HELPER AND KILLER T CELLS DO NOT EXPRESS
B CELL IMMUNOGLOBULIN JOINING
AND CONSTANT REGION GENE SEGMENTS*

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The immune system is characterized by both the specificity and breadth of its response. The generation of an immune response depends upon the specific recognition of antigens by lymphocytes. There are two classes of lymphocytes: B cells capable of secreting large quantities of immunoglobulin and T cells that can carry out facilitation or help of lymphocyte responses (1, 2), suppression of lymphocyte responses (3), or cytotoxicity directed against appropriate target cells (4). A T cell is apparently committed to a single functional subclass prior to any exposure to antigen (5, 6).

The antigen-binding receptor on the surface of B lymphocytes is an immunoglobulin molecule composed of two identical light chain and two identical heavy chain polypeptides. Both light and heavy chains have variable regions responsible for antigen binding and constant regions responsible for various effector functions such as complement fixation. The immunoglobulins are encoded by three unlinked gene families: two light chain families, κ and λ, and a heavy chain family. In mice, there are one or more constant region genes for each light chain family, and at least eight heavy chain constant region genes (μ, δ, γ_3, γ_1, γ_{2b}, γ_{2a}, α, and ϵ). Each mature B cell and its progeny can respond to only one or a few antigenic determinants because it can synthesize only one light chain and one heavy chain variable region. However, during their development, B cells switch from the expression of IgM molecules containing μ heavy chains to the expression of other immunoglobulin classes containing different heavy chain constant regions, while continuing to express the same V_H region and light chain (7, 8).

Recent experiments employing recombinant DNA techniques have clarified some of the molecular events necessary for B-cell antibody expression. Both the commitment to express single V_H and V_L regions, and the switch in expression of heavy chain constant region are characterized by DNA rearrangements. In the mouse, the light chain is encoded by three separate gene segments—V, J^1 (joining), and C (constant) (9). The V and J gene segments together code for the variable region. For mouse κ chains, there are multiple germline V_κ gene segments (≥ 200) (10) and four J_κ gene segments (11, 12). The heavy chain is encoded by four gene segments—V_H, D (diversity), J_H and C_H, and in some cases an M (membrane) exon (13–16). The V

gene segment (and presumably the D gene segment) expressed in a given B cell is rearranged from its germ-line context and becomes contiguous with a J gene segment that is located a few kilobases 5′ from a C gene (11–13, 17). DNA sequences between the V and J gene segments are deleted during this rearrangement (11, 18, 19). Heavy chain constant-region switching takes place through replacement of the μ constant-region gene and some of its flanking sequence by another C_H gene (20).[2]

Individual T cells can also respond specifically to one or a few antigenic determinants (21–23). However, the molecular properties of the T-cell antigen-binding receptor are a subject of great controversy (24). Most attempts to define the receptor have relied on antisera made to either B-cell derived immunoglobulin (25–31) or to responding T cells (32, 33) in conjunction with immunological or genetic experiments. There is considerable evidence that T cells synthesize V_H regions without light chains or any of the C_H regions expressed in B cells (29–31), but there are a number of reports of the expression of light chains and C_H regions in T cells (25–28, 34, 35).

The development of two relatively new techniques has persuaded us to reexamine the controversy regarding immunoglobulin synthesis by T cells. First, cell lines grown in T-cell growth factor or Interleukin II (36) provided us with large numbers of mature, functional T cells that are entirely free of contaminating B cells and have a well-defined antigen specificity. Second, using the methods of recombinant DNA research, we have obtained cloned DNA probes for immunoglobulin heavy and light chain J and C gene segments. We have used our DNA probes to ask whether these J and C gene segments are rearranged and transcribed into RNA by T cells. Because both the T cells and the DNA probes are homogeneous and well characterized, we hoped to avoid the ambiguities of some of the earlier experiments concerning T-cell expression of immunoglobulin constant regions. In addition, by using probes for the heavy and light chain J gene segments, we could obtain information on T-cell expression of the 3′ part of the variable region. We have obtained convincing data that the B-cell immunoglobulin light and heavy chain J and C gene segments are neither rearranged nor expressed in the monoclonal helper and killer T cells that we have analyzed.

Materials and Methods

T Cells. BALB/c Cum mice were originally obtained from Cumberland View Farms, Clinton, Tenn., and have since then been bred in our animal colony at the California Institute of Technology. C57BL/6J mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. Thymus tissue was dissected from 2- to 4-wk-old BALB/c mice killed by inhalation of ether. Mice were injected with 0.1 ml of India ink prior to sacrifice to facilitate visualization and removal of parathymic lymph nodes. The antigen specificity and cell-surface phenotype of our monoclonal T cells are presented in Table I. WEHI–22 is an irradiation-induced BALB/c T lymphoma (27). It has been reported to synthesize large amounts of a 68,000 mol wt protein that cross-reacts with an antiserum made against mouse immunoglobulin (28). WEHI-22 cells were a gift of Dr. Noel Warner, University of New Mexico, Albuquerque, N. Mex. and were grown in suspension culture in Dulbecco's modified Eagle's medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% fetal bovine serum. The helper cell line HT-1 (23) and the alloreactive killer cell line CTLLi6 (37) were grown in RPMI-1640 medium (Grand Island Biological Co.) supplemented with 10% heat-inactivated fetal bovine serum, 1 mM glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, and 5 × 10^{-5} M 2-mercaptoethanol.

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[2] Davis, M. M., S. Kim, and L. Hood. DNA sequences mediating heavy chain switching in alpha immunoglobulin genes. Manuscript submitted for publication.

