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Expression of members of immunoglobulin gene family in somatic cell hybrids between human B and T cells

(suppression/differentiation antigens/dominant traits)

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ABSTRACT **Somatic cell hybrids were obtained between human T and B cells and tested for the expression of differentiated traits of both cell lineages. The T-cell parent SUP-T1 is CD3$^-$, CD4$^+$, CD1$^+$, CD8$^+$, is weakly positive for HLA class I determinants, and has an inversion of chromosome 14 due to a site-specific recombination event between an immunoglobulin heavy-chain variable gene and the joining segment of the T-cell receptor α chain. The B-cell parent, the 6-thioguanine- and ouabain-resistant mutant GM1500, is a lymphoblastoid cell line that secretes IgG2, κ chains, and expresses B1, B532, and HLA class I and II antigens. All hybrids expressed characteristics of B cells (Ig$^+$, B1$^+$, B532$^+$, EBNA$^+$, HLA antigens), whereas only CD4 among the T-cell markers was expressed. The level of T-cell receptor β-chain transcript was greatly reduced and no RNA of the chimeric T-cell receptor α-chain joining segment–immunoglobulin heavy-chain variable region was detected. Southern blot analysis indicated that absence of T-cell differentiation markers in the hybrids was not due to chromosomal loss. Rather, some B-cell-specific factor present in the hybrids may account for the suppression.**

Glycoproteins, including immunoglobulin, T-cell receptor (TCR) complex, CD4, CD8, and HLA class I and II antigens, are involved in complex interactions between lymphocytes during immune responses. Their expression is restricted either to B or T cells with the exception of HLA antigens [HLA class I molecules are ubiquitous in somatic tissue, whereas class II determinants are present on B cells, activated T cells, macrophages, dendritic cells, and epithelial cells (1)]. Comparison of the deduced protein sequences of these genes indicates that these molecules share significant sequence and structural homology with immunoglobulins; therefore, they form the immunoglobulin supergene family (1, 2). However, little is known about the control mechanisms involved in the selective expression of these antigens during stem-cell differentiation. Somatic cell hybrids that show independent expression of differentiation antigens are generally those derived from fusions between cells that are at similar stages of differentiation and ontogeny (3–7). With few exceptions, differentiation markers tend to become "eclipsed" in somatic hybrids, reemerging later after certain chromosomes derived from the less differentiated partner have been lost. These rules can be used in somatic cell hybrids to evaluate relationships between differentiation antigens expressed on parental cells.

We report herein the establishment of somatic cell hybrids between the human lymphoblastoid cell line (LCL) GM1500 6-thioguanine- and ouabain-resistant, Ig$^+$, B1$^+$, B532$^+$, HLA class I and II positive (8), and the human T-cell lymphoma SUP-T1 [CD3$^-$, CD1$^+$, CD4$^+$, CD8$^+$ (9, 10)]. These hybrids phenotypically resemble B cells and expressed no T-cell-specific antigens except CD4. Despite the structural similarities of some of these antigens with immunoglobulin domains, our data support the notion that the expression of individual members of the immunoglobulin gene family might be regulated independently of other members (1).

MATERIALS AND METHODS

Somatic Cell Hybrids. SUP-T1 human lymphocytic leukemia cells (9) were fused with 6-thioguanine and ouabain-resistant human LCL GM1500 (8) in the presence of polyethylene glycol 4000 (Sigma) by standard procedures (11). Hybrids were selected in hypoxanthine/aminopterin/thymidine medium containing 10 μM ouabain to eliminate both parental cells.

Monoclonal Antibodies (mAb). Anti-CD3, anti-CD4, anti-CD8, anti-CD1, and anti-CD2 antibodies were purchased from Ortho Diagnostics. Anti-HLA-DR (Leu-10) and anti-HLA-DQ were purchased from Becton Dickinson. mAb anti-B1 were the generous gift of S. Schlossman (Dana–Farber Cancer Institute, Boston); anti-B532 antibodies were provided by S. M. Baird (V.A. Medical Center, San Diego, CA); and mAb W6/32, the monomorphic anti-HLA class I antigens, were the generous gift of K. Zier (Children's Hospital, Philadelphia). Fluorescein isothiocyanate (FITC)-conjugated goat F(ab')$_2$ anti-mouse IgG were purchased from Accurate Chemicals (Westbury, NY), as were FITC-conjugated rabbit anti-human immunoglobulin and FITC-conjugated goat anti-human C3 antibodies.

