

REVIEW ARTICLE

The T cell antigen receptor

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Introduction

As T lymphocytes are capable of recognizing and responding specifically to a wide variety of antigens, it seemed inevitable that, by analogy with B lymphocytes, they would express a surface protein of variable structure which would confer antigen specificity to a given T cell. Recently two independent approaches have led to the identification of such a T cell surface molecule. Firstly, clonally specific anti-(T cell) antibodies have been characterized by several laboratories. Various lines of evidence suggest that these antibodies recognize a T cell receptor for antigen and they have permitted a biochemical characterization of the protein. Secondly, subtractive screening methods have been used to isolate T cell-specific cDNA clones with immunoglobulin-like properties. These two strategies were united when partial amino acid sequence from protein components purified using the clonotypic antibodies corresponded exactly to the amino acid sequence deduced from the nucleotide sequences of two classes of cDNA clone.

It is our purpose to summarize current knowledge of the protein biochemistry and molecular biology of the T cell antigen receptor. We will then consider the implication of these structural features for two of the most important current questions in T cell biology. Firstly, how is the mature T cell repertoire generated? Further, how do mature T cells recognize antigen?

The structure of the protein

Monoclonal antibodies directed against clonotypic (idiotypic) determinants on T cells, assumed by analogy with immunoglobulins to react with a variable domain of the T cell receptor, have been used in immunoprecipitation experiments to detect a disulphide-linked heterodimer of M_r 75000–90000 in both mouse (Allison *et al.*, 1982; Haskins *et al.*, 1983) and man (Meuer *et al.*, 1983a). Upon

reduction the heterodimeric structure gives rise to an α and a β subunit. In the mouse the α and β chains both have an apparent M_r of about 40000–43000; in the human, and α and β chains have M_r values of about 50000 and 43000, respectively. Both chains contain *N*-linked glycan units (McIntyre & Allison, 1984). This clonotypic structure is identical, at least in terms of gross biochemical features, when purified from either cytotoxic or helper T cells and may also be found in some human suppressor T cells (Bensussan *et al.*, 1984). Peptide mapping analysis of the receptor purified from different T cells has detected both variable and constant peptides from the α and β chains (Kappler *et al.*, 1983b; Reinherz *et al.*, 1983). These biochemical data, together with the structural predictions from nucleotide sequence analysis of cDNA clones (see below), is combined in Fig. 1 as a model for the clonotypic structure.

The major evidence that this structure is indeed utilized by the T cell in antigen recognition comes from the use of clonotypic antibodies to inhibit, or in certain cases mimic, the effect of antigen on T cells (Kaye & Janeway, 1984; Meuer *et al.*, 1983a). However, it is clear that, while the $\alpha\beta$ heterodimer, because of its variable structure, is probably at least partially responsible for conferring antigenic specificity to a T cell, several other molecules are involved in T cell activation in response to antigen. Notable amongst these is the non-polymorphic 20–25kDa glycoprotein T3, first identified serologically in human T cells (van Wauwe *et al.*, 1980), and recently shown to have a counterpart in the mouse (Allison *et al.*, 1984; Van den Elsen *et al.*, 1985). Anti-T3 antibodies can also inhibit or mimic antigen action (Meuer *et al.*, 1983a), and T3 appears to be physically and obligatorily associated with the clonotypic molecule on the cell surface (Meuer *et al.*, 1983c; Weiss & Stobo, 1984). It is, therefore, important to remember that the $\alpha\beta$ heterodimeric structure may be only one component of the total antigen receptor complex on the cell surface. As with many other ligand-induced cellular responses, T cell activation is believed to be mediated by a ligand recognition molecule (the $\alpha\beta$ dimer) coupled to effector molecules such as T3.

Abbreviations used: cDNA, complementary DNA; kb, kilobase (pairs).

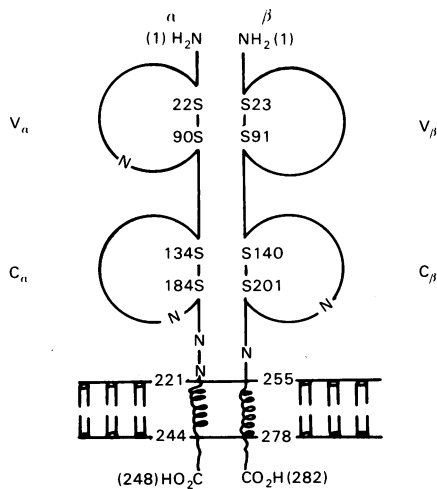


Fig. 1. Proposed structure for the T-cell antigen receptor heterodimer

The α and β chains are each represented as comprising a variable (V) and constant (C) external domain, a transmembrane segment and a short cytoplasmic tail. The positions of the cysteine residues, thought to form intrachain disulphide bonds, and potential asparagine-linked glycosylation sites (N) are indicated. The position of the interchain disulphide bond(s) is unknown.

