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CD4 and the immunoglobulin superfamily

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SUMMARY

The CD4 membrane glycoprotein was one of the first cell surface antigens to be identified using monoclonal antibodies. It was shown to have a central role in the control of the recognition of foreign proteins by T lymphocytes and later as a receptor for the human immunodeficiency virus (HIV). The analysis of the amino acid sequence of CD4 showed that the extracellular region comprised four regions with sequence similarities to immunoglobulin domains. The structure of domains 3 and 4 of CD4 has been determined by X-ray crystallography and, like domains 1 and 2 previously determined, these have typical immunoglobulin-like folds. The results are discussed with respect to the identification of other domains with immunoglobulin-like folds from amino acid sequence data, and the evolution of CD4.

1. INTRODUCTION

The term CD stands for 'clusters of differentiation' and was introduced as a simple method to group together monoclonal antibodies (mAb) that recognized the same antigen at the surface of human leucocytes (Bernard *et al.* 1984). This nomenclature is now widely used to describe both the antibody recognizing the antigen and the antigen itself. It is also used to describe homologues in species other than humans. The antigen now known as CD4, was first identified in the rat by the W3/25 mAb and was one of the first three new antigens to be defined by monoclonal antibodies (Williams *et al.* 1977). The W3/25 mAb was of particular interest as it was the first marker for the sub-population of T lymphocytes with 'helper' activity (now generally described functionally as restricted to MHC class II antigens) (White *et al.* 1978). The W3/25 mAb was also used to show the first effect of a monoclonal antibody on a functional assay *in vitro*, the inhibition of a mixed lymphocyte reaction (Webb et al. 1979) and also *in vivo* in the inhibition of the model autoimmune disease, experimental autoimmune encephalomyelitis (EAE) (Brostoff & Mason 1984). The human homologue of CD4 was identified with the mAb T4 (Reinherz *et al.* 1979) and later was shown to be the receptor for the HIV that causes AIDS (Dalgleish *et al.* 1984; Klatzmann *et al.* 1984).

The amino acid sequence of CD4 was determined at the cDNA level by transfecting human genomic DNA into a mouse cell line and isolating a CD4 positive transformant. Human cDNA prepared from this cell line was enriched by subtractive hybridization and used as a probe to isolate CD4 cDNA from cDNA libraries prepared from T lymphocytes (Maddon *et al.* 1985). This cDNA was then used as a probe to obtain the sequences of CD4 from several other species. The predicted amino acid sequence of CD4 indicated that it had a single transmembrane domain and the extracellular region comprised four domains with sequence similarities to immunoglobulin domains (Maddon *et al.* 1985; Clark *et al.* 1987; Maddon *et al.* 1987) In this article we summarize studies to determine the structure of CD4 by X-ray crystallography and their implications for the validity of the concept of the immunoglobulin superfamily (IgSF).

2. ANALYSIS OF SEQUENCE SIMILARITIES AND SUPERFAMILIES

Amino acid sequences often show similarities to those in other proteins and Dayhoff used the terms 'family' for very closely related sequences with greater than 50% amino acid identity and 'superfamily' for those below 50% identity (reviewed in Barclay *et al.* 1993). Superfamilies are predicted to be related in evolution and to have similar structures and types of function. The predicted domain structure of CD4 was controversial in that the first domain was recognized immediately as being Ig-like but the remainder was not classified (Maddon *et al.* 1985). Further analysis indicated that there were possibly four IgSF domains, but domain 2 appeared to be particularly truncated (Clark *et al.* 1987; Maddon *et al.* 1987; Williams & Barclay 1988). Claims that there were regions related to the J regions in Ig (and T cell receptor) were weak (Maddon *et al.* 1987).