Flow Cytofluorometric Analysis. Cells were incubated with the appropriate mAb followed by FITC-conjugated goat F(ab')$_2$ anti-mouse immunoglobulin (Cappel Laboratories, Cochranville, PA). Control aliquots were stained with the fluorescent reagent alone. All samples were analyzed on an Ortho Cytofluorograf System H-50 connected to a Data General MP/200 microprocessor (Ortho Diagnostics).

Biosynthetic Labeling of Immunoglobulin. Cells (2×10^6 cells per ml) were cultured for 12 hr in methionine-free medium containing 15% dialyzed fetal calf serum, 50 μM 2-mercaptoethanol, and 50 μCi of [^{35}S]methionine per ml (1004.9 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear). Cells were sedimented and lysed, and immunoglobulin from culture supernatants or lysates was precipitated with rabbit anti-human immunoglobulin (Accurate Chemicals) by the *Staphylococcus aureus* technique (12). The immune complexes were resuspended in Laemmli buffer, analyzed on 12.5% sodium dodecyl sulfate/polyacrylamide gels, and subjected to fluorography for 4 days at −70°C. Chromato-

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Abbreviations: LCL, lymphoblastoid cell line; TCR, T-cell receptor; mAb, monoclonal antibody(ies); FITC, fluorescein isothiocyanate; J_H, joining region heavy chain; C_κ, constant region κ chain; T_β and T_α, TCR β- and α-chain probes; J_α, J segment of TCR α chain; EBNA, Epstein–Barr virus nuclear antigen.

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graphically purified human IgM and IgG (Cappel Laboratories) were used as markers.

DNA Gel Electrophoresis and Southern Transfer. Agarose gel (0.7% or 1%) electrophoresis was carried out in 40 mM Tris·HCl/5 mM NaOAc/2.0 mM EDTA, pH 8.0. *Hin*dIII-digested λ phage DNA molecular weight markers (0.75 μg per lane) (Bethesda Research Laboratories) were included on every gel. DNA samples were cleaved with restriction enzymes and then subjected to electrophoresis in a horizontal agarose (Sigma) slab gel (10 μg of DNA per lane). Gels were stained for 10 min with ethidium bromide (1 μg/ml) and photographed under UV light. Transfer of DNA from the gel to nitrocellulose sheets (Millipore) was performed essentially as described (13).

Preparation of Labeled Probe DNAs. The joining (J) region heavy (H)-chain DNA (J_H) probe is a 3.3-kilobase (kb) fragment of the human genomic μ-chain DNA clone H18Cl10 (14). This fragment contains 2.2 kb of the human J_H DNA and 1.1 kb of flanking sequences at the 3′ end. The constant region κ-chain (C_κ) probe was a cDNA clone in M13mp7 (15). The clone is an *Mbo* I/*Hin*dIII fragment equivalent to codon 115 until the poly(A) tail of mRNA. The c-myc probe (Ryc 7.4) was a cDNA clone (16) derived from a K562 cDNA library. The 3′ c-*myc* genomic probe pCA1.75 contained a 1.7-kb *Sst* I fragment extending 38 kb 3′ of the c-*myc* gene (17). The joining region of TCR α-chain (J_α) genomic probe (pSKW 1.4X) was a 1.4-kb *Xba* I fragment that is 3′ to the breakpoint with t(8;14)(q24;q11) translocation in the SKW-3 cells (18). The probe for the human TCR β chain (T_β) was a 770-base-pair (bp) fragment of YT35 cDNA probe (100–870 bp) cloned in pBR322 (19). The TCR α-chain probe ($T_{\alpha 2}$) of 900 bp was derived from a Jurkat cDNA library cloned into the *Pst* I site of pBR322 (20). The DNA probes were labeled with ^{32}P by nick-translation (21) and had specific activities of $0.3–2 \times 10^8$ cpm per 0.2 μg of DNA. DNA polymerase I was purchased from Boehringer Mannheim; [^{32}P]NTPs were from Amersham.