The strategy for cDNA cloning

cDNA clones encoding the human and mouse β chains were isolated almost simultaneously. The first human cDNA clone was detected in a differential screening procedure designed to identify T cell-specific mRNAs (Yanagi *et al.*, 1984). The mouse cDNA clone was isolated using a set of assumptions designed specifically to identify mRNAs encoding the T cell receptor (Hedrick *et al.*, 1984a). The assumptions made were: (a) T cell receptor genes should be expressed in T cells but not in B cells; (b) the mRNAs for the T cell receptor should be found on membrane-bound polysomes, since the receptor should be a cell-surface glycoprotein; (c) the genes encoding the T cell receptor proteins should be rearranged in T cells as a mechanism of generating diversity; (d) they should possess, by analogy with immunoglobulin genes, constant and variable regions. The mouse and human clones were clearly homologous and also showed significant homology in predicted amino acid sequence to immunoglobulin proteins. In particular, certain residues, invariant in all immunoglobulins and necessary to maintain the Ig fold, were found in the T cell sequence. Comparison of the sequence of the initial mouse cDNA clone with those of cross-hybridizing cDNAs iso-

lated from a thymocyte library revealed the presence of a constant region and of independently assorting variable and joining regions (Hedrick *et al.*, 1984b). The predicted amino acid sequence of the human clone proved identical with the N-terminal amino acid sequence of the human β chain, demonstrating that the mouse and human cDNA clones were derived from mRNA encoding the β chain of the T cell receptor (Acuto *et al.*, 1984).

Following isolation of the β chain cDNA clones two further classes of murine cDNA clones were isolated by differential screening protocols that fulfilled the criteria of T cell specificity and gene rearrangement (Saito *et al.*, 1984a,b; Chien *et al.*, 1984b). Purification and partial amino acid sequencing of the human T cell receptor α chain (Hannum *et al.*, 1984; Fabbi *et al.*, 1984) then allowed identification of one type of these murine clones (Saito *et al.*, 1984a; Chien *et al.*, 1984b) as corresponding to the α chain defined by the clonotypic antisera, and a human α chain cDNA clone was also isolated (Sim *et al.*, 1984). The α chain nucleotide sequence shows overall homology to immunoglobulin genes, and an equal homology to the β chain in both constant and variable regions. The two murine cDNA clones sequenced were isolated from a helper hybridoma and a cytotoxic cell clone; they share identical constant regions but have different variable regions. The joining regions of the two cDNA clones show a high level of homology with β chain and immunoglobulin joining regions. mRNA for the α chain is, as would be predicted, expressed in a variety of functional T cells and the genes are correspondingly rearranged.

The third type of rearranged murine cDNA clone has become known as the γ chain (Saito *et al.*, 1984b). The predicted amino acid sequence derived from this molecule once again shows the same overall structural features as the α and β chains, and immunoglobulin genes. However its role in T cell function remains unclear, as no protein product encoded by the mRNA has yet been characterized. It is clearly not universally associated with the $\alpha\beta$ heterodimeric molecule as it is not expressed in many T_H cell lines (Kranz *et al.*, 1985a). Indeed, γ chain mRNA is present at a much lower level than that of the α and β chains in mature T cell lines and splenic T cells (Chien *et al.*, 1984b). Furthermore, γ chain rearrangements in a panel of functional mouse T cells have been shown to be identical, suggesting identical variable region usage (Saito *et al.*, 1984b; Kranz *et al.*, 1985a). Several γ chain cDNA clones from different cells do indeed contain identical variable and constant regions (Kranz *et al.*, 1985a), and thus any protein product of the γ locus would be expected to show considerably less variability than α and β chains.

Genomic organization

The cDNA clones encoding mouse and human β chains have been used to locate the corresponding gene families on chromosomes 6 and 7, respectively (Lee *et al.*, 1984; Caccia *et al.*, 1984; Collins *et al.*, 1984). The mouse β chain has been further localized to the proximal (centromeric) one-third of chromosome 6, in the same region as the immunoglobulin κ chain locus (Lee *et al.*, 1984). This linkage is, however, not maintained in the human genome, in which the κ chain locus is located on chromosome 2 (Caccia *et al.*, 1984; Collins *et al.*, 1984). The mouse and human α chain genes have both been localized to chromosome 14 (Kranz *et al.*, 1985b; Dembic *et al.*, 1985; Collins *et al.*, 1985c; Croce *et al.*, 1985). Once again there is a potential linkage, this time in the human, with immunoglobulin heavy chain (Collins *et al.*, 1985c; Croce *et al.*, 1985), which is not conserved in the mouse. The chromosomal locations of all human and mouse immunoglobulin and T cell receptor gene families are summarized in Table 1. Determination of the exact locations of the T cell receptor genes is of importance, as these regions could well be involved in chromosomal translocations in T cell tumourigenesis, by analogy with the role of immunoglobulin gene loci in Burkitts lymphoma (Perry, 1983). Indeed, the human α chain genes have been further localized to the region q11-q12 of chromosome 14, a region in which chromosomal aberrations in T cell malignancies have been observed (Croce *et al.*, 1985).

