

T AND B CELLS THAT RECOGNIZE THE SAME ANTIGEN DO
NOT TRANSCRIBE SIMILAR HEAVY CHAIN VARIABLE
REGION GENE SEGMENTS*

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The generation of a humoral immune response depends on the interaction between B lymphocytes, which synthesize immunoglobulin, and other cells, including T lymphocytes, which function to regulate the B cell response. Although an organism can respond to a large set of diverse antigens, individual T and B cells synthesize receptors that can recognize only a few related antigenic determinants. B cells bind antigen through cell-surface immunoglobulin, a molecule composed of two identical heavy (H)¹ and two identical light (either λ or κ) polypeptide chains. During differentiation, individual B cells undergo a series of DNA rearrangements, becoming committed to the synthesis of immunoglobulin of a single specificity. The light chain variable region in both the λ and κ gene families is encoded by two distinct gene segments, V_λ or V_κ and J_λ or J_κ , which become joined by a DNA rearrangement to produce a transcriptionally active light chain gene (1). Similarly, the heavy chain variable region is composed of three separate gene segments, V_H , D, and J_H , which become joined to form the expressed heavy chain gene (2).

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¹ Abbreviations used in this paper: AMV, avian myeloblastosis virus; C, constant region; CGAT, predominant idotype in murine response to GAT; D, diversity gene segment; dATP, deoxyadenosine triphosphate; dCTP, deoxycytidine triphosphate; dGTP, deoxyguanosine triphosphate; dT, deoxythymidine monophosphate; GA, synthetic polymer of glutamic acid⁶⁰, alanine⁴⁰; GAT, glutamic acid⁶⁰, alanine³⁰, tyrosine¹⁰; GT, glutamic acid⁵⁰, tyrosine⁵⁰; H, immunoglobulin heavy chain; HAT, medium containing hypoxanthine, aminopterin and thymidine; Ig, immunoglobulin; IL-2, interleukin 2 or T-cell growth factor; J, joining gene segment; κ , kappa light chain; kb, kilobase (1,000 nucleotides); λ , lambda light chain; L, leader; PC, phosphorylcholine; SET, 0.75 M sodium chloride/0.15 M Tris, pH 8/5 mM ethylenediaminetetraacetic acid; T_H, helper T cell; T_S, suppressor T cell; T_S1, subset of GAT-specific suppressor T cells that secretes T_SF₁, an antigen-binding factor composed of a single polypeptide chain; T_S2, subset of suppressor T cells that secretes T_SF₂, a factor containing two polypeptide chains, one of which binds antigen; T_SF, T-cell suppressor factor; UT, untranslated; V, variable region.

T cells also exhibit a high degree of antigen specificity, but the molecular nature of the T cell receptor for antigen is not well characterized. Previous work has shown that the antigen receptor on T cells is not a conventional immunoglobulin; many functional murine T cells do not transcribe the C_H , C_λ , C_κ , J_H , J_λ , or J_κ gene segments that are required for encoding a complete immunoglobulin molecule (3). However, serological studies have revealed that T cells involved in the responses to synthetic polypeptides (4–6), carbohydrate residues (7), alloantigens (8), haptens (9–11), and protein antigens (12) express cross-reactive idiotypic determinants characteristic of immunoglobulins that bind the same antigen. Antiidiotypic sera have been used to perturb T cell function both in vivo and in vitro (7, 11, 13, 14) and are capable of binding antigen-specific factors secreted by T cells (4, 5, 9). Furthermore, in a number of systems, the expression of idiotypic determinants by T cells is linked to the C_H locus (9, 15–18). As summarized previously (19), two conclusions often have been drawn from experiments demonstrating idiootype expression by T lymphocytes. First, it has been proposed that T cells transcribe V_H gene segments joined not to the immunoglobulin D, J_H , and C_H gene segments, but to T cell-specific constant region gene segments. Second, it has been hypothesized that T and B cells utilize the V_H gene repertoire similarly, such that the two types of lymphocyte will transcribe identical or closely related V_H gene segments in response to the same antigen.

We set out to determine whether T and B cells that respond to the same antigen and share idiotypic determinants do in fact transcribe the same V_H gene segments. We have chosen to analyze the murine immune response to the synthetic polypeptide, glutamic acid⁶⁰, alanine³⁰, and tyrosine¹⁰ (GAT), for two reasons. First, the B cell response to GAT displays limited diversity, as evidenced by a restricted isoelectric focusing pattern (20), a predominant idiootype, CGAT (21), and >90% amino acid sequence identity for the NH_2 -terminal residues of five monoclonal anti-GAT heavy chains (22). Therefore, it is likely that there are, at most, a few highly homologous V_H gene segments expressed in the murine B cell response to GAT. In fact, most of the NH_2 -terminal sequences of heavy chains from both GAT and GA-binding immunoglobulins are quite similar, regardless of the presence of the CGAT idiootype (22). Second, GAT-specific T cells of different functional classes have been maintained either as clones or hybridomas (6, 23–26, and footnote 2)² and many display CGAT idiootypic determinants (6, 25, 26). Therefore we can address directly whether a set of GAT-specific T-cell clones, some of which express idiootype, transcribe the GAT V_H gene segment.

We isolated a cDNA clone encoding the GAT V_H from a GAT-specific B cell hybridoma. Using the GAT V_H probe, we tested RNA from 10 GAT-specific T suppressor hybridomas, one GAT-specific helper hybridoma, and two GAT-specific T helper cell lines. Although six of these T cells express the CGAT idiootype on secreted, antigen-binding factors, we were unable to detect a GAT V_H transcript in any of the T cell RNAs. We therefore conclude that T and B

² Abruzzini, A. F., J. A. Kapp, and C. W. Pierce. Establishment of long term T cell lines and clones specific for L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰ and maintenance without filler cells, manuscript in preparation.