TABLE I

Monoclonal T Cells Analyzed with Cloned Immunoglobulin DNA Probes

Cell line	Strain of origin	Function	Antigen specificity	Cell surface phenotype	Description	Reference
HT-1	C57B1/6J	Helper	Sheep erythrocytes Helps H-2^b B cells	θ^+, Lyt-1$^+$	Growth factor-dependent cell line	23
CTLLi6	C57B1/6J	Killer	H-2^d	θ^+, Lyt-2$^+$	Growth factor-dependent cell line	37
WEHI-22	BALB/c	?	?	θ^+	T lymphoma	27

TABLE II

Cloned Immunoglobulin Probes

Region	Source		Reference
C_κ (C region subclone)	Subclone from BALB/c genomic DNA		38
J_κ (J region subclone)	Subclone from BALB/c genomic DNA		38
C_γ (pAbγ_1-7)	cDNA from HOPC 2020 mRNA		A. Bothwell. Personal communication.
J_H	Fragment from BALB/c genomic DNA		20; this report
Switch (p5.1)	Subclone from BALB/c myeloma DNA		20
C_μ (p104Eμ6)	cDNA from M104E mRNA		14
C_α	cDNA from S107 mRNA		20

A supernatant fraction from concanavalin A-stimulated lymphocytes enriched for Interleukin II was prepared as described (23) and added to the medium at a concentration of 10 U/ml.

Immunoglobulin Probes.   Description of the immunoglobulin DNA probes employed in these studies is presented in Table II. The C_κ and J_κ gene segments were subcloned from a liver DNA

genomic clone. These subclones were a gift of Dr. Michael Steinmetz, California Institute of Technology, Calif. (38). A C_λ cDNA clone, pAbλ_1-7, was a gift of Dr. Alfred Bothwell and Dr. David Baltimore, Massachusetts Institute of Technology, Boston, Mass. A probe containing the J_H gene segments was prepared by electroeluting a 6.2 kilobase (kb) EcoRI fragment from the ChSp μ27 clone originally isolated as a C_μ-containing clone from a collection or library of BALB/c sperm DNA clones (20). The probe containing heavy chain constant region switch sites was subcloned from the Ch603α6 clone. This clone was originally isolated from a library made from the DNA of the IgA-producing myeloma McPC603 (17). The original α6 clone contains the 603 V_H gene segment joined to a J_H gene segment, with C_α gene found 6.8 kb to the 3' side of the J_H gene segment. The subclone is a 5.1 kb EcoRI fragment containing intervening DNA sequence and the 5' half of the C_α gene (17). In the embryo, 1.5 kb of this intervening sequence is found adjacent to the C_μ gene and not the C_α gene (20). The point at which C_μ and C_α adjacent sequences are joined is denoted the switch site. Five examples of C_H switching have thus far been analyzed, including three from rearranged genes of C_α-producing myeloma tumors (17, 20),[2] one from a $C_{\gamma 1}$ producer (39, 40), and one from a $C_{\gamma 2b}$ producer (41). All the active genes from these myelomas have C_μ flanking sequence extending from a J_H gene segment towards the expressed C_γ or C_α gene. The point of deletion of the C_μ flanking sequence or switch site is not the same for every tumor, but they are all found within about 300 base pairs of one another. The 5.1 kb subclone we used as a probe should hybridize with restriction fragments containing all these known switch sites as well as several kb of surrounding DNA.

Preparation of DNA and Southern Blots. High molecular weight DNA was prepared according to the method of Blin and Stafford (42). Embryo DNA was prepared from whole 12-d BALB/c embryos. DNA digested with restriction enzymes was electrophoresed in 0.7% agarose gels, transferred to nitrocellulose filters (43), and hybridized with nick-translated probes (44). Unless otherwise noted, hybridizations were performed at 68°C in 1.0 M NaCl/0.045 M Trisodium citrate/0.2% bovine serum albumin/0.2% Ficoll/0.2% polyvinylpyrrolidone/0.1% NaDodSO$_4$/100 μg/ml denatured Salmon sperm DNA/25 μg/ml poly-rA. Subsequent to hybridization, filters were washed extensively in 0.30 M NaCl/0.03 M Trisodium citrate/0.1% NaDodSO$_4$/0.1% sodium pyrophosphate at 68°C and autoradiographed.

Preparation of RNA and Northern Blots. Purified immunoglobulin mRNA containing the α (MOPC167) and μ (M104E) heavy chain constant regions and the κ (S107) light chain constant region were prepared as described (17). Total RNA was prepared from T cells homogenized in guanidinium thiocyanate and centrifuged through cesium chloride (45). RNA preparations electrophoresed on agarose gels and stained with ethidium bromide were undegraded, free of contaminating DNA, and had a 260 nm/280 nm absorbance ratio of about 2.1:1. ^{3}H-labeled RNA from sea urachin (*Strongylocentrotus purpuratus*) embryos (a gift of Dr. Frank Costantini, California Institute of Technology) was added at the beginning of the T cell RNA preparation as a recovery marker. Between 43 and 64% of the added counts per minute were present in the final RNA solution. From the absorbance of the mouse T cell RNA at 260 nm, and the yield of labeled *S. purpuratus* RNA estimated from recovered counts per minute, we calculated that WEHI-22 has 12.7 pg of RNA/cell, HT-1 has 5.8 pg/cell, CTLLi6 has 3.7 pg/cell, and thymus has 1.6 pg/cell. Polyadenylated [poly(A)$^+$] RNA was selected by oligo(dT)-cellulose chromatography (46). Poly(A)$^+$ RNA was denatured and electrophoresed on agarose gels containing 2.2 M formaldehyde (47). Denatured RNA was transferred directly onto nitrocellulose filters (B. Seed and D. Goldberg. Manuscript in preparation.), and filters were hybridized at 42°C in 50% formamide (48). In control experiments, ^{32}P-labeled RNA from Sindbis virus-infected cells (a gift of Charles Rice, California Institute of Technology) was electrophoresed on agarose gels, transferred to nitrocellulose filters, and incubated in hybridization and wash solutions. Virla RNA as large as 13.0 kb was efficiently transferred to filters. About 45% of the RNA remained bound to the nitrocellulose as measured by densitometry of viral RNA bands present on autoradiographs of the filter exposed before and after incubation in hybridization buffers. In one experiment, diazophenylthioether paper (a gift of Brian Seed, California Institute of Technology) rather than nitrocellulose, was used as a solid support to bind RNA.