One method that has been widely used to evaluate putative IgSF sequences is to compare the test sequence with many other IgSF sequences both by visual alignment and statistical analysis as described

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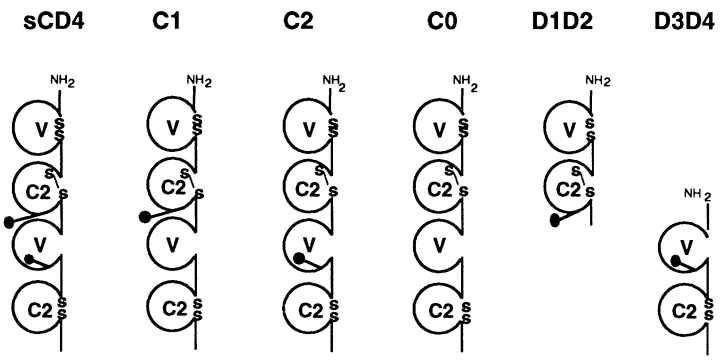

Figure 1. Schematic view of recombinant forms of soluble CD4 expressed in CHO cell lines. sCD4 corresponds to the complete extracellular region of CD4 and C1, C2, and C0 represent mutant forms lacking glycosylation at the site in domain 3, the site in domain 2 and both sites respectively. Glycosylation sites are indicated by (🍍). V and C2 represent the type of IgSF domain. These constructs were expressed at 25–150 mg l^{-1} (Davis *et al.* 1990*b*; Brady *et al.* 1993).

in (Williams 1987; Williams & Barclay 1988). The extent of the putative IgSF domain is first identified by inspection and comparison with other IgSF domains. This region is then compared with many different IgSF domains using the ALIGN programme (Dayhoff *et al.* 1983). Immunoglobulin V- and C-domains differ considerably in length making statistical analysis difficult and indeed the evolutionary relationship between V- and C-domains was controversial until their structures were determined. A second smaller domain type (C2set) was distinguished by sequence characteristics from classical C-domains (the C1set is found in Ig constant regions, T cell receptor chains, beta2-microglobulin and MHC antigens). Thus a number of residues seem to be present in virtually all C1set domains but are absent from C2set domains which themselves often have patches of sequence around predicted beta strand F similar to those found in V-domains. These sequence differences are discussed in detail in (Williams 1987; Williams & Barclay 1988). The ALIGN scores were determined with these three subsets of the IGSF and the extracellular region of CD4 was predicted to have two V and two C2set IgSF domains organized as illustrated in figure 1.

The ALIGN analysis of the sequences of domains of CD4 is given in tables 1 and 2. Domain 1 gave a high percentage of scores of 3 or more s.d. and this is typical for the analysis of IgSF members. Domains 2 and 4 gave moderate scores but domain 3 was more marginal. Examination of the sequences showed that they had many similarities with other IgSF members and these are often concentrated in the regions likely to form the beta sheets that make up the Ig fold (figures 2 and 3). Domain 3 lacks the normally conserved disulphide bond between the two beta

Table 1. *ALIGN scores for CD4 domains 1 and 3 against other Vset domains*

(The IgSF domain of CD8beta and domain 1 of CD2 are included for comparison. A sequence from the CD45 antigen that includes two Cys residues separated by a similar number of residues in IgSF domains is included as a negative control. The scores are given in standard deviations with a score of 3 representing the cut-off for likelihood of significance (Dayhoff *et al.* 1983; Williams & Barclay 1988). Good scores were obtained for CD4 domain 1, CD2D1 and CD8α but CD4D3 was marginal. The negative control CD45 gave consistently low scores as had been obtained previously (Williams & Barclay 1988). The residues aligned are shown in figure 2 together with abbreviations. rCD45, rat CD45 antigen (Swissprot database accession P04157; residues 270–371).)

	hCD4 D1	rCD4 D3	rCD2 D1	CD8beta
hCD4 D1		2.1	2.8	7.2
rCD4 D3	2.1		2.5	0.9
rCD2 D1	2.8	2.5		1.3
rCD8beta	7.2	0.9	1.3	
rCD8alpha	6.8	−0.5	1.7	7.3
Ig lambda	5.2	0.7	3.0	9.9
Ig kappa	6.5	2.2	2.2	7.0
Ig heavy	4.9	1.5	0.6	8.1
TcRbeta	4.1	1.0	2.1	8.0
TcRalpha	6.5	2.2	3.2	10.7
Thy-1	2.1	0.1	1.2	2.0
rCD45	0.5	−0.1	−0.5	0.7

