are preferentially synthesized in synaptic areas. The uneven distribution of mRNA could, in turn, arise in any of several ways—for example, transport of AChR mRNA from nonsynaptic to synaptic regions, increased stability of AChR mRNA near synapses or differential transcription of AChR subunit genes by nuclei near to and far from synapses. Whereas these and other alternatives remain to be distinguished, the latter is of particular interest. Although the 500 or so nuclei in each murine diaphragm muscle fibre are quite evenly distributed along the length of the fibre (Fig. 1*e*), a few nuclei (~5) are regularly found directly beneath the postsynaptic membrane. These synapse-associated nuclei, and the cytoplasm that surrounds them, are morphologically distinguishable from nonsynaptic nuclei and cytoplasm[13]. We speculate that AChR mRNA may be concentrated near and transcribed preferentially by these few synaptic nuclei. As only ~1% of all muscle fibre nuclei (5 per 500) are synaptic, these nuclei might transcribe orders-of-magnitude more AChR mRNA than do extrasynaptic nuclei. Thus, our results raise the possibility that synaptic and nonsynaptic nuclei within the cytoplasm of a single muscle fibre transcribe different sets of genes.

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Expression of the T-cell-specific γ gene is unnecessary in T cells recognizing class II MHC determinants

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Subtractive complementary DNA cloning combined with partial protein sequencing has allowed identification of the genes encoding the α and β subunits of T-cell receptors[1–7]. The subtractive cDNA library prepared from the cytotoxic T lymphocyte (T_c) clone 2C has been found to contain a third type of clone encoding the γ chain[5,8,9]. The γ gene shares several features with the α and β genes: (1) assembly from gene segments resembling immunoglobulin *V*, *J* and *C* (respectively variable, joining and constant region) DNA segments[10]; (2) rearrangement and expression in T cells and not in B cells[8]; (3) sequences reminiscent of transmembrane and intracytoplasmic regions of integral membrane proteins[8]; (4) a cysteine residue at the position expected for an interchain disulphide bond. The α and β genes are expressed at equivalent levels in both T_c cells and helper T cells (T_H)[4,5,9,11]. The γ gene, obtained from 2C, has been found to be expressed in all T_c cells studied[9]. Here we present evidence that strongly suggests that T_H cells do not require γ gene expression.

Despite the similarities noted above, the γ gene differs from α and β in several ways: (1) clonal diversity of γ seems to be limited to the region of the *V–J* join (refs[9,10]; also see below); (2) differential expression in T-cell development (γ, β and α genes are expressed predominantly early, consistently and late, respectively)[12]; (3) different chromosomal locations (mouse α, β and γ genes are located on chromosomes 14 (refs 13, 14), 6 (refs 15, 16) and 13 (ref. 13), respectively). On the basis of these similarities and differences, we postulated that the γ gene product may be a component of a T-cell receptor involved in the recognition of the major histocompatibility complex (MHC) molecule. An interesting question is whether T_H cells, which are ordinarily restricted by the class II MHC molecules, rearrange and express the same γ gene known to be expressed in class I-restricted T_c cells. This question is particularly intriguing in light of the findings that the two types of T cell use the same *J* and *C* gene segments and probably the same pool of *V* gene segments in their rearranged and expressed α and β genes[4,5,17,18]. We therefore analysed DNA from a panel of T_H hybridomas as well as the parental T lymphoma BW5147 for rearrangement of the γ gene (Fig. 1a). As was found with T_c-cell clones and T_H hybridomas studied previously[8,9], all demonstrated rearrangement. Northern analysis (Fig. 1*b*) revealed that the expression of the γ gene is variable in the T_H hybridomas, ranging from ~30% of the level of a cloned T_c-cell line, 2C, to levels undetectable even after prolonged exposure of film to the Northern filter (extended exposure not shown). No γ messenger RNA was detected in the fusion partner BW5147. By contrast, all of these cells produced comparable amounts of mRNA encoding the T-cell receptor α-chain (Fig. 1*c*).

