Brief Definitive Report

A SEARCH FOR MESSENGER RNA MOLECULES BEARING IMMUNOGLOBULIN V_H NUCLEOTIDE SEQUENCES IN T CELLS

By D. J. KEMP, J. M. ADAMS, P. L. MOTTRAM, W. R. THOMAS, I. D. WALKER and J. F. A. P. MILLER

From The Walter and Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, Victoria 3050, Australia

Several approaches have suggested that immunoglobulin V_H genes encode antigen receptor molecules of T cells as well as B cells (1). For example, the ability of certain anti-idiotype antisera to activate hapten-specific T and B cells is manifest only in strains of mice capable of producing hapten-specific antibodies bearing the idiotype (2). In addition the ability of T cells to express certain idiotypes maps in or near the V_H locus (3), as do the heteroclitic responses to haptens exhibited by both T cells and antibodies (4). However, convincing immunochemical evidence for expression of V_H gene products by T cells is still lacking. Whereas it is generally accepted that polypeptides encoded by C_H genes are not expressed by T cells, there are contradictory claims regarding V_H expression (5, 6). The use of cloned DNA probes derived from V_H genes offers a direct approach to determine whether these genes are expressed in T cells. We report here attempts to detect T cell mRNA molecules that hybridize with V_H probes.

Materials and Methods

Cells. Thymocytes, splenocytes, and lymph node cells were from BALB/c mice. Thymocytes were activated with concanavalin A (Con A, 5 μg/ml) for 2 h in culture, then washed and cultured for 48 h. Lymph node cells were depleted of B cells by incubation with anti-B cell monoclonal antibody 2A2 and complement (7). B lymphomas WEHI 231 and WEHI 279, T lymphomas WEHI 222, ST-1, and ST-4, and antigen-specific T cell lines P2 and 09 were described previously (8, 9). T cell hybridomas were constructed by fusion of T lymphoma EL-4 cells to BALB/c spleen cells (505), EL-4 to A/J spleen cells (2.9.1 and 2.1), and BW5147 to A/J spleen cells (C1 18). Abelson virus-transformed lymphoma 121.3 (10) was a kind gift of Dr. W. Cook of this Institute.

Detection of V_H-bearing mRNA. Poly A^+ RNA was prepared, and ~5 μg samples were fractionated on 1.5% agarose-methyl mercuric hydroxide gels, transferred to diazobenzyloxymethyl paper and hybridized with nick translated probes (specific activity $1–2 \times 10^8$ cpm/μg), as described elsewhere (8, 11), except that the formamide concentration in the hybridization mixture was 30%. The amount of a particular mRNA was estimated by comparing its autoradiographic signal to that given by a known weight of V_H-DNA restriction fragments fractionated on the same gel (e.g., Fig. 1, tracks l and m). The number of such RNA molecules per cell (n) was calculated from the formula: $n = r \cdot Dc \cdot cells^{-1} \cdot Mr^{-1} \cdot N$, where r is the ratio of the autoradiographic intensity of an mRNA band to that of a V_H-DNA standard; Dc is mass of V_H-DNA loaded (g); M_r is molecular weight of mRNA; and N is Avogadro's number (6.023×10^{23}). Except where indicated in the text and assuming no losses, RNA samples analyzed were from 10^8 cells. Assuming an average size of 2 kb (M_r ~6×10^5) for RNA molecules and a V_H-DNA loading of 1 ng per kb, a lower limit of detection can be calculated. For example,

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comparing different exposures revealed that the bands in Fig. 1 A, track 1, from the DNA standard were at least 200 times more intense than the background in track a. Thus, the number of V_H-RNA molecules per T hybridoma cell is (n) $\leq 1/2 \times 10^2 \times 1/10^9 \times 1/10^8 \times 1/6 \times 10^5 \times 6.023 \times 10^{23} = 5 \times 10^{-2}$.

The probes used were as follows: (*a*) V_H-MPC11; the 0.204 kb Pst fragment corresponding to amino acid residues 4–72 of the V_H region expressed in plasmacytoma MPC11 (12) was isolated from plasmid $pV(11)^2$, a kind gift from Dr. David Givol. (*b*) V_H-A8.2; a 1.1 kb Bgl_2-Pst fragment spanning the complete V_H and D_H regions of a rearranged V_H from Abelson lymphoma A8 (13) (isolated and sequenced by S. Gerondakis and O. Bernard). (*c*) V_H-HPC76; a sequenced 0.5 kb BamHl fragment extending from amino acid residue 16 through the V_H gene expressed in plasmacytoma HPC76 (14). (*d*) V_H-TEPC 15; a 1.5 kb BamHl-EcoRl fragment from embryonic clone M31 (13), which spans the single functional TEPC 15 gene (15).