Table 2. *ALIGN scores for CD4 domains 2 and 4 against other IgSF C2set domains*

(For comparison domain 2 of CD2 is also included. The scores are given in standard deviations with a score of 3 representing the cut-off for likelihood of significance. Both domains give several reasonable scores in agreement with the assignment of these domains to the IgSF. The residues aligned are shown in figure 3 together with abbreviations.)

	hCD4 D2	rCD4 D4	hCD2 D2
hCD4 D2		4.7	0.8
rCD4 D4	4.7		4.3
hCD2 D2	0.8	4.3	
mL1 D3	4.3	3.9	2.6
rMAG	2.9	3.3	2.8
dAMAL	1.9	3.0	3.5
cNCAM	2.5	4.2	3.1
hCEA	4.4	2.7	1.0
mFcR	3.5	3.1	4.7
hCD3E	2.0	2.2	2.3

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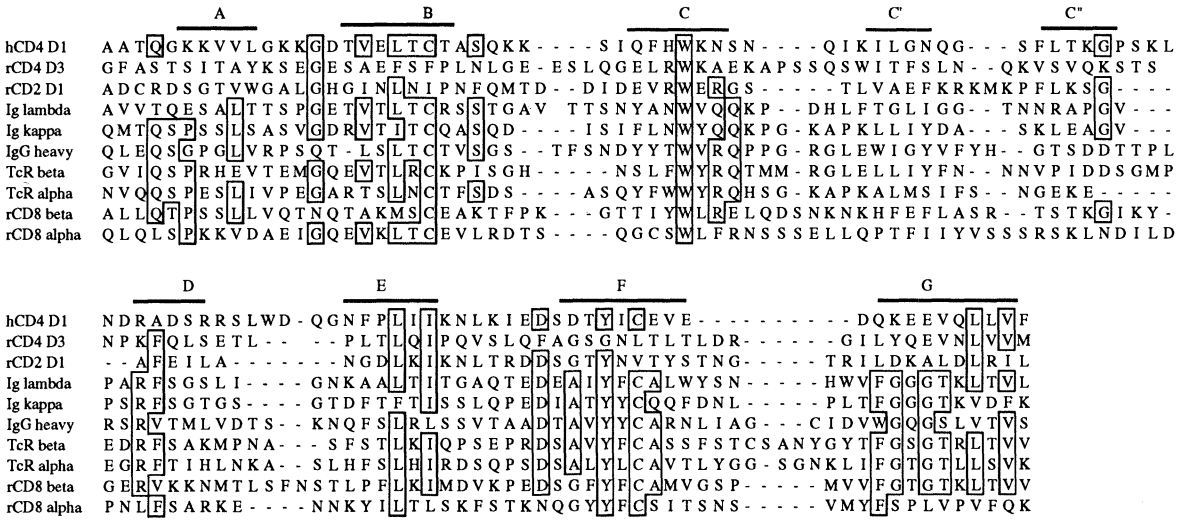

Figure 2. Alignment of CD4 domains 1 and 3 with IgSF Vset domains. Residues identical in five sequences are boxed. The approximate positions of the beta strands are indicated by the bar above the sequences. The sequences are hCD4 D1; human CD4 domain 1 (21–123); rCD4 D3, rat CD4 domain 3 (206–315) rCD2 D1, rat CD2 domain 1 (20–120); rCD8beta, rat CD8 beta (21–134); rCD8alpha, rat CD8 alpha (27–138); Ig lambda, mouse Ig V lambda MOPC 104E (21–128); Igkappa, human Ig V kappa chain (Roy) (3–107); Igheavy, human Ig heavy chain V region (Newm) (3–116); TcRbeta; human T cell receptor beta chain precursor V region (YT35) (22–35); TcRalpha; mouse T cell receptor alpha chain precursor V region (2B4) (23–132); Thy-1, rat Thy-1 membrane glycoprotein precursor (18–128).

