.1, H-2^k) thymoma BW5147 in the presence of polyethylene glycol, and HAT-resistant cell lines were isolated. From bone marrow cells of a nude mouse, 17 hybridomas were obtained and from the liver cells of the embryos, 283 hybridomas were established. The surface expression of T-cell markers on the hybridomas was assessed by immunofluorescence flow cytometry (Table 1). None of the hybridomas expressed T-cell markers such as CD3, CD4, CD8, TcR$\alpha\beta$ or TcR$\gamma\delta$. Each hybridoma expressed Thy-1.2 (Thy-1 allele of BALB/c strain) to a different extent. One-third of the cell lines were positive for Thy-1.2.

Representatives of Thy-1.2$^+$ hybridomas are listed in Table 1 and Fig. 1. FL320 expressed a minute, if any, amount of Thy-1.2. FL339 and FL4B9 showed low expression of Thy-1.2. In

Table 1. The presence of the surface antigens on the hybridomas and BW5147 determined by immunofluoresence flow cytometry

	Thy-1.2	Thy-1.1	CD4	CD8	CD3	TcR ($\alpha\beta$)	TcR ($\gamma\delta$)	MEL-14	Pgp-1	VLA-4
BW5147	–	+	–	–	–	–	–	–	+	±
FL320 (foetal liver g. 14 days)	±	+	–	–	–	–	–	–	+	±
FL339 (foetal liver g. 14 days)	±	±	–	–	–	–	–	+	+	±
BM216 (*nu/nu* bone marrow)	+	+	–	–	–	–	–	–	+	±
BM202 (*nu/nu* bone marrow)	+	+	–	–	–	–	–	–	+	+
FL3H5 (foetal liver g. 15 days)	+	+	–	–	–	–	–	–	+	+
FL4B9 (foetal liver g. 15 days)	±	+	–	–	–	–	–	–	+	±
FL350 (foetal liver g. 14 days)	+	+	–	–	–	–	–	–	+	+

–, negative; ±, dull positive; +, positive; g, gestation.