Results

Experimental Strategy. T cell DNA was analyzed for rearrangement using the Southern blot technique (43). High molecular weight genomic DNA was digested

with a restriction enzyme and the resulting fragments were separated according to molecular weight in an agarose gel. The double-stranded DNA was then denatured so that single strands were available for hybridization. The size-separated and denatured DNA was transferred from the agarose gel to a solid support, generally a nitrocellulose filter, and the filter-bound DNA was hybridized with a radioactive-cloned DNA probe. The filter was autoradiographed, and restriction fragments, having sequences complementary to the probe, appeared as bands on the film. By comparing the band pattern in T cells with the band pattern obtained from germ-line DNA, we assessed whether a particular DNA sequence was rearranged. In analyzing the DNA from WEHI-22 and BALB/c thymus, cells from 12-d BALB/c embryos and adult livers provided a source of germ-line DNA. For the HT-1 and CTLLi6 cell lines, which come from C57BL/6J mice, DNA undifferentiated with respect to immunoglobulin genes was obtained from the cells of adult liver and kidney.

We used Northern blots to detect immunoglobulin sequences in T cell RNA. The experimental strategy is very similar to that employed to detect DNA rearrangements. Poly(A)$^+$ total cell RNA from T cells was size-separated on agarose gels, transferred to nitrocellulose filters, hybridized with radioactive immunoglobulin DNA probes, and the filters were then autoradiographed.

T Cell DNA Analyzed for Gene Rearrangement Using Light Chain Probes. The results obtained from hybridizing light chain DNA probes to restriction fragments of T cell DNA are presented in Table III and Fig. 1. All the bands present in the T cell DNA are also present in the germ-line DNA from the same strain, indicating that C_κ, J_κ, and C_λ sequences are not rearranged in the T cells. In a few cases, however, C57BL/

TABLE III

Sizes of Restriction Fragments Containing Light Chain Immunoglobulin Genes in T Cell and Nonlymphoid DNA

Digests	C_κ			J_κ			C_λ		
	Bam HI	Eco RI	Hind III	Bam HI	Eco RI	Hind III	Bam HI	Eco RI	Hind III
C57B16 DNA									
HT-1	13.0	15.0	4.3, 2.7	13.0	15.0	2.7	7.3	9.0	8.4, 3.2
CTLLi6	13.0	15.0	4.3, 2.7	13.0	15.0	2.7	7.3	9.0	8.4, 3.2
Liver or kidney	13.0	15.0	4.3, 3.5 2.7	13.0	15.0	3.5, 2.7	7.3	9.0, 6.6	8.4, 3.2
BALB/c DNA									
WEHI-22	13.0	15.0	4.3, 2.7	ND‡	15.0	ND	ND	9.0	ND
Thymus	13.0	15.0	4.3, 2.7	ND	15.0	2.7	ND	9.0	ND
Liver or embryo	13.0	15.0	4.3, 2.7	ND	15.0	2.7	ND	9.0	ND

* Sizes of restriction fragments containing immunoglobulin genes were estimated from the migration of molecular weight standards that included Eco RI- and Hind III-digested λ bacteriophage DNA, and pBr322 plasmid digested with both Ava I and Hind II. In every case, T cell DNA was electrophoresed in a lane adjacent to one of the nonlymphoid DNA samples. WEHI-22 and BALB/c thymus DNA were electrophoresed on the same gels; HT-1 and CTLLi6 DNA were electrophoresed on separate gels.
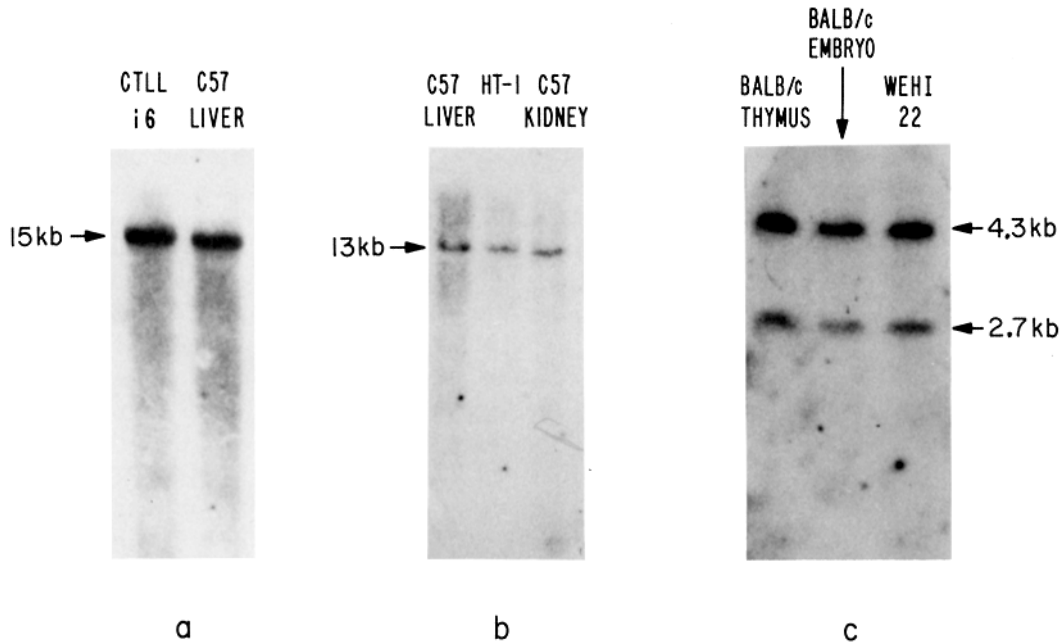
‡ ND, not determined.

Fig. 1.   T cell and nonlymphoid DNA hybridized with a radioactive C_κ probe. (a–c) are autoradiographs of DNA from separate gels. In (a) DNA samples were digested with restriction enzyme Eco RI; in (b) they were digested with Bam HI; and (c) they were digested with Hind III. The sizes of the hybridizing fragments are indicated. For experimental details see Materials and Methods.