TABLE I
Characteristics of T Cell Clones

T cell clones	Strain of normal T cell parent	Fusion partner	Immunogen	T cell functional class	Type of factor*	CGAT idiotype		Antigen specificity		
						Anti-sera [‡]	Monoclonal [‡]	GAT	GA	GT
258C4.4	DBA/1	BW5147	GAT	Suppressor	T _S F ₁	+	+	+	+	-
342B1.11	B10.S	BW5147	GAT	Suppressor	T _S F ₁	+	+	+	-	+
365C6.4	B10.S	BW5147	GAT	Suppressor	T _S F ₁			+		
367A5.2	CBA	BW5147	GT	Suppressor	T _S F ₁					+
368B1.5	BALB/c	BW5147	GT	Suppressor	T _S F ₁	+/-		+	-	+
Fc301D4.5	B10.M	BW5147	GT	Suppressor	T _S F ₁					+
Fc301A2.3	B10.M	BW5147	GT	Suppressor	T _S F ₁					+
469B5.5	B10.A	BW5147	GT and T _S F ₁	Suppressor	T _S F ₂	+	-	+	-	+
372B3.5	B10	BW5147	GAT-Mφ [‡]	Suppressor	T _S F ₁	+	+/-	+	-	+
372D6.5	B10	BW5147	GAT-Mφ [‡]	Suppressor	T _S F ₂	+	-	+	-	+
145F3511	B10.A	BW5147	GAT	Helper**				+		
BB02'	C57BL/10	None	GAT	Helper ^{‡‡}				+		
BD01'	(B10 × B10.D2)F ₁	None	GAT	Helper ^{‡‡}				+		

* The T_S hybridomas secrete either T_SF₁, an antigen-binding suppressive factor composed of a single polypeptide chain, or T_SF₂, a factor containing two chains, one of which binds antigen.

[‡] Guinea pig antisera raised against mouse GAT-binding antibodies that had been purified on antigen-affinity columns (5).

^{‡‡} Monoclonal antibody to GAT idiotypic determinants (J. Theze, manuscript in preparation).

^{‡‡‡} Injected as neonates with GAT coupled to macrophages.

** Cells synthesize IL-2 in response to antigen and syngeneic antigen presenting cells.

^{‡‡‡} Cells proliferate and synthesize IL-2 in the presence +of GAT and syngeneic macrophages and provide help to normal B cells for GAT-specific plaque forming cell responses *in vitro*.

cells responding to the same antigen do not use the same V_H gene segment. In keeping with this result, the data demonstrating the expression of idiotypic determinants on T cells may need to be reinterpreted.

Materials and Methods

B and T Cells. The GAT-specific B cell hybridoma, F9-238.9, was produced by the fusion of GAT-primed DBA/2 spleen cells and the HAT-sensitive myeloma cell line, P3-X63-Ag8 (27). The resulting hybridoma synthesizes both the MOPC21 (γ₁, κ) immunoglobulin derived from the myeloma parent and a GAT-binding immunoglobulin (μ, κ) (Fig. 1). The F9-238.9 cells were derived by Dr. Michel Pierres (INSERM-CNRS, Marseille) and kindly provided by Dr. Ronald N. Germain (National Institutes of Health, Bethesda, MD).

The suppressor T cell hybridomas were derived from splenic T cells of mice immunized either with GAT, GT, T_SF plus GT, or neonatally with GAT conjugated to macrophages. In each case, the cells were fused to BW5147, a HAT-sensitive T lymphoma of AKR origin (23-25). A GAT-specific T helper cell was cloned by culturing nylon wool nonadherent lymph node cells from GAT-primed B10.A mice on irradiated syngeneic spleen cells and GAT. The resulting T cell blasts were fused to BW5147. The hybrids were assayed for IL-2 production in the presence of GAT and syngeneic spleen cells and cloned by limiting dilution (28). Furthermore, T helper cell lines were derived from lymph node lymphocytes of mice primed *in vivo* with soluble GAT according to the techniques described by Kimoto and Fathman (29). They were maintained in culture with GAT plus supernatant from Con A-stimulated rat spleen cells without filler cells as described.³ Since T cell hybridomas and clones are genetically unstable, the cells were expanded in liquid culture from frozen stocks and assayed for GAT-specific reactivity before RNA isolation (23-25, 28, 30, and footnote 2). In all cases, the cells were harvested and the resulting pellets frozen at -70°C. The characteristics of the T cell hybrids and

³ Theze, J., manuscript in preparation.

clones that we analyzed are summarized in Table I.

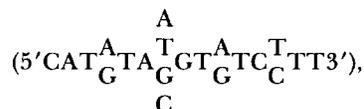
RNA Isolation. The frozen cell pellets were lysed by vortexing in 4 M guanidinium thiocyanate (Tridom, Hauppauge, NY), 25 mM sodium acetate, pH 5, 0.5% sodium sarkosyl, 0.33% antifoam (Sigma Chemical Co., St. Louis, MO), 1 M β -mercaptoethanol. The RNA was purified by centrifugation through a cushion of cesium chloride (31), and the poly(A)-containing fraction enriched by sequential passages over oligo(dT) cellulose (32) (Type 3, Collaborative Research, Waltham, MA). The RNA concentration was determined spectrophotometrically.

Construction of cDNA Library. A cDNA library was constructed using 15 μ g poly(A)-RNA from F9-238.9 GAT-specific B cells. Synthesis of the first cDNA strand by AMV reverse transcriptase (Life Sciences, Inc., St. Petersburg, FL) was carried out with minor modifications of published procedures (33–36). The synthesis was initiated at random sites along the mRNA by the addition of sheared calf thymus DNA primer (37). The primer was prepared by digesting calf thymus DNA with DNase I to an average size of 10–15 nucleotides. RNasin (Biotec, Madison, WI) was added to the reverse transcriptase reaction to inhibit degradation of the RNA template (38).