To determine the reason for the irregular expression of the γ gene in T_H hybridomas, we studied four independent T_H hybridomas of different antigenic specificities which expressed γ mRNA. (T_H hybridomas B8C3, C10 and C11A3 are described in Fig. 1 legend. Hybridoma B3C6 was obtained from a CAF_1 mouse and is autoreactive (anti-I-A^d)[19]). From these hybridomas we prepared cDNA libraries, cloned the γ cDNA and determined the nucleotide sequences. The four T_H-derived sequences, one each from the four hybridomas, were identical and were >99% homologous at the nucleotide level to the T_c-cell (2C) derived sequence. The most significant differences between the T_H-derived and the T_c-derived sequences occur at the joining region of the *V* and *J* gene segments (Fig. 2). The 2C cDNA (pHDS 4) is encoded by the germline *V*108A DNA segment up to the second base of codon 99; coding by the germline *J*10.5 segment starts at the second base of codon 100 (ref. 10). The dinucleotide AT links the *V* and *J* segment-coded regions in the proper translation frame, and is probably provided by a germline diversity (*D*) segment or a terminal transferase-like enzyme. (A similar mechanism has been proposed to explain the appearance at the joining regions of immunoglobulin gene sequences of nucleotides not encoded by the germline sequence[20,21].) Although we have not confirmed the genomic structure of the T_H hybridoma γ genes, the limited diversity observed in the germline γ-gene components justifies our proposing the following scheme. In the T_H hybridoma, cDNA (pOE15.3) coding by the germline *V*108A DNA segment ends with the first base of codon 98. Germline-encoded sequence resumes in *J*10.5 (ref. 10) at the second nucleotide of codon 99, one nucleotide before that which begins the 2C *J* segment.

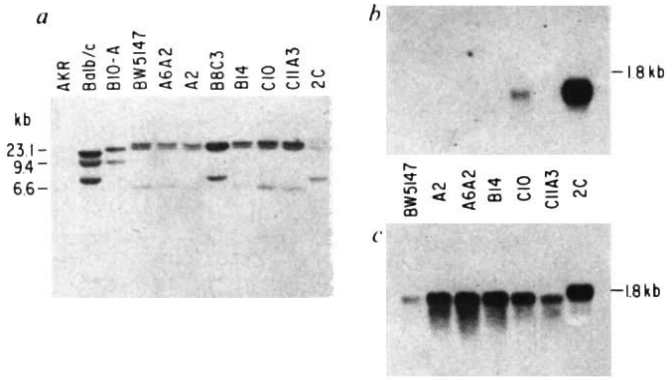

Fig. 1 *a*, Southern blot analysis[23] of T_H hybridoma DNA hybridized to a C_γ gene probe. *b*, *c*, Northern blot analyses[24] of poly(A)$^+$ RNA from T_H hybridomas hybridized to complete γ gene (*b*) or α gene (*c*) probes. The T_H hybridoma B8C3 is of BALB/c origin and is specific for pork insulin[25]. The T_H hybridomas A6A2, A2, B14, C10 and C11A3 are all from B10.A mice and are directed against hen egg lysozyme (ref. 26 and L.G. and Paul Allen, manuscript in preparation.)

Methods. *a*, DNA was isolated from T_H hybridomas, the fusion partner BW5147, BALB.B-derived T_c-cell clone 2C, and from livers (AKR and BALB/c) or kidneys (B10.A) of inbred mice. DNA (10 μg) was digested with *Eco*RI and electrophoresed through 0.8% agarose gel, transferred to nitrocellulose and hybridized with ^{32}P-labelled nick-translated probes in 50% formamide 5×SSC at 42 °C. Filters were washed at 65 °C in 0.2×SSC. *b*, *c*, Approximately 2 μg of poly(A)$^+$ RNA was denatured in 50% formamide at 65 °C and electrophoresed through a gel containing 1% agarose, 6.6% formaldehyde in 20 mM sodium phosphate buffer *p*H 7.0. The RNA was transferred to nitrocellulose and hybridized and washed as for *a*.

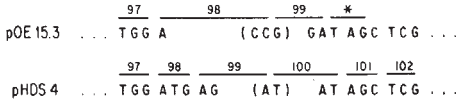

Fig. 2 Nucleotide sequences of γ cDNA clones at the *V-J* joint. The T_H hybridoma (B8C3) cDNA sequence is designated pOE15.3, that of the clone 2C, pHDS4. The nucleotides in parentheses are not encoded by germline sequences; their origin is discussed in the text. Numbers indicate codons, the asterisk a termination codon. All sequences were determined by the method of Maxam and Gilbert[27].

Between the T_H hybridoma cDNA *V* and *J* segments there are three nucleotides, CCG, which are unaccounted for by the germline sequences and which may be the product of a *D* segment or a terminal transferase-like enzyme. Compared with the previously determined sequences of productively rearranged γ gene (all from T_c cells), the *V-J* join of the T_H hybridomas alters the translational reading frame such that the open reading frame terminates immediately after codon 99. Thus, none of the four cDNA clones isolated from as many T_H hybridomas can encode a complete γ-chain.