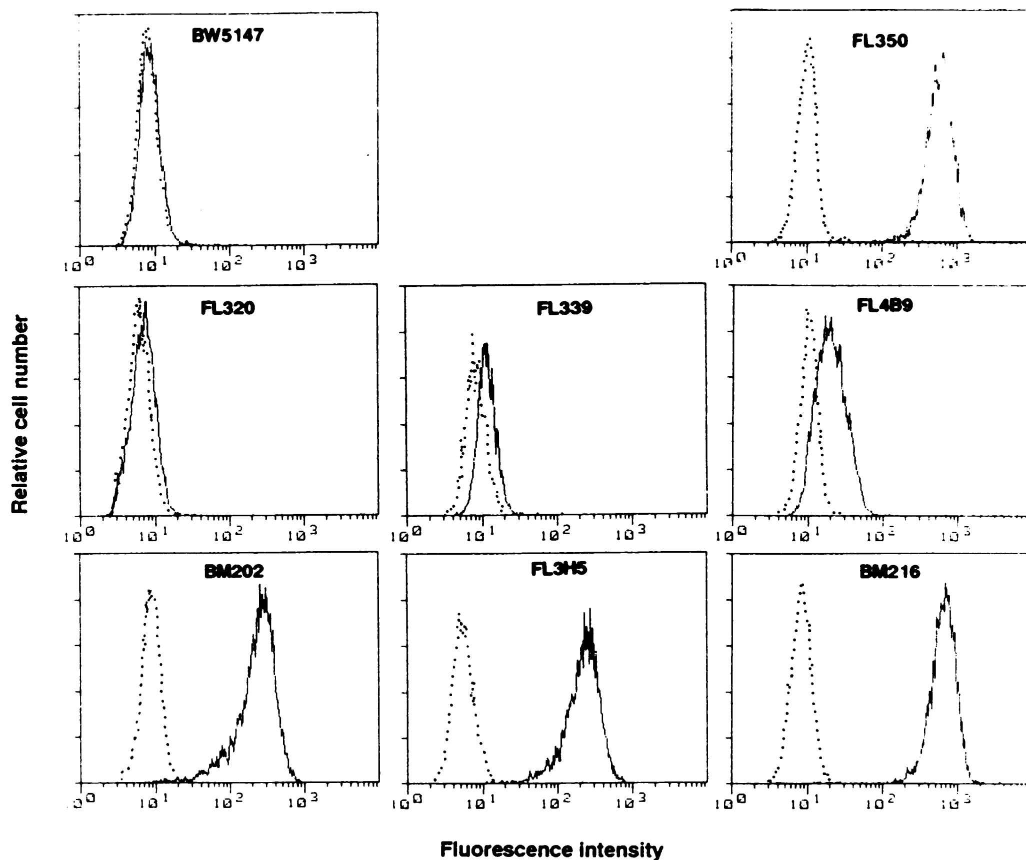

Figure 1. Different expression of Thy-1.2 in each hybridoma determined by immunofluorescence flow cytometry. Dotted lines indicate the control in which cells were treated with the second antibody (FITC-conjugated anti-mouse IgM) alone.

contrast, FL350, BM216, FL3H5 and BM202 showed high expression of Thy-1.2. The surface expression of adhesion molecules shown to be candidates for lymphocyte homing receptors on the hybridomas was then examined. The fusion partner, BW5147, expressed Pgp-1 and a little amount of VLA-4, but no MEL-14. MEL-14 expression in FL339 would thus appear to be due to the features of a BALB/c foetal liver cell. Other hybridomas were negative in the expression of MEL-14. Whether Pgp-1 and VLA-4 expression in the hybridomas was derived from the features of BALB/c cells or BW5147 could not be determined, although the VLA-4 expression in BM202, FL350 and FL3H5 was higher than in BW5147.

Expression and configuration of TcR β-, γ- and δ-genes

To obtain direct information concerning the origin of the hybridomas listed in Table 1, TcR β-, γ- and δ-genes were studied for the expression of mRNA and the genomic configuration by Northern and Southern blot analyses. Full-size mRNA for the TcR β-gene was expressed in all the hybridomas studied (Fig. 2A), which was transcribed from the rearranged TcR β-gene of BW5147[24] judging from the result of Southern blot analysis [6·8 and 5·4 kilobase (kb) in Fig. 3A]. All hybridomas except FL320 also possessed the TcR β-gene(s) of the germ line configuration of BALB/c origin (6·2 and 6·0 kb bands in Fig. 3A). This shows that TcR β-gene rearrangement did not occur in ancestor cells immortalized as these hybridomas. FL320 possessed no BALB/c cell-derived TcR β-genes, and was assumed to have lost chromosome 6 from BALB/c.

Analysis of TcR γ- and δ-genes in the hybridomas provided useful clues regarding their cell lineage and differentiation (Figs 2, 3). The fusion partner, BW5147, possesses rearranged γ-genes (Vγ7–Jγ4 rearrangement on one chromosome, and Vγ4–Jγ1 and Vγ2–Jγ2 rearrangements on the other), but is defective in the production of mRNA.[20] TcR γ-gene expression was observed in BM202, FL3H5, FL350 and FL4B9 (Fig. 2b) but not in FL339, BM216 and FL320. Genomic Southern analysis of the hybridomas indicated them to be of two major types: (1), BM202, FL339 and FL3H5, carrying germ line genes from BALB/c cells in addition to genes from BW5147, and (2), FL320, FL350 and FL4B9 without any germ line Jγ genes from BALB/c. Among the latter, hybridoma FL320 was found to contain an unknown Jγ1 positive gene corresponding to the band at 2·7 kb in Fig. 3B. Hybridoma BM216 possessed a germ line γ-gene(s) of BALB/c origin and a Vγ7–Jγ4 rearranged gene from BW5147, but lost the second chromosome from BW5147 carrying Vγ4–Jγ1 and Vγ2–Jγ2 rearrangements. The genomic configuration of the TcR γ-genes apparently did not correlate with the transcription. For example, hybridomas BM202 and FL3H5 expressed TcR γ mRNA but FL339 did not, although all three had the same

