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The role of duplication of tumour-derived chromosome 15 carrying the rearranged *pvt*-1 gene in the transformed phenotype of YACUT T-cell lymphoma × G4 T-cell line somatic cell hybrids in dictating the terminal differentiation program of the parental G4 cell

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SUMMARY

Fusion of the YACUT T-cell lymphoma with the Mls-1^a-antigen-specific non-tumorigenic T-cell line G4 was previously reported to produce growth-arrested hybrids that could be induced to proliferate in the presence of Mls-1^a antigen. The proliferation-suppressed hybrid lines exhibited phenotypic changes as follows: the usually high levels in YACUT of J11d antigen, IL-2 receptor, and c-*myb* expression, which are markers of immature T cells, were all down-regulated; the G4 T-cell function, i.e., contact helper activity for B-cell proliferation in T/B cell collaboration, was retained. Furthermore, fusion of the YACUT lymphoma with a killer T-cell line produced growth-arrested and tetraploid somatic cell hybrids having killer activity. Thus, in addition to the transformed phenotype (autonomous proliferation in vitro), the antigen-specific non-tumorigenic T-cell line genomes introduced into the YACUT lymphoma suppressed the immature phenotypes of YACUT and imposed their own programming of terminally differentiated traits on the hybrids.

Prolonged growth of the proliferation-suppressed hybrid lines by repeated antigenic stimulation was previously reported to result in the appearance of transformed hybrids, which was accompanied by both a reversion of c-*myc* expression to the levels of YACUT and an increase in the number of chromosome 15. The present study revealed that the amplification of chromosome 15 resulted from the duplication of the tumour-derived chromosome 15 carrying the rearranged *pvt*-1 gene. However, the differentiated phenotypes of the hybrids remained mostly unchanged upon cell transformation. These results indicate that the duplication of the tumour-derived chromosome 15 plays an important role in the appearance of the transformed phenotype without abrogating the imposition of the terminally differentiated traits on the YACUT × G4 hybrids.

Key words: somatic cell genetics, growth arrest, c-*myc*, gene-dosage effect, differentiation

INTRODUCTION

In previous studies we have shown that the malignant phenotype of some lymphomas such as EL4 and YACUT is suppressed at the level of cell transformation by cell fusion with the antigen-specific non-tumorigenic T-cell line G4 (Kubota and Katoh, 1990; Kubota et al., 1992). In the case of the growth-arrested YACUT × G4 hybrids, the de-regulated c-*myc* proto-oncogene expression characteristic of the YACUT lymphoma, probably due to retroviral insertions in the vicinity of the *pvt*-1 gene (Graham et al., 1985), was suppressed in the hybrids, suggesting a close link between the growth-arrested phenotype and the low levels of c-*myc* expression. The YACUT × G4 hybrids differed from other hybridization combinations such as EL4 × G4 hybrids in that prolonged passages of the proliferation-suppressed YACUT × G4 hybrids, but not the EL4 × G4 hybrids, by repeated antigenic stimulation resulted in the appearance of transformed hybrids. The transformed phenotype of the hybrids was accompanied by a reversion of c-*myc* expression to the levels of YACUT and a concomitant increase in the number of chromosome 15.

One unanswered question in the previous study was: which parental chromosome 15 was duplicated upon cell transformation? To resolve this question we have examined in the present study the ratio of germ line to rearranged *pvt*-1-carrying chromosome 15 by Southern hybridization and revealed that the amplification of chromosome 15 resulted from the duplication of the tumour-derived chromosome 15 carrying rearranged *pvt*-1.

The previous studies were aimed primarily at genetic analysis of malignancy by using somatic cell hybrids. Somatic hybrid studies, on the other hand, have also been used to analyze cellular differentiation in genetic terms; it is known that differentiated phenotypes are, as a rule, extinguished in the hybrids between different cell lineages, a phenomenon referred to as extinction (Killary and Fournier, 1984). However, hybridization of the same cell lineages but differing in their stages of

differentiation usually produces hybrids with a variety of phenotypes depending on experimental systems (Deschatrette and Weiss, 1975; Cassio and Weiss, 1979; Riley et al., 1981; Pearson et al., 1983; Lawrence and Coleman, 1983; Carbone et al., 1988).

The establishment of the proliferation-suppressed YACUT × G4 hybrid cell line that was generated by fusion between a lymphoma having immature phenotypes of a T cell and a terminally differentiated T-cell line provides an opportunity for studying T-cell differentiation in genetic terms. In the present study we also deal with this issue by examining overall phenotypic changes in the growth-arrested YACUT × G4 hybrids and report that the differentiated phenotypes of G4 parental cells, including the property of antigen-dependency for cell proliferation, dominate in the hybrids.

The 'dominant' expression of the terminally differentiated phenotypes in the YACUT × G4 hybrids raised the question of whether the appearance of the transformed phenotype that was causally associated with the duplication of the tumour-derived chromosome 15 would simultaneously extinguish the differentiated phenotypes of the hybrids. The results presented in this report indicate that the proliferation-suppressed YACUT × G4 hybrid lines undergo cell transformation without abrogating the imposition of the terminally differentiated traits on the hybrids.