The mouse and human β chain cDNA clones have also been used to isolate homologous sequences from genomic libraries. The mouse β chain locus comprises two constant region genes,

$C_{\beta 1}$ and $C_{\beta 2}$, each associated with a cluster of J_{β} gene segments (Gascoigne *et al.*, 1984; Malissen *et al.*, 1984). The gene segments are arranged tandemly within a 15-kb region (Fig. 2a). The human β chain genes are similarly organized (Sims *et al.*, 1984). The two murine constant region genes are almost identical, both genes being arranged in four exons interrupted by introns almost identical in length and position. The four exons do not obviously share the extremely close correlation between functional protein domain and exon organization found in immunoglobulins, but can nonetheless be assigned as encoding an external domain, a small hinge-like region, a transmembrane region and a cytoplasmic tail plus 3'-untranslated region (Gascoigne *et al.*, 1984; Malissen *et al.*, 1984). There are only four amino acid substitutions out of 173 between the two mouse constant regions, all of which occur in the transmembrane and cytoplasmic domains. Surprisingly,

Table 1. Chromosomal location of immunoglobulin and T cell receptor genes

Gene	Chromosome in:	
	Human	Mouse
Ig chain		
Heavy	14	12
κ light	2	6
λ light	22	16
T cell receptor antigen chain		
α	7	6
β	14	14
γ	7	13

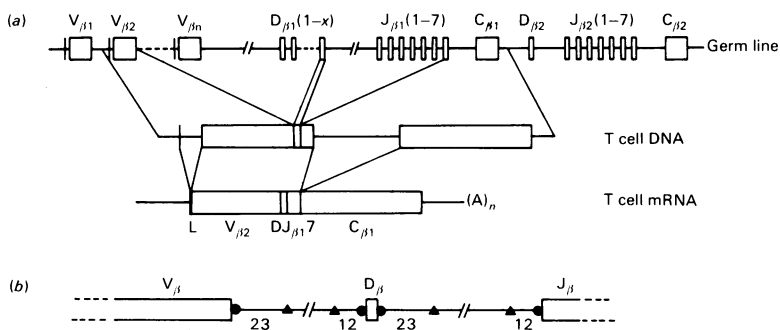


Fig. 2. Genomic organization of the β -chain gene family (a) and the organization of rearrangement signals associated with β -chain gene segments (b)

(a) The germline organization of the β -chain segments showing coding elements (boxes), is depicted together with the rearrangement events which result in the formation of a functional β -chain gene in a somatic T cell. (b) The conserved heptamer (●) and nonamer (▲) sequences separated by either 23 or 12 bp spacer sequences are shown.

this high level of conservation is also apparent at the nucleotide level (24 changes in 518 bases) (Gascoigne *et al.*, 1984). This is to be contrasted with the much greater divergence of the 3'-untranslated regions (103 changes in 217 bases) and the intron sequences, suggesting that a strong evolutionary selection pressure operates to maintain not only the protein structural similarity between the two gene products, but also the nucleotide sequence within the coding regions. Such a selection pressure at the protein level might be the requirement for multiple interactions between the β chain and the α chain and with other cell surface glycoproteins such as T3 and T4/T8. The two human C_{β} genes also show this striking similarity; however, the amino acid differences between the human $C_{\beta 1}$ and $C_{\beta 2}$ genes do not coincide with the differences between the mouse sequences (Yoshikai *et al.*, 1984b). It seems unlikely, therefore, that the few sequence differences are of biological significance.

The mouse β chain joining region segments are organized in two clusters of seven elements 5' to each constant region (Chien *et al.*, 1984a; Gascoigne *et al.*, 1984). An identical organization has been reported for the human J region clusters, although the sequence organization has not been described. In the mouse, each cluster comprises six J regions that are most probably functional and one pseudogene (Gascoigne *et al.*, 1984; Malissen *et al.*, 1984). Indeed, this large number of J regions in the β chain locus, compared with those of immunoglobulin genes, has been proposed to account for the presence of two constant region genes to allow the stable maintenance of such a large number of homologous sequences in one area of the genome (Gascoigne *et al.*, 1984). This is an attractive idea which would explain the function of the two essentially identical constant region genes. The selection of a particular J region by a V region would then determine which C region was expressed. The functional J regions all display certain similarities with one another, with the corresponding human J_{β} genes and with J regions of the immunoglobulin light and heavy chain loci. Thus, the nucleotides of the J segments code for characteristic amino acid sequences, including the nearly invariant amino acids Phe-Gly-Xaa-Gly-Thr followed by a conserved leucine/valine-rich 3' end (Gascoigne *et al.*, 1984; Malissen *et al.*, 1984). In addition, the flanking region sequences, in particular the 5' rearrangement (see below) and 3' splice donor signals, show homologies.

Comparison of the variable and joining region sequences of mouse and human β chain cDNA clones with their germline counterparts has revealed the presence of extra sequence, not present in the germline, at the V-J junction in the cDNA sequences. This was ascribed to the

presence of diversity (D) segments, a conclusion which has been confirmed by the localization of germline D region elements in mouse (Kavaler *et al.*, 1984; Siu *et al.*, 1984a) and man (Clark *et al.*, 1984). These elements have been located about 600 nucleotides 5' to each of the joining region clusters in the mouse, but to date have only been identified in association with the $J_{\beta 1}$ cluster in the human genome. The D segments sequences to date are short and are G-rich and in both these respects resemble their immunoglobulin counterparts. The variable gene segments are located an undefined but presumably huge distance from the D_{β} , J_{β} and C_{β} genes.