The cDNA libraries prepared from some of the four T_H hybridoma RNA may contain a second type of γ cDNA clone, as was found to be the case with 2C (ref. 9). To test this possibility, we exploited the fact that the tetranucleotide CCGG at the *V-J* junction of the aberrant T_H cDNA clones is the recognition sequence of endonuclease *Msp*I. We examined a total of 28 cDNA clones (7, 2, 10 and 9 from hybridomas B8C3, B3C6, C10 and C11A3, respectively) for the presence of the *Msp*I site and found that all carry this restriction site (data not shown). As this *Msp*I cleavage site is located in the highly variable inserted sequence, these results strongly suggest that none of the four T_H hybridomas contains γ mRNA other than the one represented by the sequenced cDNA clones.

The presence of a unique, non-functional *V-J* joint in the γ genes of four independently isolated T_H hybridomas of different

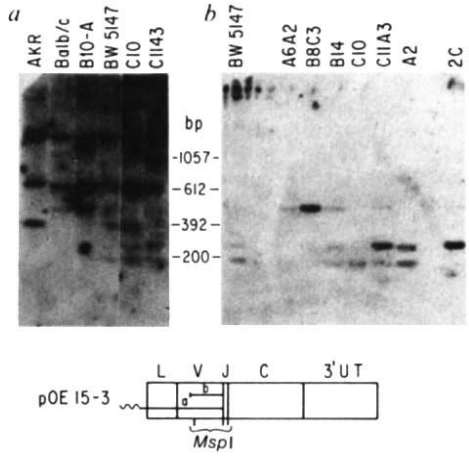

Fig. 3 Southern analyses of T_H hybridoma DNA digested with *Msp*I. The T_H hybridomas are described in Fig. 1 legend. Approximately 15 μg of DNA was digested with *Msp*I and electrophoresed in a 1.8% agarose gel. for *a* the DNA was transferred to diazophenylthioether paper (Schleicher & Schuell) and hybridized as recommended by the manufacturer. Transfer and hybridization for *b* was to nitrocellulose as described in Fig. 1 legend. The probes used are indicated, as are the relevant restriction sites in clone pOE15.3. L and 3'UT indicate the leader region and 3' untranslated region respectively.

specificity is striking and is best explained by their having a common origin. The rearranged γ alleles of the fusion partner BW5147 are the most likely source. Although this hypothesis is in apparent conflict with the Northern blot data (Fig. 1*b*), it is possible that fusion with a splenic T_H cell activated the dormant BW5147-derived allele for transcription. The following result strongly suggests that this hypothesis is correct.

The functionally rearranged V_γ gene segment expressed in 2C as well as its germline counterparts contains a single *Msp*I site at nucleotide 303 (amino-acid residues 36–37). The nucleotide sequences of the T_c-cell-derived cDNA clones, as well as the germline *J-C* clone, indicate that the next downstream *Msp*I site on the productively rearranged γ gene is located at nucleotide 551, at the boundary of the *J* region and the intron following it [9,10]. These two *Msp*I sites would yield a band of ~250 nucleotides (precisely 248 for 2C) in the *Msp*I-digested, T_c-cell clone-derived DNA hybridized to a V_γ probe. The *Msp*I site created at the aberrant *V-J* joint is at nucleotide position 488 of the coding sequence. *Msp*I digestion of DNA containing this rearrangement would result in a V_γ-hybridizing band 185 nucleotides in length. Figure 3 shows a Southern analysis of DNA from BW5147, T_H hybridomas and 2C. In BW5147 and the T_H hybridomas examined, the 185-nucleotide band characteristic of the aberrant rearrangement is present; as expected, no such band is present in the 2C DNA. These results indicate that the γ RNA seen in some T_H hybridomas probably arises from the aberrantly rearranged allele of the fusion partner BW5147. Although the significance of the activation of the BW5147 γ gene in some hybridomas is obscure, the absence in any of these T_H hybridomas of γ mRNA capable of encoding a complete γ protein, and the total absence of γ mRNA in some T_H hybridomas suggests that the putative γ polypeptide chain is not necessary for the function of T_H cells.

One way to confirm the proposal that γ gene expression is unnecessary in T_H cells is to analyse T_H clones (rather than T_H hybridomas), thereby avoiding the complications arising from the fusion partner. Although these T_H clones are relatively difficult to obtain, we derived several likely candidates from a mixed lymphocyte culture. These cells are negative for cell-surface antigen Lyt2 and positive for antigen L3T4, and proliferate and secrete interleukin-2 (IL-2) when stimulated by cells of the appropriate haplotype; all these are characteristics of T_H cells. Southern analysis of these cells (Fig. 4*a*) indicates that