6J liver and kidney DNA had a restriction fragment hybridizing with the light chain probes that was not apparent in the two monoclonal T cell lines (Table III). The extra band in C57BL/6J liver and kidney is sometimes faint, and a technical artifact probably accounts for this result. A number of much less likely explanations are formally possible, however, including a genetic polymorphism in C57BL/6J mice, a T-cell specific sequence deletion (as opposed to rearrangement), or even an immunoglobulin gene rearrangement specific to liver and kidney cells.

T Cell DNA Analyzed for Gene Rearrangement Using Heavy Chain Probes.   The results obtained from hybridizing heavy chain probes to restriction fragments of T-cell DNA are presented in Table IV and Figs. 2 and 3.

We observed no rearrangement of J_H gene segments in the T cells tested. The 6.2 kb J_H probe contains a sequence at its 5' end that is repeated in the mouse genome (P. W. Early. Unpublished observations.). To reduce the background caused by hybridization with these repeated sequences, filters hybridized with the 6.2 kb J_H probe were washed extensively in low salt conditions (20 mM cation at 68°C), that favor melting of imperfectly matched hybrid molecules. After the low salt washes, one (EcoRI and Hind III digests) or two (Bam HI digest) dark bands remained on the filter, and the sizes of these major bands are presented in Table IV. The Eco RI fragment from BALB/c DNA that hybridizes with the probe is the same size as the EcoRI fragment used to isolate the probe from a sperm DNA clone, suggesting that this major band is not simply a large tandem array of repeated sequences or some other artifact. A 5.1 kb subclone containing sequences involved in B-cell heavy chain

TABLE IV

Sizes of Restriction Fragments Containing Heavy Chain Immunoglobulin Genes in T Cell and Nonlymphoid DNA

Digests	J_H			Switch			C_μ membrane			C_α		
	Bam HI	Eco RI	Hind III	Bam HI	Eco RI	Hind III	Bam HI	Eco RI	Hind III	Bam HI	Eco RI	Hind III
C57B16 DNA												
HT-1	11.0	6.2	2.0	23.0	15.0	6.2	11.0	15.0	2.65	22.5	10.0	5.7
	1.0		0.9	14.0	10.0	5.7			1.25			
CTLLi6	11.0	6.2	2.0	23.0	15.0	6.2	11.0	15.0	2.65	22.5	10.0	5.7
	1.0		0.9	14.0	10.0	5.7			1.25			
Liver or kidney	11.0	6.2	2.0	23.0	15.0	6.2	11.0	15.0	2.65	22.5	10.0	5.7
	1.0		0.9	14.0	10.0	5.7			1.25			
BALB/c DNA												
WEHI-22	10.0	6.2	2.0	ND*	12.5	ND	11.0	12.5	2.65	22.5	9.5	3.7
	1.0		0.9		9.5, 4.8				1.25			
Thymus	10.0	6.2	2.0	ND	12.5	ND	11.0	12.5	2.65	22.5	9.5	3.7
	1.0		0.9		9.5, 4.8				1.25			
Liver or embryo	10.0	6.2	2.0	ND	12.5	ND	11.0	12.5	2.65	22.5	9.5	3.7
	1.0		0.9		9.5, 4.8				1.25			

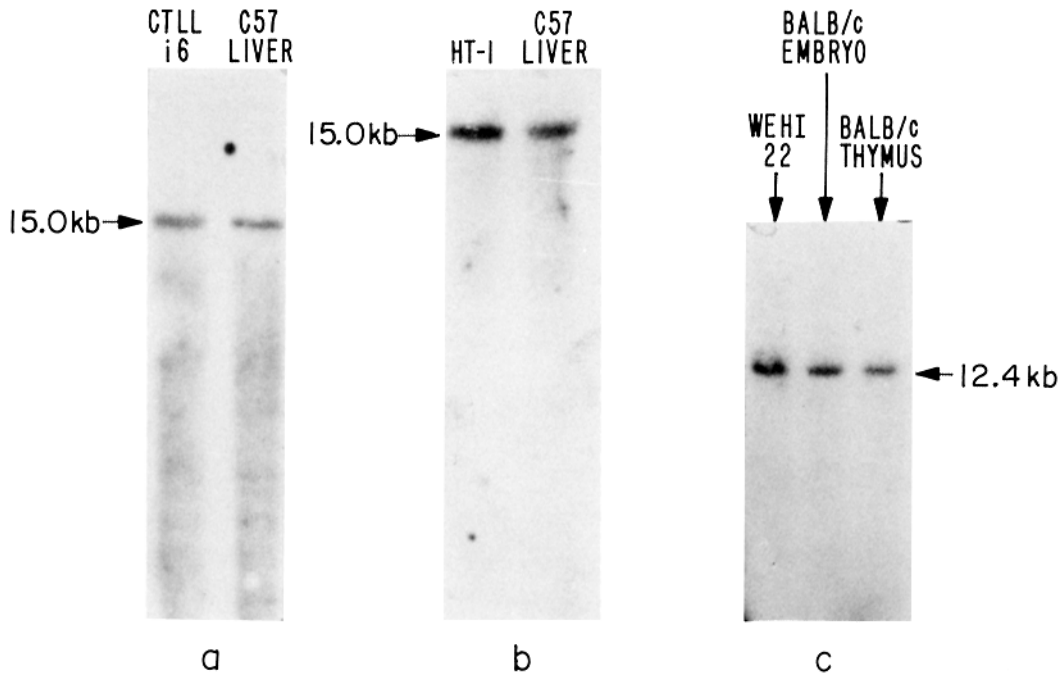For details see the legend for Table III.
* ND, not determined.