sheets that used to be considered the hallmark of the Ig domain. However, subsequently many other IgSF domains have been predicted to lack this disulphide bond and these are particularly common in cytosolic members of the IgSF such as twitchin and titin (Benian *et al.* 1989; Labeit *et al.* 1990). A functionally active antibody lacking this disulphide bond has also been characterized (Rudikoff & Pumphrey 1986). The assignment of domain 2 was problematical because it seemed to be separated from domain 1 by more sequence than usual and was very truncated. This was resolved by predicting an abnormal disulphide bond within the sheet rather than between the sheets (Williams *et al.* 1989). This prediction was based on the sequence analysis of other IgSF members where this was likely, e.g. in CD33 and the myelin associated glycoprotein (Williams *et al.* 1989). With this alignment, moderate scores were obtained with the ALIGN programme (table 2) and typical IgSF sequences patterns were identifiable (figure 3).

3. STRUCTURE OF CD4

The determination of the atomic structure of CD4 was an important step towards understanding the functions of CD4 in antigen recognition in MHC class II

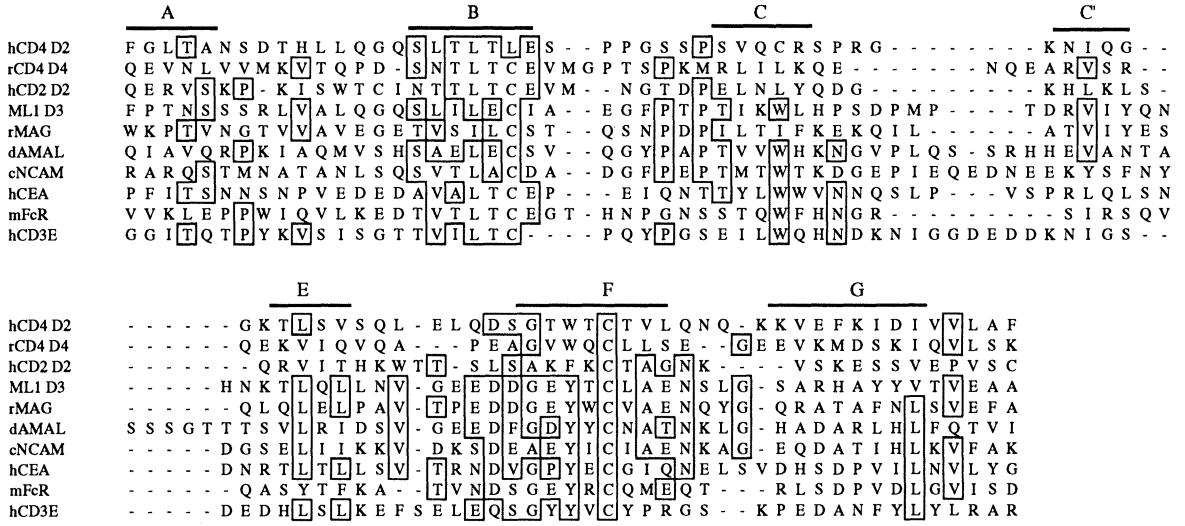

Figure 3. Alignment of CD4 domains 2 and 4 with IgSF C2set domains. Residues identical in four sequences are boxed. The approximate positions of the beta strands are indicated by the bar above the sequences. The sequences are CD4 D2, human CD4 domain 2 (123–204); rCD4 D4, rat CD4 domain 4 (309–391), hCD2 D2, human CD2 domain 2 (127–203); mL1D3 mouse neural L1CAM protein (243–331); rMAG, rat myelin associated glycoprotein (327–412); dAMAL, drosophila amalgam domain 3 (231–327); cNCAM, chicken NCAM (203–295); hCEA, human carcinoembryonic antigen (325–414); mFcR, mouse FcR II (37–117); hCDE, human CD3 epsilon chain (29–117).