MATERIALS AND METHODS

Cell lines

YACUT is a hypoxanthine-guanine phosphoribosyltransferase-negative and ouabain-resistant sub-line selected from the Molony MuLV-induced YAC lymphoma cell line (Klein and Klein, 1964). Details of the establishment, culture conditions and antigenic profiles of the Mls-1^a antigen-responsive helper T-cell line G4 (Kubota and Katoh, 1990) and the K^d antigen- and IL-2-dependant killer T-cell line 1G8-H7 (Kubota, 1986) were previously reported.

Cell fusion and hybrid selection

These methods were described previously (Kubota et al., 1992).

Chromosome preparation

Metaphase spreads were prepared from cultures by standard technique and stained with Giemsa.

Analysis of surface antigens

Monoclonal antibodies (mAbs) specific to J11d antigen (J11d.2), IL-2 receptor (7D4) and CD4 (GK1.5) were obtained from the ATCC. mAbs to mouse T-cell receptor (TCR) (H57) and CD3 (2C11) were prepared by Dr R. Kubo and Dr J. A. Bluestone, respectively. Anti-H-$2D^k$, anti-H-$2D^d$, anti-Lyt-2.1, and anti-Lyt-2.2 mAbs were purchased from Meiji Institute of Health Science (Tokyo, Japan). Anti-TCRVβ8 mAb (F23.1) was obtained from Tokai University via Dr H. Tamauchi. Ascites of anti-Thy-1.2 (HO-13) was prepared in our laboratory. Cells were first incubated with mAbs then stained with fluorescein-isothiocyanate (FITC)-labeled secondary antibodies and analyzed using a fluorescein-activated cell sorter (Becton-Dickinson FACS system 420, Mountain View, CA).

RNA preparation and northern hybridization

Total RNA was isolated by the guanidine isothiocyanate method and total cytoplasmic RNA was prepared as reported previously (Pearse and Wu, 1988). Northern hybridization was performed by the method described previously (Kubota et al., 1992). v-*myb* probe was purchased from Takara Shuzo (Kyoto, Japan) and was ^{32}P-labeled by the random primer method.

DNA preparation and Southern hybridization

Total DNA was prepared according to the method of Pearse and Wu (1988). Briefly, 5×10^5 cells in siliconized microcentrifuge tubes were pelleted by centrifugation and then resuspended in 150 μl of ice-cold membrane lysis buffer (150 mM NaCl, 10 mM Tris-HCl, pH 8.0, 2 mM $MgCl_2$, 0.5% NP-40, 10 mM vanadyl ribonucleoside complexes). After vortexing for 10 seconds the lysate was centrifuged for 7 minutes at 2,000 ***g*** to pellet the intact nuclei, which were then resuspended in 10 μl of nuclei lysis buffer (0.5 mM EDTA, pH 9.5, 1% sodium lauroylsarcosine). To the lysate, 10 μl of Sea Plaque low-gelling-temperature agarose (2% agarose in 0.5 mM EDTA, pH 9.5, 1% sodium lauroylsarcosine containing 0.5 mg/ml of proteinase K) was added and incubated at 50°C for 2 hours. After centrifugation the tubes were placed on ice to solidify the agarose and then filled with TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) and incubated at room temperature with gentle rocking. The buffer was changed after 1, 4 and 18 hours, and then the tubes were rinsed with cold TE buffer and spun briefly to enable the last traces of TE to be removed. The tubes were heated to 65°C until the agarose was completely melted then transferred to 37°C and the following added: 4 μl of restriction enzyme buffer, 4 μl bovine serum albumin (2 mg/ml), 2.5 μl RNase A (10 μg/ml) and H_2O to 40 μl. This mixture was incubated at 37°C for 15 minutes. The total DNA in each tube was completely digested with restriction endonuclease *Kpn*I (Takara, Kyoto, Japan) and run on 0.8% agarose gels and transferred to Hybribond-N^+ membrane (Amersham, Aylesbury, UK). The 0.6 kb *Eco*RI fragment (fragment e) (Graham et al., 1985) of the *pvt*-1 genomic clone was ^{32}P-labeled by the random primer method and used as a probe.

Preparation of resting B cells

Small resting B cells were prepared as previously described (Defranco et al., 1982). Briefly, DBA/2 or C3H/He mouse spleen cells were dissociated into a single cell suspension and erythrocytes were lysed by treatment with Tris-buffered ammonium chloride. Then, spleen cells were depleted of T cells using anti-Thy-1.2 (HO-13) and anti-CD4 (GK1.5) and rabbit C (Cedarlane, Ontario, Canada). The cells were washed once with RPMI 1640 medium containing 10% FCS and then layered on top of a discontinuous gradient composed of 70, 60 and 50% Percoll. The gradient was centrifuged at 2,300 ***g*** for 12 minutes at 4°C and then the cells at the interface of the 60 and 70% Percoll were collected.

In vitro contact helper assay

Small resting B cells (4×10^4) and an equal number of YACUT cells, growth-arrested G4 cells, growth-arrested hybrid cells or transformed hybrid cells, which had all been pretreated with mitomycin C (50 μg/ml) for 45 minutes at 37°C, were added to flat-bottomed microculture plates in the presence or absence of 100 U/ml human rIL-2 (Shinogi, Osaka, Japan) and the plates were cultured for 3 days in a CO_2 incubator. B cell proliferation was assessed by measuring uptake of [^{3}H]thymidine (New England Nuclear, Boston, MA) into DNA during the last 8 hours of culture.

Cytotoxic assay

This method was described previously (Kubota, 1986).