FIG. 2. T cell and nonlymphoid DNA hybridized with a radioactive C_μ probe. All DNA samples were digested with Eco RI. See Fig. 1 legend and Materials and Methods for details.
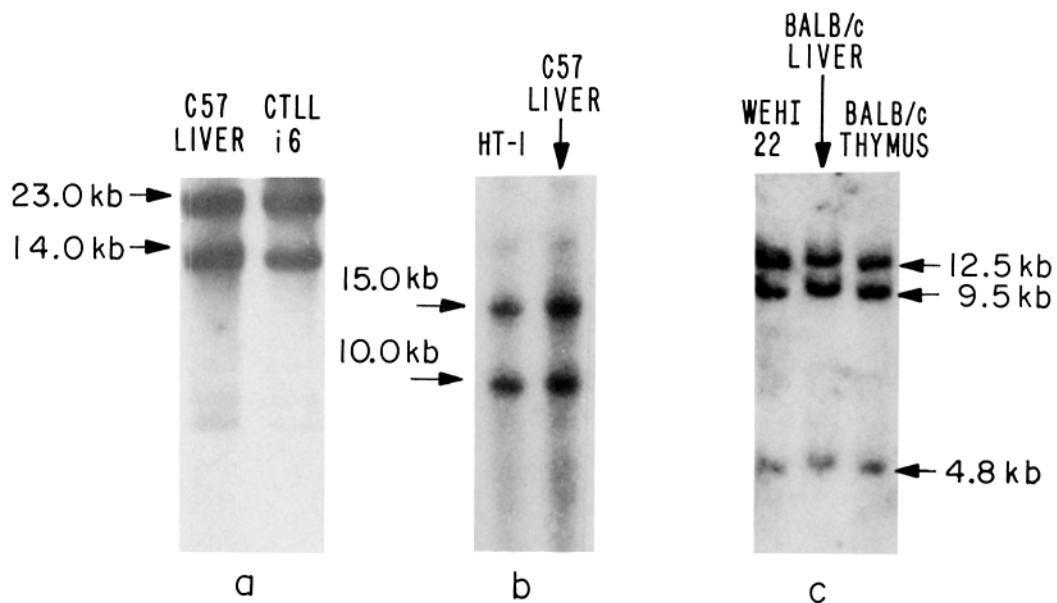
Fig. 3. T cell and nonlymphoid DNA hybridized with a radioactive probe containing heavy chain constant region switch sites. In 3a, DNA was digested with Bam HI, whereas in Figs. 3b and c the DNA was digested with Eco RI. See Fig. 1 legend and Materials and Methods for details.
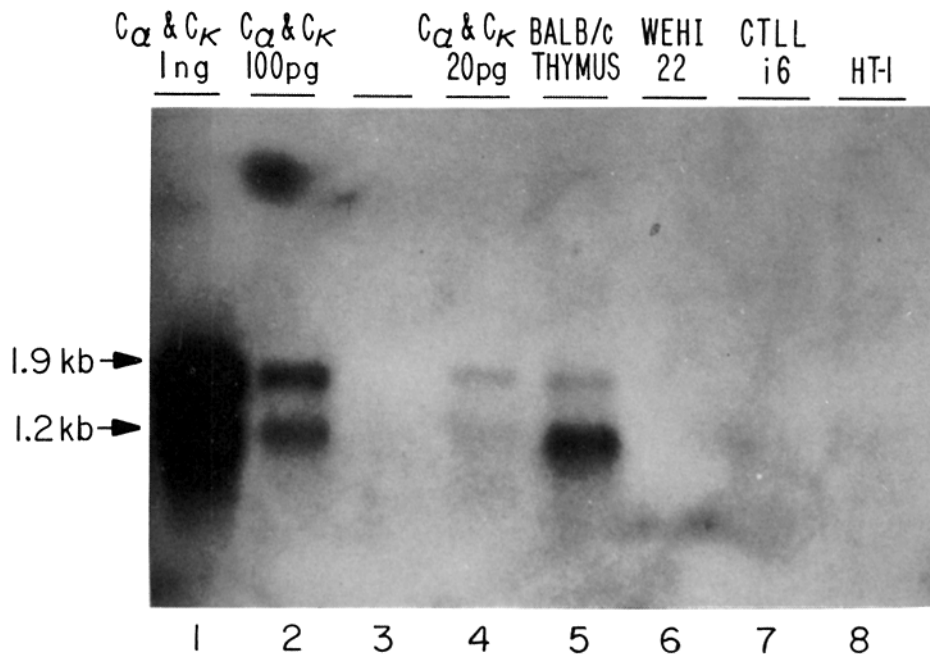