While there is to date little information on the α chain genomic organization, although preliminary evidence from Southern blotting analysis suggests that there is only one α chain constant region (Collins *et al.*, 1985c), that of the murine γ chain has been investigated. Three constant region genes, split into three exons, have been identified, each with a single 5' J region (Hayday *et al.*, 1985). The exon structure of the C region is similar to that of the β chain, except that the putative transmembrane and cytoplasmic domains are encoded in a single exon. Three genomic variable region genes, which cross-hybridize with a cDNA probe, have also been identified (Hayday *et al.*, 1985). The facts that one of the constant region genes is defective and that one of the variable genes is in the wrong orientation to undergo rearrangement may explain the observation that an identical rearrangement has been detected in all cytotoxic T cell clones so far examined (Kranz *et al.*, 1985a). It therefore seems that any diversity in γ chain gene rearrangements is generated by junctional diversity at the point of V/J joining, which could involve a D segment or nucleotide insertion (Kranz *et al.*, 1985a).

Rearrangements of β chain gene segments

The T cell receptor β chain gene, like immunoglobulin heavy chain genes, is assembled in a somatic T cell from four separate gene segments, V_{β} , D_{β} , J_{β} and C_{β} , following V-D and D-J rearrangements. This has been formally proved by sequence analysis of the β chain locus from both T cells and other tissues (Chien *et al.*, 1984a; Siu *et al.*, 1984a). Similar V-J rearrangements of the γ chain have been detected in T cell DNA (Hayday *et al.*, 1985). The mechanism by which this rearrangement occurs for immunoglobulin genes in B cells was first proposed by Early *et al.* (1980) as the so-called '12-23' rule, allowing recombination only between a conserved heptamer-nonamer sequence separated by a 12-nucleotide spacer and one with a 23-nucleotide spacer. Recombination in

β chain genes is postulated to be mediated by the conserved flanking sequences homologous to those in the immunoglobulin genes, found on the 3' side of the V_β gene segment, the 5' and 3' sides of the D_β segments and the 5' side of the J_β segments (Fig. 2b) (Gascoigne *et al.*, 1984; Malissen *et al.*, 1984). These are also observed in the γ chain locus (Hayday *et al.*, 1985). The arrangement of the recognition sequences of immunoglobulin heavy chain gene segments permits only D_H - J_H and V_H - D_H joining. β -Chain genes, however, because of the position of their recognition sequences, appear much more versatile in the ways in which their segments can recombine. Thus, direct V_β - J_β joining is possible as is D_β - D_β joining, in addition to V_β - D_β - J_β and D_β - J_β joining. The predominant type of recombination which takes place in the generation of a functional β chain gene appears to be of the V-D-J-C type. However, in at least one case a direct V-J-C joining has most probably occurred (Yoshikai *et al.*, 1984b).

Northern blotting analysis of mouse and human T cell mRNA with β chain constant region probes has revealed two transcripts of about 1.3 kb and 1.1 kb in size (Siu *et al.*, 1984b; Collins *et al.*, 1985a). The 1.3 kb transcript results from a productive rearrangement and encodes a functional β chain with a V, D, J and C region. The 1.1 kb transcript lacks a variable region, since V region probes hybridize only to the 1.3 kb transcript (Clark *et al.*, 1984). The majority of 1.1 kb transcripts are thought to arise from non-productive D-J joining events in the absence of V_β joining (Kavaler *et al.*, 1984; Siu *et al.*, 1984b; Collins *et al.*, 1985a); in at least one case a 1.1 kb transcript appears to initiate at a promoter immediately 5' to the D region (Collins *et al.*, 1985a). This has a direct parallel in immature B cells in which incomplete heavy chain rearrangements are frequently found (Alt *et al.*, 1981). D-J aberrant transcription is more frequent in the thymus (see Fig. 4); indeed, several of the transcripts characterized during the initial isolation of mouse β chain cDNA clones are most probably non-functional (Hedrick *et al.*, 1984b; Kavaler *et al.*, 1984).

There are several ways in which the frequent aberrant joining events may be of significance in the generation of the T cell repertoire. Firstly, it is likely that the inefficiency of the rearrangement process contributes to the mechanism by which allelic exclusion operates. Thus, although rearrangements are usually found on both chromosomes, only one productively rearranged transcript is ever found in a cloned T cell line (Kronenberg *et al.*, 1985). Secondly, the junctional variation in the joining process, which frequently results in the generation of a non-productively rearranged β chain gene, introduces a large degree of variability

into the β chain protein. Thirdly, it has been proposed by Siu *et al.* (1984b) that at least some D-J-C transcripts may be translated to produce a protein that can be expressed at the cell surface [although this clearly cannot be the case for the D-J-C $_{\beta 1}$ transcript of one line (Collins *et al.*, 1985a)]. The function of these truncated polypeptides is unknown; however, if they exist, they may perform some function associated with the generation of the T cell repertoire.