The alkaline hydrolysis of the RNA and subsequent synthesis of the second cDNA strand by *Escherichia coli* DNA polymerase I (Boehringer Mannheim Biochemicals, Indianapolis, IN) were performed as previously described (33, 34, 39). Following digestion of the single-stranded loop with *Aspergillus* S1 nuclease (Sigma Chemical Co.) (33, 34), the double-stranded cDNA was fractionated by gel filtration on a Biogel A150 (Bio-Rad Laboratories, Richmond, CA) column. The cDNA greater than 500 nucleotides in length, with an average size of \sim 800 bases, was tailed at each 3' end with 10–15 dCTP nucleotides using terminal deoxynucleotidyl transferase (New England Nuclear, Boston, MA) (40).

The cDNA was then annealed to the tetracycline-resistant plasmid, pBR322, which had been digested with the restriction enzyme PstI (New England Biolabs, Beverly, MA) and tailed at the resulting 3' ends with 8–10 nucleotides of dGTP. The annealed DNA was used to transform *E. coli* strain MC1061 (41), as described (42) and the resulting transformants were selected by plating on nitrocellulose filters (Millipore, Bedford, MA) placed on media containing 15 μ g/ml tetracycline. Replica filters were prepared for hybridization or frozen for storage as described (43).

Isolation of pGAT50, the GAT V_H cDNA Clone. The cDNA library was screened with a mixture of 32 pentadecamers



which are complementary to the mRNA predicted to encode residues 30–34 of the GAT immunoglobulin heavy chain. The oligonucleotides were synthesized at Hoffmann-LaRoche (Nutley, NJ) and kindly provided by Dr. Kenneth Wieder (formerly at Hoffman-LaRoche, currently at DuPont Co., Wilmington, DE). The probe mixture was labeled at the 5' end with T4 polynucleotide kinase and [γ - 32 P]dATP (ICN, Irvine, CA) (44). Before screening with the radioactive probe, plasmid DNA was amplified for 36 h on plates containing 12.5 μ g/ml chloramphenicol. The bacterial cells then were lysed and prepared for hybridization with the probe as described (43). Duplicate filters were prehybridized at 23°C for 3 h in hybridization buffer, consisting of 5X SET (0.75 M sodium chloride/0.15 M Tris, pH 8/5 mM ethylenediaminetetraacetic acid), 5X Denhardt's solution (45), 10 μ g/ml each of poly(rA), poly(rC), and poly(rG), 0.5% sodium dodecyl sulfate, and 250 μ g/ml denatured, sheared salmon sperm DNA. The probe was added to the filters in fresh hybridization buffer at 0.5 pmol/ml or 5.5×10^6 cpm/ml. Following 16 h of hybridization at 23°C, the filters were washed in 5 X SET, 0.1% sodium dodecyl sulfate, 0.1% sodium pyrophosphate at 23°C and exposed to Kodak XAR-5 film with an intensifying screen at -80°C .

DNA Sequencing. DNA digested with restriction endonucleases (New England Biolabs, Inc., Beverly, MA), was subcloned into the bacteriophage vector, M13mp8 (Bethesda

Research Laboratories, Gaithersburg, MD) and sequenced by the dideoxy method (46). Alternatively, DNA fragments labeled at the 5' ends with T4 polynucleotide kinase were digested internally with a second restriction endonuclease and the labeled fragments purified and sequenced by the Maxam-Gilbert technique (47).

Northern Blots. Poly(A)-containing RNA was denatured, electrophoresed in agarose gels containing formaldehyde (48) and blotted to nitrocellulose (49). *E. coli* 16S and 23S rRNAs and murine 18S and 28S rRNAs were electrophoresed in parallel as molecular weight standards. The filters were hybridized at 42°C in formamide and dextran sulphate (Pharmacia Fine Chemicals, Piscataway, NJ) as previously described (49), with the addition of 5 µg/ml liver poly(A)-minus RNA. The blots were washed in 0.3 M sodium chloride, 0.03 M sodium citrate, and 0.1% sodium dodecyl sulfate, pH 7, at 50°C and then exposed to Kodak XAR-5 film using an intensifying screen.

Hybridization Probes. The following cloned DNAs were labeled to a specific activity of $2-8 \times 10^8$ cpm/µg by nick-translation (50). (a) *C_μ probe.* Plasmid pSpμA1 (51) was digested with four restriction endonucleases, *Bam*HI, *Kpn*I, *Eco*RI and *Xba*I to produce a 2.5-kb fragment containing the exons encoding C_μ3, C_μ4 and half of C_μ2. This DNA fragment was purified from a preparative agarose gel by electroelution (52). (b) *MOPC21 probe.* A cDNA containing virtually the entire MOPC21 heavy chain variable region gene was cloned into pBR322 (53). (c) *V11 probe.* A 3.5-kb *Sau*III A restriction fragment, containing the genomic V11 gene segment (54), was subcloned into pBR322. V11 is a member of the TEPC15 gene family. DNA was kindly provided by Dr. Johanna A. Griffin (University of Alabama, Birmingham, AL). (d) *V14A probe.* A *Bam*HI-*Eco*RI restriction fragment containing most of the genomic V_H gene segment, V14A, was subcloned into pBR322 (Stephen Crews, unpublished observation). The translation of the V14 DNA sequence is very similar to the protein sequence of the J606 myeloma heavy chain. The DNA was given to us by Dr. Stephen Crews (California Institute of Technology, Pasadena, CA). (e) *S107 probe.* A cDNA clone containing the V_H gene segment expressed by the S107 myeloma was previously isolated (2). S107 is a member of the TEPC15 gene family. The DNA was prepared by Dr. J. A. Griffin. (f) *GAT50 probe.* The derivation of the GAT V_H cDNA clone, pGAT50, is described in this manuscript. (g) *H-2 class I probe.* pH2-IIa was subcloned from a cDNA encoding a class I gene of the d haplotype (55). The DNA was provided by Dr. Michael Steinmetz (Basel Institute, Switzerland). (h) *J_H probe.* A 2-kb fragment containing all four J_H gene segments was excised from a plasmid subclone of the lambda clone μ27 (51), as described elsewhere (53).