Table 3. *Summary of crystallizations*

protein	crystals	resolution of diffraction
rat sCD4		
1 sCD4	no	—
2 sCD4.C1	no	—
3 sCD4.C2	no	—
4 sCD4.C0	no	—
5 sCD4.C0 domains 3,4 papain fragment	no	—
6 sCD4D1D2	no	—
7 sCD4D3D4	yes	2.8 Å
rat sCD4–Fab complexes		
1 sCD4:W3/25 IgG1 Fab	yes	
Type 1		3.5–5.0 Å[a]
Type 2		8 Å
2 sCD4:W3/25 IgG2A Fab	yes	
Type 1		3.5–5.0 Å[a]
3 sCD4.C1:W3/25 Fab	no	—
4 sCD4.C2:W3/25 Fab	yes	
Type 2		8 Å
5 sCD4.C0:W3/25 Fab	no	—
6 sCD4:OX35 Fab	no	—
7 sCD4:OX38 Fab	no	—
8 sCD4:OX67 Fab	no	—
9 sCD4:OX68 Fab	no	—
10 sCD4D1D2:W3/25 Fab	no	—
human sCD4		
1 native	yes	> 7 Å
2 desialated	yes	4.5 Å

[a] Crystal disorder restricts resolution along 1 axis.

restricted responses, in HIV binding and in regard to the validity of structural predictions based on its inclusion in the IgSF. The production of crystals suitable for X-ray crystallography is not a straightforward process and satisfactory crystals of the complete extracellular part of CD4 have so far not been obtained (Davis *et al.* 1990*a*; Kwong *et al.* 1990). We have expressed soluble forms of rat CD4 (sCD4) in eucaryotic cells and in order to maximize the chances of getting good crystals, mutants were made that lacked one or other or both of the glycosylation sites. The sCD4 proteins were produced by Chinese hamster ovary cell lines using the glutamine synthetase expression system developed at Celltech Ltd., Slough by C. Bebbington and colleagues. This expression system gives the rapid production of stable cell lines expressing very high levels of recombinant protein (Bebbington & Hentschel 1987; Cockett *et al.* 1990; Davis *et al.* 1990*b*). Several different constructs were expressed at around 100 mg l^{-1} when the cell cultures were grown to exhaustion (Davis *et al.* 1990*b*). The various different constructs expressed are summarized in figure 1.

Crystallization was attempted on the variants of CD4 and to maximize chances of obtaining suitable crystals for X-ray diffraction, complexes of sCD4 with Fab from various monoclonal antibodies were made and crystallization attempted. The various conditions are summarized in table 3 which shows that most protein forms did not give suitable crystals for X-ray diffraction. It had been hoped that the removal of carbohydrate sites would help crystallization but in this case it actually prevented crystallization. One complex of sCD4 with Fab gave moderate diffraction but good diffraction was obtained with the two domain form sCD4 D3D4. A structure for this protein