The T cell receptor in T cell development

The ability of T cells to recognize antigen in the presence of self MHC (see the following section) is 'learnt' in the thymus (Bevan & Fink, 1978). During foetal development, and in the adult in lesser numbers, T cell precursors from the bone marrow enter the thymus. The cells within the thymus are destined to a large extent to proliferate and die without leaving; however, a small number migrate from the thymus as mature T cells (Moore & Owen, 1976). Our knowledge of the interrelationships and functions of the various phenotypically defined thymocyte subsets is at present very limited. The study of T cell receptor expression in the thymus has therefore provided valuable information on the process of T cell differentiation.

It is now believed that T cells in the murine thymus which lack either of the surface markers L3T4 or Lyt2 (corresponding to T4 and T8 in the human) present on the separate helper (L3T4) and cytotoxic (Lyt2) subsets are the least mature cells and are precursors of other thymocytes (Fowlkes, 1984), though whether the 'single positive', i.e. L3T4 or Lyt 2 positive, mature cells are derived directly from the 'double negative' cells or from a 'double positive' cell, remains unclear. A schematic representation of human thymic subpopulations is shown in Fig. 3. In this case the surface antigen T3 provides a further useful marker. Because of its co-ordinate expression with the antigen receptor (see protein structure) it can be regarded as a potential marker for $\alpha\beta$ heterodimer surface expression and is present on about 40% of the phenotypically more mature cells (M. A. Ritter, unpublished work).

A monoclonal antibody which binds to a subset of murine $\alpha\beta$ heterodimeric receptors (about 20% of those on a mixed population of mature T cells) has also been used to observe expression of this epitope in murine thymocytes (Roehm *et al.*, 1984). This also is expressed at a higher level on the more mature medullary thymocytes. However, the lack of an antibody which recognizes an epitope common to all T cell receptors prevents a definitive answer to the question of which thymocyte populations express surface T cell receptors. Further-

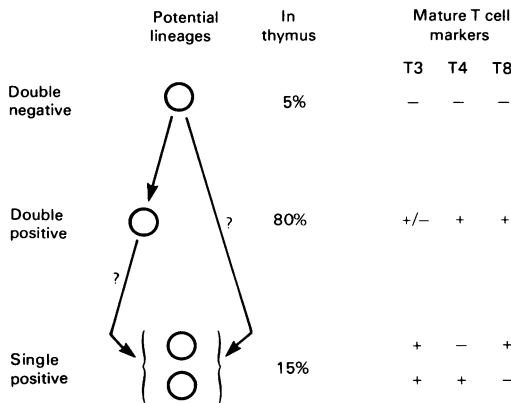


Fig. 3. Schematic representation of human thymic subpopulations

The relevant T-cell surface markers (defined by monoclonal antibodies) and possible lineage relationships between the thymocyte subpopulations defined by these markers are indicated.

more, by analogy with immunoglobulin genes it can be predicted that mRNA for one T cell receptor chain will be expressed prior to the other in T cell development. This has been postulated as a mechanism to ensure a higher chance of productive rearrangements at two loci in the same cell, if cells with one productive rearrangement, expressing functional mRNA, are subject to a period of selective advantage. To study potential temporal differences in α and β chain gene rearrangement and expression and, by inference, which thymic populations are capable of surface receptor expression, molecular biological techniques have now been applied.

The most complete study of murine thymocyte T cell receptor mRNA expression is that of Raulet *et al.* (1985). They find that, in the adult, the 'double negative' cell population expresses β chain mRNA but reduced levels of α chain mRNA. Previous results had shown that β chain mRNA was expressed in cortical thymocytes, which include less mature cells (Snodgrass *et al.*, 1985), and that β chain mRNA including aberrant transcripts is present at a much higher level in thymocytes than in mature T cells (Yoshikai *et al.*, 1984a). Raulet *et al.* (1985) have also demonstrated that β chain transcripts are present early in murine foetal thymic development, in agreement with another report (Snodgrass *et al.*, 1985), with α chain mRNA appearing later. Interestingly, they find that γ chain transcripts are present in 'double negative' adult thymocytes and early in foetal development, and that the level of these decreases when α chain mRNA appears. They propose a model of a $\gamma\beta$ surface heterodimeric protein present early in T

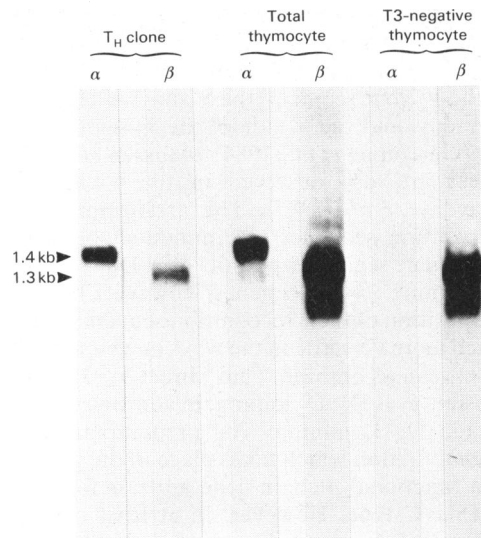


Fig. 4. Northern hybridization analysis of thymocyte and T-cell RNA

RNA from T3-negative thymocytes, total thymocytes and a human helper T-cell clone was electrophoresed, transferred to nitrocellulose and hybridized to human α and β chain transcripts in these populations (taken with permission from Collins *et al.*, 1985b).

cell development and involved in T cell repertoire generation. This remains purely speculation as a protein product of the γ chain locus has not been identified.