Hybridization. DNA on nitrocellulose sheets was hybridized to ^{32}P-labeled probe DNA in a hybridization solution containing 50% (vol/vol) formamide. After hybridization, the filters were washed, air-dried, and exposed to XRP-5 film for various periods.

RNA Transfer Analysis. Cytoplasmic RNA was extracted by the cesium chloride method as described (22). RNA was denatured in 1 M glyoxal in 10 mM sodium phosphate buffer (pH 6.5) at 50°C for 1 hr, electrophoresed in 1% agarose gel, transferred to nitrocellulose, prehybridized, and hybridized to 0.2 μg of nick-translated probes ($\approx 4 \times 10^7$ cpm) according to the method of Thomas (23). Twenty micrograms of RNA was loaded in each lane. Prehybridizations and hybridizations were performed as reported (24). Molecular weight markers were electrophoresed in each gel.

ELISA. Supernatants from cultures (cell density, 10^6 cells per ml) were tested for antibody by the ELISA technique (25). For detection of class-specific immunoglobulin, microtiter wells were coated with 10 μg of the $F(ab')_2$ fragment of goat anti-human immunoglobulin per ml (Cappel Laboratories), and antibodies that bound were detected with H-chain-specific, goat anti-human IgG or anti-IgM conjugated to alkaline phosphatase (Sigma).

RESULTS

The antigenic phenotype of the parental cells and the hybrids is summarized in Table 1. The human lymphoblastoid B-cell line (B-LCL) GM1500 (γ2, κ-producer) is deficient in hypoxanthine phosphoribosyltransferase activity and resistant to ouabain as a result of selection for 6-thioguanine and ouabain as described (8, 11). This cell line expresses the B-cell-specific differentiation antigen B1 (M_r, 30,000) (29) (99.9% positive cells), an early B-lymphocyte activation antigen B532 (M_r, 45,000) (30) (99.4% positive cells), as well as HLA class I and II antigens (>97%). Human T-cell lymphoma SUP-T1 cells (9, 10) are $CD1^+$ (84.3%) with very high coexpression of CD4 (99.9%) and CD8 (99.3%) surface glycoproteins, a characteristic of rather immature T cells (31). SUP-T1 expresses no HLA class II antigens (<5% positive cells) and very little total HLA class I determinants (50% positive cells with low intensity of expression). The CD3 antigen is not expressed on the surface of these cells as assessed by immunofluorescence with CD3-specific mAb (1.4% positive cells), and by immunoprecipitation of ^{125}I cell-surface-labeled protein using this antibody. The TCR complex is also not detected by immunoprecipitation with βF1 mAb [β framework 1 reactive with shared determinants on the human TCR β chain (32)]. SUP-T1 cells contain a paracentric inversion of chromosome 14 (q11.2;q32.3), which is the result of site-specific recombination uniting a TCR α-chain joining segment (J_α) from band q11.2 with the immunoglobulin H-chain variable-region gene (*IGHV*) from band q32.3. Their juxtaposition produces a transcriptionally active gene (10).

GM1500 cells were fused with SUP-T1 cells and hybrids were selected in HAT medium containing 10 μM ouabain. Eight hybrids were analyzed for the expression of the B- and T-cell differentiated traits, particularly with respect to the

Table 1. Phenotypic analysis of the parental and hybrid cells

	Antigen-positive cells, %										
									HLA		
Cells	sIg	B1	B532	EBNA	CD3	CD4	CD8	CD1	W6/32	DR	DQ
Parental											
GM1500	97.6	99.9	99.4	100	1.4	1.4	1.2	1.5	99.1	97.8	97.7
SUP-T1	1.6	3.3	1.5	0	5.1	99.9	99.3	84.3	50.1	5.0	2.5
Hybrid											
DC5	83.8	100.0	99.7	100	4.7	94.5	12.9	2.7	99.6	99.5	98.1
AD3	72.5	68.8	71.7	100	2.7	74.9	19.6	9.8	97.1	77.9	67.0
AD1	95.5	99.9	99.7	100	2.1	81.8	8.7	14.2	96.5	99.8	98.5
DD3	94.1	99.8	99.6	100	9.9	79.5	2.4	10.2	99.0	98.6	98.9
CA5	98.8	99.5	99.0	100	3.3	96.6	3.1	3.5	98.1	98.4	99.4
CA2	87.0	100.0	99.7	100	4.6	91.3	15.1	12.1	97.5	98.7	98.6
DD2	69.5	98.4	99.7	100	3.2	82.2	11.3	15.4	95.2	99.1	96.1
CA6	95.7	99.6	99.5	100	3.5	88.3	7.5	4.5	94.6	98.1	99.3