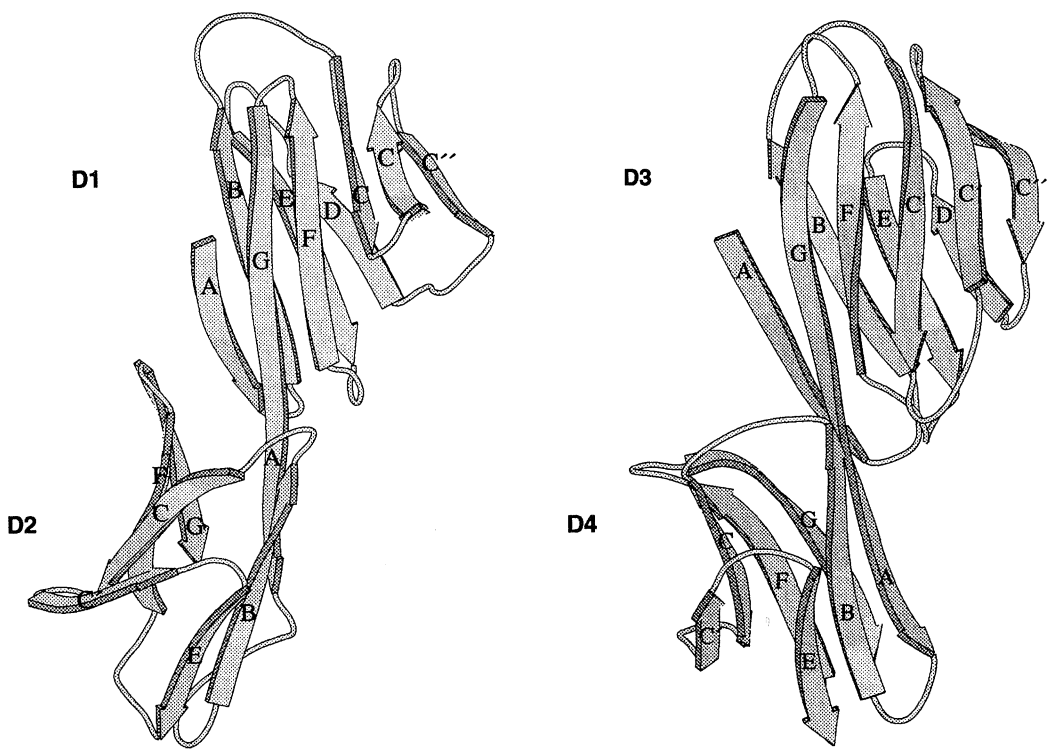

Figure 4. Ribbon diagram showing the folding pattern of human CD4 D1D2 compared to rat CD4 D3D4. Data from Ryu *et al.* (1990); Wang *et al.* (1990); Brady *et al.* (1993). The beta strands are labelled as in figures 2 and 3.

at 2.8 Å resolution has been determined (Brady *et al.* 1993). This confirmed that these two domains, like those determined for human CD4 domains 1 and 2 (Ryu *et al.* 1990; Wang *et al.* 1990), have Ig-like folds and are all members of the IgSF (figure 4).

As discussed above, the assignment of domains 2 and 3 into the IgSF was controversial because domain 2 required the prediction of a novel disulphide within the sheet and domain 3 lacked any disulphide and also gave ALIGN scores of marginal significance. The finding that these domains have Ig-like folds adds considerable weight to the argument that members of the IgSF predicted by this type of sequence analysis will have Ig-like folds. This argument is strengthened further by other recent IgSF members where structures have been determined. These include the T cell surface antigen CD2 which also contains a domain lacking the intersheet disulphide bond, the T cell antigen CD8 alpha chain (see also table 1) and the first structure for a cytosolic member of the IgSF, telokin (Holden *et al.* 1992; Jones *et al.* 1992; Leahy *et al.* 1992).

4. THE EVOLUTION OF CD4

The solution of the structure by X-ray crystallography of domains 3 and 4 of CD4 (Brady *et al.* 1993) shows that there is a striking similarity between the structures of the pairs of domains, that is human D1D2 and rat D3D4 (figure 4). For instance there is a continuous beta strand between domains 1 (G strand) and 2 (A strand) and a similar strand between domains 3 and 4 and this arrangement has not been found in any other IgSF structures. There are other structural similarities between the pairs of domains as discussed in Brady *et al.* (1993) and these are compatible with evolution of the four domains of CD4 via a two domain precursor. This had been suggested on the basis of some patches of sequence similarity between domain 2 with domain 4 and domain 1 with domain 3 (Williams *et al.* 1989). For instance the sequence in beta strand F in the IgSF almost invariably contains a Tyr Xaa Cys sequence. However in CD4 domains 2 and 4 the Tyr is replaced with a Trp in CD4 in all species except cat CD4 D2 (Norimine *et al.* 1992). To the best of our knowledge the only other IgSF domain where this is found is the LAG-3 protein which is present on activated T lymphocytes and is a transmembrane glycoprotein with four IgSF domains and like CD4, interacts with MHC class II antigen (Triebel *et al.* 1990; Baixeras *et al.* 1992). LAG-3 shows limited sequence similarity to CD4 in the extracellular domains but none in the transmembrane and cytoplasmic regions. However the extracellular domains of CD4 have diverged rapidly in evolution and there is only 53% identity between rat and human sequences for domains 1–4. The presence of this unusual sequence in both domains 2 and 4 together with the localization of both genes to the same region of chromosome 12 is compatible with the evolution of both CD4 and LAG-3 from a common four IgSF domain precursor (Triebel *et al.* 1990). It seems probable that in evolution a two IgSF domain structure was duplicated to give a precursor for both CD4 and LAG-3.