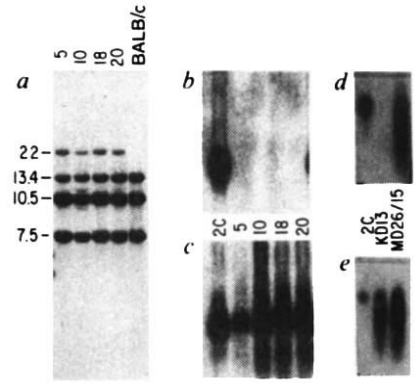

Fig. 4 *a*, Southern analysis of T_H-cell clones. The cloned T_H-cell lines 5, 10, 18 and 20 were obtained from a BALB/c anti BALB.B mixed lymphocyte culture. DNA was digested with *Eco*RI and treated as described in Fig. 1*a* legend. The filter was hybridized with a C_γ probe. *b*, *c*, Northern analysis of T_H-cell clones and T_c-cell clone 2C. The methods used were as described for Fig. 1*b*. Probes used: *b*, C_γ; *c*, C_β. *d*, *e*, Northern analysis of T_c hybridomas KD13 and MD26/15 and T_c-cell clone 2C. The KD13 hybridoma resulted from the fusion of BW5147 with a product of a C3H anti-BALB/c mixed lymphocyte culture. KD13 expresses antigens L3T4 and Lfal but not Lyt2. Following stimulation by irradiated I-E^d-bearing stimulator cells, KD13 lysed I-E^d-bearing target cells and did not produce detectable levels of IL-2. MD26/15 is described in ref. 22. Probes used were: *d*, C_γ, *e*, C_β.
Methods. A modification of the methods of Goldberg[28] and Bond and Farmer[29] was used. Approximately 15 μg of total cellular RNA was electrophoresed in a gel containing 1.8% agarose, 2.2% formaldehyde in a buffer of 40 mM MOPS, *p*H 7.2 and 50 mM sodium acetate, *p*H 7.0. After electrophoresis the gel was soaked briefly in 10×SSC and transferred to nitrocellulose. Hybridization was performed as described in Fig. 1*a* legend. Probes used: *c*, C_γ, *d*, C_β.

each clone contains an *Eco*RI fragment of 22 kilobases (kb), resulting from rearrangement of the γ genes. The other fragments were identical to those found in the embryonic configuration. Previous hybridization analyses of T_c-cell clones[10] indicated that this 22-kb band, present in all T_c cells, contains *C*-region sequences but no detectable *V*-region sequence and that a 16-kb fragment, also present in all T_c cells, is representative of a productively rearranged γ gene. This 16-kb band was not found in the clones with T_H phenotype. More significantly, Northern analysis (Fig. 4*b*, *c*) of the RNA derived from these T_H clones showed normal levels of β mRNA but no indication of γ mRNA, supporting the contention that γ gene expression is not essential in mature T_H cells.

The disparity of expression observed in the two major types of T cells, T_c and T_H, of γ mRNA capable of yielding a full-length polypeptide is in striking contrast to the ubiquitous and comparable expression of the α and β gene in the two cell types. It is consistent, however, with the hypothesis that the role of the γ polypeptide chain is to recognize the class I MHC gene product. An alternative possibility is that the γ-chain is involved in effector function (that is, cytotoxicity). As a first attempt to distinguish between these two possibilities, we analysed RNA from a rare class II MHC-specific T_c hybridoma, KD13 (J.L.G. *et al.*, manuscript in preparation; see Fig. 4 legend), as well as a T_c hybridoma (MD 26/15)[22] conventionally restricted to class I; neither hybridoma expresses Lyt2. As shown in Fig. 4*d*, the class II-specific hybridoma contained no detectable γ mRNA whereas the class I-restricted hybridoma did. As a control, the same RNA blot was analysed, after washing, with a β-gene probe. The autoradiogram showed a comparable level of β mRNA in both hybridomas (Fig. 4*e*). This result is consistent with a role of the γ-chain in the recognition of the class I MHC gene product rather than in the effector function, although more class II-specific T_c cells and also some class I-specific T_H cells must be analysed before a firm conclusion can be drawn.

Thus, the γ gene, productively rearranged and expressed in T_c cells and immature thymocytes, is not necessarily expressed in class II MHC-restricted T_H hybridomas, alloreactive T_H clones and in a class II MHC-specific T_c hybridoma. These results, together with the limited clonal diversity observed in this gene, suggest that the role of its gene product is in the recognition of class I MHC molecules. This recognition may occur during antigen presentation (MHC restriction) or during the intrathymic selection of self-MHC-restricted T-cell subpopulations (thymus education) or both. One implication of this hypothesis is that the class II-restricted T cells (mostly T_H cells) and/or their precursor thymocytes express a fourth type of gene, the product of which is involved in recognition of the class II MHC gene products. If this is so, this gene has diverged too much to be detected with the probes available.

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Note added in proof: We have recently found a new V_γ gene segment which does not cross-hybridize with the previously described V_γ gene segments. This new V_γ gene segment is rearranged to a J_γ gene segment in the 22-kb *Eco*RI fragment.

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