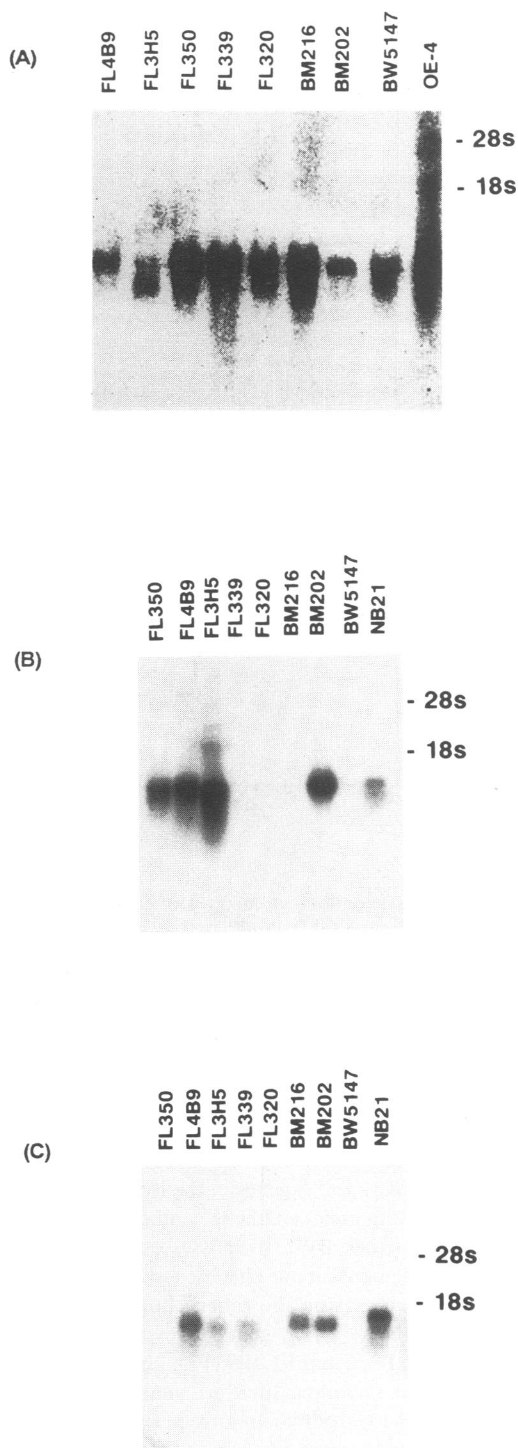

Figure 2. Northern blot analysis of TcR β, γ-, and δ-gene transcripts. Twenty micrograms of total RNA for TcR β-transcripts and 1 μg of mRNA fraction for TcR γ- and δ-transcripts prepared from each hybridoma were analysed. (A) TcR β-gene expression. The probe used in this analysis was 86T5, a cDNA clone of TM86[21] which included Cβ2 segment and cross-hybridized with Cβ1 gene segments. (b) TcR γ-gene expression. 8/10-2γ1.1 cDNA was digested with *Ava*I and the resulting 3′-side fragment (Jcγ10.5, including Cγ2 segment which is cross-reactive with Cγ1 and Cγ3 gene segments) was used as a probe.[22] (c) TcR δ-gene expression. A Cδ probe prepared from TcR $\gamma\delta$-bearing hybridoma KN21[20] was used.

genomic configuration at TcR γ-genes. It could not be determined in this study whether TcR γ mRNA expressed in BM202, FL3H5, FL4B9 and FL350 was transcribed from TcR γ-genes of BW5147 activated by the interaction with BALB/c genes or from those of BALB/c cells rearranged in the same manner as BW5147.

TcR δ-genes present in the hybridomas are derived from BALB/c cells, since TcR δ-gene segments of BW5147 were eliminated from its genome during TcR α-gene rearrangement. FL339, FL3H5, FL4B9 and BM202 possessed the TcR δ-gene(s) of germ line configuration, based on the result of Southern blot analysis shown in Fig. 3D. BM216 possessed a rearranged TcR δ-gene, which gave a 6·6 kb band representing a TcR δ-gene with partial rearrangement between Dδ1 and Dδ2 as reported by Chien *et al.*[25] and this was assured by the polymerase chain reaction (PCR) experiment using 5′ Dδ1 and 3′ Jδ1 probes, in which Dδ1–Dδ2 rearranged 1·2 kb DNA fragment was expanded (data not shown). FL320 and FL350 lost their TcR δ-genes. Molecular size of TcR δ mRNA expressed in BM216, FL3H5, FL4B9, FL339 and BM202 was somewhat smaller (1.3 kb) than that of mature TcR δ mRNA expressed in NB21 (1·7 kb).