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REFERENCES

Baixeras, E., Huard, B., Miossec, C., *et al.* 1992 Characterization of the lymphocyte activation gene 3-encoded protein. A new ligand for human leukocyte antigen class II antigens. *J. exp. Med.* **176**, 327–337.

Barclay, A.N., Beyers, A., Birkeland, M.L., *et al.* 1993 *Leucocyte antigens factsbook*. (424 pages.) London: Academic Press.

Bebbington, C.R. & Hentschel, C.C.G. 1987 The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells. In *DNA cloning* (ed. D. M. Glover), pp. 163–188. Oxford: IRL press.

Benian, G.M., Kiff, J.E., Neckelmann, N., Moerman, D.G. & Waterston, R.H. 1989 Sequence of an unusually large protein implicated in regulation of myosin activity in *C. elegans*. *Nature, Lond.* **342**, 45–50.

Bernard, A., Boumsell, L., Dausset, J., Milstein, C. & Schlossman, S.F. 1984. *Leucocyte typing*. (814 pages.) Berlin: Springer-Verlag.

Brady, R.L., Dodson, E.J., Dodson, G.G., *et al.* 1993 Crystal structure of domains 3 and 4 of rat CD4 and their relationship to the N-terminal domains. *Science, Wash.* **260**, 979–983.

Brostoff, S.W. & Mason, D.W. 1984 Experimental allergic encephalomyelitis: successful treatment *in vivo* with a monoclonal antibody that recognizes T helper cells. *J. Immunol.* **133**, 1938–1942.

Clark, S.J., Jefferies, W.A., Barclay, A.N., Gagnon, J. & Williams, A.F. 1987 Peptide and nucleotide sequences of rat CD4 (W3/25) antigen: evidence for derivation from a structure with four immunoglobulin-related domains. *Proc. natn. Acad. Sci. U.S.A.* **84**, 1649–1653.

Cockett, M.I., Bebbington, C.R. & Yarranton, G.T. 1990 High level expression of tissue inhibitor of metalloproteinases in Chinese hamster ovary cells using glutamine synthetase gene amplification. *Biotechnology* **8**, 662–667.

Davis, S.J., Brady, R.L., Barclay, A.N., Harlos, K., Dodson, G.G. & Williams, A.F. 1990*a* Crystallization of a soluble form of the rat T-cell surface glycoprotein CD4 complexed with Fab from the W3/25 monoclonal antibody. *J. molec. Biol.* **213**, 7–10.

Davis, S.J., Ward, H.A., Puklavec, M.J., Willis, A.C., Williams, A.F. & Barclay, A.N. 1990*b* High level expression in chinese hamster ovary cells of soluble forms of CD4 T lymphocyte glycoprotein including glycosylation variants. *J. biol. Chem.* **265**, 10410–10418.

Dayhoff, M.O., Barker, W.C. & Hunt, L.T. 1983 Establishing homologies in protein sequences. *Meth. Enzymol.* **91**, 524–545.

Holden, H.M., Ito, M., Hartshorne, D.J. & Rayment, I. 1992 X-ray structure determination of telokin, the C-terminal domain of myosin light chain kinase at 2.8 Å resolution. *J. molec. Biol.* **227**, 840–851.

