THREE T CELL HYBRIDOMAS DO NOT CONTAIN DETECTABLE HEAVY CHAIN VARIABLE GENE TRANSCRIPTS*

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There is considerable controversy as to whether or not the genes encoding the T cell antigen receptor are homologous to immunoglobulin gene segments. Results from a number of different experiments have been interpreted as evidence that T lymphocytes utilize heavy chain variable (V_H)[1] regions to bind specifically to antigen. For example, some antiidiotypic and anti-V_H framework sera interfere with T cell function and/or bind to antigen-specific factors secreted by T cells (1–3). In several cases, the gene encoding the cross-reactive determinant expressed by the T cells is linked to the immunoglobulin heavy chain gene cluster (4–9). Also, the genes encoding a series of T cell alloantigens have been mapped to chromosome 12, between the C_H gene locus, Igh-1, and the prealbumin gene (9, 10). Recently, these alloantigens have been detected on antigen-binding factors secreted by T cells (11–13). It has been proposed that the antigenic determinants encoded by C_H-linked genes are T cell isotypes that may be expressed in conjunction with V_H gene segments (10, 14). Furthermore, some T lymphocytes contain rearranged J_H gene segments or a C_μ transcript (15–23). This may indicate that the mechanisms controlling V_H-D-J_H joining and immunoglobulin transcription also operate upon homologous sequences in the synthesis of T cell antigen-binding receptors.

Experiments that report the expression of V_H serologic determinants by T lymphocytes have provided the most extensive and convincing data in support of V_H gene transcription by T cells. However, the serologic data are indirect, and there are three possible ways to interpret them. First, T and B cells

responding to the same antigen may express highly similar or identical V_H gene segments. This is unlikely since, in several experimental systems the receptor synthesized by T cells responding to an antigen does not share all the serologic determinants present on the immunoglobulin synthesized by B cells responding to the same antigen (1, 24). In addition, we and others have demonstrated that idiotype-positive T and B lymphocytes that respond to the same antigen do not transcribe highly similar V_H gene segments (25, 26). Second, it is possible that T cells use the repertoire of V_H genes differently than B cells do. This might occur because T lymphocytes do not express light chain genes (3, 4) or because T cells recognize antigen in the context of syngeneic MHC gene products. If this were true, then T cells responding to an antigen may transcribe V_H gene segments that have limited structural similarity to those transcribed in B cells responding to the same antigen, although these different V_H gene products could share some idiotopes. Finally, it is possible that the V_H cross-reactive determinants present on T cells and T cell factors are not the products of V_H genes.

In this paper, we report our attempts to determine whether any V_H gene segments are expressed in T lymphocytes. cDNA libraries were constructed from a suppressor T cell hybridoma specific for the synthetic polypeptide GAT, and from two helper T cell hybridomas, one specific for HGG and the second responding to KLH. The cDNA libraries were hybridized with two sets of probes; each set capable of detecting a wide range of V_H gene segments. In constructing the probes, no assumptions were made concerning the degree of homology between the B cell heavy chain variable regions binding GAT, HGG, or KLH and V_H gene transcripts that might be present in the T cell hybridomas. One set of probes was a synthetic oligonucleotide complementary to a conserved sequence found at the 3' end of many mouse V_H gene segments and a single-stranded cDNA synthesized primarily from the heavy chain variable genes present in spleen RNA. The second set of probes was two cloned V_H gene segments, one from the V_HII gene subgroup and one from the V_HIII gene subgroup. The cDNA libraries were sufficiently large so that the chance of detecting a sequence found in the nonabundant messenger RNA class (10–20 copies per cell) was excellent. Since no V_H-containing cDNA colonies were found, we conclude that V_H gene segments are not likely to encode the T cell antigen-binding receptor.

Methods

RNA Preparation. T cell hybridomas were grown in liquid culture and harvested. The cell pellets were lysed in guanidinium thiocyanate, and the RNA was prepared by centrifugation through a cushion of cesium chloride (27). The percent yield and the amount of RNA per cell were estimated using a recovery marker as previously described (20). RNA was similarly prepared from spleens of 6-mo old BALB/c Cum mice. Poly(A)$^+$ RNA was purified by two cycles of oligo(dT)-cellulose chromatography (28).

cDNA Synthesis. Double-stranded cDNA was synthesized as described (29, 30). First strand synthesis was initiated by random priming using sheared calf thymus DNA (31). The double-stranded cDNA was fractionated by gel filtration and the material ranging in size from 400 to 1,500 base pairs was pooled. The average length of the cDNA was ~800 base pairs. The cDNA was cloned into the Pst I site of the tetracycline-resistant plasmid pBR322 by annealing dC-tailed cDNA to dG-tailed vector (32). Bacterial strain MC1061 (33) was transformed with cDNA and the transformants were selected with tetracycline

(34). We obtained $\sim 10^6$ colonies per μg of cDNA. Transformation with vector alone (dG-tailed pBR322) yielded a 2% background.

Synthetic Oligonucleotides. Two undecamers were synthesized separately by Dr. S. Horvath (California Institute of Technology) by the phosphite coupling method (35, 36). The sequences were verified by the method of Maxam and Gilbert (37). The oligonucleotide probes were labeled with $5'$-$[\gamma$-^{32}P]dATP to a specific activity of >2 μCi/pmol using T4 polynucleotide kinase (38). Filters were prehybridized and hybridized with the radioactive oligonucleotides at 4°C as previously described (25). The filters were washed with several changes of 5X SET (0.75 M NaCl/0.15 M Tris, pH 8.0/5 mM EDTA) with 0.1% sodium pyrophosphate between 12 and 20°C and were then exposed to film.