The percentage of positive cells for expression of a given antigen was estimated by flow cytofluorometric analysis using mAb or antisera. The EBNA was stained by anti-complement immunofluorescence as described (26). Antibody W6/32 is against a determinant common to all the M_r 43,000 chains of HLA-A, -B, and -C antigens but is also conformationally dependent on β_2-microglobulin (27, 28). sIg, surface immunoglobulin.

expression of the members of the immunoglobulin gene family: immunoglobulin, CD4, CD8, HLA determinants, $TCR_{\alpha\beta}$ subunits. Table 1 summarizes the immunophenotyping characteristics of the parental and hybrid cells. Hybrids displayed a B-cell-type morphology with a high percentage of cells strongly positive for surface immunoglobulin (87.1 ± 3.9 SEM), B1 (95.7 ± 3.8 SEM), B532 (96.07 ± 3.4 SEM), and HLA class I (97.2 ± 0.6 SEM) and II [DR (96.2 ± 2.6 SEM); DQ (94.4 ± 3.9 SEM)] antigens. Of the T-cell differentiation antigens, only CD4 (84.8 ± 2.4 SEM) was highly expressed, whereas both CD8 (10.0 ± 2.0 SEM) and CD1 (9.0 ± 1.7) were eclipsed. The Epstein–Barr virus nuclear antigen (EBNA) always associated with LCLs (26) was expressed in all hybrids. Hybrids secreted (10–30 ng/ml) γ,κ chains of immunoglobulin as determined by ELISA or immunoprecipitation with [^{35}S]methionine-labeled immunoglobulin (Fig. 1), and had a growth rate and pattern similar to that of GM1500 parent (doubling time, 20–24 hr).

Fig. 2*A* shows the results of RNA blot analysis to determine the expression of the TCR subunits in the hybrids using a cDNA probe that was a 770-base-pair (bp) fragment of YT35 cDNA clone containing V, D (diversity), J, and C regions at TCR β chain (19). RNA from Jurkat [T-cell leukemia cell line used as a positive control (33)] contains a 1.3-kb (V–D–J–C rearrangement) and a 1.0-kb (J–C transcript) mRNA (34) (lane 1), whereas SUP-T1 does not show the 1.0-kb mRNA (lane 2). GM1500 does not express any T_β RNA (lane 3). The T_β transcript can be detected in four of six hybrids analyzed: CA6, CA2, AD1, and DC5 (lanes 4, 6, 8, and 9, respectively) but with an intensity <5% of the parental transcript.

The (1.5 kb) T_α RNA expressed in Jurkat cells detected with a cDNA probe, $T_{\alpha 2}$, containing V, J, C, and 3′ untranslated regions of α chains (Fig. 2*B*, lane 1) is a faint band in SUP-T1 (Fig. 2*B*, lane 2) and represents the chimeric *J_α–IGHV* gene (10). None of the hybrids shows expression of this gene. However, RNAs hybridized with a human C_κ cDNA probe and a c-myc cDNA probe (Ryc 7.4) (Fig. 2*C*) showed transcripts of 1.3 kb and 2.4 kb, respectively, in all hybrids with similar levels of intensity.