RESULTS

Transformed YACUT × G4 hybrids had a high ratio of tumour-derived chromosome 15 carrying rearranged *pvt*-1 to normal chromosome 15

The results of karyotype analysis of the growth-arrested and

Table 1. Chromosome numbers of parental and hybrid cells

Cells	Modal chromosome number and range immediately after 3rd panning	Clones*	
		Designation	Mean chromosome number
Parental cells			
YACUT	41 (42-36)		
G4	39 (40-38)		
1G8-H7	40 (40-37)		
Fused cells			
1 YACUT × G4	80 (83-74)	2G6	79.6
2 YACUT × G4	80 (85-76)	2H7	80.8
3 YACUT × G4	nt	1A11	78.7
		1G5	79.2
		1F11	78.2
		2D7	79.2
4 YACUT × 1G8-H7	80 (83-73)	E11	81.5
		F12	78.0

*After the 3rd panning, fused cells were cloned by limiting dilution in the presence of irradiated (2,000 rad) DBA/2 spleen cells and 50% ConA-stimulated spleen supernatant fluid. nt, not tested.

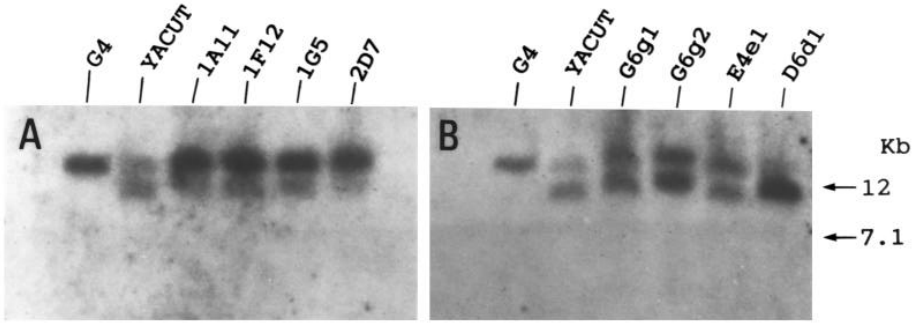

Fig. 1. Southern hybridization of the *pvt*-1 locus in *Kpn*I-cleaved genomic DNA. (A) Total DNA was isolated from G4 cells 3 days after antigenic stimulation, from YACUT cells and from 4 growth-arrested YACUT × G4 hybrid cell lines 3 days after antigenic stimulation. DNA was digested with *Kpn*I and run on a 0.8% agarose gel. (B) Total DNA was isolated from G4 cells 3 days after antigenic stimulation, from YACUT cells and from 4 transformed YACUT × G4 hybrid cell lines, digested with *Kpn*I and run on a 0.8% agarose gel. *Kpn*I-digested fragments: 19 kb, germ line *pvt*-1; 12.5 kb, rearranged *pvt*-1.

the transformed YACUT × G4 hybrid cell lines used in the present study are summarized in Table 1. They were all near tetraploid cells. The chromosome constitution of the growth-arrested hybrid line 2G6 was also reported previously (Kubota et al., 1992).

The *pvt*-1 gene of YACUT is rearranged due to insertions of Mo-MuLV in this locus, and the rearranged *pvt*-1 can be detected as a 12.5 kb *Kpn*I fragment by Southern hybridization with *pvt*-1 probe e (Graham et al., 1985). To compare the ratio of rearranged chromosome 15 to normal chromosome 15, total DNA from equal numbers of exponentially growing YACUT, G4, and hybrid cells was prepared, digested with restriction endonuclease *Kpn*I, and Southern hybridization was performed. Fig. 1 shows autoradiographs of Southern blots probed with *pvt*-1 probe e. The T-cell line G4 had a 19 kb germ line fragment and, as previously reported (Graham et al., 1985), the YACUT cells had a 12.5 kb rearranged fragment in addition to a 19 kb germ line fragment. In the 4 growth-arrested hybrids, as expected by the fact that the hybrids had both YACUT and G4 cell-derived chromosome 15, the intensity of the rearranged fragment was lower than that of the germ line fragment (Fig. 1A). By contrast, in 3 transformed hybrid cell lines (G6g1, G6g2 and E4e1), which had been shown previously to have increased copy numbers of chromosome 15 (Kubota et al., 1992), the intensity of the rearranged fragment increased to levels almost identical to the intensity of the germ line fragment (Fig. 1B). In the transformed hybrid line D6d1, which had been shown previously to have two copies of a new translocation T(12;15) (A3;D2), the intensity of the rearranged fragment was much stronger than that of the germ line fragment (Fig. 1B). The latter result strongly suggests that the new translocation occurred between chromosome 12 and the tumour-derived chromosome 15 carrying rearranged *pvt*-1.

Together with the previous chromosome analysis that showed a non-random increase in the number of chromosome 15 in the transformed YACUT × G4 hybrids (Kubota et al., 1992), we conclude from the above results that the amplification of chromosome 15 resulted from the duplication of the tumour-derived chromosome 15 carrying the rearranged *pvt*-1 gene.

Surface phenotypic changes in growth-arrested and transformed hybrids

The YACUT cells were characterized by immunofluorescein for surface staining of antibodies specific for a variety of T-cell markers. The YACUT cells were J11d$^+$ (Fig. 2A), IL-2 receptor (IL-2R)$^+$ (Fig. 3A), thy-1$^+$, TRCVβ8$^+$, CD3$^+$, but they were CD4$^-$, and CD8$^-$ (data not shown for the latter 5 antigens). Thus, the YACUT lymphoma had the phenotype of an immature T cell.