Specially Primed cDNA Made from Spleen RNA. The primer was prepared by purifying a 2-kb Hpa II fragment containing the four J_H coding sequences from a subclone derived from the bacteriophage lambda clone ChSp $\mu27$, which contains germline BALB/c DNA. This 2-kb fragment was then digested with the restriction enzymes Dde I, Hae III, Pst I, and Rsa I. This results in a number of restriction fragments, including four that contain part of the J_H coding sequences (21). The restriction fragments were denatured by boiling and annealed to poly(A)$^+$ RNA from spleen. cDNA synthesis primed with the annealed J_H fragments was carried out as described (29). The concentration of α-^{32}P-labeled and unlabeled deoxynucleotide triphosphates was adjusted so that the synthesized material had a specific activity of 2–3 × 10^8 cpm/μg. RNA in the reaction was hydrolyzed with alkali and the single-stranded cDNA was separated from unincorporated nucleotides by gel filtration. The yield of cDNA was $\sim 0.5\%$ the mass of spleen RNA in the reaction; a fourfold stimulation over a reaction with no added primer. Filters were prehybridized at 50°C and hybridized at the same temperature with 5 ng/ml of J_H-primed cDNA for 48 h. Conditions were otherwise as previously described (25). Filters were washed at 50°C in several changes of 5X SET/0.1% sodium pyrophosphate/0.1% SDS before exposure to film.

V Region Probes. The plasmid p107V1 contains the entire gene segment coding for the heavy chain variable region expressed in the S107 myeloma (39). The V_H gene can be separated from the pBR322 vector DNA by digestion with Pst I. The plasmid pVH3 obtained by the laboratory of Dr. Sam Strober, was provided by Dr. Michael McGrath, Stanford University. This plasmid has a 1-kb Bam HI fragment that contains the heavy chain variable gene expressed by the BCL1 lymphoma (40). The 1-kb Bam HI fragment of pVH3 and the 445 base pair Pst I fragment of p107V1 were nick translated (41) to a specific activity of 1–8 × 10^8 cpm/μg. Filters hybridized with these probes were handled as described for the J_H-primed cDNA except that the probe was present at a concentration of 0.2–1.0 ng/ml.

Colony Hybridization. Nitrocellulose filters (HATF 13750, Millipore, Bedford, MA) were replica plated and prepared for in situ hybridization as described (42). Duplicate filters were annealed with each probe. For each screening, a positive control filter with colonies containing a heavy chain variable gene segment (MOPC21) was hybridized in parallel (Fig. 1).

Southern Blots. Plasmid DNA was prepared from clones isolated from the cDNA libraries. This DNA was digested with various restriction endonucleases, separated by molecular weight in 1% (wt/vol) agarose gels and transferred to nitrocellulose (43). The filters were then hybridized with the synthetic oligonucleotides or the J_H-primed spleen cDNA as described above.

DNA Sequencing. Restriction fragments were labeled at the $5'$ end with ^{32}P-γ-dATP using polynucleotide kinase (37) or labeled at the $3'$ end with ^{32}P-α-cordycepin-$5'$-triphosphate using terminal deoxynucleotidyl transferase (44). The labeled fragments were cut internally with a second enzyme and those isolated fragments were sequenced according to the method of Maxam and Gilbert (37).

Results

cDNA libraries were constructed from three different T cell hybridomas. Some features of the three hybrid cell lines are summarized in Table I. These
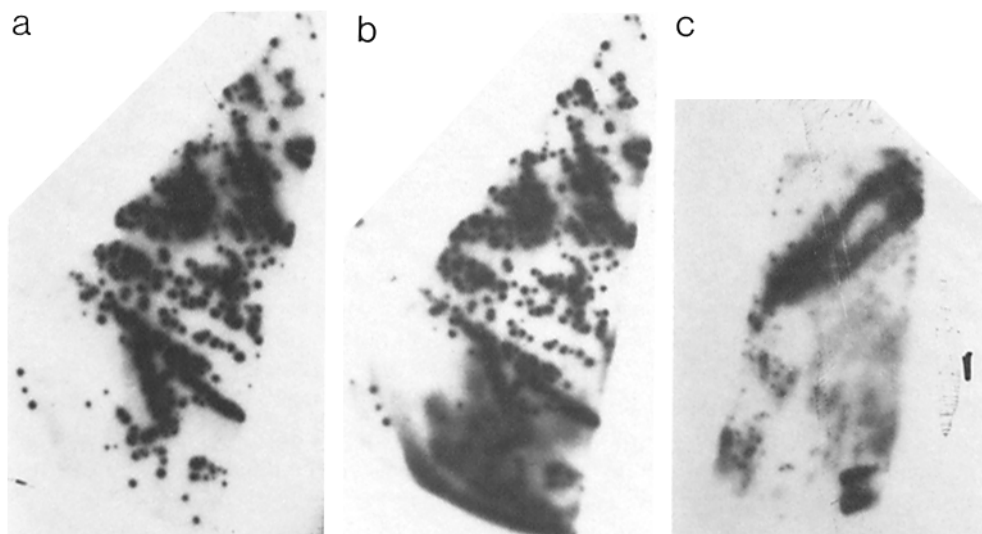
FIGURE 1. Positive control hybridizations to cDNA colonies containing a cloned V_H gene segment. A circular nitrocellulose filter containing DNA from several thousand identical pF9V21 (MOPC21) V_H cDNA clones was cut into sections. Hybridization conditions are described in the Methods section. The hybridization probes were: (a) Radiolabeled synthetic oligonucleotides. The filter was exposed to film for 12 h. (b) Radiolabeled J_H-primed spleen cDNA. The filter was exposed for 24 h. (c) pVH₃ plasmid containing the BCL1 V_H gene. The filter was exposed for 10 h. The same section of filter was hybridized to the synthetic oligonucleotides washed to remove the probe and was then hybridized with the J_H-primed spleen cDNA.

TABLE I

T Cell Hybrids Used to Construct cDNA Libraries

Hybridoma	Normal T cell parent	Fusion partner	Antigen specificity	Functional class	Reference
AODK 10.4	KLH immune B10.D2	AO40.10AG1	KLH + I-A^d	Helper	60
AODH 7.1	HGG immune DBA/2	AO40.10AG1	HGG + I-E^d	Helper	60
395A4.4	GT immune B10.S	BW5147	GT and GAT	Suppressor	59

cells were chosen for several reasons. First, they have retained function that is antigen specific, and in two cases MHC restricted. Second, they grow continuously in the absence of irradiated feeder spleen cells that, if present, could contribute contaminating immunoglobulin sequences to the T cell RNA preparations (21). Third, the hybridomas are specific for structurally unrelated antigens and are therefore likely to employ rather different antigen-binding receptors. If T lymphocytes do transcribe V_H gene segments, this should increase the probability that the probes will have sufficient homology to hybridize with a V_H gene transcript from at least one of the lines. Finally, two hybridomas help B cells secrete antibody and the third secretes a specific suppressor factor. Since most reports of idiotype expression by T lymphocytes involve the helper or suppressor functional subclasses, these types of cells may be best suited for the detection of V_H transcripts (2, 3, 7). To detect virtually any V_H gene transcript present in these cells, we employed two experimental strategies for screening the

cDNA libraries.

Hybridization with Synthetic Oligonucleotides and J$_H$-Primed cDNA. In the first attempt, two types of probes, synthetic oligonucleotides and a J$_H$-primed cDNA, were used to screen the libraries. Each probe will hybridize to a variety of V$_H$ gene segments, as well as to some sequences that do not contain V$_H$ genes. The frequency of such non-V$_H$ hybridizing sequences is low, so that a clone hybridizing with both types of probes probably contains a V$_H$ gene segment.

The sequences of the eleven-base synthetic oligonucleotides are 5' GCA CAG TAA/G TA 3'. These probes are complementary to a highly conserved sequence found at the 3' end (amino acids 95–98) of mouse heavy chain variable region gene segments. A sequence perfectly complementary to either oligonucleotide is found in 50% (17/34) of the murine V$_H$ genes for which DNA sequence data are available. The degree of homology of the cloned murine V$_H$ gene segments to the oligonucleotides is listed in Table II. Furthermore, in those cases for which no DNA sequence but amino acid sequence data are available, 78% (18/23) of the mouse immunoglobulin variable regions have tyr-tyr-cys-ala at positions 95–98, and therefore may share complete homology to one of the probes (45, 46). However, the oligonucleotide is not long enough to identify unambiguously a V$_H$ gene segment. Given the number of nucleotides of genomic DNA transcribed into RNA, and the random chance of occurrence of an 11-nucleotide sequence, we calculate that any mouse cell, whether it synthesizes immunoglobulin or not, should contain about five species of messenger RNA that will hybridize with each oligonucleotide.[2]

The second type of probe was a cDNA synthesized from spleen RNA (Fig. 2). The spleen contains a relatively high proportion of B cells that should express many different immunoglobulin heavy chains. Therefore, it was possible to use J$_H$ DNA as a primer to stimulate the synthesis of a radioactive single-stranded cDNA complementary to many V$_H$ genes. Using either Southern blots or hybridization to cloned DNA spotted onto nitrocellulose filters, the specifically primed cDNA hybridized to all five V$_H$ gene segments that were tested. These include T15, MOPC21, V$_H$B2, V14A, and V14B (V14B did not hybridize on one blot [21], but did on a second attempt). Since two of the hybridizing gene segments, T15 and V$_H$B2, share <60% homology, this probe should hybridize to a large number of different V$_H$ sequences. In addition, the specifically primed cDNA does hybridize to a few cloned DNA segments that do not contain V$_H$ genes (M. Kronenberg, unpublished observations). There are two explanations for hybridization to sequences lacking V$_H$ genes: (*a*) There is a significant amount of cDNA synthesis in the absence of added primer (see Methods) that should not be enriched for immunoglobulin sequences. (*b*) The primer DNA is a mixture of restriction fragments from both the J$_H$ gene segments and the intervening and

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[2] A number of measurements have indicated that most mammalian cells, including lymphocytes, contain $\sim$10^4 different sequences or species of mRNA of average length 2×10^3 nucleotides (48–51). Thus, $\sim$2 $\times$ 10^7 base pairs ($= 10^4 \times 2 \times 10^3$) of genomic DNA are transcribed into mRNA. The random chance of any one of the four nucleotides occurring at a particular place in a DNA sequence is 1/4; therefore, if we ignore the effects of base composition and nearest neighbors, the probability that an 11-nucleotide sequence will occur is $(1/4)^{11} = 2.5 \times 10^{-7}$. Multiplying the probability of occurrence for the undecamer by the number of nucleotides in the mRNA gives the number of different mRNA species expected to be perfectly complementary to the oligonucleotide, $(2 \times 10^7)(2.5 \times 10^{-7}) = 5$.

TABLE II

Homology of Murine V_H Gene Segments to the Hybridization Probes

Cloned V_H gene segment	Derivation of clone*	Homology of cloned V_H gene segments to the hybridization probes			Comments	Reference
		Synthetic un-decamers‡	S107V1§	BCL1§		
$V_HT15(V1)$	G	11/11	100	56	T15 gene family[I]	39, 70
V11	G	11/11	90	59	T15 gene family	70
V13	G	11/11	86	56	T15 gene family	70
S107V1	R	11/11	100	56	T15 gene family	39
M603	R	11/11	98	56	T15 gene family	39
MOPC167	R	10/11	96	56	T15 gene family	71
V14A	G	10/11	75	55	—	S. Crews, unpublished
V14B	G	10/11	73	54	—	S. Crews, unpublished
V_H76	R	10/11	74	55	—	72
V102	G	11/11	58	77	S43 gene family	73
V23	G	10/11	58	78	S43 gene family	73
V3	G	11/11	59	76	S43 gene family	73
V186-1	G	9/11	58	76	S43 gene family	73
V186-2	G	10/11	58	76	S43 gene family	73
S43	R	10/11	56	74	S43 gene family	73
B1-8	R	10/11	58	76	S43 gene family	73
pCH105	G	10/11	60	78	MPC11 gene family	74
pCH108A	G	11/11	59	82	MPC11 gene family	75
pCH108B	G	11/11	57	80	MPC11 gene family	75
MPC11	R	10/11	59	74	MPC11 gene family	76
V_H101	G	10/11	59	57	—	77
V_H101	R	11/11	60	57	Related to V_H101-G	77
PJ14	G	11/11	57	56		78
M141	R	10/11	57	57	Related to PJ14	78
V_H441	G	11/11	71	57		79
UPC10	R	10/11	70	57	Related to V_H441	80
BCL1	R	8/11	56	100	—	40
J558	R	11/11	—**	—**	—	Unpublished
MOPC21	R	11/11	71	58	—	73; this paper
V_HB2	R	11/11	54	74	—	81
V_HB49	R	11/11	56	50	—	81
V_HGAT	R	10/11	57	71	—	25
93G7	R	10/11	56	78	—	82
G5B2.2	R	11/11	58	73	—	83, 84

* G, germline V_H gene segment; R, rearranged V_H gene segment. Five gene segments considered to be pseudogenes have not been included in this compilation.

‡ Synthetic oligonucleotides we synthesized are: 5'GCACAGTA^A/$_G$TA3'. The degree of homology is expressed as a fraction, the denominator being the length of the oligonucleotides (11) and the numerator being the maximal number of residues homologous to either undecamer.

§ Percent homology of V_H gene segments to the S107V1 and BCL1 probes. Gaps were introduced where appropriate to compensate for the different lengths of the hypervariable regions. The complete amino acid sequence of the protein produced by the S107 myeloma and the nucleotide sequence of the germline gene (V1) encoding this protein are both available. However, the nucleotide sequence of the rearranged S107V1 cDNA we used as a probe is not complete. Based on the protein sequence we have assumed the rearranged and germline genes are identical, although the possibility of a few silent nucleotide substitutions has not been ruled out.

I Gene family denotes a set of closely related sequences. Some members of the T15 family are involved in the response to phosphorylcholine. Some members of the S43 family are involved in the response to NP (4-hydroxy-3-nitrophenylacetyl).

** Insufficient nucleotide sequence data are available.

nearby flanking sequences. Some of the fragments from the noncoding DNA may prime cDNA synthesis from nonimmunoglobulin sequences in spleen RNA. It is possible that some of the nonimmunoglobulin sequences that hybridize with the probe are repeated DNA sequences that are transcribed abundantly in spleen cells. We have not, however, characterized these hybridizing sequences.

To demonstrate that these probes can detect a V_H sequence we screened a cDNA library made with RNA extracted from a B cell hybridoma (25). A colony
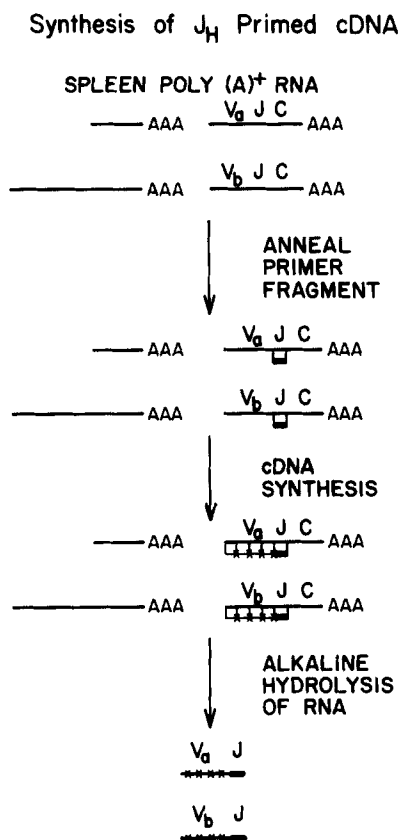
Synthesis of J_H Primed cDNA

SPLEEN POLY (A)$^+$ RNA

FIGURE 2. Synthesis of J_H-primed cDNA from spleen RNA. Coding sequences on the immunoglobulin heavy chain messenger RNAs are indicated as follows: C, constant region; J, joining gene segment; V_a and V_b, two different V_H gene segments. The J_H primer is indicated by the thick horizontal line. The short vertical lines represent hydrogen-bonded base pairs and the asterisks represent radioactive nucleotides incorporated into cDNA.

that hybridized with both the oligonucleotide and the J_H-primed cDNA (21) was characterized further. The nucleotide sequence of this cDNA clone (Fig. 3) indicates that it encodes the MOPC21 heavy chain variable region synthesized by the P3-X63-Ag8 myeloma parent cell. The clone includes almost the entire V_H gene segment beginning at the codon for amino acid 2 as well as the entire D and J_H4 gene segments.

Having determined that these probes were able to detect B cell V_H gene segments, we screened the three T cell cDNA libraries with the synthetic undecamers. The filters were hybridized and washed under conditions such that 11/11 homology was required to give a positive signal. 54 positive colonies were found (Table III). A single filter containing three hybridizing colonies is shown (Fig. 4). The frequency of positives was low, indicating that these cells do not contain abundant RNA molecules with sequences complementary to the undecamers. Northern blots hybridized with the synthetic oligonucleotide gave a similar result (M. Kronenberg, unpublished observations). In fourteen cases, the colony that hybridized with the oligonucleotide was isolated and the plasmid

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    Q  L  V  E  S  G  G  G  L  V  Q  P  G  G  S  R  K  L  S  C  A  A  S  G  F
1   TGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAGGGTCCCGGAAACTCTCCTGTGCAGCCTCTGGATTC   77

     T  F  S  S  F  G  M  H  W  V  R  Q  A  P  E  K  G  L  E  W  V  A  Y  I  S
78  ACTTTCAGTAGCTTTGGAATGCACTGGGTTCGTCAGGCTCCAGAGAAGGGGCTGGAGTGGGTCGCATACATTAGT     152

     S  G  S  S  T  L  H  Y  A  D  T  V  K  G  R  F  T  I  S  R  D  N  P  K  N
153 AGTGGCAGTAGTACCCTCCACTATGCAGACACAGTGAAGGGCCGATTCACCATCTCCAGAGACAATCCCAAGAAC     227

     T  L  F  L  Q  M  T  S  L  R  S  E  D  T  A  M  Y  Y  C  A  R  W  G  N  Y
228 ACCCTGTTCCTGCAAATGACCAGTCTAAGGTCTGAGGACACGGCCATGTATTACTGTGCAAGATGGGGTAACTAC     302
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FIGURE 3. Nucleotide sequence of a V_H cDNA clone (F9V21) detected with the synthetic oligonucleotides and J_H-primed spleen cDNA. The predicted amino acid sequence is shown above the DNA sequence. The sequence agrees with one previously reported for the MOPC21 V_H gene (73), except for the 209th nucleotide, which was cytidine instead of adenosine. This difference is silent with respect to the predicted amino acid sequence. As noted previously, there are six discrepancies between the published amino acid sequence for MOPC21 and the nucleotide sequence (45, 73). The F9V21 cDNA clone begins in the middle of the codon for the second amino acid, and contains the entire V_H, D, and J_H4 gene segments. A portion of the D and the entire J_H gene segment are not shown. The 11-nucleotide sequence complementary to one of the synthetic nucleotides is underscored.

TABLE III

Colony Hybridization Results

cDNA Library	Synthetic oligonucleotides			J_H-Primed spleen DNA			S107 V_H and BCL1 V_H		
	Colonies hybridized	Positive colonies	Frequency	Colonies hybridized	Positive colonies	Frequency	Colonies hybridized	Positive colonies	Frequency
AODK10.4	140,000	7	1/20,000	26,000	55	1/500	140,000	0	—
AODH7.1	200,000	4	1/50,000	18,000	10	1/1,800	200,000	0	—
395A4.4	920,000	43	1/21,000	200,000	145	1/1,400	920,000	0	—

DNA prepared from the bacterial clone. Southern blots of this plasmid DNA also hybridized with the oligonucleotide, thereby confirming the colony hybridization results (Fig. 5).

Following hybridization with the oligonucleotides, the three cDNA libraries were screened with the J_H-primed cDNA. To test for colonies homologous to both probes, all filters that contained a colony that annealed with the oligonucleotide were hybridized with the cDNA probe. The frequency of positive colonies was 15- to 40-fold higher with the J_H-primed spleen cDNA than was obtained with the synthetic probe (Table III). However, none of the colonies that hybridized with the spleen cDNA also hybridized with the oligonucleotide (Fig. 4). Plasmid DNA isolated from fourteen colonies that hybridized with the undecamer was also tested with this probe. None of the isolated plasmid DNA hybridized with the J_H-primed cDNA (Fig. 5).

Hybridization with Cloned V_H DNA Sequences. Using cloned V_H DNA probes and hybridization conditions of decreased stringency, it is possible to detect cDNA colonies containing V_H genes that are only 55–60% homologous to the probe (S. Crews, unpublished observations, Fig. 1). We therefore screened the three T cell cDNA libraries with V_H probes from the S107 (V_H subgroup III) and BCL1 (V_H subgroup II) tumors. These were chosen because the complete DNA sequences of these V_H gene segments are available and because fragments of the appropriate sizes are easily prepared without contaminating vector or
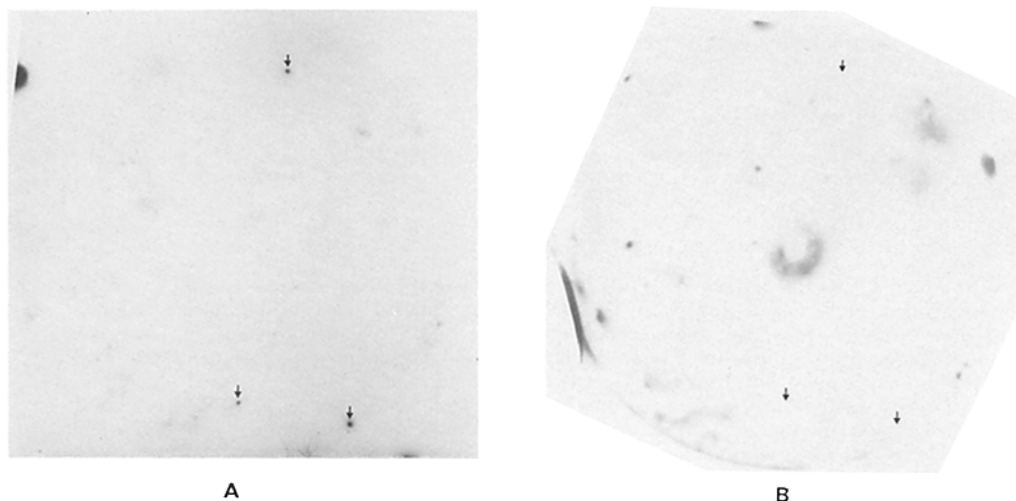
FIGURE 4.   Hybridization of the synthetic oligonucleotides and J_H-primed spleen cDNA to T cell cDNA colonies. A single circular filter containing several thousand colonies from the 395A4.4 suppressor T cell cDNA library is shown. (A) Hybridization with the synthetic oligonucleotide. The arrows indicate three colonies to which hybridization of the radiolabeled synthetic oligonucleotides was detected in a 72-h exposure. A duplicate filter gave the same pattern of oligonucleotide-positive colonies. (B) Hybridization with the J_H-primed spleen cDNA. After incubation of the filter at 50°C to remove the hybridized oligonucleotides, the same filter as shown in (A) was hybridized with the radiolabeled J_H-primed spleen cDNA. The signal from positive colonies varies and some nonspecific background is present. The filter was exposed for 48 h. The arrows mark the position of the three oligonucleotide-positive colonies. None of the colonies hybridized with both probes.

much flanking DNA. However, there were no colonies in the three T cell cDNA libraries that hybridized with either of these probes. A positive control filter with colonies containing the MOPC21 V_H gene segment hybridized with pVH3, which contains the V_H gene expressed in BCL1 (Fig. 1). The MOPC21 and pVH3 V_H gene segments share only 58% homology. Table II indicates that the V_H gene segments for which nucleotide sequences are available have greater than 56% homology to at least one of our probes. Therefore, it is likely that we could have detected all of these V_H genes.

Discussion

We have utilized several strategies to evaluate whether T cells express any V_H gene segments. To accomplish this, we had to construct DNA probes capable of detecting many different V_H genes. The Northern blot hybridization is the most direct method to test for a particular transcript present even at the level of a single copy per cell (20). For the detection of possible V_H transcripts in T cells, we decided instead to screen large cDNA libraries. There are two reasons for doing this. First, we have found that it is possible to detect V_H sequences <60% homologous to the probe in hybridizations to cDNA colonies (Fig. 1), while >80% homology is required when hybridizing under conditions of moderate stringency to Northern blots (25). This difference may reflect a number of factors, including the concentration of the filter-bound nucleic acid. In addition,
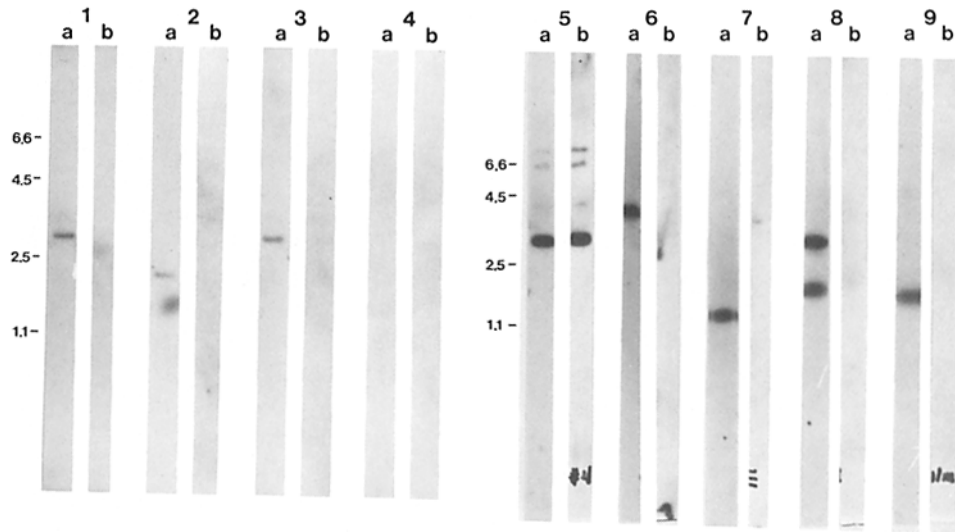
FIGURE 5. Southern blots of some oligonucleotide-positive T cell cDNA colonies. Plasmid DNA was prepared from several T cell cDNA clones that hybridized with the synthetic oligonucleotides. Restriction mapping of the plasmids indicated they contain an average of 800 base pairs of mouse cDNA inserted into the pBR322 cloning vector (4.36 kb). The purified plasmid DNA was digested with the restriction enzymes Eco RI and Pvu II. Digested DNA was electrophoresed on 1% agarose gels and blotted onto nitrocellulose sheets. Migration distances of some molecular weight markers, and their lengths in kilobases, are indicated. Lanes 1–3 and 6–9 contain DNA from separate oligonucleotide-positive colonies isolated from the 395A4.4 library. Lane 4 contains pBR322 vector DNA. Lane 5 contains the MOPC21 V_H cDNA. (a) Hybridization with the radiolabeled synthetic oligonucleotides. Exposure was for 3 h. (b) Hybridization with the radiolabeled J_H-primed cDNA. The filter was exposed for 24 h.

we have achieved a greater hybridization signal to filter-bound DNA as opposed to RNA, even when both nucleic acids contained identical sequences, were electrophoresed in parallel, and were hybridized to the same probe in the presence of 50% formamide (M. Kronenberg, unpublished observations). Second, the initial strategy to detect a V_H gene segment involved the use of two probes that could hybridize to a number of sequences, including those not containing V_H genes. Since there are multiple sequences present in the poly(A)$^+$ RNA that hybridize to these probes, we detect diffuse smears rather than discrete bands on Northern blots. By contrast, each cloned cDNA in the library is physically separate and could be analyzed individually for the possibly rare sequences that hybridize with both probes.

The cDNA libraries were screened with a synthetic oligonucleotide and a J_H-primed cDNA made from spleen RNA. This method has been characterized extensively by test hybridizations (21) and by the cloning and sequencing of the MOPC21 V_H cDNA from a B cell hybridoma (Fig. 3). Of the ~1.2 × 10^6 colonies from three T cell cDNA libraries hybridized with the synthetic oligonucleotide, 54 clones were positive. Because of the relatively short length of the probe, we would have predicted, on statistical grounds, the existence of such colonies even in cDNA made from nonlymphoid RNA. None of these colonies also annealed with the specifically primed spleen cDNA when tested in situ (54

cases) or after Southern blotting the purified plasmid DNA (14 cases). A relatively large number of colonies did, however, hybridize with the spleen cDNA probe. The identification of colonies that hybridized with either one of the two probes provided an internal control and indicates that there was no technical problem with the screening that would have prevented us from identifying clones of interest. Positive control hybridizations with V_H-containing colonies support this conclusion. Since no colonies hybridized with both probes, we conclude that V_H gene segments are absent from these libraries. Hybridization with cloned V_H DNA gene segments under conditions whereby sequences <60% homologous to the probe could be detected constituted a second, independent test for the presence of V_H genes. By hybridizing the cDNA libraries with just two V_H sequences from different heavy chain subgroups, we should be able to detect all of the well-characterized V_H genes. No T cell cDNA colonies hybridized with these probes, confirming the result obtained by the first method.

While B lymphocytes expressing cell-surface IgM contain 100–200 copies of C_μ RNA per cell (17, 47), a messenger RNA for the T cell receptor may not be this prevalent. The bulk of the 5,000–15,000 sequences found in most eucaryotic cells, including lymphocytes, are in the low abundance class (10–20 copies/cell) (48–51). Our calculations indicate that we had a good chance of detecting V_H sequences transcribed at this level (Table IV). For example, if there were a V_H sequence homologous to the probes and present at 10 copies per cell, the probability of detection would be 77% for the AODK 10.4 cDNA library, 88% for the AODH 7.1 cDNA library, and >99% for the 395A4.4 library. The probabilities of detecting a sequence present at 15 or 20 copies per cell are higher. If each library construction and screening were an independent event, then the overall probability of not obtaining a V_H clone that is present at 10 copies per cell becomes extremely low (0.23 × 0.12 × 0.01 = 0.00028). This analysis cannot exclude the possibility of expression of V_H genes at one or even a few copies per cell. However, there is some indication that these hybrids synthesize a significant amount of receptor protein. The T cell hybridoma AODH 7.1 binds antigen avidly in the presence of the proper antigen-presenting cells

TABLE IV

Probability of Cloning a Rare mRNA from the T Cell cDNA Libraries

Hybridoma	pg RNA per cell (yield)*	pg RNA per cell (total)‡	pg Poly(A)+ RNA per cell (yield)*	N (Colonies)§	1 Copy/cell		10 Copies/cell		15 Copies/cell	
					f∥	P**	f	P	f	P
AODK10.4	5.8	9.3	0.15	105,000	1/715,000	0.14	1/71,500	0.77	1/46,500	0.90
AODH7.1	3.3	5.0	0.05	150,000	1/715,000	0.19	1/71,500	0.88	1/46,500	0.96
395A4.4	3.1	4.8	0.13	690,000	1/715,000	0.62	1/71,500	<0.99	1/46,500	<0.99

* RNA yield estimated from absorbance at 260 nm.

‡ Calculated by multiplying the yield of RNA by the recovery of a small amount of ³H-labeled sea urchin RNA added to the preparation. Recovery was at least 60% in each case.

§ N is the number of colonies in the library hybridized; N has been corrected on the assumption that 25% of the RNA is ribosomal or other nonpolyadenylated species. This fraction was estimated following gel electrophoresis of the poly(A)+ RNA.

∥ f is the fraction of the mass of total cell poly(A)+ RNA present in a given sequence. We assumed each cell contains 0.3 pg of poly(A)+ RNA. We considered a sequence of 800 nucleotides, the average length of the cDNA clones, present at either 1, 10, or 15 copies per cell.

** Probability of cloning a gene calculated from the formula. $P = 1 - (1-f)^N$; assumes the probability of detecting a clone is a function of its abundance in the RNA population. See discussion for details.

and almost all the cells in the culture retain this ability (J. Kappler and P. Marrack, unpublished observations). In addition, the 395A4.4 hybridoma constitutively synthesizes both an antigen-binding suppressor factor and an antigen-binding receptor. We therefore consider it unlikely that the receptor mRNA could be present at an extremely low-copy number.

The calculated detection limit depends on a formula that assumes that the probability of detecting a cDNA is solely a function of the abundance of its RNA template in the population (52, 53). This will be true only if the synthesis and cloning of cDNAs uses all templates with equal efficiency. Although factors such as secondary structure of an RNA (54) may influence the ultimate cloning efficiency, there does not appear to be a selection against cloning of heavy chain variable region cDNA. In addition, since the bulk of the first strand of cDNA synthesis is shorter than the average 2-kb length of an mRNA, there is an intrinsic bias towards obtaining cDNA clones containing sequences close to the point of initiation of synthesis. By using a sheared calf thymus DNA primer to initiate cDNA synthesis randomly at many points along the RNA templates, as opposed to an oligo(dT) primer that will initiate synthesis only at the 3' end, we eliminated any bias towards obtaining clones corresponding to only one end of the messenger RNA. Given these considerations, we feel justified in using the formula shown in Table IV. Finally, in order to calculate the probability of cloning a particular messenger RNA, we needed to estimate the amount of poly(A)$^+$ RNA per cell. Each of the hybrid cells contains between 5 and 10 pg of total RNA (Table IV). We assumed that 0.3 pg of this total is in the poly(A)$^+$ fraction, although our actual yield was substantially lower, between 0.05 and 0.15 pg per cell. Poly(A)$^+$ RNA selected by oligo(dT)-cellulose chromatography will contain a residual poly(A)$^-$ component that is mostly ribosomal RNA. Since this material may give rise to a proportionate fraction of the cDNA colonies in Table IV, we corrected N, the number of colonies screened, to account for the contaminating poly(A)$^-$ RNA.

The calculations are based upon reasonable estimates of the purity of the poly(A)$^+$ RNA and the amount present per cell. However, if we assume there is somewhat more than 0.3 pg of poly(A)$^+$ RNA per cell, or if the poly(A)$^+$ fraction of the RNA preparation is <75% of the total mass, the basic conclusion remains valid. For example, if the oligo(dT)-passaged RNA were only 50% pure, the probability of detecting a homologous V_H sequence present at 10 copies per cell becomes 63% for the AODK 10.4 cDNA library, 75% for the AODH 7.1 library, and >99% for the 395A4.4 library.

Although unlikely, it is possible that the three T lymphocytes might express a V_H gene that could not be detected by either of our two screening methods. The DNA sequence homology of mouse V_H gene segments to our probes is presented in Table II. The J_H-primed spleen cDNA hybridized with all of the five V_H gene segments tested (21). In addition, homology with the undecamers does not appear to be restricted to any particular type of heavy chain. Some murine V_H genes coding for proteins in subgroups I, II, and III, as well as three out of four human V_H gene segments (all subgroup III) have complete homology to these probes (55, 56). Even two mouse germline V_λ gene segments have 10/11 matching nucleotides (57, 58). However, the synthetic oligonucleotides will not

hybridize with half of the known murine V_H gene segments. In principle, none of these genes would have been detected by our first method. However, all of the V_H gene segments listed in Table II are >56% homologous to one of the two cloned V_H probes and probably could be detected by the second method.

Estimates of the ability of the probes to detect different V_H gene segments depend upon comparison with the known DNA sequences of relatively few variable gene segments. Almost all of these sequences come from V_H subgroups II and III and a large proportion are members of the gene families involved in binding the NP and PC haptens. It is not certain how well these sequences represent the total germline V_H gene repertoire. To increase the probability of detecting V_H expression, we constructed cDNA libraries from three T cell hybridomas responding to presumably dissimilar antigens. Since each of these cells maintained antigen-specific function, if V_H genes encode the T cell antigen-binding receptor, each should synthesize an RNA containing a V_H gene segment. Because the hybrids are the product of two (395A4.4) or more parental T cells (59, 60), they might be expected to express two or more V_H genes if, as in B cell hybrids, V_H gene segments are transcribed from several chromosome 12 homologues. In addition, it has been suggested that even a single diploid T cell may synthesize more than one V_H RNA (61, 62). Thus, if T cells use the entire V_H gene repertoire, we believe there would have been a V_H segment sufficiently homologous to have been detected by one of our two screening methods. Although it remains formally possible that T cells express selectively a portion of the B cell V_H gene repertoire containing sequences only distantly related to our probes, no such V_H genes have been characterized.

We have presented strong evidence in favor of the proposition that the helper and suppressor T cells tested do not contain RNA with V_H gene segments. This negative conclusion is not, however, completely compelling and two major objections concerning the detection limit and the range of our library screening, have been discussed. A number of unlikely possibilities, including selection against cloning the V_H-containing sequences or a V_H mRNA that is present mostly in the poly(A)$^-$ fraction, have also not been eliminated. However, using different methods, another laboratory has reported that T lymphocytes do not transcribe any V_H gene segments (63).

Many of the genes that have dominated our thinking about the immune response including β_2-microglobulin and the class I and class II products of the MHC, show clear homology to immunoglobulin genes (64–68). The T cell and B cell antigen-binding receptors presumably have somewhat homologous functions. We would be surprised, therefore, if the genes encoding the T cell antigen receptor were to have no homology to immunoglobulin genes. Since multigene families are known to duplicate and diverge (69), it is possible that gene families important for B cell and T cell antigen recognition diverged before or relatively early in vertebrate evolution. Attempts to clone T cell receptor genes using V_H probes might therefore not be feasible, somewhat analogous to attempting to clone V_κ gene segments using V_λ probes. At this point, we can only speculate on the selective forces that may have resulted in separate V gene families expressed in B and T cells.

Summary

We attempted to determine whether T cells express any V_H gene segments. cDNA libraries were constructed from one suppressor and two helper T cell hybridomas. Both the library construction and screening were designed to maximize detection of a wide range of V_H gene segments. One screening method should detect about half of the sequenced V_H genes, while the second should detect most of these genes. The probability of detecting a V_H gene homologous to the probes and present at 10 copies per cell was 77% for one helper cell cDNA library, 88% for the second helper cell library, and >99% for the suppressor cell library. No cDNA clones with V_H gene segments were detected. From this result, we conclude that V_H gene segments are not likely to encode the antigen-specific receptor in the cells we tested.

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