Low level expression of the T_β transcript and lack of detectable T_α transcript in these hybrids might reflect suppression of T_β gene expression by, for example, B-cell-specific transacting factors that might be present in the hybrids or, alternatively, by loss of the relevant chromosomes. To determine the presence of the rearranged T_β and T_α genes in the hybrids, DNAs were digested with *Bam*HI (Fig. 3*A*) or *Hin*dIII (Fig. 3*B*) and hybridized with T_β cDNA and genomic J_α probes, respectively. This 16.5-kb rearranged T_β chain can be detected in six of eight hybrids with different intensities, indicating that some chromosomal segregation had occurred that might be at least in part responsible for the low expression of T_β RNA in some of the hybrids. However, four hybrids [CA6 (lane 3), CA2 (lane 6), AD1 (lane 8), and DC5 (lane 9)] showed high intensity of the T_β rearranged band, comparable to that of the SUP-T1 T_β band. On the other hand, T_β mRNA in these hybrids was almost undetectable (Fig. 2, lanes 4, 6, 8, and 9), indicating suppression of the T_β RNAs. Similarly, the 12-kb T_α rearranged band (Fig. 3*B*) was present in six of eight hybrids, but no transcript of this gene was detected. The rate of chromosome loss in the hybrids can be monitored based on the presence of chromosomes carrying other genes such as immunoglobulin J_H, C_κ,

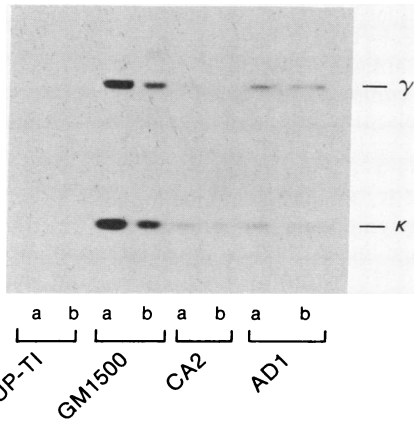

FIG. 1. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis of immunoprecipitated immunoglobulin chains synthesized by hybrid and parental lines. Cells were cultured at a density of 2×10^6 cells per ml for 12 hr and 24 hr in methionine-free medium in the presence of [^{35}S]methionine (50 μCi/ml). The human immunoglobulin chains were precipitated with rabbit anti-human IgG and anti-IgM by the *Staphylococcus aureus* technique. Aliquots of immunoprecipitates were reduced and analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis on a 12.5% polyacrylamide slab gel. Gels were developed by fluorography. Lanes a and b, secreted and cytoplasmic immunoglobulin, respectively.