We next addressed the question of whether the surface membrane phenotypes of the YACUT cell were altered after

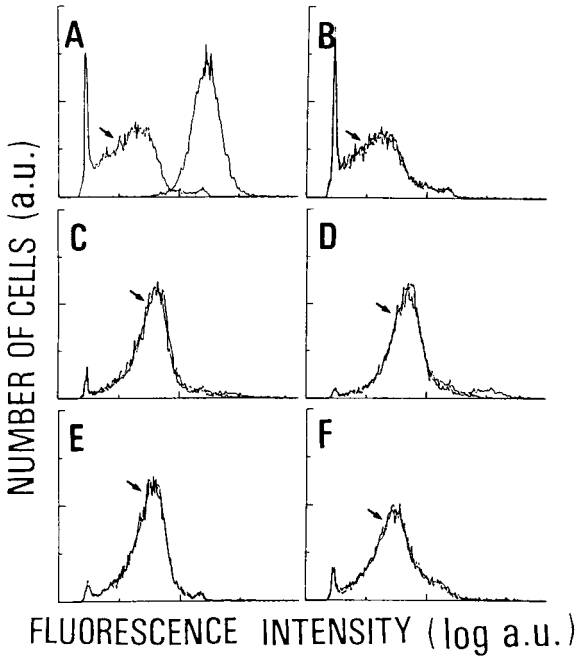

Fig. 2. J11d antigen expression on YACUT (A), G4(B), 2 growth-arrested YACUT × G4 cell lines 2G6(C) and 2H7(D), and 2 transformed hybrid cell lines G6g4(E) and D6d1(F). Cells were incubated with anti-J11d mAb and subsequently stained with FITC-labeled goat anti-rat IgM antibody. The lines indicated by arrows show control staining with FITC-labeled antibody only; a.u., arbitrary units.

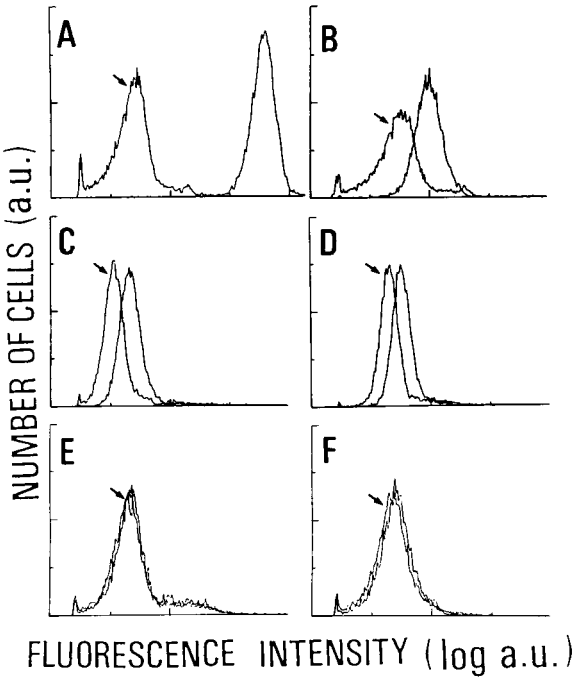

Fig. 3. IL-2R expression on YACUT(A), G4(B), 2 growth-arrested hybrid cell lines 2G6(C) and 2H7(D), and 2 transformed hybrid cell lines E4e1(E) and D6d1(F). The G4, 2G6 and 2H7 cells were collected 10 days after antigenic stimulation. Other cells were all exponentially growing cells. Cells were incubated with mAb 7D4 and subsequently stained with FITC-labeled goat anti-rat IgM antibody. The lines indicated by arrows show control staining with FITC-labeled antibody only; a.u., arbitrary units.

cell fusion with the terminally differentiated helper T-cell line G4. The FACS profiles of J11d antigen expression on the parental cells, and 2 growth-arrested and 2 transformed hybrid lines are shown in Fig. 2, and all the data are summarized in Table 2. The YACUT lymphoma expressed J11d antigen (Fig. 2A), but the terminally differentiated G4 parental cell did not (Fig. 2B). All the growth-arrested and transformed hybrids examined did not express J11d antigen (Fig. 2C-F, Table 2), indicating that J11d antigen expression was suppressed in the hybrids. J11d antigen expression remained suppressed in 5 transformed hybrid cell lines after more than 6 months of culture (data not shown).

Fig. 3 shows IL-2R expression by the parental cells and hybrid cell lines. The YACUT lymphoma expressed high levels of the IL-2R. The expression of the IL-2R on YACUT was of interest, because immature thymocytes were reported to express the IL-2R transiently, and this was down-regulated in mature T cells (Shimonkevitz et al., 1987; Pearse et al., 1989). The T-cell line G4 also constitutively expressed the IL-2R, but its expression levels were substantially lower than those of the YACUT lymphoma. All the growth-arrested hybrid lines examined expressed the IL-2R at levels similar to those of the G4 cells (Fig. 3C and D; Table 2). The transformed hybrid lines also exhibited down-regulation of the IL-2R expression (Table 2) and, moreover, the 2 transformed hybrid lines E4e1 and D6d1 did not express the IL-2R (Fig. 3E and F). The down-regulation of the IL-2R expression in exponentially growing hybrids means that the detection of low levels of the IL-2R expression on the growth-arrested hybrids, though it was examined at the G_0 phase of the cell cycle, was not due simply to the difference in the state of proliferation. These

Table 2. Summary of phenotypes of parental and hybrid cell lines

	Phenotypes		
	Surface membrane molecule		c-*myb*
Cell lines	J11d	IL-2 receptor	mRNA
Parental cells			
YACUT	+	high	high
G4	–	low	low
Growth-arrested hybrids			
2G6	–	low	low
2H7	–	low	low
1A11	–	low	low
1G5	–	low	low
1F12	–	low	low
2D7	–	low	low
Transformed hybrids			
G6g1	–	low	low
G6g2	–	low	low
G6g3	–	low	low
G6g4	–	low	low
E4e1	–	–	low
D6d1	–	–	low

+, detected; –, not detected; high, expressed in high amounts; low, expressed in low amounts. See Fig. 3 to compare high and low expressions of the IL-2R.

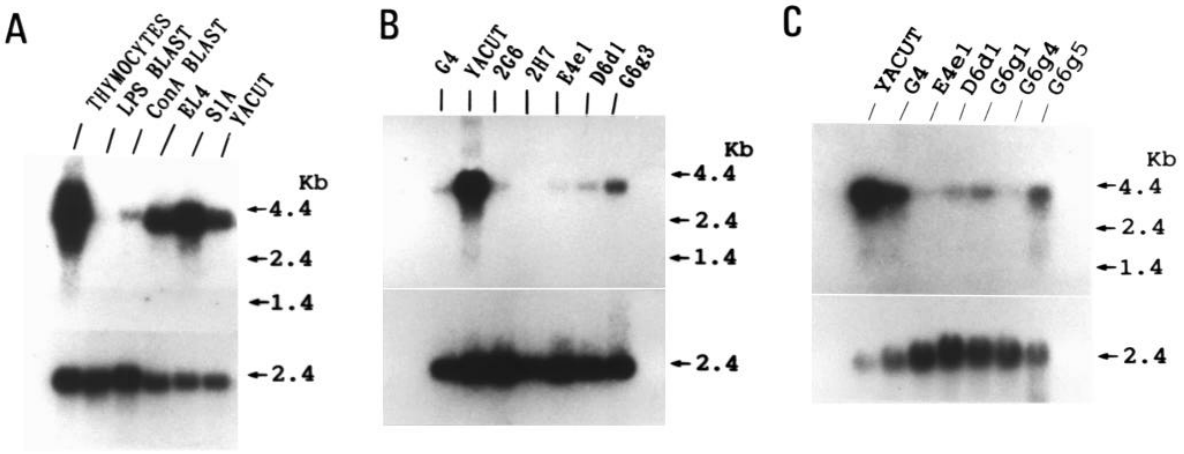

Fig. 4. c-*myb* mRNA levels of various cells. (A) Total RNA was isolated by the guanidine isothiocyanate method from DBA thymocytes, from DBA spleen cells 2 days after LPS or ConA stimulation, and from 3 T-lymphomas EL4, S1A and YACUT. Total poly(A)$^+$ RNA was prepared by using Oligotex dT30. Samples (2 μg) of poly(A)$^+$ RNA were run on 1% agarose gels. (B) Total RNA and total poly(A)$^+$ RNA were isolated as above (A) from G4 cells 3 days after antigenic stimulation, from YACUT cells, from 2 growth-arrested hybrid cell lines 2G6 and 2H7 3 days after antigenic stimulation and from 3 transformed hybrid cell lines. Samples (2 μg) of poly(A)$^+$ RNA were run on a 1% agarose gel. (C) Total cytoplasmic RNA was isolated by the method of Pearse and Wu (1988) from 1×10^6 cells of YACUT and of G4 3 days after antigenic stimulation and from 5 transformed hybrid cell lines that had been maintained in culture more that 6 months. Total RNA samples were run on a 1% agarose gel. Re-hybridization of the same blot with an actin probe after removing the previous v-*myb* probe is shown at the bottom of each panel.

results indicate that the constitutive expression of the IL-2R in YACUT was down-regulated in both the growth-arrested and the transformed hybrids.

High levels of c-*myb* expression in YACUT were down-regulated in growth-arrested and transformed hybrids

The c-*myb* expression in 3 mouse lymphomas, concanavalin A (Con A)-stimulated T-cell blasts, lipopolysaccharide (LPS)-stimulated B-cell blasts, and thymocytes are shown in Fig. 4A. As previously reported (Sheiness and Gardinier, 1984), thymocytes expressed c-*myb* mRNA in high amounts and there was a significant difference between the steady-state levels of c-*myb* mRNA in the exponentially growing 3 lymphomas and normal lymphocytes (Fig. 4A). As shown in Fig. 4B and Table 2, the c-*myb* mRNA level in the YACUT lymphoma contrasted with the low levels found in both the G4 cells and in the growth-arrested hybrid lines. These results indicate that the high c-*myb* expression in the YACUT cells was down-regulated in the hybrids. The 6 transformed hybrid lines displayed c-*myb* mRNA levels similar to that of the G4 cells (Fig. 4B, Table 2), indicating that the c-*myb* expression remained suppressed upon cell transformation. Fig. 4C shows the c-*myb* expression of the 5 transformed hybrid lines that had been cultured for more than 6 months. None of the 5 transformed hybrid lines expressed c-*myb* mRNA at the levels of YACUT, indicating that the down-regulation of c-*myb* expression in the hybrids was very stable as well as the suppression of the J11d antigen expression

The ability of constant T-cell helper activity of parental G4 cells was retained in both growth-arrested and transformed hybrids

In the T/B cell collaboration, B cells present antigen associated with class II MHC to helper T cells, which subsequently trigger the activation and differentiation of specific B cells (see review by Parker, 1993). This process requires close physical contact between T and B cells to enable CD40 on B cells to associate with CD40 ligand induced on activated helper T cells (Grabstein et al., 1993).

In order to evaluate this T-cell contact-dependent helper activity, the parental cells and hybrid cell lines were co-cultured with small resting B cells from DBA/2 mice (Mls-1^a) in the presence or absence of exogenous IL-2, and [^{3}H]thymidine uptake into proliferating B cells was measured (Fig. 5). While the YACUT lymphoma did not have the capacity to stimulate B cells, the G4 cell had this helper function. The growth-arrested hybrid lines 2G6 and 2H7 also exhibited the contact help capacity, indicating that this highly differentiated phenotype expressed by the G4 cells was retained in the YACUT × G4 hybrids. Small resting B cells from C3H/He mice (Mls-1^b) did not receive a contact help signal either from the G4 cells or the hybrid cells (data not shown), which was compatible with the observation that both of the cells were not activated by the C3H/He spleen cells (Kubota et al., 1992). The 3 transformed hybrid lines (G6g1, G6g3, E4e1) also had contact helper activity, but only in the presence of rIL-2 in culture medium, indicating that the transformed hybrids could induce the surface structure that was necessary for contact help, but they were somewhat impaired with respect to the production of IL-2.

Fusion of YACUT lymphoma with a killer T-cell line produced growth-arrested hybrids having cytotoxic activity

To exclude the possibility that the YACUT lymphoma itself might be a cell that is committed to differentiate to a helper T cell so that the YACUT × G4 hybrids might be able to express

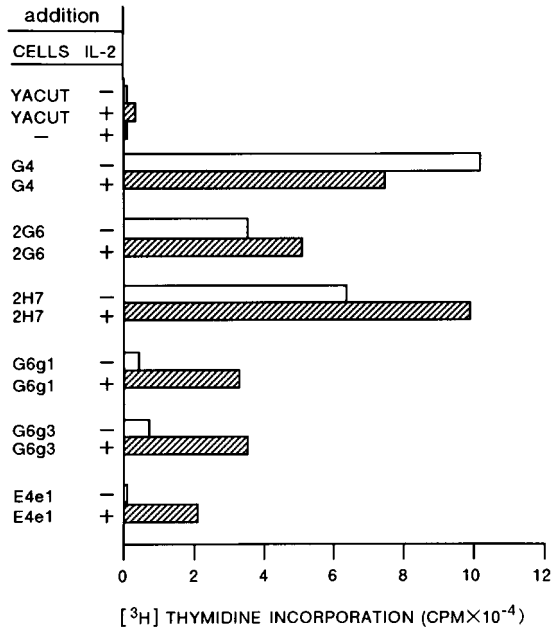

Fig. 5. Contact help-dependent B-cell proliferation. Small resting B cells (4×10^4) were cultured in 96-well microculture plates with an equal number of YACUT cells, G4 cells, 2 growth-arrested YACUT × G4 hybrid cells lines (2G6 and 2H7), and 3 transformed YACUT × G4 hybrid cell lines (G6g1, G6g3, E4e1), which had all been pretreated with mitomycin C (50 μg/ml). After 3 days of culture, 1 μCi of [^{3}H]thymidine was added to each well during the last 8 hours.

the contact helper function, we next addressed the question of whether the YACUT T-lymphoma would produce growth-arrested hybrids when fused with a killer T-cell line and, if so, whether the hybrids allowed the expression of killer function for which different gene expressions were necessary. Fusion of the YACUT lymphoma with killer T cell line 1G8-H7 did yield growth-arrested cells. The FACS profile of H-2 antigen expression (Fig. 6A) and the result of karyotype analysis (Table 1) of 2 YACUT × 1G8-H7 hybrid lines show that the growth-arrested cells were hybrids. The 1G8-H7 killer T cells were Lyt-2.1^+ and J11d^-, while the YACUT cells were Lyt-2.1^- and J11d^+ (Fig. 6B). The Lyt-2.1 antigen expression by the parental 1G8-H7 cells was not suppressed in the hybrids (Fig. 6Bg,h) in contrast to the result of different T-lymphoma × Lyt-2^+ lymphocyte hybrids reported by other investigators (Carbone et al., 1988). The expression of J11d antigen in YACUT was again suppressed in these hybrids (Fig. 6Bc,d). Fig. 7 shows the cytotoxic activity of the parental cells and the 2 hybrid cell lines against P815Y cells. While the YACUT lymphoma did not have killer activity, the 1G8-H7 cells and the 2 hybrid cell lines E11 and F12 exhibited cytotoxic activity against P815Y cells. Thus, the killer function of the 1G8-H7 cells was retained in the YACUT × 1G8-H7 hybrids.

DISCUSSION

T lymphocytes differentiate and mature in the thymus. The markers that are associated with immature phenotypes of the intrathymic T-cell development have been defined (Nikolic-Zugic, 1991); these include J11d antigen (Bruce et al., 1981; Crispe and Bevan, 1987; Kay et al., 1990), IL-2R (Shimonkevitz et al., 1987; Pearse et al., 1989) and Myb (Shen-Ong, 1990). Down-regulation of these gene expressions is associated with maturation of T cells. The results of the present study demonstrated that the YACUT lymphoma had these three markers and that in addition to the suppression of the transformed phenotype, the immature phenotypes of YACUT were suppressed in all of the growth-arrested hybrids examined

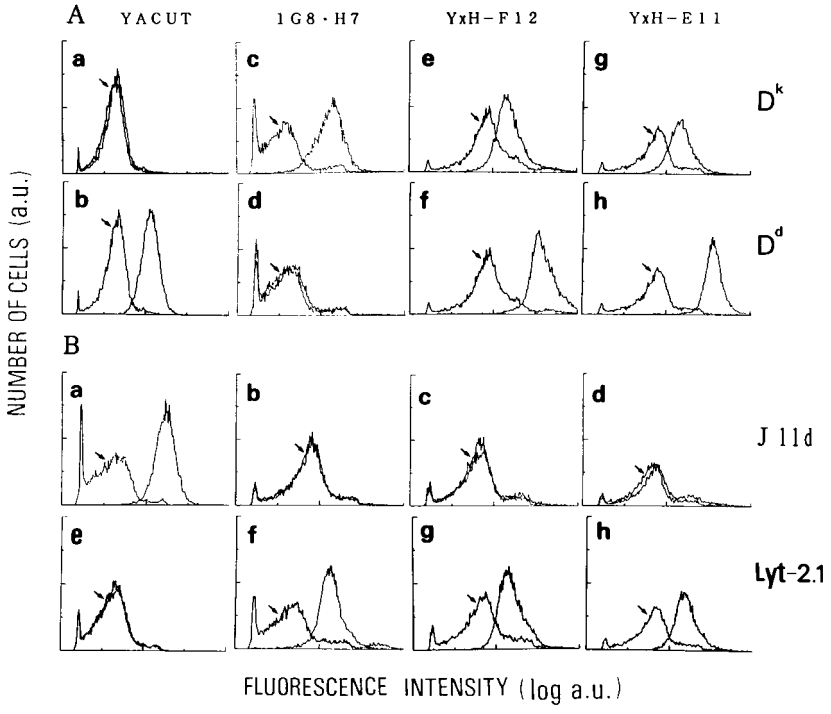

Fig. 6. FACS profiles of H-2(A), J11d antigen (Ba-d) and Lyt-2.1 (Be-h) expressions on YACUT (Aa,b; Ba,e) and killer T-cell line 1G8-H7 (Ac,d; Bb,f), and 2 growth-arrested hybrid cell lines E11 (Ag,h; Bd,h) and F12 (Ae,f; Bc,g). Cells were incubated with mAbs and subsequently stained with FITC-labeled secondary antibody. The lines indicated by arrows show control staining with FITC-labeled antibody only; a.u., arbitrary units.

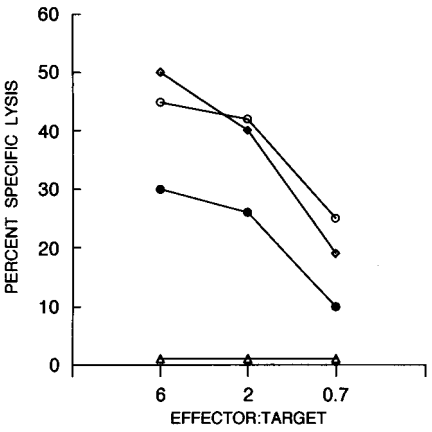

Fig. 7. Cytolytic activity of hybrid cells. Various numbers of 1G8-H7 cells (○-○), YACUT cells (△-△), or 2 YACUT × 1G8-H7 hybrid cell lines, E11 (□-□) and F12 (●-●), were incubated with 1×10^4 cells of ^{51}Cr-labeled P815Y for 4 hours. The percentage specific release was determined by the following formula: (release in test-spontaneous release)/(maximum release-spontaneous release)×100. Spontaneous release was below 20% of maximum release.

(Table 2). The results suggested that the mature phenotype of the parental G4 cells dominated in the hybrids. This notion was further substantiated by the observation that the highly differentiated T-cell functions of parental T-cell lines, i.e. contact helper activity for B-cell proliferation (Fig. 5), and cytotoxic activity (Fig. 7), were retained in the growth-arrested hybrids. Thus, the mature T-cell line genomes introduced into YACUT down-regulate the immature phenotypes of YACUT and impose their own programming of the terminally differentiated traits on the hybrids.

Fusions between cells of different cell lineages usually result in extinction of differentiated phenotypes, and diffusible *trans*-acting factors are suggested to play a key role in regulating tissue-specific gene activity, since the extinction is reversible following loss of chromosomes (Killary and Fournier, 1984). Our fusion reported here showed an opposite result: immature phenotypes, not differentiated phenotypes, were suppressed in the hybrids. This result is probably attributable to our particular hybridization combination, i.e. fusion of the same T-cell lineages, but differing in their stages of differentiation. Since the immature phenotypes are down-regulated in mature T cells, the suppression of the immature phenotypes in the hybrids may reflect as yet undefined mechanisms of the epigenetic change that occurs during normal T-cell development. The present results suggest negative control of the immature phenotypes by means of *trans*-acting regulatory factors formed in mature T cells. Their suppressive effects on the immature phenotypes, unlike *trans*-acting factors in the tissue-specific gene regulation, may not be reversible, since so far no segregants expressing the immature phenotypes have appeared in the transformed YACUT × G4 hybrid cell population. However, it is also possible that the suppression of the immature phenotypes in the transformed YACUT × G4 hybrids in culture remained unchanged over a long extended period of time, because the maintenance of the differentiated phenotypes might have been advantageous for the growth of the hybrids in culture.

In previous studies (Kubota and Katoh, 1990; Kubota et al., 1992), we have shown that the transformed phenotype of mouse lymphomas, such as EL4, YACUT and S1A, are suppressed by the non-malignant G4 cell genome. The mechanism of suppression of the transformed phenotype of lymphomas is unknown. The results of the YACUT × G4 hybrids reported here, however, indicate that there may be an inter-relationship between the suppression of the transformed phenotype and the imposition of the terminal differentiation program on the hybrids. The cell growth arrest of the YACUT × G4 hybrids was previously reported to be correlated with the suppression of the de-regulated c-*myc* expression of YACUT. Furthermore, the facts that the growth-arrested YACUT × G4 hybrids were capable of proliferating in response to antigenic stimulation and that the kinetics of c-*myc* expression in the hybrids upon antigenic stimulation was similar to that in the non-malignant T-cell line G4 (Kubota et al., 1992) suggested that the G4 cell genome imposed its own antigen-dependent proliferation program on the hybrids. The antigen-dependent cell proliferation is also one of the properties that are developmentally regulated in the T-cell differentiation pathway (Rothenberg, 1992), and the autonomous proliferation of YACUT is thought to be a trait acquired probably at an immature stage of T-cell development. Therefore, we conclude that the suppression of the autonomous proliferation of YACUT may be associated with the overall genetic features of the YACUT × G4 hybrids to suppress immature phenotypes and dictate the terminal differentiation program of the parental G4 cell.

The notion mentioned above is reminiscent of the reports of other investigators (Stanbridge et al., 1982; Peehl and Stanbridge, 1982; Harris, 1990), which demonstrated that the non-tumorigenic hybrids between malignant cells and normal diploid parental cells were executing in vivo the differentiation program of a normal diploid cell, whereas the malignant segregants lost this capability. An important difference between those experiments and ours lies in the fact that our fusion deals with the suppression of the transformed phenotype (autonomous proliferation of lymphomas) in vitro. Whereas in the other studies, it is the suppression of the tumorigenic phenotype in vivo that is examined. Nevertheless, the concept of association of cell multiplication arrest with the execution of terminal differentiation programs in hybrids may explain both cases; in the experiments hitherto done in vivo, the non-tumorigenic hybrids, mostly intertypic hybrids manifesting the transformed phenotype in culture, probably require some signals from the microenvironment in vivo for executing the differentiation program of the normal diploid parental cell, while our growth-arrested hybrids, which are intratypic hybrids of the same T-cell lineages but differing in their stages of differentiation, probably dictate the terminal differentiation program by reflecting processes of the epigenetic change that occurs during T-cell development.

It is not well understood how repeated antigenic stimulation abrogates the suppressive mechanism in the YACUT × G4 hybrids to generate the phenotype of autonomous growth, or why the growth-arrested phenotype of other hybrid combinations such as EL4 × G4 hybrids (Kubota and Katoh, 1990) remains unchanged. We have shown previously that the transformation of the YACUT × G4 hybrids was accompanied by a reversion of c-*myc* expression to the levels of YACUT and a concomitant increase in the number of chromosome 15

(Kubota et al., 1992). This result suggested a causal relationship between the reversion of c-*myc* expression and the amplification of chromosome 15. The data presented here (Fig. 1) have reinforced this conclusion; the amplified chromosome 15 was the tumour-derived chromosome 15 carrying the rearranged *pvt*-1 gene due to the retroviral insertion, which was assumed to bring about, in *cis*, the up-regulation of c-*myc* expression of YACUT (Lazo et al., 1990). The duplication of this changed chromosome 15, probably due to a non-disjunction event during antigen-dependent proliferation, may abolish in a gene-dosage-dependent manner the putative negative regulatory effect of the normal genome on the de-regulated c-*myc* expression, leading eventually to the autonomous growth of the hybrids.

The results presented here also show that although the appearance of the transformed phenotype of the YACUT × G4 hybrids was causally associated with the duplication of the tumour-derived chromosome 15, the transformed phenotype was not accompanied by the extinction of the differentiation phenotypes of the hybrids. Thus, the duplication of the tumour-derived chromosome 15 plays an important role in the appearance of the transformed phenotype of the YACUT × G4 hybrids without abrogating the imposition of the terminally differentiated traits on the hybrids.

The importance of the duplication of the changed chromosome 15 in the appearance of the transformed YACUT × G4 hybrids is basically consistent with the previous conclusion that the duplication of tumour-derived chromosome 15 endowed T-lymphoma × lymphocyte or T-lymphoma × fibroblast hybrids with a malignant phenotype (Spira et al., 1981; Uno et al., 1989). However, it remains to be determined whether the duplication of the changed chromosome 15 is sufficient or whether other chromosomal changes are required for the tumorigenic phenotype of the YACUT × G4 hybrids.

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