Results and Discussion

The rationale of the experiments is that, under low stringency hybridization conditions, a single V_H probe will identify not only its exact mRNA complement but any mRNA molecule derived from a large family of related V_H genes. For example, such conditions permit cross hybridization between a V_H-M11 probe and TEPC 15 DNA (Fig. 1, track m) even though their nucleotide sequences (12, 15) are only 64% homologous in framework regions. Significantly, pairwise comparisons of the nucleotide sequences of our V_H probes with four other published sequences of V_H genes from distinct families show that each exhibits considerable framework homology (66–87%) with at least one of our probes. This suggests that a large number of V_H sequences can be detected at low stringency with a given V_H probe. To further maximize the probability of cross hybridization, mRNA preparations from both cloned (lymphomas, hybridomas) and uncloned (peripheral lymphocytes, T cell lines) sources were examined using one or more V_H probe.

Four distinct V_H probes were used. Fig. 1 A is an autoradiogram of a typical experiment in which a number of T cell RNA preparations were screened for mRNA complementary to the M11 V_H gene. No specific hybridization is evident in any track containing RNA from thymocytes, peripheral T cells, or T cell-derived tumor cell lines. Fig. 1 B shows that the A8 genomic V_H probe gave weak hybridization with 18S ribosomal RNA and other RNA species present in all of the cells. These irrelevant signals reflect weak homology to repetitive sequences flanking the V_H gene in the probe. Two other faintly hybridizing RNA species of ~2.4 and 2.7 kb are also evident in track h, which contains poly A^+ RNA from a transformed line obtained by intrathymic injection of Abelson virus, but this line was entirely Thy-1^- (10) and hence probably is not of the T lineage. Equivalent experiments were carried out with V_H-HPC76 (16) and V_H-TEPC15 (15) probes and, as a control, a probe for the Cμ sequence. Results are shown in Table I, and several other T cell lymphomas and hybridomas have also been examined (8, and our unpublished results). The general conclusion is clear. In no case could mRNA from a T cell source, either normal or neoplastic, be shown to contain sequences complementary to any V_H probe used.

Each of the four V_H probes used detects a family of 4-22 V_H genes under stringent hybridization conditions, and most or all of these are nonoverlapping (15, 16). Several observations suggest that, under the low stringency conditions used here, they would detect a substantial fraction of the V_H genes expressed in the B lineage. (*a*) Spleen B cell mRNA was shown to contain sequences hybridizable with both Cμ and V_H

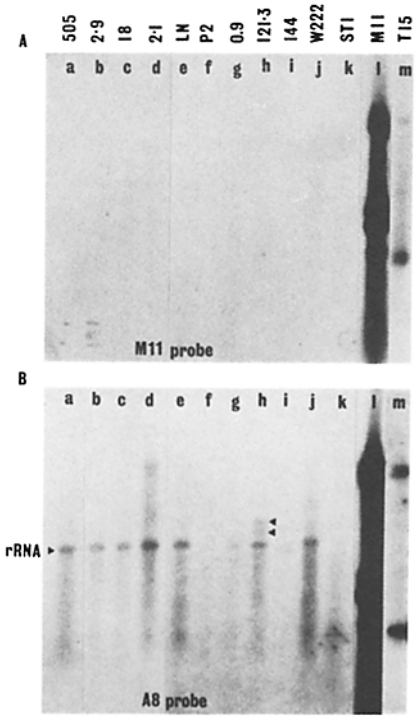

FIG. 1. Hybridization of mRNA samples with cloned V_H probes. Poly A^+ RNA samples were separated by electrophoresis, transferred to diazobenzyloxymethyl paper, and hybridized with a ^{32}P-labeled V_H-MPC11 in (A) and a V_H-A8.2 probe in (B). RNA samples were from T cell hybridomas 505 (a), 2.9 (b), Cl18 (c), and 2.1 (d), lymph node T cells (e), antigen-specific T cell lines P2 (f) and 0.9 (g), Abelson-virus transformed lines (h and i), and T lymphomas WEHI 222 (j) and ST1 (k). Tracks l and m contain restriction digests of plasmids containing MPC11 and TEPC 15 V_H regions, respectively (1 ng/kb). The presence of minor components, such as partial digestion products in these tracks, has been exaggerated by autoradiographic overexposure.

