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

By D. J. KEMP, J. M. ADAMS, P. L. MOTTTRAM, 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 VH genes encode antigen receptor molecules of T cells as well as B cells (1). For example, the ability of certain anti-idiotypic antisera to activate hapten-specific T and B cells is manifest only in strains of mice capable of producing hapten-specific antibodies bearing the idiotypic determinant (2). In addition, the ability of T cells to express certain idiotypes maps in or near the VH locus (3), as do the heteroclitic responses to haptens exhibited by both T cells and antibodies (4). However, convincing immunochemical evidence for expression of VH gene products by T cells is still lacking. Whereas it is generally accepted that polypeptides encoded by the Cn genes are not expressed by T cells, there are contradictory claims regarding VH expression (5, 6). The use of cloned DNA probes derived from VH 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 VH 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 VH-bearing mRNA. Poly A+ RNA was prepared, and ~5 μg samples were fractionated on 1.5% agarose-methyl mercuicide gels, transferred to diazobenzyloxy-methyl paper and hybridized with nick translated probes (specific activity 1–2 × 10⁶ 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 VH-DNA restriction fragments fractionated on the same gel (e.g., Fig. 1, tracks 1 and 2). The number of such RNA molecules per cell (n) was calculated from the formula: n = r × Dc × cells⁻¹ × Mr⁻¹ × N, where r is the ratio of the autoradiographic intensity of an mRNA band to that of a VH-DNA standard; Dc is mass of VH-DNA loaded (g); Mr is molecular weight of mRNA; and N is Avogadro’s number (6.023 × 10²³). Except where indicated in the text and assuming no losses, RNA samples analyzed were from 10⁶ cells. Assuming an average size of 2 kb (Mr ~6 × 10⁵) for RNA molecules and a VH-DNA loading of 1 ng per kb, a lower limit of detection can be calculated. For example,
comparing different exposures revealed that the bands in Fig. 1A, track 1, from the DNA standard were at least 200 times more intense than the background in track a. Thus, the number of VH-RNA molecules per T hybridoma cell is \( n \approx \frac{1/2 \times 10^9 \times 1/10^9 \times 1/10^6 \times 1/6 \times 6.023 \times 10^{23}}{5 \times 10^{-2}} \).

The probes used were as follows: (a) \( \text{VH-MPC}11 \); the 0.204 kb Pst fragment corresponding to amino acid residues 4–72 of the VH region expressed in plasmacytoma MPC11 (12) was isolated from plasmid pV(11)2, a kind gift from Dr. David Givol. (b) \( \text{VH-A8.2} \); a 1.1 kb Bgl2-Pst fragment spanning the complete VH and DH regions of a rearranged VH from Abelson lymphoma A8 (13) (isolated and sequenced by S. Gerondakis and O. Bernard). (c) \( \text{VH-HPC76} \); a sequenced 0.5 kb BamHI fragment extending from amino acid residue 16 through the VH gene expressed in plasmacytoma HPC76 (14). (d) \( \text{VH-TEPC}15 \); a 1.5 kb BamHI-EcoRI fragment from embryonic clone M31 (13), which spans the single functional TEP15 gene (15).

**Results and Discussion**

The rationale of the experiments is that, under low stringency hybridization conditions, a single VH probe will identify not only its exact mRNA complement but any mRNA molecule derived from a large family of related VH genes. For example, such conditions permit cross hybridization between a VH-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 VH probes with four other published sequences of VH 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 VH sequences can be detected at low stringency with a given VH 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 VH probe.

Four distinct VH probes were used. Fig. 1A is an autoradiogram of a typical experiment in which a number of T cell RNA preparations were screened for mRNA complementary to the M11 VH gene. No specific hybridization is evident in any track containing RNA from thymocytes, peripheral T cells, or T cell-derived tumor cell lines. Fig. 1B shows that the A8 genomic VH 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 VH 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 VH-HPC76 (16) and VH-TEPC15 (15) probes and, as a control, a probe for the C\(_\mu\) 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 VH probe used.