Jones, E.Y., Davis, S.J., Williams, A.F., Harlos, K. & Stuart, D.I. 1992 Crystal structure at 2.8 Å resolution of a soluble form of the cell adhesion molecule CD2. *Nature, Lond.* **360**, 232–239.

Kwong, P.D., Ryu, S.E., Hendrickson, W.A., *et al.* 1990

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Molecular characteristics of recombinant human CD4 as deduced from polymorphic crystals. *Proc. natn. Acad. Sci. U.S.A.* **87**, 6423–6427.

Labeit, S., Barlow, D.P., Gautel, M., *et al.* 1990 A regular pattern of two types of 100-residue motif in the sequence of titin. *Nature, Lond.* **345**, 273–276.

Leahy, D.J., Axel, R. & Hendrickson, W.A. 1992 Crystal structure of a soluble form of the human T cell coreceptor CD8 at 2.6 Å resolution. *Cell* **68**, 1145–1162.

Maddon, P.J., Littman, D.R., Godfrey, M., Maddon, D.E., Chess, L. & Axel, R. 1985 The isolation and nucleotide sequence of a cDNA encoding the T cell surface protein T4: a new member of the immunoglobulin gene family. *Cell* **42**, 93–104.

Maddon, P.J., Molineaux, S.M., Maddon, D.E., *et al.* 1987 Structure and expression of the human and mouse T4 genes. *Proc. natn. Acad. Sci. U.S.A.* **84**, 9155–9159.

Norimine, J., Miyazawa, T., Kawaguchi, Y., Tohya, Y., Kai, C. & Mikami, T. 1992 A cDNA encoding feline CD4 has a unique repeat sequence downstream of the V-like region. *Immunology* **75**, 74–79.

Reinherz, E.L., Kung, P.C., Goldstein, G. & Schlossman, S.F. 1979 Separation of functional subsets of human T cells by a monoclonal antibody. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4061–4065.

Rudikoff, S. & Pumphrey, J.G. 1986 Functional antibody lacking a variable-region disulphide bridge. *Proc. natn. Acad. Sci. U.S.A.* **83**, 7875–7878.

Ryu, S.E., Kwong, P.D., Truneh, A., *et al.* 1990 Crystal structure of an HIV-binding recombinant fragment of human CD4. *Nature, Lond.* **348**, 419–426.

Triebel, F., Jitsukawa, S., Baixeras, E., *et al.* 1990 LAG-3, a novel lymphocyte activation gene closely related to CD4. *J. exp. Med.* **171**, 1393–1405.

Wang, J.H., Yan, Y.W., Garrett, T.P., *et al.* 1990 Atomic structure of a fragment of human CD4 containing two immunoglobulin-like domains. *Nature, Lond.* **348**, 411–418.

Webb, M., Mason, D.W. & Williams, A.F. 1979 Inhibition of mixed lymphocyte response by monoclonal antibody specific for a rat T lymphocyte subset. *Nature, Lond.* **282**, 841–843.

White, R.A., Mason, D.W., Williams, A.F., Galfre, G. & Milstein, C. 1978 T-lymphocyte heterogeneity in the rat-separation of functional subpopulations using a monoclonal antibody. *J. exp. Med.* **148**, 664–673.

Williams, A.F. 1987 A year in the life of the immunoglobulin superfamily. *Immunol. Today* **8**, 298–303.

Williams, A.F. & Barclay, A.N. 1988 The immunoglobulin superfamily-domains for cell surface recognition. *A. Rev. Immunol.* **6**, 381–405.

Williams, A.F., Davis, S.J., He, Q. & Barclay, A.N. 1989 Structural diversity in domains of the immunoglobulin superfamily. *Cold Spring Harb. Symp. quant. Biol.* **LIV**, 637–647.

Williams, A.F., Galfre, G. & Milstein, C. 1977 Analysis of cell surfaces by xenogeneic myeloma-hybrid antibodies: differentiation antigens of rat lymphocytes. *Cell* **12**, 663–673.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES