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FRANK J. DIXON

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The Antigen-Specific, Major Histocompatibility Complex-Restricted Receptor on T Cells

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I. Introduction

The receptor on T cells responsible for clonal recognition of and response to antigen by these cells has been long sought, not only because of the insights an understanding of these molecules might shed on human health and disease, but also because of scientific curiosity about the way in which such molecules might function. Early attempts to isolate these proteins relied heavily on the idea that T cell receptors might be similar, if not identical, to immunoglobulin. In retrospect although this idea was not unreasonable, it certainly created a good deal of confusion in the field.

Thus, conflicting data were generated about the presence on T cells, or T cells' secretion, of proteins cross-reacting with anti-immunoglobulin antibodies (Marchalonis and Cone, 1973; Vitetta *et al.*, 1973; Eichmann and Rajewsky, 1975; Binz and Wigzell, 1977; Ben Neriah *et al.*, 1980). Similarly, attempts to pursue T cell receptors based on the assumption that, like antibody molecules, they would bind free antigen molecules, also led to opposing results (Hunter *et al.*, 1972; Feldmann, 1972; Binz and Wigzell, 1975;

Krawinkel *et al.*, 1977). Meanwhile data accumulated which suggested that the receptors for antigen on most T cells might not be immunoglobulins.

Perhaps the first indications of this came from experiments which showed that T cells and B cells from the same animals did not recognize the same determinants on antigens. For example, many experiments showed that T cells do not distinguish between native and denatured antigens, whereas antibody commonly does (Parish, 1971; Mason *et al.*, 1972; Schirrmacher and Wigzell, 1972; Chesnut *et al.*, 1980). Moreover, T cells and B cells primed to a particular antigen frequently cross-react differently on related antigens (Hoffmann and Kappler, 1972; Playfair, 1972), although exceptions to this rule were also noted (Rajewsky and Mohr, 1974). In addition, the confusing phenomena of immune response (Ir) genes also had to be explained, since these major histocompatibility complex (MHC)-associated genes were known to affect T cell reactivity without much direct effect on B cells (Katz *et al.*, 1973b; Press and McDevitt, 1977).

The explanation for these findings was founded on the work in the early 1970s of Kindred and Shreffler, Katz and his colleagues, and Shevach and Rosenthal (Kindred and Shreffler, 1972; Katz *et al.*, 1973a,b; Rosenthal and Shevach, 1973; Shevach and Rosenthal, 1973). Later Zinkernagel and Doherty and others built on the findings of these groups, and demonstrated directly for the first time that cytotoxic T cells only kill antigen-bearing cells if the antigen is presented to them in association with products of the MHC on the surfaces of antigen presenting cells (Zinkernagel and Doherty, 1975; Shearer *et al.*, 1975; Gordon *et al.*, 1975; Bevan, 1975). Numerous workers have since confirmed this finding for T cells responsible for B cell help, delayed hypersensitivity, and lymphokine secretion (Kappler and Marrack, 1976, 1977; Singer *et al.*, 1978; Sprent, 1978; Schwartz *et al.*, 1978; Waldmann, 1978; von Boehmer and Haas, 1981).

The discovery that many T cells recognize antigen only in association with products of the MHC led to the conclusion that at least a component of the receptor on T cells was not immunoglobulin. This, coupled with numerous observations that many T cells do not secrete immunoglobulins (Roehm *et al.*, 1984b) or usually have mRNA encoding these molecules (Kronenberg *et al.*, 1980, 1983; Kurosawa *et al.*, 1981), suggested that the isolation of T cell receptors might be most easily accomplished by methods which did not depend on the idea that these molecules were related to immunoglobulins.

In fact the two methods which were most successful in identifying receptors were both based on a minimum number of assumptions. The first experiments depended on the idea that receptors would bear antigenic determinants which would not necessarily cross-react between different T cell clones, and the second was based on the assumption that receptor mRNA would be transcribed only in T cells.

II. Identification and Properties of T Cell Receptor Proteins

A number of investigators identified molecules of interest on T cells by injecting bulk, uncloned T cells or T cell tumors from one strain or species into other animals and studying the properties of the antisera and/or monoclonal antibodies so identified. By these means many important proteins on the surfaces of T cells were identified, some of which appeared to have a role in T cell recognition of antigen, because antibodies against them either blocked or stimulated T cell activation (Itakura *et al.*, 1972; Cantor and Boyse, 1975; Shiku *et al.*, 1976; Nakayama *et al.*, 1979; Davingnon *et al.*, 1981; Swain, 1981; Biddison *et al.*, 1982; Reinherz *et al.*, 1982). Since none of these molecules appeared to vary in amino acid sequence between one T cell clone and another, however, it was concluded that they did not contribute to specific recognition of antigen and MHC by T cells. These molecules are of great interest, however. Some of their properties are discussed in detail below.

A major advance occurred with the description by Infante *et al.* (1982) of antisera raised in mice against alloreactive T cell clones. These antisera stimulated division of the immunizing T cell clone and had no effect on other, closely related T cells, suggesting that the functional antibodies involved were recognizing some clone-specific determinant on the surface of the T cell clone used for immunization, a determinant which might be the analog for T cell receptors of an immunoglobulin idiotype. This work was followed by the description of other antisera with similar properties, and finally by reports from a number of laboratories of monoclonal antibodies which recognized clone-specific determinants bourne by the T cell clones, tumors, or hybridomas used for immunization (Allison *et al.*, 1982; White *et al.*, 1983; Meuer *et al.*, 1983b; Haskins *et al.*, 1983; Samelson and Schwartz, 1983; Kaye *et al.*, 1983; Bigler *et al.*, 1983; Lancki *et al.*, 1983; Staerz *et al.*, 1984; Kranz *et al.*, 1984).

Many properties of these monoclonal antibodies indicated that they bound receptors on T cells. First, their specificity for particular T cell clones showed, of course, that the molecule bound had determinants that were unique to a particular clone. Of all T cell surface proteins, only the receptor for antigen and MHC would have this characteristic. Second, these antibodies inhibited the responses or binding to antigen plus MHC of target T cell clones (Haskins *et al.*, 1983). Alternatively, under certain circumstances the antibodies stimulated responses of target T cells. This was particularly apparent if the antibodies were made polyvalent, by coupling to Sepharose beads for example (Kaye *et al.*, 1983; Kappler *et al.*, 1983b; Meuer *et al.*, 1983c). Again, these are characteristics that would be expected of antibodies binding part of the T cell receptor. Third, we demonstrated that a mono-

clonal antibody, KJ1-26, raised against an ovalbumin-specific, IA^d-restricted T cell hybridoma, DO-11.10, failed to bind to subclones of this hybridoma which had lost the ability to bind to or respond to ovalbumin plus IA^d, again suggesting that the antibody concerned did indeed bind all or part of the receptor for ovalbumin plus IA^d (Haskins *et al.*, 1983). Fourth, this same clone-specific anti-receptor antibody, KJ1-26, was used to screen a large panel of independent T cell hybridomas prepared from T cells of BALB/c ($H\text{-}2^d$) mice primed with ovalbumin. The antibody bound to a single hybridoma of about 400 tested, and this T cell hybridoma turned out to have the same rare fine specificities, both for antigen and MHC, as the T cell hybrid. DO-11.10, against which the antibody was raised. This result could only be compatible with the reaction of the antibody with the antigen- and MHC-specific receptor on the T cell (Marrack *et al.*, 1983b).

Finally, all monoclonal antibodies so far described with properties of the type listed above precipitate similar surface molecules from target T cells. These molecules are disulfide-bonded heterodimeric glycoproteins with characteristics that are both reminiscent of yet distinguish them from immunoglobulins.

Although like antibodies these molecules are constructed of two different kinds of polypeptide chains, each molecule contains a single chain of each type. The two polypeptide chains are of similar molecular weights, being both about 43,000 in mouse (Allison *et al.*, 1982; Kappler *et al.*, 1983b). In man the more acidic, α, chain is between 45,000 and 50,000, and the more basic, β, chain is lower in molecular weight at about 40,000 (Meuer *et al.*, 1983a; Kappler *et al.*, 1983a). Both α and β chains are extensively glycosylated. In mouse both chains bear both N-linked and O-linked sugar residues. The polypeptide backbones, stripped by endoglycosidases or synthesized in the presence of tumicamycin, have been reported to be 31–32K (β chain) and 27–31K (α chain) (McIntyre and Allison, 1984; Kaye and Janeway, 1984). The molecular weights of the amino acid sequences predicted from cDNA clones suggest the lower of these molecular weights for the α chain is probably more accurate (Chien *et al.*, 1984a; Saito *et al.*, 1984a). The difference in molecular weights between human α and mouse β chains seems to be entirely due to more extensive glycosylation of the α chain in this species (Oettgen *et al.*, 1984). Probably because of different amino acid sequences in their variable regions, however, both chains vary in molecular weight and charge when isolated from different T cell clones (Kappler *et al.*, 1983b). In addition, peptide fingerprinting showed that α chains from different clones had peptides which distinguished them, and also peptides which they shared. This was also true for β chains, indicating that both chains had immunoglobulin-like variable and constant regions. Finally, peptide fingerprinting showed that α and β chains had no peptides in common, proving

that they were not derived from the same collection of genes (Acuto *et al.*, 1983; Kappler *et al.*, 1983a).

Apart from the disulfide bond between the two chains, data from experiments in which receptor polypeptides were reduced with limiting concentrations of 2-mercaptoethanol indicated that intrachain disulfide bonds also existed (Samelson and Schwartz, 1983). The interchain disulfide bond is the most sensitive to reduction (Samelson and Schwartz, 1983; J. Kappler, unpublished observations). Interestingly this parallels results with immunoglobulins in which the interheavy chain bonds are most easily destroyed by reducing agents.

Contrary to expectations the gross structural properties and peptide fingerprints of α and/or β chains could not be used to distinguish receptors on T cells specific for class I MHC products from those on class II specific cells. Class I- and class II-restricted cells all shared the same ^{125}I surface labeled peptides after digestion with trypsin or pepsin (Acuto *et al.*, 1983; Kappler *et al.*, 1983a). The possibility remained, however, that some part of the receptor buried deeply in the membrane, or in the cell cytoplasm, which would be inaccessible to the ^{125}I-labeling methods used in these experiments, might be different in cells with these different specificities. This idea has not been supported by molecular biological analyses (see below).

In summary the receptor on T cells for antigen plus MHC has been isolated using monoclonal antibodies. The molecule is a disulfide-bonded heterodimer of 85–90K. The two glycopolypeptide subunits appear unrelated to each other and to immunoglobulin. Each has variable and constant peptides. The overall structure of the molecule is therefore similar in design to the antibody molecule.

III. Properties of cDNA and Genomic Clones Encoding T Cell Receptor Proteins

Early in 1984 two groups reported the sequences of cDNA clones isolated from subtraction libraries of a human T cell leukemia (Yanagi *et al.*, 1984) or of mouse T cell hybridomas (Hedrick *et al.*, 1984a,b). The mRNAs which gave rise to these clones are expressed only in T cells, and the cDNA clones bind DNA which rearranges between the germ line and mature T cells. The amino acid sequences predicted by these clones are immunoglobulin-like, having two domains with properties very similar to those found in immunoglobulins, containing intrachain disulfide loops and other residues such as Trp-32, Gln-35, and Trp-178 at conserved positions. Each of these sequences also contains a leader sequence, a J-like region, a hinge-like portion concerning a cysteine residue, which is presumably involved in covalently linking the α and β chains, a transmembrane region and a short cytoplasmic tail (diagrammed in Fig. 1). Sites for N-linked glycosylation are also present.

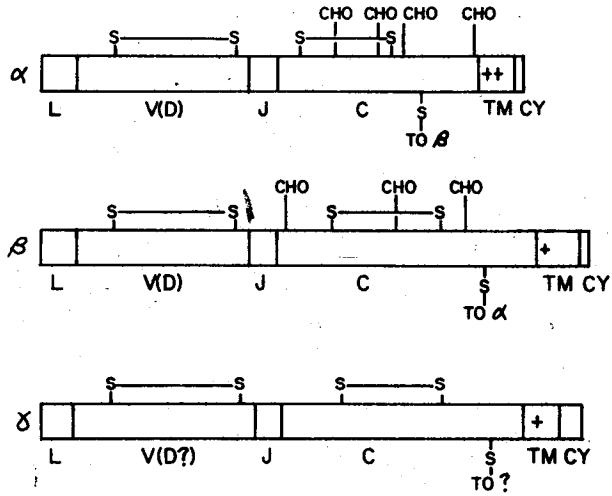

FIG. 1. Structure of mouse receptor and receptor-like polypeptides. The diagrams show to scale the predicted protein structures of mouse α, β, or γ polypeptides. The leader, variable plus D, J, constant domain, transmembrane, and cytoplasmic regions are indicated for each chain. Also shown are some of the cysteine residues, with their probable disulfide linkages. Sites for N-linked glycosylation (CHO) are shown for the constant regions only. Data are from Hedrick *et al.* (1984b), Saito *et al.* (1984a,b), and Chien *et al.* (1984a).

When the sequences of cDNA inserts isolated from different T cell hybridomas are compared it is clear that these genes are immunoglobulin-like in one other crucial respect since the predicted amino acid sequences of the first domain are clearly variable, whereas the second domain, hinge, transmembrane, and cytoplasmic region sequences appears (almost) constant. Extensive homology between the nucleotide and predicted amino acid sequences of the human and mouse cDNA clones shows that they code for the same chain of the T cell receptor. N terminal protein sequencing later showed that the chain encoded is that of the β chain (Acuto *et al.*, 1984; Hannum *et al.*, 1984a,b).

Many interesting conclusions have been drawn already from studies on these recently discovered β chain genes. First, the structure of these genes in the germ line has been analyzed (diagrammed in Fig. 2). The germ line structure of mouse and man is very similar. In each case there are two closely linked genes with very similar structures, exon sequences, and predicted amino acid sequences encoding two constant regions. Each constant region gene is constructed from four exons. The positions and lengths of the introns interrupting the two C region genes are almost identical. The exons

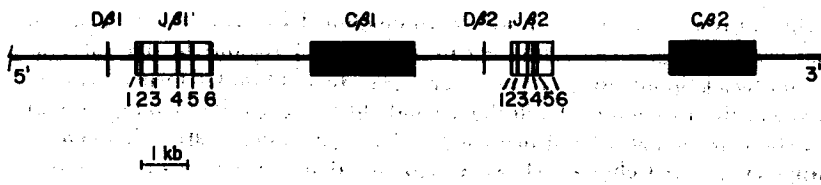

FIG. 2. Germ line structure of the mouse β chain locus. This is shown to scale without indication of the intron/exon structure of Cβ1 and Cβ2. Data are from Gascoigne *et al.* (1984), Malissen *et al.* (1984), Siu *et al.* (1984a,b), and Kavaler *et al.* (1984).

of each gene consist of one coding for the second, constant region, extracellular domain, a very small exon coding for the hinge-like region, one exon encoding the transmembrane region and finally an exon coding for the cytoplasmic tail and 3′ untranslated region (Gascoigne *et al.*, 1984; Malissen *et al.*, 1984). Most interestingly in mouse the coding regions of the two constant region genes differ by only 24 of their 318 nucleotides, whereas the 3′ untranslated regions of the two genes are different lengths, and also differ considerably in DNA sequence, being less than 50% homologous. The 24 base differences between the coding sequences for the two constant regions lead to only 4 amino acid changes, all in or near the transmembrane and cytoplasmic domains (Gascoigne *et al.*, 1984). The fact that the 3′ untranslated regions of these two genes are so dissimilar, coupled with the fact that the genomic organization of these genes in man is similar to that in mouse, suggests that duplication of the Cβ genes occurred before speciation of mouse and man. Gascoigne *et al.* (1984) have suggested a time about 120 million years ago. In that case, the conservation of nucleotide and coded amino acid sequences of the two constant regions is really remarkable, and implies strong, selective pressures on the sequences encoded, or perhaps very recent or very frequent gene conversions in the coding sequences. The idea that the amino acid sequence of these genes is strongly conserved by pressure for it to function in a particular way is supported by the fact that the translated amino acid sequences of human and mouse Cβ regions are also very similar; only 37 amino acids of 177 encoded by these four exons differ.

There is no evidence that one or other C region is selectively expressed by one type of T cell, since class I- and class II-restricted cells have been found expressing Cβ1, and likewise other cells of both specifities have been found expressing Cβ2 (Royer *et al.*, 1984; Kronenberg *et al.*, 1985; Hedrick *et al.*, 1985). The genomic structures of the two constant regions are similar in other ways. Both genes are preceded by a cluster of 6–7 functional J region genes, and about 500–600 bases upstream from each of these a D region segment is located (Gascoigne *et al.*, 1984; Malissen *et al.*, 1984; Siu *et al.*, 1984a,b; Kavaler *et al.*, 1984; Clark *et al.*, 1984). The approximate structure

of the entire locus is shown in Fig. 2. In functional T cells it has been shown that each D region can rearrange to any of the J regions in the adjacent cluster by a looping out-insertion mechanism. In addition Dβ1 can rearrange to any of the J region genes in the second cluster, again by looping out and insertion. In immature and mature T cells V region genes also rearrange to either D and/or J cluster. There is evidence that in some cases rearrangements may occur by mechanisms such as sister chromatid exchange or inversion (Chien *et al.*, 1984b; Siu *et al.*, 1984a,b; Kronenberg *et al.*, 1985; Born *et al.*, 1985).

In any case the mechanisms of rearrangement for these genes seem to be very similar to those used for immunoglobulin gene rearrangement in B cells. Thus D segments and the 5′ sides of J regions are flanked by heptamer/nonamer sequences similar to those which are used to indicate rearrangement sites for heavy and light chains (Siu *et al.*, 1984b; Kavaler *et al.*, 1984; Sakano *et al.*, 1979; Early *et al.*, 1980; Sakano *et al.*, 1980; Tonegawa, 1983). One difference was noted, however, in that if the rules worked out for immunoglobulin genes apply here, there is evidence that Vβ genes may have the option of rearranging directly to a J region gene. Thus D region genes are flanked by heptamer/nonamer sequences with spacers of 12 nucleotides on their 5′ sides, and 23 nucleotides on their 3′ sides. The heptamer/nonamer sequences of J region genes all have 12 base spacers on their 5′ sides. This implies that a V region gene with 3′ heptamer/nonamer sequences having a 23 base spacer could rearrange either to D or J (Siu *et al.*, 1984b; Kavaler *et al.*, 1984). Indeed there is some evidence from the sequencing of a β chain of a cDNA clone from the human T cell leukemia HPB-MLT that D region genes may sometimes be missing, though of course such sequences may theoretically be so small as to be invisible (Jones *et al.*, 1985).

Again as in the case of immunoglobulins there is evidence that non-germ line encoded nucleotides can be introduced at the point of V to D or D to J joining, leading to so-called N region diversity (Siu *et al.*, 1984b; Sakano *et al.*, 1981; Alt and Baltimore, 1982). All in all, it is clear that β chains use many of the strategies previously described for immunoglobulin genes to achieve diversity. This will be discussed at greater length below.

cDNA clones encoding α chain polypeptides have been isolated, like β chain cDNAs, from T cell-specific cDNA subtraction libraries (Chien *et al.*, 1984a; Saito *et al.*, 1984b) and also by the use of amino acid sequences of tryptic peptides from the α chain of the human leukemia, HPB-MLT (Sim *et al.*, 1984). In this latter case degenerate oligonucleotide probes were built to match the known amino acid sequences, and were used to isolate cDNA clones which encoded the α chains in question. Like β chains, both in mouse and man, α cDNA probes bind to mRNA expressed only in T cells and detect

genomic DNA which rearranges between the germ line and mature T cells. Again, there is extensive homology between the coding DNA sequences and translated amino acid sequences of mouse and man, though this is less marked than for β chains.

As for β chains, the amino acid sequences known or predicted for α chains suggest a strong relationship to the immunoglobulin family (Fig. 1). α chain polypeptides each have a leader sequence of about 20 amino acids, two immunoglobulin-like domains defined by intrachain disulfide bonds and other conserved residues, a J-like sequence, and hinge, transmembrane, and cytoplasmic regions (Chien *et al.*; 1984a; Saito *et al.*, 1984b; Sim *et al.*, 1984). There is evidence that D region sequences are optional, as for β chains (Sim *et al.*, 1984). The first immunoglobulin like domain varies in sequence from clone to clone as expected for a V region. The second, C region domain is somewhat surprising since it is unexpectedly short, the disulfide-bonded intrachain loop includes only 50 rather than the usual 70 amino acids, and lacks residues that are otherwise universally conserved in the immunoglobulin family such as Trp-148. On the other hand, the so-called hinge region, containing a cysteine residue presumably involved in cross-linking to the β chain, is more reminiscent of an immunoglobulin hinge than its counterpart in the β chain.

The supposed transmembrane region of human and mouse α chains contains 2 positively charged amino acids. This was surprising, since β chain transmembrane regions had previously been shown to contain one basic amino acid, and it had been suggested that α β dimers might be stabilized in the membrane by the presence of a complementary negatively charged amino acid in the α chain transmembrane region. The significance of the fact that the two chains possess jointly 3 basic amino acids in what is thought to be that portion of their sequences which spans the lipid bilayer of the cell will be discussed in greater detail below.

So far very little of the germ line structure of α chain genes has been reported. It is already clear, however, that, unlike Cβ, the locus contains only one functional C region gene, and there must be a very large intron between the Jαs and Cαs since no restriction enzyme used by us has shown rearrangements involving Jα on Southern blots using a Cα probe (J. Yagüe and W. Born, personal communication).

In summary cDNA clones encoding the α and β chains of the T cell receptor have now been isolated. They demonstrate that these genes are expressed exclusively in T cells and have properties very similar to those of immunoglobulins. The functional gene is composed of rearranged V, (D), and J segments and a constant region portion. The mechanisms causing rearrangement seem to be similar to those used by immunoglobulin genes in B cells.