Results

Isolation of pGAT50, a GAT V_H cDNA Clone. To isolate a cDNA clone containing the GAT immunoglobulin V_H coding region, we had to overcome two technical problems. First, the μ RNA is long, ~2.4 kb in length and the V_H region is located at its 5' end. Conventionally, cDNA synthesis is initiated by an oligo(dT) primer hybridized to the poly(A) tail found at the 3' end of most eucaryotic messenger RNAs. Therefore, to obtain a clone containing the entire GAT V_H would require synthesis of a cDNA >2 kb in length. Since cDNAs this long are rare, we initiated synthesis using short fragments of calf thymus DNA as a primer. This primer could anneal at various points along the mRNA and the cDNA synthesized would derive from the 5' end of the RNA more frequently than with an oligo(dT) primer. Therefore, the probability of cloning the entire V_H coding sequence would be increased.

The second technical problem arose because the B cell hybridoma producing anti-GAT antibody, F9-238.9, contains ~10 times more γ₁ heavy chain mRNA derived from the P3-X63-Ag8 myeloma parent than μ heavy chain mRNA containing the GAT V_H. This is demonstrated by hybridizing a blot containing

the B cell RNA with a germline J_H probe capable of detecting all heavy chain messenger RNAs (Fig. 1). Hybridization to the 1.9-kb MOPC21 γ mRNA species is significantly more intense than hybridization to the 2.4-kb μ RNA encoding the GAT heavy chain. In addition, the F9-238.9 cells secrete approximately one-tenth as much μ as γ heavy chain (Kraig, data not shown). To distinguish between the clones containing the GAT V_H and the more prevalent clones encoding the MOPC21 V_H , initially we screened the cDNA library with a C_μ probe. Although several clones containing portions of the V_H sequence were isolated, no cDNA clones containing the entire GAT V_H gene were identified. Since the NH_2 -terminal amino acid sequence of several GAT-binding immunoglobulin heavy chains had been determined (22), it was possible to synthesize an oligonucleotide probe, complementary to the RNA sequence predicted to encode amino acid residues 30–34 (Fig. 2). Because there exist ambiguous positions in the reverse translation from amino acid into nucleotide sequence, the complements of all 32 possible coding sequences were synthesized. We hybridized the pool of labeled pentadecamers to the cDNA library under conditions which would distinguish between MOPC21 V_H , which has no more than 12 of 15 nucleotides identical to any of the 32 pentadecamers, and the GAT V_H , which should be identical to one of the probes at all 15 positions. Of 6,000 cDNA clones screened, one, designated pGAT50, hybridized to the pool of radioactive pentadecamers and was characterized further.

The nucleotide sequence of pGAT50 and the sequencing strategy used are

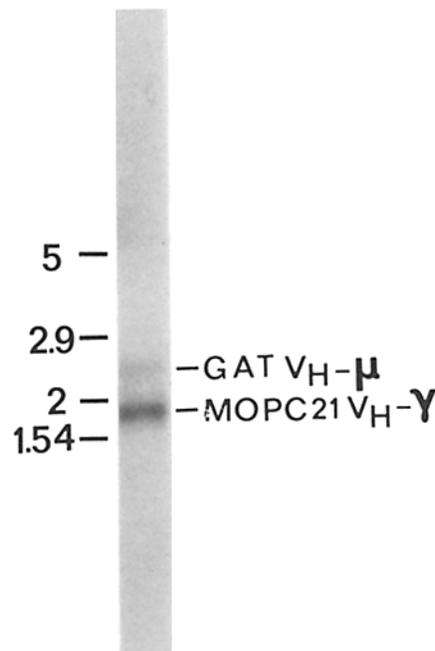


FIGURE 1. Hybridization of a probe containing the four J_H gene segments to a Northern blot of poly(A)-RNA from the F9-238.9 GAT B cell hybridoma. The migration distances of the RNA molecular weight standards and their sizes in kilobases are indicated. The positions of the bands containing MOPC21- γ_1 RNA and GAT V_H - μ RNA are shown.

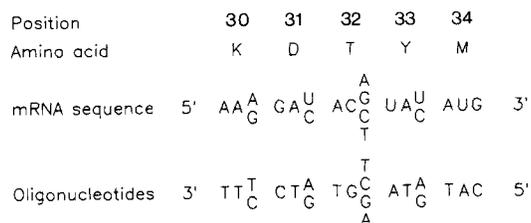


FIGURE 2. Oligonucleotide probes for the GAT V_H sequence. The GAT V_H amino acid sequence, the predicted mRNA sequences and the pentadecamers used in this study as probes complementary to the mRNA are shown.

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1  TTCTGGTAGTTCANGTGAAGGAAATGGTGCTGGGCAGGAPGTCCCGGGCCAPGCAGCCC  80
-19  ATGAGCCCTATCTTCTCTACAGACACTGAATCTCAAGGTCCTTACAATGAAATGCAGC  -16
61  W V I F F L M A V V T G V N S | E V Q L Q  5
119  TGGGTTATCTTCTTCTGATGGCAGTGGTTACAGGGTCAATTACAGAGGTTACAGCTGCAG  178
6  Q S G A E L V K P G A S V K L S C T A S  25
179  CAGTCTGGGGCAGAGCTTGTGAAGCCAGGGSCCTCAGTCAAGTTGTCTGCACAGCTTCT  238
26  G F N I K D T Y M H W V K Q R P E Q G L  45
239  GGCTTCAACATTAAAGACACCTATATGCACTGGGTGAAGCAGAGGCCTGAACAGGGCCTG  298
46  E W I G R I D P A N G N T K Y D P K F Q  65
299  GAGTGGATTGGAAGGATTGATCCTGCGAATGGTAATACTAAATATGACCCGAAGTTCCAG  358
66  G K A T I T A D T S S N T A Y L Q L S S  85
359  GGCAAGGCCACTATAACAGCAGACACATCCTCCAACACAGCCTACCTGCAGCTCAGCAGC  418
86  L T S E D T A V Y Y C A S | Y Y G S S Y | Y  105
419  CTGACATCTGAGGACACTGCCGTCTATTATTGTGCTAGTTACTACGGTAGTAGCTACTAC  478
106  Y W G Q G T T L T V S S | E S Q S F P N V  125
479  TACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAGAGAGTCAGTCCCTCCCAATGTC  538
126  F P L V S C E S P L S D K N L V  140
539  TTCCCCCTCGTCTCCTGCGAGAGCCCCCTGTCTGATAAAGAACTGGT  585