Fig. 4. Filter-bound poly(A)$^+$ RNA hybridized with C_κ and C_α probes. RNA molecular weight standards included *Escherichia coli* 16, and 23S ribosomal RNA, and murine 18 and 28S ribosomal RNA. Purified myeloma RNA is present in lanes 1, 2, and 4. When the probes are hybridized to separate filters, the C_κ RNA sequence in thymus is 1.2 kb, whereas the larger C_α RNA is 1.9 kb. We could detect 1.4 copies/cell of κ RNA and <1.0 copy/cell of α RNA on this autoradiograph.
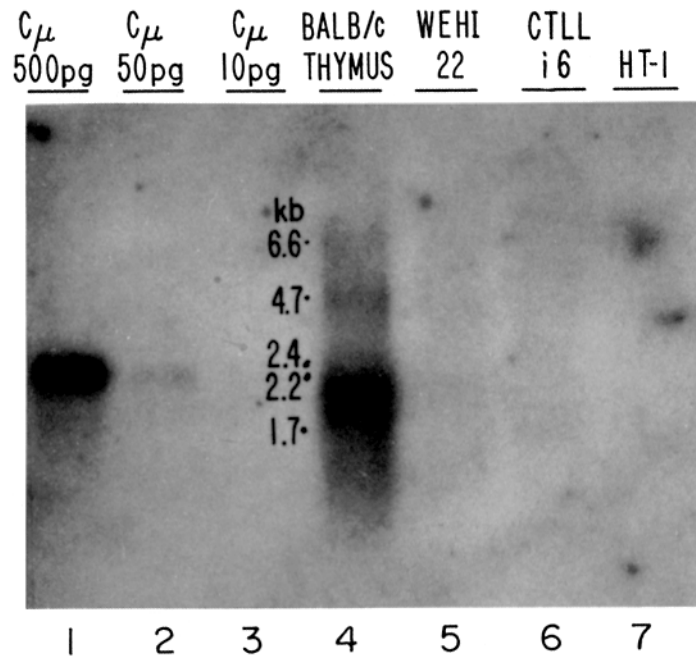
FIG. 5.   Filter-bound poly(A)$^+$ RNA hybridized with a C_μ probe. RNA molecular weight standards included 26S and 42S Sindbis virus RNA and *E. coli*, and murine ribosomal RNA. Purified myeloma RNA standards are present in lanes 1, 2, and 3. Molecular weights of thymus RNA bands are indicated. We could detect 1.8 copies/cell or less of μ RNA on this autoradiograph. Detection limit varied with different samples because of different amounts of poly(A)$^+$ RNA (1–10 μg) added to the gel.
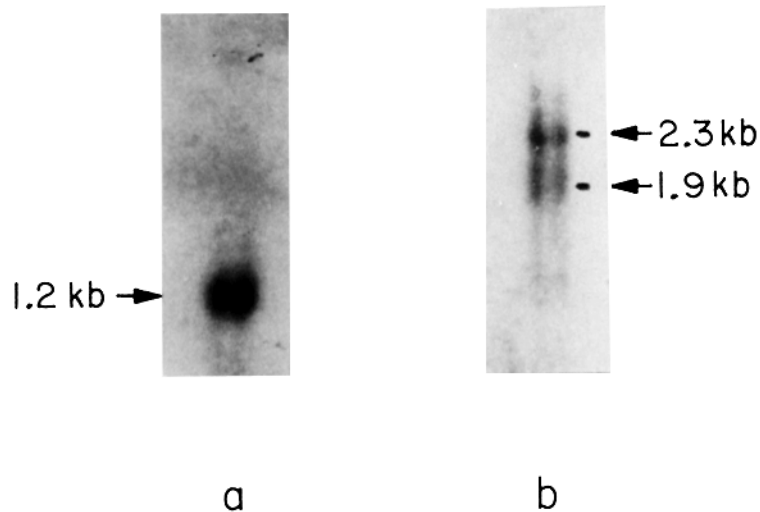

FIG. 6.   Poly(A)$^+$ thymus RNA hybridized to C_κ and C_μ probes. (a) Poly(A)$^+$ thymus RNA was size-separated on an agarose gel and transferred to diazotized paper. RNA covalently bound to the diazotized paper was hybridized with a radioactive C_κ probe, and the paper was autoradiographed. (b) The paper with bound thymus RNA, as in (a), was washed in 95% formamide at 68°C to remove the radioactive C_κ probe. It was then hybridized with a C_μ probe and autoradiographed.

constant region switching was hybridized to the T cell DNA. No rearrangement of these sequences was observed (Fig. 2). The C_μ probe we used is a cDNA clone made from RNA coding for the membrane form of μ heavy chain. We have detected no rearrangement of the C_μ gene in the T cells (Fig. 3). The C_α gene was also present in the germline or undifferentiated configuration in all the T cells analyzed.

In addition, because of reports that T cells make a polypeptide serologically cross-reactive with the μ chain (25, 26), we hybridized the C_μ probe at 55°C to BALB/c and C57Bl/6J liver DNA cut with various restriction enzymes. The filter was subsequently washed at 55°C. All other conditions were as described in Materials and Methods. These low temperature conditions should permit hybridization of two nucleotide sequences that are only 70% homologous (see Discussion). No bands besides those known to contain the C_μ gene hybridized with the probe, therefore the gene for the putative C_μ homologue was not detected in this experiment.

Northern Blot Analysis of T Cell RNA. Although gene rearrangement is closely associated with immunoglobulin gene expression in B cells, it is possible that T cells could express immunoglobulin genes without rearranging them. We therefore analyzed T-cell RNA for the presence of immunoglobulin sequences. Size-separated poly(A)$^+$ RNA from T cells was hybridized with ^{32}P-labeled C_κ (Fig. 4), C_α (Fig. 4), C_μ (Fig. 5), and C_λ (data not shown) probes. Purified immunoglobulin RNA were run on the same gels, and provided us with an estimate of our detection limit, which ranged from 10 to 50 pg of RNA in a series of five gels. Given the detection limit, the amount of poly(A)$^+$ RNA/cell (which is about 1% of the total cell RNA calculated in Materials and Methods) and the micrograms of RNA added per gel lane, we estimate that we could detect less than two molecules of immunoglobulin RNA/cell in every case. No immunoglobulin RNA was detected in three monoclonal T-cell lines. Whole thymus, however, contained ~150 pg of C_κ RNA (7 copies/cell), 30 pg of C_α RNA (0.7 copies/cell), and 680 pg of C_μ RNA (16 copies/cell) as judged by densitometric comparison of thymus RNA bands to the bands obtained with purified immunoglobulin RNA. In addition, a 4.7 kb species that hybridized with the C_μ probe was present on some gels at ~0.4 copies/cell. Whole thymus was not tested for the presence of C_λ RNA. The C_κ and C_α RNA in the thymus were found in the poly(A)$^+$ fraction only (data not shown), and were approximately the same molecular weight as the polysomal C_κ and C_α mRNAs found in B lymphocytes. The C_μ RNA was found in a diffuse band ranging from ~2.3 to 1.7 kb in size. The μ mRNA species in B cells are 2.7 and 2.4 kb (13). The relatively low molecular weight for C_μ sequences in thymus was observed in two separate RNA preparations, and a control experiment, in which both the κ and μ probes were hybridized to the same RNA, verified that degradation was not responsible for the diffuse band and decreased size. The thymus RNA sequences hybridizing with the κ probe appeared to be a single species of about 1.2 kb (Fig. 6a), whereas the sequences hybridizing with the C_μ probe had a characteristic broad molecular weight distribution, ranging in this case from ~2.6 to 1.7 kb, with major bands visible at ~2.3 and 1.9 kb (Fig. 6b).