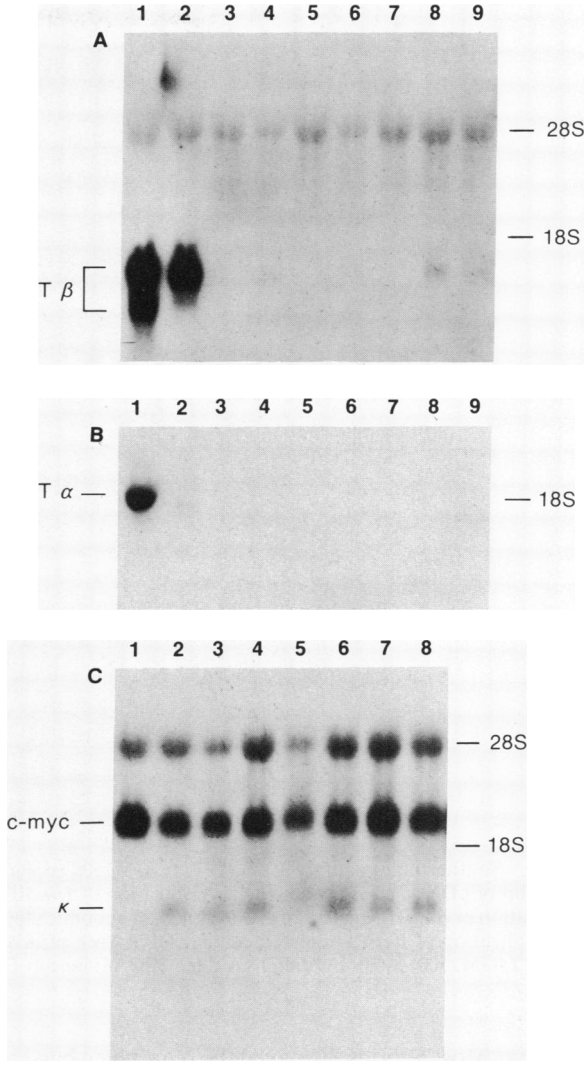

FIG. 2. RNA blotting analysis of SUP-T1 × GM1500 somatic cell hybrids for the presence of TCR β-chain transcript with cDNA probe 100–870 bp of YT35 clone (*A*), TCR α-chain transcript with $T_{\alpha 2}$ cDNA probe (*B*), c-myc (Ryc 7.4) and C_κ transcripts (*C*). (*A* and *B*) Lanes: 1, Jurkat; 2, SUP-T1; 3, GM1500; 4–9, hybrid RNAs (lane 4, CA6; lane 5, DD3; lane 6, CA2; lane 7, CA5; lane 8, AD1; lane 9, DC5). (*C*) Lanes: 1, SUP-T1; 2, GM1500; 3–8, hybrid RNAs (lane 3, CA6; lane 4, DD3; lane 5, CA2; lane 6, CA5; lane 7, AD1; lane 8, DC5).

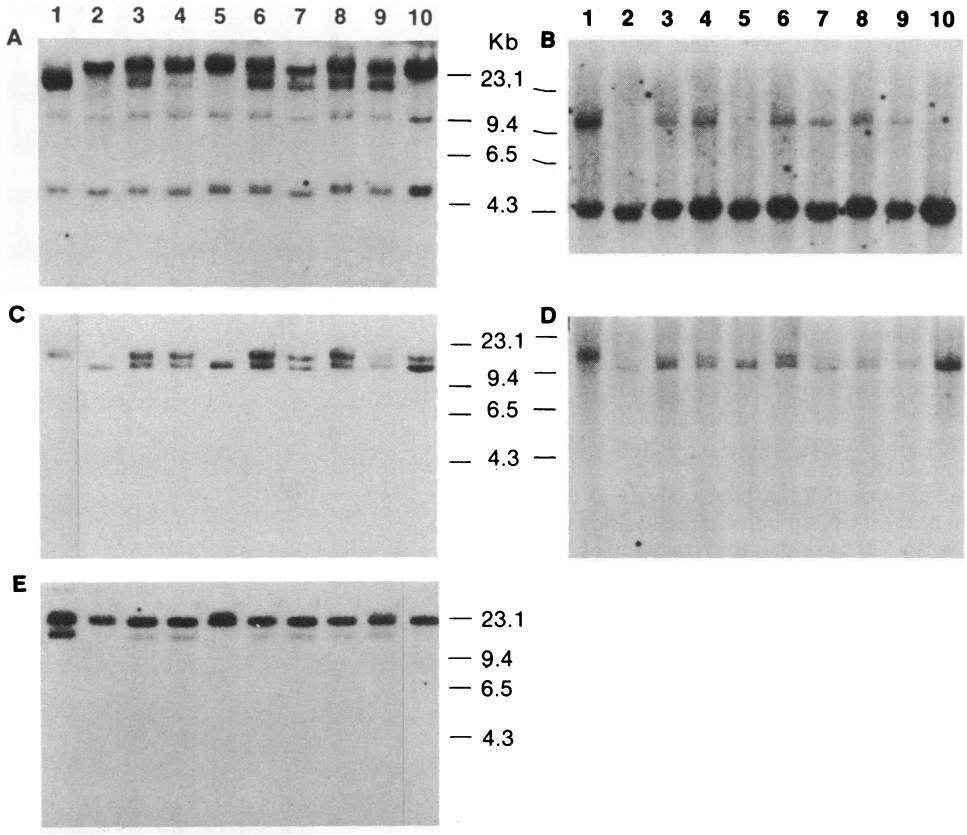

FIG. 3. Southern blot analysis of SUP-T1 × GM1500 somatic cell hybrids. (*A*) Hybridization of *Bam*HI-digested DNAs with TCR β chain 100–870 bp of cDNA YT35 clone. (*B*) Hybridization of *Hin*dIII-digested cellular DNAs with TCR α-chain genomic probe (pSKW 1.4X). (*C*) Hybridization of *Bam*HI-digested DNAs with J_H of genomic clone (H18Cl10). (*D*) Hybridization of *Bam*HI-digested DNA with cDNA C_κ probe. (*E*) Hybridization of *Eco*RI-digested cellular DNA with pCA1.75 probe specific for the flanking region 3′ to the c-*myc* oncogene. Lanes: 1, SUP-T1; 2, GM1500; 3–10, hybrid DNA (lane 3, CA6; lane 4, DD2; lane 5, CA5; lane 6, CA2; lane 7, AD3; lane 8, AD1; lane 9, DC5; lane 10, DD3).