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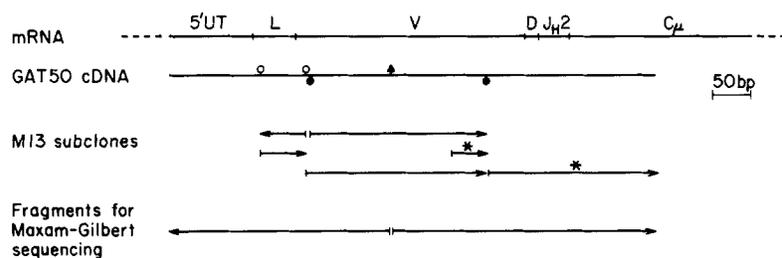


FIGURE 3. Characterization of pGAT50. The pGAT50 cDNA clone is shown with the recognition sites for the restriction endonucleases, Pst I (\blacktriangle), PvuII (\circ), and StuI (\triangle) indicated. The specified restriction fragments from pGAT50 and pGAT40 (*), a second cDNA clone containing only part of the GAT V_H sequence, were subcloned into M13mp8, a bacteriophage vector, and sequenced in the direction indicated by the arrows by the dideoxy method (46). The sequence of pGAT50 was confirmed and extended by the Maxam-Gilbert method (47) from the StuI site, as shown. The resulting DNA sequence of pGAT50 and its translation into protein sequence are given. The position of the pentadecamer used as probe is underscored. Vertical lines delineate the 5' UT, L, V_H, D, and J_H portions.

summarized in Fig. 3. The cDNA clone contains 106 nucleotides from the 5' untranslated (UT) region, a sequence capable of encoding a hydrophobic 19 amino acid leader (L) peptide, complete V_H, D, and J_H gene segments and 72 nucleotides of the C_μ gene. The V_H, D, and J_H gene segments have rearranged in a continuous translational reading frame, indicating that the cDNA clone encodes a functional V region coding sequence. The amino acid sequence predicted from pGAT50 (Fig. 3) agrees with the 33 residues of published NH₂-terminal protein sequence data for the F9-238.9 heavy chain (22). The D is identical to D_{FL16.1} (56) and the J gene segment used is J_H2 (57). The clone also contains 72 nucleotides whose translated sequence is identical to that published for the most NH₂-terminal portion of the first domain of the C_μ region (58).

GAT-specific T Cells Do Not Transcribe the GAT V_H Sequence. RNA was extracted from 11 different GAT-specific T cell hybridomas and two T cell lines (Table I) and the poly(A)-containing RNA purified by two or three sequential passages over oligo(dT)-cellulose. 10 μg of each T cell hybridoma RNA were separated by size on a denaturing gel, blotted to nitrocellulose, and hybridized with the radioactive pGAT50 probe. Each RNA was examined on three independent Northern blot hybridizations, yet there was no evidence of a T cell mRNA species that hybridized to the GAT V_H probe (a characteristic blot is shown in Fig. 4). Similarly, there was no GAT V_H sequence in 3–6 μg of poly(A) RNA from two T_H lines, BB02' and BD01' (data not shown). The GAT V_H sequence could, however, be detected when as little as 50 ng of poly(A)-containing RNA from the B cell hybridoma was loaded on the gel (Fig. 4).

The T cell RNA was shown to be present and undegraded by a positive control hybridization using pH2-IIa, a cDNA clone encoding an H-2^d class I polypeptide (Fig. 5). All of the T cell RNAs contained an mRNA ~1.9 kb in length that hybridized to the H-2 probe and encodes the H-2 class I polypeptide localized in the cell membrane. In addition, several of the T_S hybridoma RNAs had a second mRNA of ~1.8 kb that hybridized to the class I probe. This smaller species of mRNA had been observed previously in liver and possibly encodes a secreted H-2 class I polypeptide (59).

If the T cell RNA contained a transcript from a gene related, but not identical, to the GAT V_H gene, the hybridization signal would be less intense than expected for identical sequences. To determine the degree to which nucleotide sequence divergence would affect the hybridization signal, several V_H gene probes were hybridized to 2 ng of purified heavy chain RNA from the myeloma S107. For a particular messenger RNA present at 50 copies per cell, 2 ng would approximate the amount of that RNA present in 10 μg poly(A)-RNA. All hybridizations were done using conditions identical to those described for the analysis of GAT V_H expression by T cells. The Northern blots are shown in Fig. 6. Hybridization of the S107 RNA to the VH11 probe, which shares 90% sequence identity, showed a three- to fivefold loss of signal when compared with hybridization with the completely identical S107 probe. Furthermore, the V14A probe, which shared only 75% sequence similarity with the S107 RNA, gave no detectable signal. So, if the T cell hybridomas or cell lines contained a V_H sequence, sharing <80% sequence similarity with the GAT V_H probe, this transcript would most likely not have been detected.

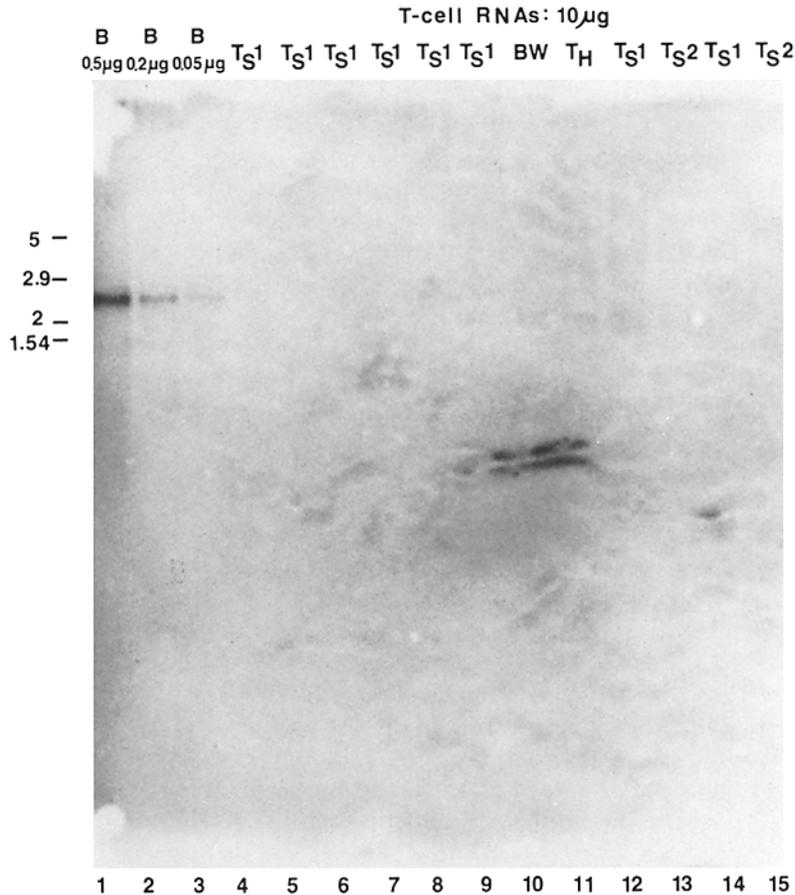


FIGURE 4. Hybridization of a Northern blot containing RNA from GAT-specific T and B cells with the pGAT50 probe. Varying amounts of poly(A)-RNA from F9-238.9, the GAT-B hybridoma, were loaded as follows: 500 ng (lane 1), 200 ng (lane 2) and 50 ng (lane 3). 10 μ g of poly(A) T cell RNA from BW5147 (lane 10) and the T cell hybridomas were loaded as follows: lane 4, Fc301D4.5; lane 5, 342B1.11; lane 6, 365C6.4; lane 7, 258C4.4; lane 8, 367A5.2; lane 9, 368B1.5; lane 11, 145F3511; lane 12, Fc301A2.3; lane 13, 469B5.5; lane 14, 372B3.5; and lane 15, 372D6.5. The spot in the center of the autoradiogram is a background artifact. It does not correlate with a lane and was not observed in other Northern blots of these RNAs. The positions and sizes (in kilobases) of the rRNA markers are indicated.

Discussion

We wished to determine whether T and B cell receptors that bind the same antigen and share idiotypic determinants are encoded by similar V_H gene segments. Since GAT-specific T cells have been cloned and since the murine B cell response to GAT is restricted in diversity we have looked for V_H expression in GAT T cells. To do so, we obtained and characterized a cDNA clone (pGAT50) containing the entire V_H coding sequence from F9-238.9, a GAT-specific B cell hybridoma. The cDNA clone has been sequenced and is identical to the mRNA predicted from the 34 NH₂-terminal amino acid residues determined for the GAT-binding antibodies (22). Since the B cell response to GAT is relatively

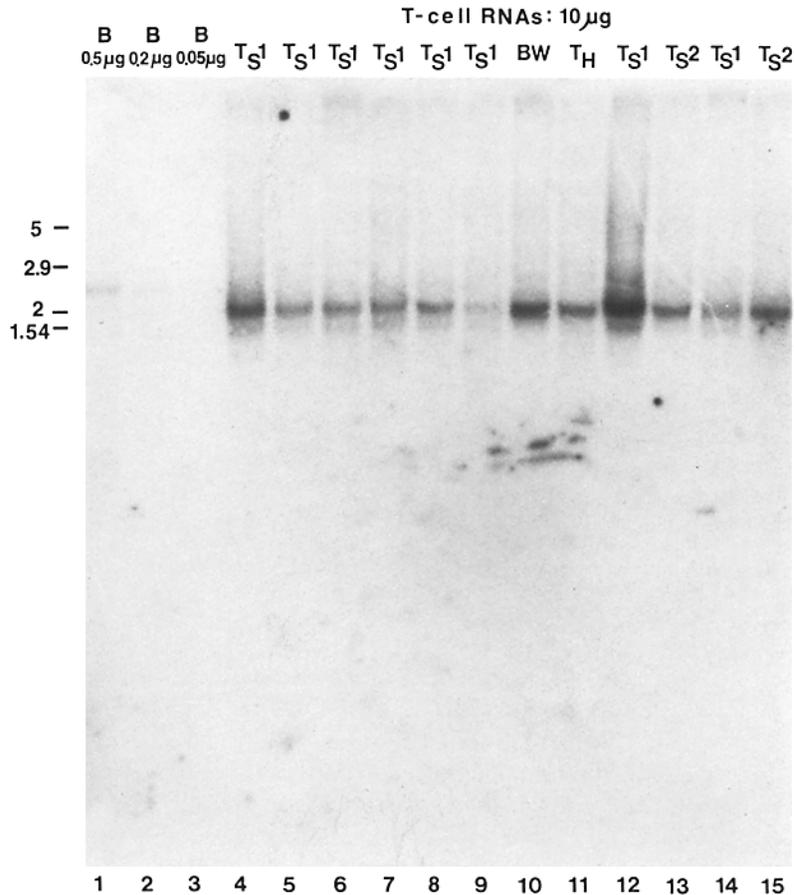


FIGURE 5. Hybridization of a Northern blot of T cell RNAs with an H-2 class I probe. The blot used in Fig. 4 was rehybridized with pH2-IIa, a murine class I probe. Residual hybridization of pGAT50 to the B cell RNA is observed. The positions and sizes (in kilobases) of the rRNA markers are indicated.

homogeneous, pGAT50 certainly should hybridize to mRNA encoding any of the five sequenced heavy chains present in antibodies which bind GAT. Therefore, if B and T cells responding to the same antigen use the V_H gene repertoire in a similar way, we would expect the GAT V_H probe to hybridize to transcripts from the GAT-specific T cell hybridomas or clones.