Thymus colonization by hybridomas

Hybridomas, classified based on phenotype and TcR gene status, were examined for their ability to colonize the thymus following intravenous transfer. Each cell line was injected into the tail vein of a recipient mouse [F_1 of BALB/c (Thy-1.2) × C3H/He (Thy-1.2) having H-2 haplotypes d × k which is compatible with the injected cells]. The presence of donor-derived cells (Thy-1.1$^+$) in thymus was determined by immunofluorescence flow cytometry 3 weeks later and the results are shown in Fig. 4 and Table 2. BM216, BM202, FL3H5 and FL4B9 were found capable of colonizing the thymus. It is a point of particular significance that all these cell lines expressed TcR δ mRNA and more or less Thy-1.2. In contrast, the fusion partner, BW5147, and cell lines FL320 and FL350, from which TcR δ-genes had been deleted, failed to show any capacity for colonizing the thymus. FL339, which also did not colonize the thymus, expressed TcR δ mRNA to a slight degree, but its TcR γ-genes were silent. The relationship between the thymus colonization capacity to Thy-1.2 expression and TcR gene status in each hybridoma is summarized in Table 3. The surface expression of reported lymphocyte homing receptors (MEL-14, Pgp-1, VLA-4) appeared not to be correlated with the potential to home to the thymus in hybridomas. The specific mechanism for the activation of these molecules or other unknown receptors may thus be involved in the thymus migration of these hybridomas.

DISCUSSION

The production of haematopoietic cell lines with characteristics of pro-T cells was attempted by the hybridoma method. TcR gene status in the produced hybridomas was thus examined to obtain information about the cell lineage of the fused cells since TcR gene activation occurs generally in T-committed cells.[26]

Each hybridoma possessed TcR genes of different status with respect to their expression and rearrangement. The expression of immature mRNA of TcR genes in the germ line