Each of the four VH probes used detects a family of 4–22 VH 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 VH genes expressed in the B lineage. (a) Spleen B cell mRNA was shown to contain sequences hybridizable with both C\(_\mu\) and VH
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

| Gene probes | HPC 76 | AB | TEPC 15 | MPC 11 | Cu |
|-------------|--------|----|---------|--------|----|
| T cell lines |        |    |         |        |    |
| P2          |        |    |         |        |    |
| 09          |        |    |         |        |    |
| T cell hybridomas |        |    |         |        |    |
| 505         |        |    |         |        |    |
| 2.9.1       |        |    |         |        |    |
| C1 18       |        |    |         |        |    |
| T lymphomas | WEHI 222 |    |         |        |    |
| ST 1        |        |    |         |        |    |
| ST 4        |        |    |         |        |    |
| B lymphomas | WEHI 231 | 2-10 | 2-10    | ND     | ND |
| WEHI 279    | 2-10   | ~100| ND      | ND     | ~100|
| Abelson lymphoma | 121.3 |    | 0.1-1.0 |        | 5-10|
| Thymocytes§ |        |    |         |        |    |
| Con A thymocytes§ |        |    |         |        |    |
| Purified LN T cells§ |        |    |         |        |    |
| Cultured LN T cells (1 wk) |        |    |         |        |    |
| Unfractionated spleen§ | 5-10 |    | ND      | ND     | ~100|
| T cell depleted spleen§ | 5-10 |    | ND      | ND     | ~100|
| Nude spleen cells§ | 5-10 |    | 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 Con A, 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 Cu probe, Table I), probably ~10% of B cells contain VH regions capable of efficient hybridization with the VH-HPC76 probe (Table I). If 10% of T cells also used VH 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 VH-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 VH genes at the same frequency.

Our results seriously question the proposition that T cells use the conventional VH 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 CH, DH, or JH 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" DH or JH rearrangements that could not encode any functional polypeptide have been reported (22, 23). Although such results do not rule out the possibility that the VH 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 VH 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.

We are grateful to Dr. John Schrader and Dr. Ken Shortman for providing Con A-activated T cells and purified lymph node cells.

Received for publication 28 July 1982.

References

1. Eichmann, K., and K. Rajewsky. 1975. Induction of B and T cell immunity by anti-idiotypic antibody. Eur. J. Immunol. 5:661.
2. Strassman, G., R. Lifshitz and E. Mozes (1980) Elicitation of delayed-type hypersensitivity responses to poly(L Tyr, L Glu)-poly(DL Ala)-poly(L Lys) by anti-idiotypic antibodies. J. Exp. Med. 152:1448.
3. Bach, B. A., M. I. Greene, B. Benacerraf and A. Nisonoff. 1979. Mechanisms of regulation of cell mediated immunity. IV. Azobenzenearsonate specific suppressor factor(s) bear cross reactive idiotypic determinants the expression of which is linked to the heavy chain allotype linkage group of genes. J. Exp. Med. 149:1084.
4. Weinberger, J. Z., M. I. Greene, B. Benacerraf, and M. E. Dorf (1979) Hapten-specific T-cell responses to 4-hydroxy-3-nitrophenylacetyl. I. Genetic control of delayed-type hypersensitivity by $V_H$ and Ia region genes. J. Exp. Med. 149:1336.
5. Eichmann, K., Y. Ben-Neriah, D. Hetzelberger, C. Polke, D. Givol, and P. Lonai. 1980. Correlated expression of $V_H$ framework and $V_H$ idiotypic determinants on helper T cells and on functionally undefined T cells binding group A streptococcal carbohydrate. Eur. J. Immunol. 10:105.
6. Wilder, R. L., C. C. Yuen, I. Seher, and R. G. Mage. 1979. Are $V_H$ framework antigenic determinants expressed on both rabbit B and T lymphocytes? Eur. J. Immunol. 9:777.
7. Bartlett, P. F. 1982. Identification of T lymphocyte progenitor cells-similarity to pluripotential haemopoietic cells. J. Immunol. In press.
8. Kemp, D. J., A. W. Harris, and J. M. Adams. 1980. Transcripts of the immunoglobulin C$\beta$ gene vary in structure and splicing during lymphoid development. Proc. Natl. Acad. Sci. U. S. A. 77:7400.
9. Thomas, W. R., P. Mottram and J. F. A. P. Miller. 1982. Hapten-specific T cell lines mediating delayed hypersensitivity to contact sensitising agents. J. Exp. Med. 156:300.
10. Cook, W. 1982. Rapid thymomas induced by Abelson virus leukemia virus. Proc. Natl. Acad. Sci. U. S. A. 79:2917.
11. Alwine, J. C., D. J. Kemp, and G. R. Stark. 1977. Method for detection of specific RNAs in agarose gels by transfer to diazobenzyloxy-paper and hybridisation with DNA probes. Proc. Natl. Acad. Sci. U. S. A. 74:5330.
12. Zakut, R., J. Cohen, and D. Givol. 1980. Cloning and sequence of the cDNA corresponding to the variable region of immunoglobulin heavy chain MPC 11. Nucleic Acid Res. 8:3591.
13. Kemp, D. J., B. Tyler, O. Bernard, N. Gough, S. Gerondakis, J. M. Adams, and S. Cory. 1982. Organization of genes and spacers within the mouse immunoglobulin $V_H$ locus. J. Mol. Biol. Gen. 1:243.
14. Bernard, O., and N. Gough. 1980. Nucleotide sequences of immunoglobulin heavy chain joining segments between translocated V\(_H\) and in constant region genes. *Proc. Natl. Acad. Sci. U. S. A.* 77:3630.