Discussion

After 10 years of intensive investigation, the structure of the T-cell antigen receptor remains controversial. Approaches to T-cell antigen recognition molecules based on the use of antisera to immunoglobulins have failed to yield definitive results. In this

study we have employed cloned probes containing immunoglobulin coding and flanking genomic DNA sequences to test for rearrangement and expression of immunoglobulin genes in T cells. The experiments depend upon the ability of our labeled probes to hybridize with genomic DNA or poly(A)$^+$ RNA containing immunoglobulin sequences. This approach has two distinct advantages: (a) The physical and chemical basis of nucleic acid hybridization is well understood and we can estimate the degree of sequence homology required for hybridization. (b) We can detect events, such as the synthesis of RNA molecules, which occur at two copies/cell or less. Most of our experiments were performed on three cloned cell lines, eliminating the possibility of any B cell contamination of our DNA and RNA preparations. In addition, HT-1 and CTLLi6 are bona fide mature T cells with a defined antigen specificity and functional subclass.

There is No Rearrangement of Immunoglobulin J and C Gene Segments in the Monoclonal T Cells. We used the Southern blot technique to determine whether T cells rearrange immunoglobulin J and C gene segments. Such rearrangements are clearly involved in V gene expression and heavy chain constant region switching in B cells. A rearranged DNA sequence could, however, give a band pattern identical to embryo DNA on Southern blots for two reasons: (a) The sequence is fortuitously rearranged to a new restriction fragment the same size as the embryonic fragment. (b) The restriction enzyme employed cuts a fragment that does not span the part of the DNA sequence rearranged. For example, the restriction enzyme might cut a fragment from the middle of a C_H gene stretching several kb to the 3' side of this gene. This cleavage would not detect DNA rearrangement in B cells because when the V and J gene segments are joined in these cells, all the sequences 3' to the constant region remain in the germ-line configuration. To reduce the possibility of missing a rearrangement, we generally hybridized each of our probes to separate T cell DNA samples cut with three different restriction enzymes. This is particularly important for the C57BL/6J-derived T cells because the position of restriction enzyme sites with respect to the immunoglobulin genes is not as well characterized in this inbred strain as it is in BALB/c mice. In total, we have carried out 45 separate comparisons of the T-cell DNA with liver, kidney, and embryo DNA using the heavy chain probes, and another 27 with the light chain probes (Table III, Table IV, M. Kronenberg. Unpublished observations.). All the restriction fragments that hybridize with immunoglobulin probes in T cells also are present in the nonlymphoid DNA. The J_κ, C_κ, C_λ, J_H, C_μ, and C_α gene segments, as well as the defined heavy chain constant region switch sites, are therefore not rearranged in the T cells analyzed. Although we have not directly tested for the rearrangement of the C_δ or C_γ heavy chain genes, it seems unlikely that T cells express these sequences because the J_H gene segments and heavy chain constant region switch sequences are not rearranged. Because the J_λ gene segment should be on the same EcoRI restriction fragment as the C_λ gene in BALB/c, we can also infer that the J_λ gene segment is not rearranged in WEHI-22.

It is difficult to draw definitive conclusions from Southern blots of thymus DNA. If some thymus cells rearrange immunoglobulin genes, we would expect each clone of cells to generate a new restriction fragment when it joins a J gene segment to a particular V gene segment. The detection limit on our Southern blots was between 0.1 and 0.5 copies/cell. The concentration in thymus DNA of any rearranged fragment particular to a given clone of cells would therefore be too low to detect on Southern

blots. Blots on thymus DNA then can only rule out a rearrangement common to most thymus cells that might, for example, be involved in the inactivation of the immunoglobulin gene loci. There is no evidence for joining of variable gene segments to J_H and J_κ gene segments in thymus, and our data clearly indicate that monoclonal, mature T cells, which certainly developed in the thymus, do not rearrange these gene segments.

Monoclonal T Cells Do Not Synthesize RNA Containing Immunoglobulin Sequences. In addition to testing for gene rearrangement, we analyzed T cells for transcription of immunoglobulin genes. We could not detect any C_κ, C_λ, C_μ, or C_α RNA in the monoclonal T cells. Because of the susceptibility of RNA molecules to enzymatic degradation, conclusions based upon negative data from Nothern blots must be viewed with caution. However, poly(A)$^+$ T-cell RNA electrophoresed on agarose gels and stained with ethidium bromide appeared intact as judged by general size distribution and the staining of some residual 18 and 28S ribosomal RNA bands. In addition, spleen and S117 myeloma RNA, prepared and handled exactly as the T-cell RNA were, and run in parallel on the same gels, gave strong hybridization with the radioactive immunoglobulin probes (data not shown). Finally, Southern blot analysis has indicated that the C_κ, C_λ, C_μ, and C_α gene segments are found in the germ-line configuration. Thus, if we did miss a small amount of RNA synthesis, the transcribed constant region gene was not close to a V gene segment, and this transcription is therefore unlikely to be involved in antigen-receptor biosynthesis. It has recently been reported that WEHI-22 cells synthesize about three copies of C_μ RNA/cell (49). We retested our poly(A)$^+$ RNA from WEHI-22, under conditions where we should have been able to detect ~0.4 copies/cell, and found no hybridization with the C_μ probe (M. Kronenberg. Unpublished observation.). This discrepancy is most probably caused by some heterogeneity in the WEHI-22 cell line.

Thymocytes May Synthesize Some RNA with C_μ Sequences. In preparations of total cell RNA from thymus, we find small amounts of C_κ, C_μ, and C_α RNA in the poly(A)$^+$ fraction. We have no information as to whether the immunoglobulin RNA is in the nucleus, free in the cytoplasm, or on polysomes, nor is it entirely clear which cell type synthesizes the RNA.

The sizes of the κ and α RNA are similar to that found in antibody-secreting B cells. It is therefore unlikely that this RNA originated from cells with unrearranged κ and α genes. In our experiments, B-cell contamination may be a likely explanation for RNA containing κ and α sequences. Given the amount of immunoglobulin RNA in plasmacytomas (50), if our thymus preparations contained ~0.01% plasma cells, we would have obtained the C_κ and C_α hybridization that was observed on Northern blots. Storb et al. have previously reported the presence of C_κ and C_α RNA in thymocytes, although they detected about 50-fold more RNA/cell than we did (51, 52). Conflicting data have been obtained on the question of whether or not the C_κ RNA is synthesized by contaminating B cells (53, 54).

The unusual molecular weight distribution observed for C_μ RNA in the thymus is similar to that previously observed for thymocytes (53), and for 7 out of 13 different T lymphomas ([49]; and D. Kemp. Personal communication.). This suggests that the C_μ RNA that we have detected may originate from thymocytes rather than contaminating B cells. Because most thymocytes are not immunologically competent (55), one could speculate that immature T cells synthesize μ heavy chain before switching

to expression of T cell constant regions. T cell-derived μ RNA is transcribed from unrearranged DNA and does not contain either V_H or J_H gene segments (D. Kemp. Personal communication.). The relationship of this C_μ sequence transcription to eventual expression of antigen receptors that presumably contain V regions is unclear.

The Gene for a Putative T Cell Constant Region Must Have Diverged by at Least 30% from the C_μ Gene Segment. There are reports in the literature that T cells synthesize a polypeptide serologically cross-reactive with μ heavy chain (25, 26). In DNA cleaved with several restriction enzymes, we have found only one band containing a single gene (15) that hybridizes with the C_μ probe. We can obtain a rough estimate of how far a homologous C_μ gene must have diverged in order to have not cross-hybridized with the C_μ probe. Hybridization of a C_μ probe to a homologous gene will depend upon a number of conditions including salt, temperature, percent guanine and cytosine content of the DNA sequence, and the length of the hybridizing fragments (56). Under our standard hybridization conditions, we are ~30°C below the temperature at which 50% of hybrid molecules formed will separate (T_m) for perfectly matched sequences. We therefore estimate that a gene 25% divergent in DNA sequence from a probe can be detected, and this has been empirically verified (S. Crews. Unpublished observations.). For a 55°C hybridization, we might hope to detect a gene that is another 10% (i.e., 35%) divergent from our C_μ probe. Although there are uncertainties in the estimates, given our Southern blot results, if there is a polypeptide homologous to μ made by T cells, it is likely that the gene coding for this protein diverged by at least 30% from the B cell C_μ gene sequence.

The T Cell Receptor Genes May Have J and C Gene Segments that are Distinct from Those of Their B Cell Counterparts. In Fig. 7 is given our model of the organization of the genes encoding the B and T cell antigen receptors. Evolutionary considerations indicate that multigene families can duplicate to generate new families that can acquire different functions and interact with gene products of the old family (57). This suggests that the gene families encoding the B and T cell antigen receptors could have evolved from a common ancestral gene family. If so, they should share common or homologous gene elements and mechanisms of DNA rearrangement for gene expression. This has been demonstrated for the B-cell κ, λ, and heavy chain gene families (9, 11–13, 57). Our model is based upon these evolutionary considerations, data drawn from the literature indicating that B and T cells express the same V_H gene segments (29–33), and the data presented in this paper demonstrating that T cells do not express B-cell J and C gene segments. Several points should be emphasized. (*a*) We presume that T cells express V_H gene segments through a mechanism similar to the mechanism that has been defined for B cells. This implies that T cells express V_H gene segments in conjunction with T cell-specific constant region (C_T) genes, and that there are multiple C_T genes that are expressed differentially on the functional subclasses of T cells. We believe that the rearrangement of a V_H gene segment, with
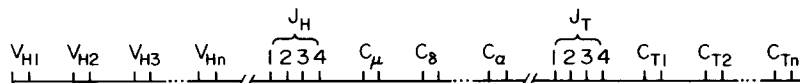

Fig. 7. A model of the genes encoding the B and T cell antigen binding receptors. Exons and intervening DNA sequences are not drawn to scale. Subscript T denotes gene segments expressed in T cells only. The position of the postulated J_T and C_T gene segment cluster with respect to the other indicated gene segments is unknown.

or without a D segment, to a T cell J gene segment (J_T) will place that V_H gene segment in the proper context for expression along with C_T genes. (*b*) The J_H gene segment codes for much of the third hypervariable region of B-cell derived antibody molecules (13). If T cells employ different J_H gene segments along with the B-cell V_H gene segments, then we might expect to find some differences in the antigen-binding specificities of T cells. This may partially explain experiments indicating that T cells recognize different antigenic determinants than B cells (58). In addition, some idiotypic markers that depend upon specific residues in the J region should be absent from T cell antigen binding receptors. This has implications for experiments employing anti-idiotypic reagents as probes for antigen-binding receptors and for the proposed regulation of immune responses via idiotypic antiidiotypic interactions. (*c*) Although there is much evidence for V_H expression, there is little evidence for V_L gene segment expression by idiotype-positive T cells (31, 32, 59). If T cells do express V_L gene segments, then the statements we make concerning the heavy chain gene family can be readily extended to include the light chain family. (*d*) If there is a C_T gene product that cross-reacts serologically with the μ chain, we have obtained data indicating the gene for this protein is not likely to be $>70\%$ homologous to the C_μ heavy chain gene sequence. This is not surprising because C_T genes probably diverged from B-cell C_H genes about the time of appearance of vertebrates with circulating immunoglobulin, and we would therefore expect only limited sequence homology between the B and T cell C gene clusters.

Summary

We have analyzed four kinds of T cells for rearrangement and expression of immunoglobulin genes. These cells include: (*a*) whole thymus; (*b*) WEHI-22, a T-cell lymphoma; (*c*) HT-1, an major histocompatability complex-restricted T helper line; and (*d*) CTLLi6, an H-2 alloreactive killer cell line. None of the B-cell joining and constant gene segments are rearranged in the T cells. The monoclonal cells do not express any C_κ, C_λ, C_μ, or C_α RNA species. Small amounts of C_κ, C_α, and C_μ sequences are present in RNA prepared from the thymus, although the significance of this RNA for T-cell antigen receptor synthesis is uncertain. The data support the hypothesis that expression of B-cell joining and C gene segments is unnecessary for T-cell helper and T-cell killer activity.

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