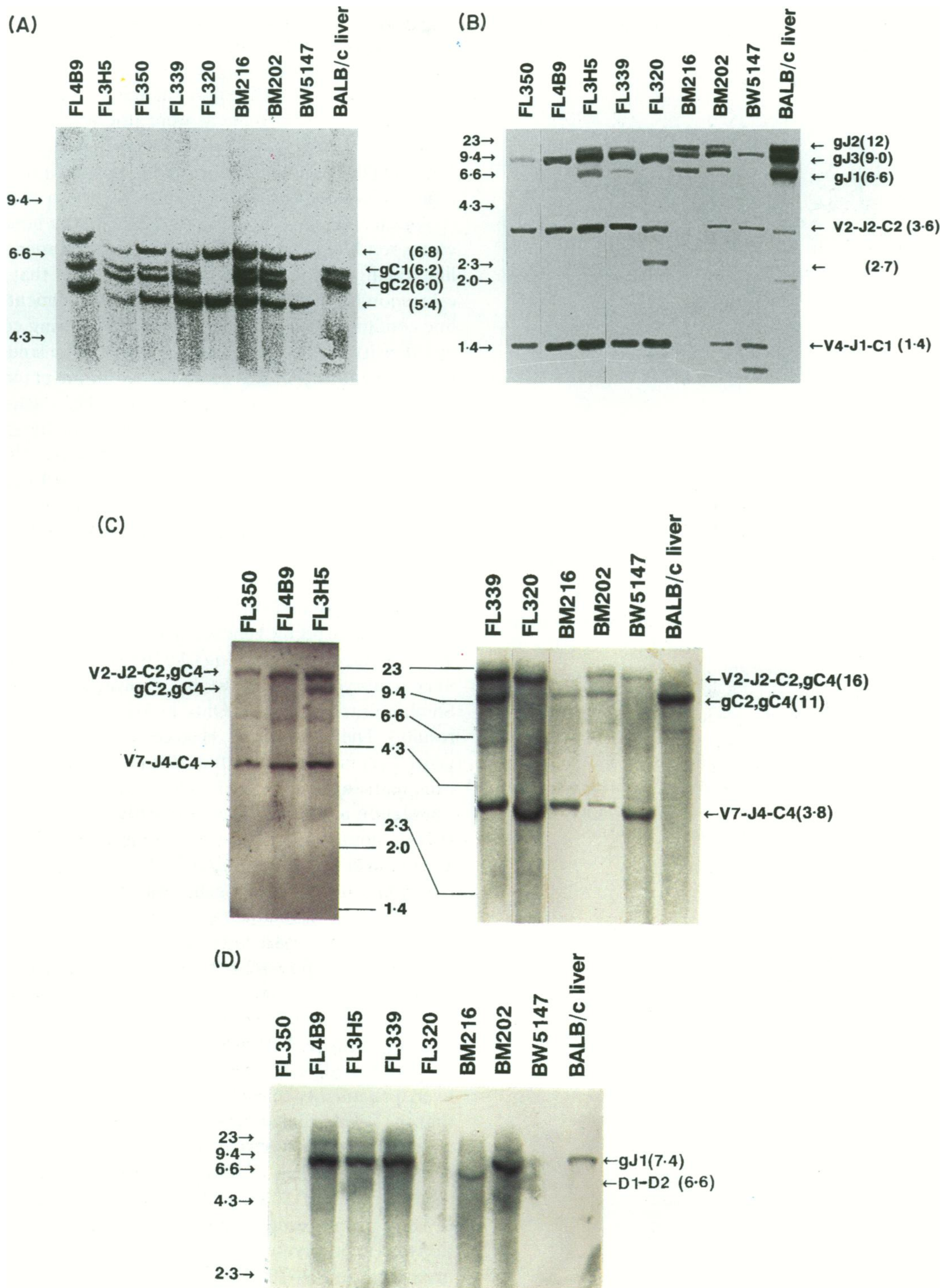

Figure 3. Analysis by Southern blot hybridization of the configuration of the β (A), γ (B, C) and δ (D) genes in the hybridomas. (a) *Pvu* II-digested genomic DNA was analysed by the Cβ2 probe.[21] (B) *Hind*III-digested genomic DNA was hybridized with the Jγ1 probe[20] which also cross-hybridized with Jγ2 and Jγ3. The 3·5 and 2·0 kb bands in the lane of BALB/c liver and the 1·0 kb band in that of BW5147 may be generated by the star activity of *Hind*III. (C) *Eco*RI-digested genomic DNA was analysed by the Cγ4 probe.[23] The 16 and 11 kb bands represent the TcR γ-genes having Cγ4 in the germ line configuration and simultaneously Cγ2 with Vγ2–Jγ2 rearrangement or in the germ line configuration, respectively. (D) *Eco*RI-digested genomic DNA was analysed by the Jδ1 probe.[20] Molecular mass of *Hind*III-digested phage DNA fragment are indicated as markers.

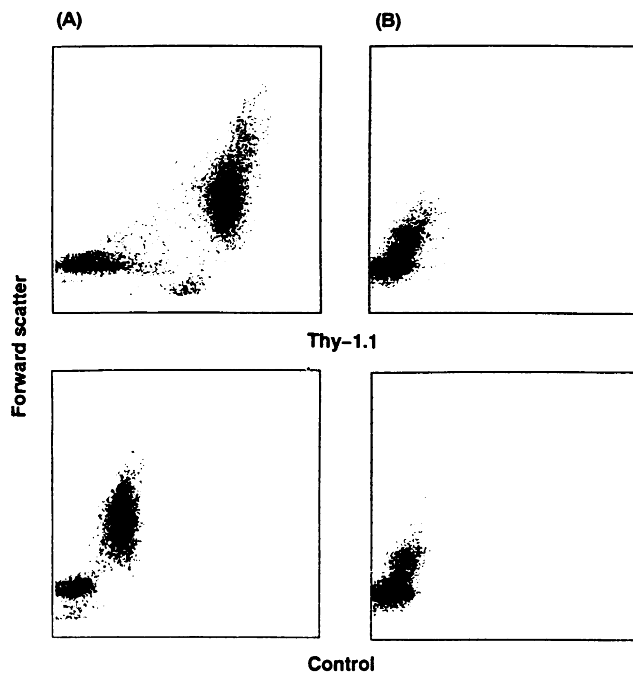

Figure 4. Homing of hybridomas to thymus following *in vivo* transfer. Donor-derived cells in the recipient thymus (BALB/c × C3H/He F_1) were immunostained with biotinylated anti-Thy-1.1 antibody followed by streptavidin-FITC, and analysed by flow cytometry (see Materials and Methods). As control experiments, cells stained only with streptavidin-FITC were analysed. (A) Thymus cell suspension of a FL4B9-injected recipient demonstrated as a thymus homing-positive case. The Thy-1.1$^+$ population with large forward scatter composed of thymus-colonizing donor cells. (B) Thymus cell suspension of a BW5147-injected recipient. No donor cells were detected in this case.

Table 2. Thymus homing of the hybridomas*

Donor	Number of positive animals/total
BW5147	0/6
FL320	0/5
FL339	0/5
BM216	2/8
BM202	4/7
FL3H5	3/13
FL4B9	5/10
FL350	0/5

* Donor-derived cells were determined after 3 weeks following intravenous transfer to the recipient mice. The results are shown as the ratio of the mice positive in the donor cells to the mice tested.

configuration was observed in FL3H5 (TcR β and δ), BM202 (TcR δ), FL4B9 (TcR δ) and FL339 (TcRδ). The immature TcR δ mRNA expression found in these cell lines may be the first cases, although immature TcR γ mRNA expression has recently been reported in an IL-3-dependent T-cell line.[27] The transcription of genes in the germ line configuration was origincally observed in immunoglobulin genes.[28] Such TcR gene activation in these hybridomas might possibly be due to the initial switching to the T-cell lineage or at least to the lymphoid lineage.

TcR gene rearrangements were also noted in some hybridomas. BM216 possessed a TcR δ-gene with partial rearrangement (Dδ1–Dδ2). FL4B9 was suggested to possess a rearranged TcR β-gene based on the findings from Southern blot analysis as shown in Fig. 3A. The band at 6·2 kb in the lane for FL4B9, corresponding to the Cβ1-including gene segment in the germ line configuration appeared weaker than that at 6·0 kb, corresponding to the Cβ2-including gene segment in the germ line configuration (ratio of about 1:2 following compensation by intensity of the corresponding bands in the lane for BALB/c liver). One of the TcR β-genes carried in the parental BALB/c chromosomes would thus seem to have been rearranged. This was actually confirmed by Southern blot analysis using a Jβ2 probe for which an extra band was observed in addition to that of the non-rearranged gene (data not shown). FL320 contained a TcR γ-gene of unknown rearrangment (Fig. 3B). A TcR γ-gene with the same rearrangement has been reported by Pelknonen *et al.*[19] FL320 lost BALB/c-derived TcR β- and δ-genes and only the γ-gene could be shown to have derived from a BALB/c cell. However, genomic DNA of this cell line hybridized with the Y chromosome-specific gene segment AC11,[29] but that of BW5147 did not, thus clearly showing FL320 to be derived from a cell of a male embryo and not to be a revertant of BW5147 (M. Shimamura, M. Oku, S. Ohta and T. Yamagata, unpublished results). The presence of these cell lines with rearranged TcR gene(s) strongly suggests that *T*-lineage commitment and TcR gene rearrangement have already begun, at least in the limited population of T-cell progenitors within haematopoietic organs such as adult bone marrow and foetal liver. This finding is in accord with the recent report[30] that expression of mRNA of rearranged TcR γ- and δ-genes was detected in the later foetal livers by PCR technique.

Hybridomas were intravenously transferred to recipient mice to confirm possible potency for colonizing the thymus. The results in Table 3 indicate that only some of the cell lines possessed potential to colonize the thymus but fusion partner BW5147, in spite of its tumour origin, did not at all, while all the cell lines tested possessed ability to enter and grow in the lung or liver, presumably due to the metastasis. These findings imply that the thymus colonizing capacity observed for the particular hybridomas is derived from the intrinsic feature of the parental haematopoietic cells of BALB/c mice. The correlation between the expression of TcR δ mRNA and the ability to colonize the thymus appears quite significant ($P = 0{\cdot}071$), while the correlations between thymus colonization capacity and other phenomena are low ($P = 0{\cdot}21$–$0{\cdot}51$). Thus, it is tempting to speculate that activation of transcription factors for the expression of the immature mRNA is an initial signalling for T commitment and the expression is a further reflection of phenotype of normal pro-T cells present in the haematopoietic organs. TcR δ gene segments are present between Vα and Jα gene segments; activation of TcR δ-genes may, therefore, be critical for the T-cell developmental regulation.

The somewhat low percentage of the mice subjected to thymic colonization following the hybridoma transfer (BM216, 25%; BM202, 57%; FL3H5, 23%; FL4B9, 50%) may be explained by the genetic incompatibility between the host mice

Table 3. Relationship between Thy-1.2 expression, TcR gene status and homing capacity to thymus in the hybridomas

Cell line	Thy-1.2	TcR mRNA expression			TcR β-genes			TcR γ-genes				TcR δ-genes		Homing capacity to thymus
		β	γ	δ	g*	BW†	Additional	V7-J4	V4J1/V2J2/ gJ3/gJ4	g	Additional	g	−D1−D2−	
BW5147	−	+	−	−	−	+		+	+	−		−	−	−
FL320	±	+	−	−	−	+		+	+	−	V2-J2/?**	−	−	−
FL339	±	+	−	+§	+	+		+	+	+		+	−	−
BM216	+	+	−	+§	+	+		+	−	+		−	+	+
BM202	+	+	+	+§	+	+		+	+	+		+	−	+
FL3H5	+	+‡	+	+§	+	+		+	+	+		+	−	+
FL4B9	±	+	+	+§	+	+	(V)-D-J¶	+	+	−		+	−	+
FL350	+	+	+	−	+	+		+	+	−		−	−	−
P††		0·21	0·23	0·071	0·21				0·51	0·23		0·23	0·5	

−, negative; ± dull positive; +, positive.

* Germ line configuration.

† BW5147 type, Vβ1-Dβ2.1-Jβ2.5-Cβ2.

‡ The immature short mRNA was expressed in addition to the full-size mRNA.

§ The immature short mRNA was expressed.

¶ Partial rearrangement between (V)-D1 or D2-J2 gene segments (M. Shimamura, M. Oku, S. Ohta and T. Yamagata, manuscript in preparation).

** Unknown rearrangment with V2-J2 rearrangement, corresponding to the 2·7 kb band in the Southern blot analysis shown in Fig. 3B.

†† *P*-values of χ^2-test for correlation between homing capacity to thymus and each TcR gene status or Thy-1.2 expression were calculated according to the Fischer's modified method.

(BALB/c × C3H/He F_1) and donor cells (BALB/c × AKR hybrid) although their major histocompatibility complex (MHC) haplotypes were identical (d × k); another reason may be the large size of the injected hybridomas which causes them to be lodged in the capillary venules of lung or liver. However, these hybridomas were suggested to possess affinity to thymus (presumably to thymus vascular endothelium)[15,31] since BM216 and FL4B9 were detected from the thymus more than four times as much but from the spleen or lung to the same degree as BW5147 2 h after the intravenous injection (M. Shimamura, M. Oku, S. Ohta and T. Yamagata, unpublished results). In addition, FL4B9 was found to rearrange its TcR δ-gene following thymus colonization (M. Shimamura, M. Oku, S. Ohta and T. Yamagata, manuscript in preparation). These observations are additional support for the possibility that thymus colonization noted in some cell lines is in fact based on thymus-directed homing which is derived from the features of pro-T cells immortalized as the hybridomas. The pro-T-cell clones, C4, established by Palacios *et al.*[2] were reported to be unable to repopulate the thymus following intravenous transfer,[32] thus, the haematopoietic cell lines produced in this study may only be available to study the pro-T cell migration to thymus. Further examination of these cell lines will thus lead to greater clarification of the homing of pro-T cells to the thymus.

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