probes, whereas both thymic and lymph node T cell RNA hybridized only to the Cμ probe (Table I). The Cμ RNA detected in some T cells is not mRNA, as it encodes no polypeptide and contains no V_H sequence (8, 17, 18); (*b*) the mRNA of two arbitrarily selected B lymphomas, WEHI 231 and 279, hybridized with both V_H probes tested, albeit more efficiently with one than the other (Table I); (*c*) Southern hybridization results show that the four probes detect a minimum of 52 distinct V_H genes under high stringency conditions, out of an estimated ~160 V_H genes in the V_H locus (13). Under the low stringency conditions used here, an even greater proportion of V_H genes would hybridize. Taken together, these observations suggest that our probes can detect a sizeable proportion of the B cell V_H repertoire. If even one molecule per cell of mRNA entirely complementary to the V_H probes was present in any of the T cell lymphomas, T cell hybridomas, or antigen-specific T cell lines, its detection by one or more of the V_H probes used would have been anticipated. Although the lymphomas and hybridomas could conceivably lack antigen-specific receptors, this is certainly not the case with the T cell lines (9).

The results of Table I set an upper limit upon the amount of RNA coded by HPC76-"like" V_H genes in thymocytes and peripheral T cells. Because B cells typically

TABLE I

mRNA Copies per Cell Complementary to V_H Gene Probes

Gene probes		HPC 76	A8	TEPC 15	MPC 11	Cμ
T cell lines	P2	—*	—	—	—	ND
	09	—	—	—	—	ND
T cell hybridomas	505	—	—	—	—	ND
	2.9.1	—	—	—	—	ND
	C1 18	—	—	—	—	ND
T lymphomas	WEHI 222	—	—	—	—	~30
	ST 1	—	—	—	—	—
	ST 4	—	—	—	ND	~30
B lymphomas	WEHI 231	2–10	2–10	ND	ND	~100
	WEHI 279	2–10	~100	ND	ND	~100
Abelson lymphoma	121.3	—	0.1–1.0	—	—	5–10
Thymocytes‡		—	ND	ND	ND	5–10
Con A thymocytes§		—	—	—	—	ND
Purified LN T cells‖		—	ND	ND	ND	5–10
Cultured LN T cells (1 wk)		—	—	—	—	5–10
Unfractionated spleen‡		5–10¶	ND	ND	ND	~100
T cell depleted spleen‡		5–10¶	ND	ND	ND	~100
Nude spleen cells‡		5–10¶	ND	ND	ND	~100

* Dash signifies <1 molecule per cell.

‡ Calculated from data in ref. 20.

§ Thymocytes cultured for 2 h in serum-free medium with 5 μg/ml CoA, then washed and cultured for a further 48 h.

‖ Prepared by treatment of lymph node cells with an anti-B cell hybridoma antibody, 2A2, plus complement, followed by removal of dead cells.

¶ Detected using 0.5 μg RNA/track whereas all other samples were 5 μg; hence the sensitivity for the T cell RNA should be 10 times greater.

contain ~100 copies of H-chain mRNA per cell (determined using the Cμ probe, Table I), probably ~10% of B cells contain V_H regions capable of efficient hybridization with the V_H-HPC76 probe (Table I). If 10% of T cells also used V_H genes of this family and expressed them at even 10 molecules per cell, a clear signal would have been obtained and even two molecules per cell would have been detected. Thus, the V_H-H76 gene family appears to be expressed rarely, if at all, in T cells, contrary to the conclusion (19) that B and T cells use the same V_H genes at the same frequency.

Our results seriously question the proposition that T cells use the conventional V_H gene repertoire. It remains conceivable that some partial overlap in the T and B repertoires exists, for example, for some T cell subset, but an entirely independent repertoire must be considered.

There is also mounting evidence that T cell receptor polypeptides are not encoded by C_H, D_H, or J_H region gene segments. In some cloned cytotoxic effector and helper cell lines and in T cell hybridomas, these gene segments are not rearranged (20–25). In others, "abortive" D_H or J_H rearrangements that could not encode any functional polypeptide have been reported (22, 23). Although such results do not rule out the possibility that the V_H genes code for T cell receptor molecules, they do deprive these genes of all known molecular elements required to assemble a functional antibody. Thus, the available data from molecular genetics provides no support for sharing of the V_H genes repertoire in T and B cells.

Summary

Expression of V_H-coded mRNA molecules in T cells, antigen-specific T cell lines, or T cell hybridomas was not detected using four different V_H DNA probes under conditions that permitted cross-hybridization between distantly related V_H genes. In contrast, V_H gene expression was readily detected in two B cell lymphomas and in splenic B cells. Less than one molecule per cell of RNA, exactly complementary to the DNA probes used, would have been detected in these T cell populations. The results thus seriously question the proposition that T cells use the B cell V_H repertoire to code for antigen receptors.

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