15. Crews, S., Griffin, J., Huang, H., Calame, K., and L. Hood. 1981. A single V\(_H\) gene segment encodes the immune response to phosphorylcholine: somatic mutation is correlated within class of the antibody. *Cell.* 25:59.

16. Givol, D, Zakat, R., Eftan, K., Rechavi, G., Ram, D., and Cohen, J. B. 1981. The diversity of germline immunoglobulin V\(_H\) genes. *Nature (Lond.)*. 292:426.

17. Walker, I. D., and A. W. Harris. 1980. Immunoglobulin in T lymphoma cells is not translated. *Nature (Lond.)*. 288:290.

18. Alt, F. W., N. Rosenberg, V. Enea, E. Siden, and D. Baltimore. 1982. Multiple immunoglobulin heavy chain gene transcripts in A-MuLV-transformed lymphoid cell lines. *Mol. Cell. Biol.* 2:386.

19. Julius, M. H., H. Cozenza and A. A. Augustin. 1977. Parallel expression of new idiotypes on T and B cells. *Nature (Lond.)*. 267:437.

20. Cayre, Y., M. A. Palladino, K. B. Marcu, and J. Stavenezer. 1981. Expression of an antigen receptor on T cells does not require recombination at the J\(_H\)-C\(_\mu\) locus. *Proc. Natl. Acad. Sci. U. S. A.* 78:3814.

21. Kronenberg, M., M. M. Davis, P. W. Early, L. E. Hood, and J. D. Watson. 1980. Helper and killer cells do not express B cell immunoglobulin joining and constant region gene segments. *J. Exp. Med.* 152:1745.

22. Kurosawa, Y., H. Von Boehmer, W. Haas, H. Sakano, A. Traunecker, and S. Tonegawa. 1981. Identification of D segments of immunoglobulin heavy chain genes and their rearrangement in T lymphocytes. *Nature (Lond.)*. 290:565.

23. Cory, S., J. M. Adams, and D. J. Kemp. 1980. Somatic rearrangements forming active immunoglobulin \(\mu\) genes in B and T lymphoid cell lines. *Proc. Natl. Acad. Sci. U. S. A.* 77:4943.

24. Forster, A., M. Hobart, H. Hengartner, and T. H. Rabbits. 1980. An immunoglobulin heavy chain gene is altered in two T-cell clones. *Nature (Lond.)*. 286:897.

25. Zuniga, M. C., P. D'Eustachio, and N. H. Ruddle. 1982. Immunoglobulin heavy chain gene rearrangement and transcription in murine T cell hybrids and T lymphomas. *Proc. Natl. Acad. Sci. U. S. A.* 79:3015.
Author/s: KEMP, DJ; ADAMS, JM; MOTTRAM, PL; THOMAS, WR; WALKER, ID; MILLER, J

Title: A SEARCH FOR MESSENGER-RNA MOLECULES BEARING IMMUNOGLOBULIN VH NUCLEOTIDE-SEQUENCES IN T-CELLS

Date: 1982-01-01

Citation: KEMP, D. J., ADAMS, J. M., MOTTRAM, P. L., THOMAS, W. R., WALKER, I. D. & MILLER, J. (1982). A SEARCH FOR MESSENGER-RNA MOLECULES BEARING IMMUNOGLOBULIN VH NUCLEOTIDE-SEQUENCES IN T-CELLS. JOURNAL OF EXPERIMENTAL MEDICINE, 156 (6), pp.1848-1853. https://doi.org/10.1084/jem.156.6.1848.

Persistent Link: http://hdl.handle.net/11343/258364

License: CC BY-NC-SA