Human thymocyte T cell receptor mRNA expression using a panel of thymic leukaemia cell lines has also been studied (Collins *et al.*, 1985b). Lines of an immature surface phenotype, corresponding to 'double negative' cells, contain high levels of β chain mRNA, but very little or no α chain transcripts. However cells of a more mature 'double positive' phenotype, which expressed surface T3, contain mRNA for both α and β chain. It was therefore postulated that α chain gene expression might control the surface appearance of the receptor-T3 complex. Confirming this hypothesis, RNA from T3-negative human thymocytes was shown to contain β chain but not α chain mRNA (Fig. 4). This result, while supporting α chain control of Ti/T3 surface expression, does not exclude the possibility that mRNA for any component of the T3 complex is also absent from the T3-negative population.

It therefore appears that, in both mouse and human T cell development, β chain gene rearrangement and transcription occurs early and is followed by that of the α chain. Indeed, the data from human thymocytes show that approx. 60% of

the least mature cells, presumably including the 5% 'double negative' cells, lack α chain mRNA. Surface T cell receptor expression is then clearly an event which occurs during the process of T cell development in the thymus.

Part of the high level of cell proliferation and death in the thymus might be explained by selective proliferation of cells with functional β chain rearrangements, and the high level of β chain mRNA, compared with peripheral T cells or α chain in the thymus (Fig. 4), could also reflect its role in this process. Following productive α chain gene rearrangements the machinery required for this process can be 'switched off' and this may therefore explain the apparent lack of non-functional α chain transcripts resulting from aberrant rearrangements in this locus, compared with those commonly observed in the β chain (Fig. 4). Indeed, the deletions of β chain alleles frequently observed in mature T cells (Kronenberg *et al.*, 1985) may also stem from the period prior to α chain rearrangement; these deletions appear to be absent from early thymocytes (Royer *et al.*, 1984).

Once the α chain gene is rearranged and a surface receptor is expressed then the thymus must carry out the process of selection against cells with high affinity for self MHC molecules and selection for cells able to recognize self MHC plus antigen. Analysis of the pattern of variable region expression in thymic subsets, compared with the periphery, may shed some light on the mechanism of this step. Information on the exact stage in T cell development, for example in relation to the functional subset markers T4 and T8, at which receptor expression occurs is also vital to explain the correlation between HLA Class I or Class II recognition and T cell function (see the following section).

Implications for the T cell repertoire

The main issue that must be tackled by any review on a cell surface receptor is the nature of the binding reaction by which this molecule recognizes its ligand, and how this recognition leads to triggering of cell functions. In the case of the T cell receptor both these steps involve complexities not encountered when binding of a soluble hormone, such as insulin, to its receptor on a given target cell is considered. The population of mature T cells can be divided into diverse sets of cells that participate in the regulatory and effector phases of the immune response (Paul & Benacerraf, 1977) (see Fig. 5).

Not only is the response of these subsets on contact with their particular ligand different, but they also recognize a spectrum of antigens in the context of different classes of self MHC molecules, Class II in the case of helper cells (Kappler &

Marrack, 1976), Class I for cytotoxic cells (Zinkernagel & Doherty, 1975) and possibly without MHC in the case of suppressor cells (Germain *et al.*, 1979). Into the framework of knowledge that we have reviewed on the nature of α and β chain molecules, we must therefore try to fit these requirements for combined recognition of multiple antigens and MHC and differential responses following this recognition.

Two mechanisms have been proposed whereby a T cell might recognize both foreign antigen and self MHC involving either two separate receptors, or a single receptor recognizing the two in combination; the latter is supported by a variety of experiments (Kappler *et al.*, 1981; Heber-Katz *et al.*, 1982; Hunig & Bevan, 1982). There is no definitive evidence that the $\alpha\beta$ heterodimer is such a single receptor for any T cell. However, this is suggested by its variable nature, the ability of clonotypic antibodies to mimic or block the effect of antigen (Kaye & Janeway, 1984; Meuer *et al.*, 1983a), and alterations in the α and β protein chains observed in mutant alloreactive T cell hybridomas with altered MHC specificities (Augustin & Sim, 1984). Indeed, β genes are rearranged and expressed in a large number of cytotoxic and helper T cell lines and hybridomas, in fact in all those studied so far (Hedrick *et al.*, 1985; Kronenberg *et al.*, 1985). The limited evidence available for the α chain suggests that it also can be expressed in T cells of both helper and cytotoxic subsets (Saito *et al.*, 1984b; Chien *et al.*, 1984b). The γ chain, however, is expressed in the periphery almost exclusively in mature cytotoxic T cells and at a very low level. Any potential protein encoded by this gene must therefore fulfil a role within this subset, if indeed it has any function in mature T cells (see the previous section). An $\alpha\beta$ heterodimer is therefore very probably capable of recognition of both Class I and Class II MHC molecules in combination with antigen. There is no direct correlation between the expression of a given β chain variable region and the recognition of unique antigen or MHC molecules (Goverman *et al.*, 1985), favouring the idea of combined antigen/MHC recognition by the $\alpha\beta$ dimer, although an α chain variable region has not been similarly analysed.

While helper and cytotoxic T cells rearrange and express β chain genes it appears that some suppressor T cells may recognize antigen via a receptor other than the $\alpha\beta$ heterodimer. Some human suppressor cell lines do express β chain mRNA (Yoshikai *et al.*, 1984a), and similar heterodimeric surface proteins (Bensussan *et al.*, 1984). However, two studies with mouse suppressor cell hybridomas show no β chain gene expression (Hedrick *et al.*, 1985; Kronenberg *et al.*,

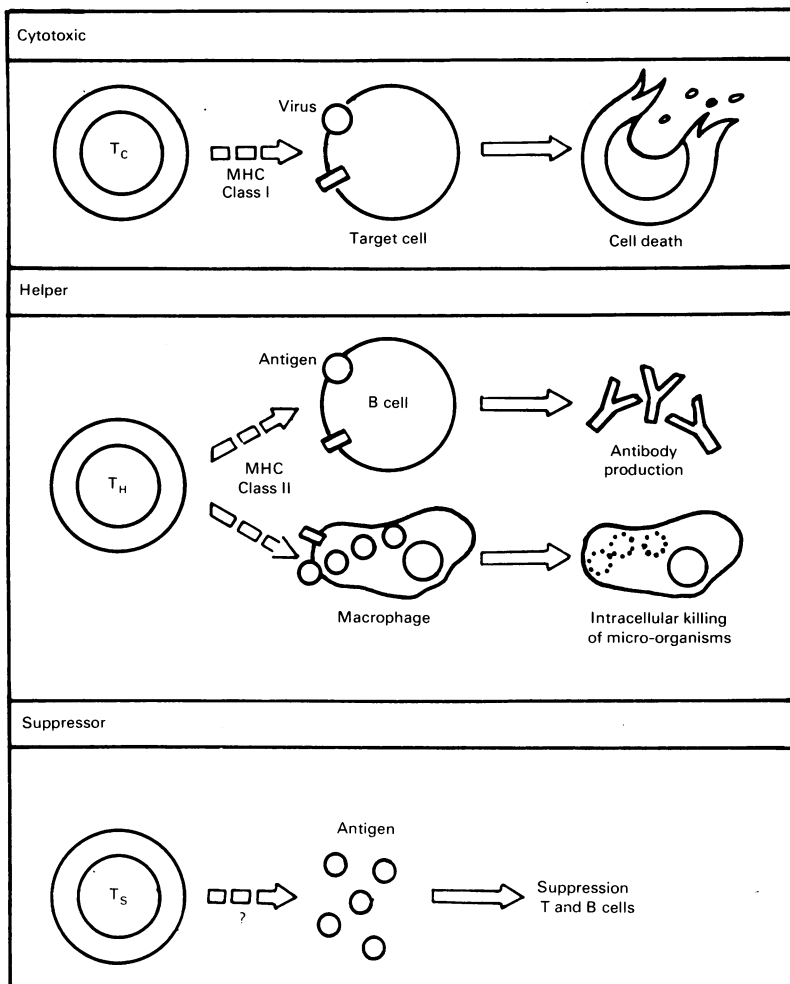


Fig. 5. The functions of the three T-cell subsets are shown together with the requirement for joint recognition of antigen plus MHC gene products by cytotoxic and helper T-cells

There is some evidence that suppressor T-cells may be 'B cell-like' and recognize antigen alone.

1985) and in many cases deletion of the entire β chain gene locus. This might reflect rearrangement of a β chain variable region to a constant region located downstream of $C_{\beta 2}$. However, as the murine IgK chain locus, which is situated some considerable distance away, is also deleted in suppressor cells (Hedrick *et al.*, 1985; Kronenberg *et al.*, 1985), a large deletion on chromosome 6, not confined to the β chain locus, must have occurred. It is clear that there may be much variability in the definition of a suppressor cell in culture in terms of its specificity within the suppressive pathway. Whether those that lack β chain genes and mRNA utilize the α chain locus will be of interest. If they indeed recognize antigen by a further unidentified receptor molecule, then more rearranged gene families must be activated in T cells.

The existence of two constant region genes in the β chain locus has raised the possibility that these may be functional isotypes, in some way performing different functions upon antigen recognition. It is however clear that the obvious differential response, that between cytotoxic and helper cells, does not stem from different C_{β} expression, as both subsets can utilize either $C_{\beta 1}$ or $C_{\beta 2}$ (Kronenberg *et al.*, 1985). If these functional subsets can be separated on $\alpha\beta$ dimer structure, then different usage of V_{β} or V_{α} gene pools remains a possibility, but it seems probable that other invariant subset-specific molecules will determine the different effector functions that follow triggering of a common receptor. There is conflicting data on differential expression of the two β chain constant regions during T cell maturation. While $C_{\beta 1}$ clones have

been observed to be more frequent than $C_{\beta 2}$ clones in mouse thymocyte or spleen cDNA libraries (Gascoigne *et al.*, 1984), $C_{\beta 2}$ usage has been reported as higher in mouse thymus and lymphoid organs (Kronenberg *et al.*, 1985) and in human thymus, though not in peripheral T cells (Yoshikai *et al.*, 1984b), and $C_{\beta 2}$ is clearly more commonly employed among all functional cultured T cells analysed (Kronenberg *et al.*, 1985). Some of this discrepancy may be due to a lack of distinction between functional and non-functional transcripts. As the two constant region genes are very similar in their coding region it seems likely that any differential expression occurs as an outcome of the mechanism of β chain rearrangement during T cell development, rather than from functional necessity at the protein level.

Having considered features common among T cell receptor molecules, it is important to turn to the mechanisms whereby a T cell repertoire, capable of recognizing a large number of foreign antigens, is constructed. However, very little data are available on the relative contributions of various potential mechanisms to the diversity of T cell recognition. No comparison of V_{α} sequences can yet be made. Seven murine β chain variable regions have been compared (Patten *et al.*, 1984); they proved to be extremely heterogeneous with between 18% and 51% homology at the amino acid level. This is consistent with the lack of murine V region families observed by Southern hybridization analysis, making it difficult to estimate the number of V_{β} genes (Patten *et al.*, 1984) although such a family has been observed for a human variable region (Sims *et al.*, 1984). Analysis of amino acid homologies between the V_{β} genes by the method of Wu & Kabat (1970) revealed three regions of hypervariability in similar positions to those in immunoglobulin variable regions (Patten *et al.*, 1984). Further detailed analysis of hypervariability is difficult in such a small sample. When the pattern of expression of five of these variable region genes was analysed in the thymus, four were estimated to comprise 40% of the functional transcripts, the fifth being undetectable (Patten *et al.*, 1984). This latter gene has, however, been observed in functional T cells (Chien *et al.*, 1984a; Goverman *et al.*, 1985) and may, therefore, be under-represented in immature T cells. These results suggest that T cells use a small number of V_{β} genes very frequently, although an analysis of thymic expression might detect predominant early rearrangements not characteristic of the mature repertoire.

As previously discussed the T cell receptor β chain has possibilities of combinatorial associations of V, D and J regions not seen in immunoglobulin genes. Receptor diversity can be gener-

ated not only by V, D and J region multiplicity, but also by direct V-to-J joining, and by $D_{\beta 1}$ to $J_{\beta 2}$, both of which have been observed (Yoshikai *et al.*, 1984b; Goverman *et al.*, 1985). N region diversity, first detected in B cells (Alt & Baltimore, 1982) refers to extra nucleotides inserted between V and D and D and J segments during the joining process by terminal deoxynucleotidyltransferase, which is expressed at high levels in immature thymocytes. Evidence of this mechanism in operation is suggested (Kavaler *et al.*, 1984; Siu *et al.*, 1984b; Goverman *et al.*, 1985) although the presence of an additional D_{β} segment to those previously identified could also explain the results. Junctional diversity is caused by imprecision of joining events, and the variation in length of sequence between the heptamer recognition sequence and the expected start of J_{β} regions allows considerable scope for this (Gascoigne *et al.*, 1984). Indeed, it leads to translation of the $D_{\beta 1}$ and $D_{\beta 2}$ segments in all three reading frames in various β chain cDNA clones (Goverman *et al.*, 1985). The mechanism which is known to generate a high level of diversity in immunoglobulin molecules, somatic hypermutation (Kim *et al.*, 1981), has so far not been detected in the two studies where expressed β chain variable regions have been compared with their germline counterparts (Chien *et al.* 1984a; Goverman *et al.*, 1985) and the role of this mechanism in T cells remains an open question. Indeed, while these results with the β chain suggest potential mechanisms that may be involved in generation of diversity of T cell recognition, our understanding remains limited until similar data become available for the α chain.

Conclusion

While this review has described recent major advances in the T cell antigen receptor field, it is clear that many important questions remain to be answered. Firstly, conclusive evidence that an $\alpha\beta$ dimer is capable of both antigen and MHC recognition can only come from transfection of genes encoding α and β chains, from a donor T cell of known specificity. The recipient cell, which presumably would have to be capable of at least T3 expression, should then acquire the MHC and antigen recognition pattern of the donor. It is also impossible at present to measure the affinity of a T cell receptor for antigen plus MHC, and its presumably lower affinity for either component alone. These important parameters cannot be determined in the multiple molecule reaction that occurs when a T cell interacts with an MHC Class I bearing target cell. Here also genetic engineering could provide an answer, by generating a 'soluble' T cell receptor, upon transfection of α and β chains to an

appropriate cell lacking their transmembrane portions, binding of which can then be measured. Clearly the interaction between the $\alpha\beta$ heterodimer and the T3 molecule is of great importance, as it is believed that T cell receptor occupancy leads to cell triggering by T3 activation. Site-directed mutagenesis of α and β chain genes, to generate variant proteins incapable of interaction with T3, might enable mapping of the site on the T cell receptor molecule which interacts with T3 and furthermore reveal any responses which are not T3 mediated. Thus it should be evident, from these few examples, that while we now have some basic information on the nature of the T cell receptor for antigen, the reagents are now available for a much more detailed analysis of its properties and function.

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