In addition, six of the suppressor T cell hybridomas secreted factors that had determinants detected by a heterologous anti-CGAT idiotype serum. Of these, three were also positive with a monoclonal antiidiotypic reagent (J. Kapp, unpublished observations). The two T cell lines, although they showed no reactivity with the monoclonal antibody, might have CGAT determinants detectable with the heterologous anti-CGAT serum, since similar clones that have been tested were positive (6). The remaining T cell hybridomas have not been examined for CGAT idiotype expression. Using pGAT50 as a probe, no hybridizing RNA species were detected on Northern blots of 13 different T cell RNAs.

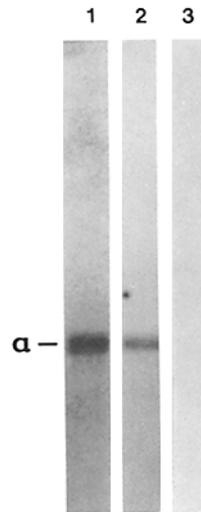


FIGURE 6. Hybridization of S107 heavy chain-encoding RNA with V_H probes of varying sequence similarity. 2 ng of sucrose gradient purified S107 heavy chain RNA was hybridized with the S107 probe, 100% identical (lane 1), the VH11 probe, 90% sequence similarity, (lane 2), and the VH14A probe, 75% sequence similarity (lane 3).

The GAT-specific T cell hybridomas represented three different functional classes, T_S1¹, T_S2¹, and T_H; the two T cell lines have T_H function.² To ensure that the cells tested had maintained antigen-specific function, all lines were assayed for GAT-specific reactivity after expansion in culture and before RNA purification. Positive control hybridizations with an H-2 class I probe argue that the RNA was intact and capable of hybridizing to complementary DNA probes.

Although the GAT heavy chain transcript is only moderately abundant (Fig. 1), the GAT V_H sequence was detected in as little as 50 ng of poly(A)-RNA from the B cell hybridoma producing anti-GAT antibody. Since 10 μg of each T cell hybridoma poly(A)-RNA was tested, we conclude that there must be at least 200 times (10 μg/50 ng) less GAT V_H-encoding RNA in the T cell hybridomas than in the B cell hybridoma. We estimate the detection limit of this analysis to be ~1.5 copies of GAT V_H sequence per T cell for the hybridomas.⁴ A similar calculation yields an estimated detection limit of 5–10 copies per cell for the 3–

⁴ By comparing the signal obtained with J_H, MOPC21 V_H, GAT50 V_H, and C_μ probes hybridized to F9-238.9 RNA and purified myeloma RNAs, we estimate that ~0.2% of the F9-238.9 poly(A)-RNA encodes the GAT Ig heavy chain (Kraig and Kronenberg, unpublished observation). The 5' untranslated (5' UT) region plus V_H comprise 21% = (500/2,400) of the mass of the entire μ mRNA. Therefore, the V_H plus 5' UT should be present at 0.04% of the mass of the F9-238.9 poly(A) RNA. A sequence this abundant could be detected in 50 ng of B cell RNA (Fig. 4, lane 3), so in the 10 μg of each T hybridoma poly(A) RNA tested, we could have detected an homologous V_H sequence present in T cells at 0.00021% of the mass of the poly(A) RNA. Assuming 0.2 pg poly(A) RNA per T cell (53), we could have detected as little as 4 × 10⁻⁷ pg V_H/cell. One molecule of 5' UT-V_H weighs 2.76 × 10⁻⁷ pg = (500 nucleotides) (330 daltons/nucleotide) (1.67 × 10⁻¹² pg/dalton). Therefore the calculated detection limit is 1.5 molecules/cell = (4 × 10⁻⁷ pg detectable/cell)/(2.76 × 10⁻⁷ pg/5' UT-V_H). This number is a best estimate, but there is a degree of uncertainty in determining both the percentage of the F9-238.9 poly(A)-RNA that encodes the GAT immunoglobulin heavy chain and the amount of poly(A)-RNA per cell in the T-cell clones. Since less RNA from the T_H cell lines was tested, the detection limit is two- to threefold higher than for the T cell hybridomas.

6 μ g of RNA from the two T cell lines tested. With the sensitivity of the Northern blots, we easily could have identified transcripts as abundant as the mRNA encoding μ heavy chain in B lymphomas, \sim 100 copies per cell (60). Furthermore, most mRNAs in the low abundance class are present in mammalian cells at 5–20 copies per cell (61), so we could have detected even a transcript in this class. Nevertheless, the T cells analyzed do not contain detectable levels of a messenger RNA similar to GAT V_H . This result is consistent with a report that a T_S clone that secretes a $T_S F$ that binds phosphorylcholine and shares the predominant idiotype (TEPC15) of PC-binding antibodies, fails to transcribe or rearrange the TEPC15 V_H gene segment (62). Therefore, we conclude that shared idiotypic determinants need not imply transcription of similar V_H genes.

There are two limitations to the analysis we have presented. First, although unlikely, the homologous RNA could be present, with an average abundance of <1.5 molecules per cell. For example, despite the fact that antigen-specific function was assayed, it is possible that only 10% of the T lymphocytes were synthesizing antigen-receptor mRNA at the time of harvest. We consider this improbable, since function was tested after the cells were expanded in culture and 10 different suppressor hybrids were analyzed. Furthermore, for the helper T hybridoma, the amount of IL-2 released per cell was unchanged during expansion, so receptor-negative variants had not emerged as a dominant population. However, if for example, the half-life of the $T_S F$ protein were sufficiently long, mRNA need not have been continually present. Second, the degree of hybridization detected on a Northern blot is greatly affected by the amount of sequence similarity shared between the probe and the RNA tested. Thus, if a GAT-specific T cell contained a V_H gene segment that shared only 80% sequence similarity with pGAT50, this T cell transcript probably would not have been detected. However, in the accompanying manuscript (53), we report the screening of T cell cDNA libraries directly with probes capable of hybridizing to virtually any known V_H sequence. These experiments demonstrate that T cells probably do not transcribe any gene segments from the B cell V_H repertoire.

Since there is no evidence for transcription of similar V_H gene segments by T and B cells responding to the same antigen, how does one explain the occurrence of shared idiotypic determinants on some of these cells? First, it should be noted that serological cross-reactivity between molecules does not imply that they share extensive similarities in primary structure. In fact, there are numerous cases in which either complex antisera or monoclonal reagents detected cross-reactions between molecules lacking extensive sequence similarity (63–66). For example, antiidiotypic antibodies raised against antibodies to insulin interfere with the binding of insulin to its receptor (65).

Generally there are two ways to consider the expression of idiotypic determinants by T lymphocytes. The cross-reactivities observed may be biologically irrelevant. For example, in some cases, it is possible that T and B cell antigen receptors reported to share idiotypic determinants actually express no common serological specificity. If the immunogen used to raise the antiidiotypic reagent contained contaminating T cells or T cell-derived antigen-binding material, the resulting sera would detect different determinants on both B and T cells. In other cases, detection of idiotype expression by T lymphocytes depended on the

use of complex antisera, containing multiple serological specificities. In such a complex sera, a minor subset of antibodies might recognize a determinant on both B and T cell receptors, although these molecules share little or no structural similarity. Since the assay for T cell idiotype is often the ability to disrupt T cell activity *in vitro*, perturbations in T cell function due to a minor subset of antibodies would not be easily distinguishable from inhibition by the major components of the antiidiotypic sera. No doubt other explanations are possible and no single rationale can account for every report of idiotype expression by T cells.

However, the sharing of idiotypic determinants by B and T cells may be instead a biologically relevant phenomenon. It may either reflect a selection mechanism, like networks (67), proposed to act similarly on B and T cell antigen receptors, or may be due to constraints on antigen recognition itself. We feel the data are most consistent with a common regulatory mechanism for several reasons. First, it has been demonstrated that monoclonal antiidiotypic reagents can react with T lymphocytes, so in these cases, the reactivity probably is not due to minor components in a complex sera (10, 68; J. Kapp, unpublished observation). These data demonstrate that T and B lymphocytes probably can share a single serologic determinant. However, even monoclonal antibodies may recognize multiple specificities. Second, the presence of idiotypic determinants on T lymphocytes generally is correlated with antigen specificity. Third, in a number of cases, the expression of idiotype by T lymphocytes is linked to the genes encoding immunoglobulin heavy chain allotypes (9, 15–18). It has been suggested that the T cell receptor for antigen also is encoded on chromosome 12. Alternatively, if the putative regulatory mechanism were dominated by B cell antigen receptors, the genes encoding the T cell antigen receptor would appear to map to chromosome 12 even though they are actually located elsewhere (10).

Although it is not certain how the presumptive selection mechanism operates, it is clear that such selective pressures do exist at least for the B cell compartment. For example, mice responding to anti-p-azophenylarsonate primarily synthesize antibodies bearing a cross-reactive idiotype (CRI). However, if suppressed for CRI production, the mice nevertheless can generate a good idiotype-negative antibody response (69). Therefore, for B lymphocytes, the heterogeneity of receptors expressed in response to some antigens reflects both the diversity of the receptor repertoire and the selective forces that operate on it. Since T cells involved in many immune responses also express determinants, it is possible that some antiidiotypic sera recognize determinants involved in the selection or regulation of receptors.

We conclude that T cells and B cells that recognize the same antigen and share idiotypic determinants, need not transcribe similar V_H gene segments. Understanding the structural and genetic bases for idiotype expression by T cells awaits a thorough characterization of the antigen-binding molecules themselves and the genes which encode them.

Summary

We have attempted to determine whether T cells and B cells that have the same antigenic specificity and whose receptors share idiotypic determinants in

fact express similar V_H gene segments. To do this, we have obtained and characterized a cDNA clone containing the entire coding sequence for the V_H gene from a glutamic acid⁶⁰/alanine³⁰/tyrosine¹⁰ (GAT)-binding immunoglobulin that carries the CGAT idiotype. The GAT- V_H clone was hybridized to Northern blots of GAT-specific T cell RNAs; there was no evidence of a T cell transcript that hybridized to the GAT- V_H probe. The T cells analyzed included: (a) 10 GAT-binding suppressor T cell hybridomas, 6 of which secreted factors with CGAT idiotypic determinants, (b) one GAT-specific helper T cell hybridoma, and (c) two GAT-specific helper T cell lines grown in the absence of feeder cells. The detection limit of the Northern blot analysis was 1–2 copies of a particular mRNA species per cell for the hybridomas and 5–10 copies per cell for the T cell lines. Therefore, we conclude that T and B lymphocytes responding to GAT do not utilize similar V_H gene segments. Furthermore, the presence of idiotypic determinants on T lymphocytes does not necessarily imply close structural similarity between T and B cell antigen receptors.

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