and c-*myc*. Most hybrids displayed the rearranged band of J_H (Fig. 3*C*), C_κ (Fig. 3*D*), and c-myc (Fig. 3*E*). The (14 kb) rearranged J_H band was present in all hybrids, as was the (10 kb) rearranged C_κ band, which was consistent with IgG κ-chain production by the hybrids. The 16.5-kb rearranged c-myc band derived from the SUP-T1 parent was present in the majority of the hybrids. These data suggest that suppression of TCR T_β and T_α subunits in the B–T-cell hybrids is mediated by some B-cell-specific factor(s) rather than by chromosomal loss. Similar mechanisms might be responsible for suppression of the CD1 and CD8 antigens in these cells. CD8 has been mapped to the 2p1 region in close proximity to the immunoglobulin κ light-chain gene (35). Our Southern analysis with C_κ probe showed that chromosome 2 was preferentially retained in these hybrids, which agreed with the karyotyping results (data not shown).

DISCUSSION

Our analysis of B–T-cell hybrids reveals that a B cell can suppress T-cell-specific antigen expression. All of the hybrids obtained morphologically and phenotypically resembled the B-cell parent. EBNA, as well as B-cell-specific differentiation antigens B1 or B532, or class I or II determinants of the HLA complex, were autonomously expressed at high levels. Hybrids were highly positive for surface immunoglobulin and secreted lower amounts of immunoglobulin (10–30 ng per ml of culture medium) than the parental GM1500 (60 ng per ml of culture medium) cells.

Unlike B-cell markers, the T-cell differentiation antigens present on the parental SUP-T1 cells (CD1 and CD8) were suppressed in the hybrids with the exception of CD4. CD4, a M_r 62,000 glycoprotein, has been shown to be present on T lymphocytes exhibiting inducer/helper activities for T–T, T–B, and T–macrophage interactions (31, 36, 37). Because CD4 is primarily present on helper cells and only rarely on cytotoxic cells (38, 39), it has been suggested that $CD4^+$ cells recognize antigen associated with class II major histocompatibility complex molecules on antigen-presenting cells. CD4 reveals structural homology with the V and J regions of immunoglobulin light chains (40) as well as similar mechanisms of expression. CD4 expression has been reported in a LCo-B LCL, the U937 monocytic cell line derived from histiocytic lymphoma, some normal cells at the monocyte–macrophage lineage (41), some subline mutants of GM1500 LCLs, and HL-60 promyelocytic cells (Jim Hoxie, personal communication). Together, these data show that CD4 expression is different from T-cell-specific antigen expression and is perhaps more closely related to B-cell-specific genes than other T-cell antigens.

As with other T-cell markers, expression of the TCR α and β subunits becomes "eclipsed" on the B-cell background. Trace amounts of β-chain RNA could only be detected in a few hybrids, and α-chain RNA representing the chimeric gene J_α–*IGHV* was not detectable in any of the hybrids. The same J_α/immunoglobulin rearrangement has recently been described in the B-cell line VP (42). Based on the assumption that the α chain is the last of the TCR loci to rearrange and to be expressed during T-cell development (43), and thus might not recombine in a pre-B or pre-T cell, Denny (42) suggested that the rearrangement of the immunoglobulin loci may have occurred in SUP-T1 cells after commitment to a

T-cell lineage. The fact that the J_α–*IGHV* complex present in SUP-T1 cells is suppressed in the hybrids—i.e., is expressed as a T-cell-specific gene—supports this hypothesis. Finally, the fact that J_α is rearranged with the immunoglobulin locus may account for the absence of TCR–CD3 expression on the surface of SUP-T1 cells. The structural organization of the TCR–CD3 complex molecules in humans is not yet known. However, reconstitution of an active surface TCR–CD3 antigen receptor by cDNA β-chain transfer (44) suggests that only cells expressing both of these products appear to have surface TCR or CD3.

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