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Analysis of structural similarities between brain Thy-1 antigen and immunoglobulin domains

Evidence for an evolutionary relationship and a hypothesis for its functional significance

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The Thy-1 membrane glycoprotein from rat brain is shown to have structural and sequence homologies with immunoglobulin (Ig) domains on the basis of the following evidence. 1. The two disulphide bonds of Thy-1 are both consistent with the Ig-fold. 2. The molecule contains extensive β-structure as shown by the c.d. spectrum. 3. Secondary structure prediction locates β-strands along the sequence in a manner consistent with the Ig-fold. 4. On the basis of rules derived from known β-sheet structures, a three-dimensional structure with the Ig-fold is predicted as favourable for Thy-1. 5. Sequences in the proposed β-strands of Thy-1 and known β-strands of Ig domains show significant sequence homology. This homology is statistically more significant than for the comparison of proposed β-strand sequences of β_2-microglobulin with Ig domains. An hypothesis is presented for the possible functional significance of an evolutionary relationship between Thy-1 and Ig. It is suggested that both Thy-1 and Ig evolved from primitive molecules, with an Ig fold, which mediated cell–cell interactions. The present-day role of Thy-1 may be similar to that of the primitive domain.

Rat Thy-1 antigen, a polypeptide of 111 amino acid residues, is a major membrane molecule of thymocytes and brain, with unknown function [see the preceding paper (Campbell *et al.*, 1981) for sequence and review]. Preliminary studies suggested that Thy-1 antigen might be structurally and evolutionally related to an Ig domain (Campbell *et al.*, 1979).

Immunoglobulin chains are made up of a repeating domain structure of about 110 amino acids (Edelman, 1970). IgG light (L) chains consist of two such domains and heavy (H) chains of four or five depending on the Ig class. These chains are believed to have evolved by duplication of a primordial gene coding for a single domain. The determination of the three-dimensional structures of immunoglobulins has confirmed the domain hypothesis and established the existence of a characteristic folding pattern for each domain (reviewed in Amzel & Poljak, 1979). The polypeptide backbone for the Ig-fold (Fig. 1) consists of a core of two β-sheets made up of at least four, and three, β-strands each (there are one or two extra β-strands in V regions). Usually, the amino acids in these strands alternate between hydrophobic and hydrophilic residues, such that each β-sheet has a hydrophobic surface on one side and a hydrophilic one on the other. The two sheets interact through the hydrophobic surfaces and this inter-

Abbreviations used: Ig, Immunoglobulin; IgG, immunoglobulin G; C_L, C_H1, C_H2 and C_H3, immunoglobulin domains of constant regions of IgG L chain and H chain respectively; V_L and V_H, variable region domains of L chain and H chain; Thy-1, Thy-1 antigen or glycoprotein (see Williams *et al.*, 1976); the one-letter code for amino acids: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln, R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr; Z, pyrrolidone-2-carboxylic acid.

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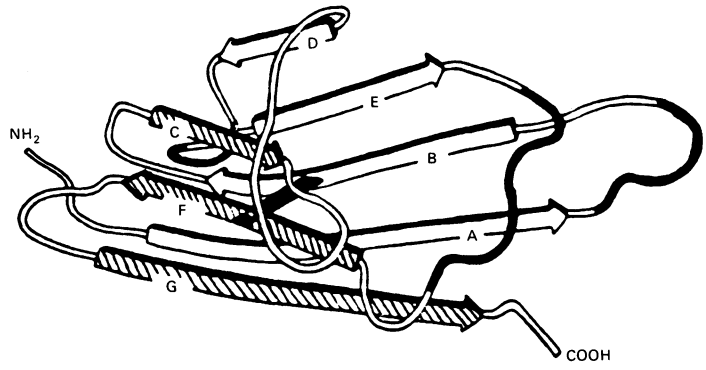


Fig. 1. *Ig domain structure*
The diagram (adapted from Beale & Feinstein, 1976) shows the fold of the polypeptide chain in constant region domains. The stretches making up the β-strands are named A to G as in Table 1. The disulphide bond joining the half-cystine residues in the four-strand and three-strand β-sheets is shown as a filled rectangle.

action is stabilized by an intrachain disulphide bond between the two β-sheets, which is characteristic of all Ig domains. The sequence homology between different Ig domains occurs mostly in the residues forming the β-strands and particularly around the half-cystine residues forming the intrachain disulphide bond (Beale & Feinstein, 1976).

Certain types of sequences are necessary to form β-sheet structures and studies on β-sheets in proteins of known structure have led to the development of algorithms to predict both the secondary structure, and recently the tertiary fold. Secondary structure prediction (see review by Schulz & Schirmer, 1979) locates the regions of the polypeptide chain that are likely to form α-helices and β-strands. Tertiary structure prediction (Cohen *et al.*, 1980) is concerned with the packing of β-strands on the basis of burial of hydrophobic residues and other stereochemical constraints.

In the present paper we examine the possibility that Thy-1 is related to immunoglobulin on the basis of secondary structure, tertiary structure and sequence homology.

Procedures and results

Disulphide bonds

The disulphide bonds of Thy-1 (Campbell *et al.*, 1981) are compared with those of rabbit IgG C_H1 in Fig. 2. The bond from Cys-19 to Cys-85 in Thy-1 has the spatial characteristics of the conserved disulphide bond found in all Ig domains. In these the *N*-terminal half-cystine is usually at about residue 20 of the domain and the *C*-terminal half-cystine about 50–75 residues away, with the distance between greater in V-region domains.

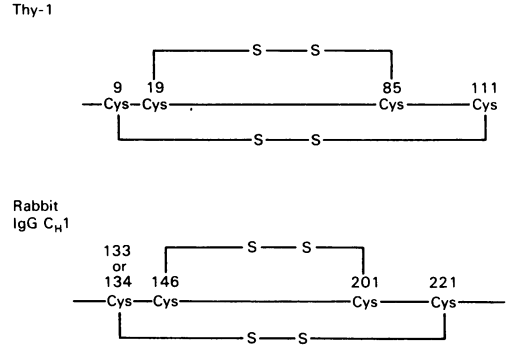


Fig. 2. *Disulphide bonds in Thy-1 glycoprotein and rabbit IgG C_H1 domain*
The diagram shows the positions and sequence numbers of half-cystine residues in Thy-1 and rabbit IgG C_H1 domain which form intrachain disulphide bonds.

The other disulphide bond in Thy-1 antigen is from Cys-9 to Cys-111. These residues must be close to each other if the molecule has an Ig-fold (Fig. 1). Furthermore, half-cystine residues are commonly found in Ig domains at positions analogous to Cys-9 and Cys-111. In most C_H1 domains cysteine residues are found in both positions, but C_L domains have a *C*-terminal half-cystine. These residues form the H–L and H–H interchain disulphide bonds (Beale & Feinstein, 1976). In the rabbit IgG C_H1 domain (O'Donnell *et al.*, 1970) and human IgE C_H1 domain (Bennich & Bahr-Lindström, 1974) there exists an extra intrachain disulphide bond which seems directly homologous with the Cys-9–Cys-111 bond in Thy-1 (Fig. 2).

Structural data

Six IgG domains with known three-dimensional structures representing an entire IgG molecule were used for the sequence and structural comparisons with Thy-1. The C_L, C_H1, V_L and V_H domains were from the Fab' fragment of human myeloma IgG (New) (Saul *et al.*, 1978) and the C_H2 and C_H3 domains were from a human Fc fragment (Huber *et al.*, 1976).

The β-sheets of these domains, given in Table 1, were assigned from the three-dimensional structures as in Cohen *et al.*, (1980). The seven β-strands that are conserved in the 'Ig-fold' are lettered sequentially A to G (Fig. 1). The additional β-strands of the variable domains are coded C' and C''.

Secondary structure prediction

The method of Garnier *et al.* (1978) was used. The advantages of this method over others (e.g. Chou & Fasman, 1974*a*,*b*; Lim, 1974*a*,*b*) are that the prediction can be performed automatically by a computer algorithm, and that the accuracy of the prediction can be improved if it is known that the protein contains mainly α-helices or β-sheets. In this case a bias is built into the algorithm towards the appropriate structures by the use of cut-off parameters.

The c.d. spectrum of Thy-1 indicates a high content of β-sheet structure and an absence of α-helices, and is very similar to the c.d. spectrum of Ig (Campbell *et al.*, 1979). Accordingly the cut-off parameters used for the secondary structure prediction were ones that located most of the observed β-strands in the Ig domains (Table 1). With the same parameters the algorithm predicted that Thy-1 was 59% β-strand and 5% α-helix. The postulated secondary structure of Thy-1 can be roughly aligned with the Ig domains to yield a similar pattern of β-strands and connecting regions. This alone helps to confirm the suggestion that Thy-1 has a similar fold to Ig domains.

Tertiary structure predictions

In making a tertiary structure prediction, β-strand regions are first identified and then their arrangement into β-sheets is predicted by an algorithm described by Cohen *et al.* (1980).

In making β-strand assignments the following points were considered.

(1) The results of the secondary structure prediction.

(2) Stereochemical constraints which have been identified by studies on known structures with a β-sandwich. Analysis of β-sheet stacking in Ig domains and other proteins (Cohen *et al.*, 1980) shows that there is a distinct pattern of non-polar residues that mediate the sheet–sheet interaction (boxed residues in Fig. 3*a*). These alternate along the strands to form the in-pointing hydrophobic surface, which stabilizes the 'sandwich' between the two β-sheets. Furthermore, the interacting non-polar residues tend to progress from left to right as one proceeds down the top sheet and from right to left down the bottom sheet. This anticomplementary pattern is a consequence of the stacking of two twisted β-sheets (Cohen *et al.*, 1980).

(3) Sequence homologies between Thy-1 and Ig domains.

(*i*) *Assignment of strands B and F.* The disulphide bond of Thy-1 that is homologous with the conserved Ig intrachain bond defines the centres of strand B (Cys-19) and strand F (Cys-85). In strand B a suitable non-polar patch for sheet–sheet interaction is formed from Leu-17 and Cys-19. In the six Ig domains shown in Table 1, only Leu, Ile and Val are aligned with Leu-17 of Thy-1. In strand F Tyr-83, Cys-85 and Leu-87 form a suitable sheet–sheet-interaction site and non-polar residues are at these positions in most Ig domains.

(*ii*) *Strand E.* The above alignment of strand F extends back towards the *N*-terminus to locate the position of strand E by homology with the Ig sequences. Not only is the sheet–sheet interaction site conserved (in Thy-1, Leu-70 and Leu-72) but Thr-71 of Thy-1 aligns with Thr and Ser in Ig constant domains.

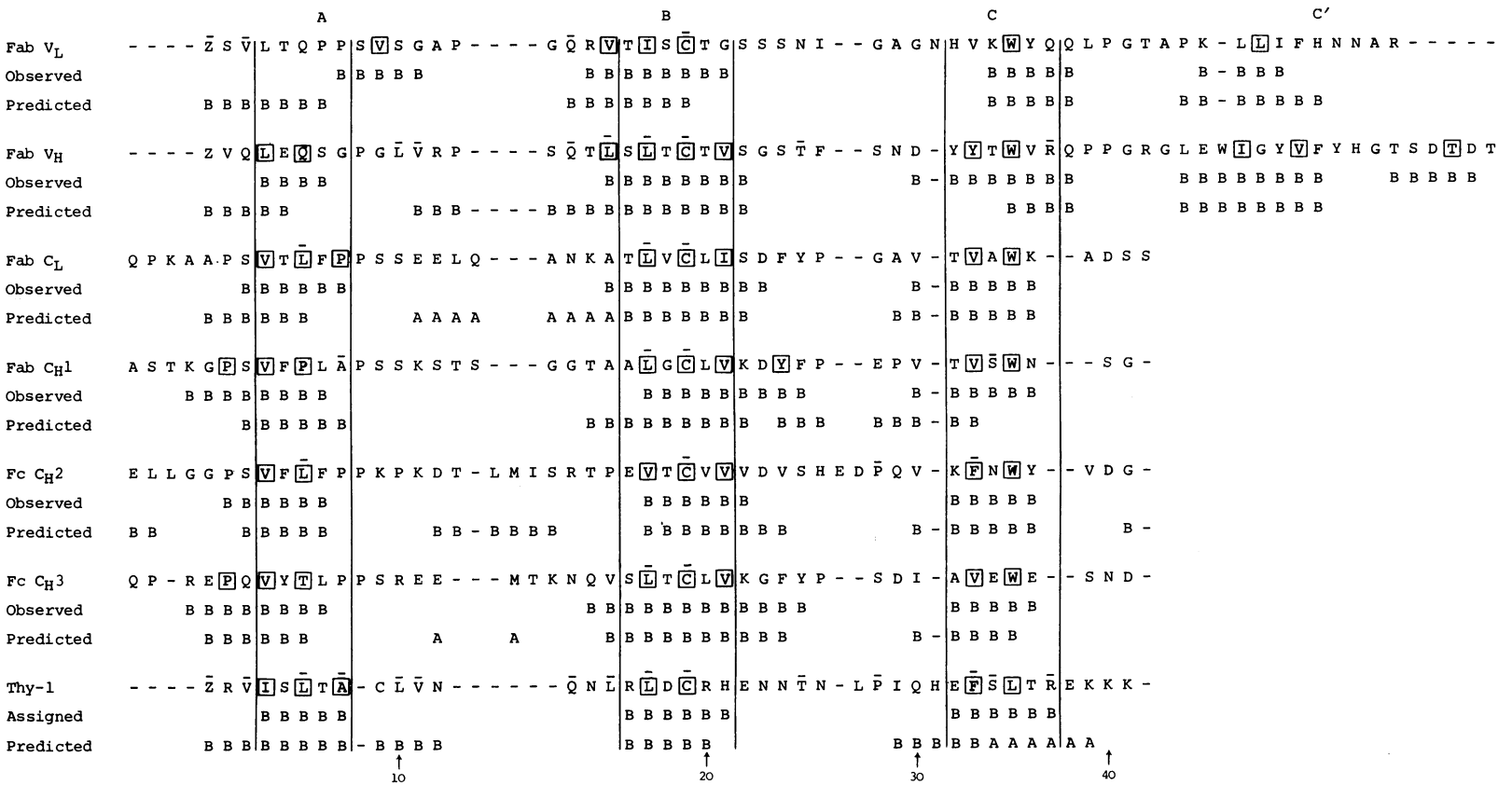
(*iii*) *Strand G.* If the alignment of β-strands E and F in Thy-1 and the Ig domains is extended towards the *C*-terminus, then Lys-99 of Thy-1 is aligned with the Lys of the Ig constant domains. Thus strand G of Thy-1 will have to start at or before Lys-99, although the secondary structure prediction suggests that the β-strand starts at Thr-100. A suitable sheet–sheet interaction site would be formed by Lys-99 and Ile-101. Lys is considered effectively a non-polar side chain if the β-strand lies at the edge of the β-sheet, as the charged ε-amino group can be exposed to solvent whereas the non-polar C^{β}–C^{γ}–C^{δ}–C^{ϵ} region acts as a hydrophobic side chain.

(*iv*) *Strand A.* The proposed alignment suggests that Ile-4, Leu-6 and Ala-8 mediate the sheet–sheet interaction.

(*v*) *Strand C.* The secondary structure prediction locates a β-strand region which could form strand C. In this region there are two candidates for the strand C sheet–sheet interaction site, Leu-27 and Ile-29, or Phe-33 and Leu-35. The second pair has been selected mainly because residue 28 in Thy-1 is a proline, which rarely occurs inside β-strands (Chou & Fasman, 1974*a*,*b*). When it does if often forms a β-bulge (Richardson *et al.*, 1978) which would disrupt the site Leu-27 and Ile-29. Moreover, Pro-28 of Thy-1 would occur just before strand C in a similar way to proline residues in C_H1 and C_H2, and Ser-34 could be aligned with the Ser in C_H1. This alignment was also supported by some other

Table 1. *Sequence alignments of Thy-1 with six immunoglobulin domains*

For each Ig domain the observed and predicted α-helical (A) and β-strand (B) regions are shown. The observed β-strands were defined by first delineating the β-sheet hydrogen bonding (amide NH–carboxyl oxygen distances <0.33 nm) and then extending each β-strand if a subsequent residue had its main chain dihedral angles in the range $-170° < \phi < -90°$, $100° < \psi < 170°$. α-Helices were located by inspection of the hydrogen bonding. The secondary structure was predicted by the method of Garnier *et al.* (1978) with cut-off parameters for α-helix, β-structure, turn and coil of 158, 50, 0, 0. For Thy-1 the α-helix and β-strands predicted by the same procedure are shown. The final assignment of the β-strands in Thy-1 is shown. The sequence of β-strand regions of Thy-1 was aligned with the immunoglobulin domains as described in the text. The alignment of the connecting loops in Thy-1 with the Ig sequence was performed by eye to suggest possible similarities and should only be taken as a suggestion of further resemblances. The boxed residues of the β-strands are those which are observed (Ig domains) or proposed (Thy-1) to be inpointing residues which mediate the sheet–sheet interactions. The residues with a bar above them are those which are identical between Thy-1 and the sequence in any of the Ig domains. The sequence numbering is for Thy-1. Hyphens between residues have been omitted for clarity.



D
E
F
G
Fab V_L
Observed
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Fab V_H
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Fab C_H1
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Predicted
Fc C_H2
Observed
Predicted
Fc C_H3
Observed
Predicted
Thy-1
Assigned
Predicted
50
60
70
80
90
100

homologies around the Trp of strand C (see the collated sequences in Kabat *et al.*, 1979). For example, strand C from dog IgM C_H3 domain (Wasserman & Capra, 1978):

dog IgM C_H3 -Ile-Ser-Trp(382)-Thr-Arg-Glu-Glu-Asn-

Thy-1 -Phe-Ser-Leu(35) -Thr-Arg-Glu-Lys-Lys-

The only contradiction to choosing strand C as shown in Table 1 is that the secondary structure prediction suggests part of this region should be in α-helix. However, those predictions are not entirely reliable and the Thy-1 c.d. spectrum does not show any α-helix.

The Trp of strand C is invariant in Ig domains but the alignment of β_2-microglobulin in this region (Beale & Feinstein, 1976) places Leu in homology with the Trp, as is suggested for Thy-1.

(*vi*) *Strand D.* This is the hardest section of Thy-1 to align with the Ig domains. The additional strands of the V_L and V_H domains (strands C′ and C″) lie between strands C and D. Furthermore, strand D does not markedly contribute to the sheet–sheet interaction. One possible assignment is shown in Table 1, based on the alignment of Gly-49 and Val-50 of Thy-1 with these residues in strand D of C_L and C_H1. Val-50 in Thy-1 could be involved in sheet–sheet interaction in an analogous way to the aligned non-polar residues in the Ig.

Alternatively, this region could be aligned with V-region sequences. For example, if the sequence Arg-Ser-Arg-Val in Thy-1 beginning at residue 56 were aligned with the same sequence prior to strand D in the V_H domain, then Thy-1 residues 42–55 could be involved in β-strands analogous to C′ and C″ of V-region domains.

Algorithm prediction of tertiary structure

The assigned strands can be assembled into a pair of β-sheets which are analogous to those found in Ig domains (Fig. 3*b* compared with Fig. 3*a*). The Thy-1 sheet diagram has both the alternating non-polar residues and the characteristic progression of non-polar residues in both sheets.

A computer algorithm can be used to test whether the pattern shown in Fig. 3(*b*) is a likely one for this set of strands. There are about 10^7 possible structures that could be formed from seven β-strands segregating into two β-sheets. The computer algorithm first reduces this to about 10^3 possible structures on the basis of reducing the exposed surface area of non-polar residues, distance constraints due to disulphide bonds and on other stereochemical constrainst. Structures are then rank-ordered on the basis of the number of hydrogen bonds. In tests of the method with four Ig sandwiches with seven β-strands a structure close to the correct one was obtained at positions 6, 10, 36 and 327 on the rank-ordered list (Cohen *et al.*, 1980). When the algorithm was applied to the seven β-strands assigned for Thy-1 the structure shown in Fig. 3(*b*) was in the top eight when both disulphide bonds were considered as constraints, and in the top twelve if only the Cys-19 to Cys-85 bond was used. All the other structures (not shown) did not have an Ig fold. Thus the postulated structure emerges as favourably as would be expected were it the correct one, given the results obtained with the Ig domains.

Statistical evaluation of sequence homologies of the β-strands

The structural analysis above suggests that Thy-1 antigen is folded as in Ig domains. However, this does not establish that the molecules derive from a common ancestor. An evolutionary relationship must be based on sequence homology. Within a family of structurally and functionally related proteins the amino acid sequence is better conserved between aligned secondary structures than in the connecting loop regions (Shotton & Hartley, 1970; Novotný & Franek, 1975; Beale & Feinstein, 1976). Accordingly the sequence similarity between Thy-1 and Ig domains was evaluated in the six β-strands (ABCEFG) whose alignment is most reliable. β_2-Microglobulin, with β-strands assigned as in Cohen *et al.* (1980), was included in this analysis since this is commonly accepted to be homologous to Ig (Poulik & Reisfeld, 1975). The minimum number of codon nucleotide base changes required to make any two sequences identical is reported as an average per residue (Fitch, 1966*a*,*b*). The results are given above the diagonal in Table 2, and show that the scores for Thy-1 versus Ig domains are at least as good as those for the comparison between β_2-microglobulin and Ig.

One way to assess the significance of these results is to consider the probability that these scores could be obtained by a random alignment of two sequences (Moore & Goodman, 1977). These data, shown below the diagonal in Table 2, suggest that both Thy-1 and β_2-microglobulin have significant sequence homologies with constant region domains. However, this apparent homology could be due to structural constraints (convergent evolution) rather than divergence from a common ancestor. To control for this in the statistical analysis, a β-sheet

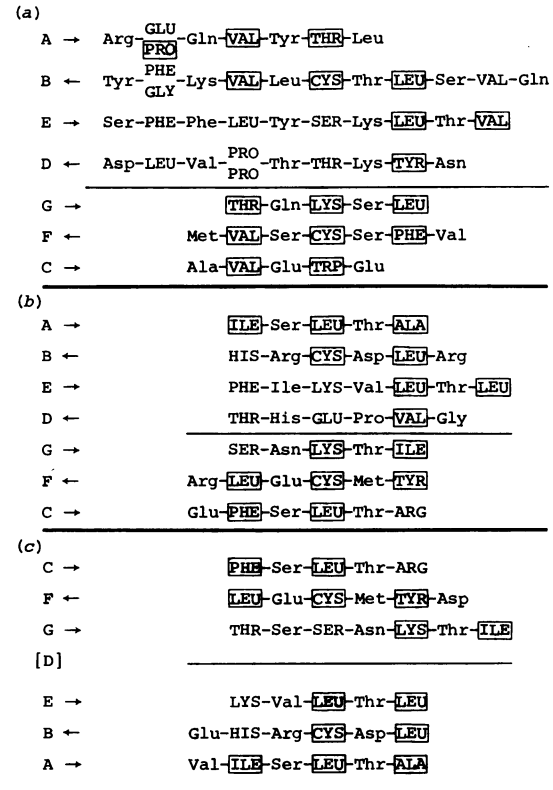

Fig. 3. *Strand alignment diagrams*
(*a*) C_H3 domain: the positions of the β-strands that form the two β-sheets are shown. In strands A, B and D, Glu-Pro, Phe-Gly and Pro-Pro form β-bulges. Residues vertically below one another in the same sheet can form intrasheet hydrogen bonds. Residues in capitals are in-facing (i.e. towards the other sheet) and boxed residues mediate the sheet–sheet interaction. (*b*) Thy-1 predicted: the non-polar residues that are suggested to be involved in the sheet–sheet interaction are boxed. Note that they have the same anticomplementary direction as the boxed residues in C_H3. (*c*) Thy-1 wrong core: the diagram of Thy-1 with the wrong core is a structure used as a control for the evaluation of sequence resemblance between β-sandwiches. This was generated by placing boxed residues of strands A, B, E in the equivalent boxed positions in strands C, F, G and *vice versa*. Additional residues on the ends of the strands were adjusted to produce an equivalent set of residues.

sandwich was constructed from the β-strands in the proposed Thy-1 domain, such that the structural requirements were met but with the β-strands in a different order. The incorrect structure for Thy-1 is shown in Fig. 3(*c*). Strands A and C, B and F, and E and G have been interchanged with adjustments in terminal residues to give a satisfactory pattern of hyrophobic residues and hydrogen bonds. The sequence comparisons were then repeated between this incorrect sequence alignment and correct sequences for Thy-1, V_L, V_H, C_L, C_H1, C_H2, C_H3 and β_2-microglobulin. The percentage probabilities of a random association were respectively 10, >10, >10, 1, 10, 5, 10 and >10. This strongly suggests that the comparisons between correct Thy-1 and correct C_L, C_H1, C_H2 and C_H3 domains (see Table 2) are not likely to be due to chance. For β_2-microglobulin there is also evidence for homology with the constant domains, but at a less significant level than for Thy-1.

In comparisons of V-region domains with Thy-1, β_2-microglobulin or C domains, the statistical evidence for homology is poor [with the exception of a good score for V_L versus C_L and C_H1 (Table 2)]. A statistically significant homology between V and C domains is difficult to establish (Edelman, 1970; Moore & Goodman, 1977) and the argument for homology is based on the folding of the polypeptide and on sequence homologies in residues which are highly conserved amongst the Ig domains (Beale & Feinstein, 1976; Amzel & Poljak, 1979).

Patches of sequence homology between Thy-1 and V regions

Although the above statistical studies only show significant homology between Thy-1 and constant Ig domains, some stretches of sequence homology with variable domains are intriguing. These occur around Cys-19 and Cys-85 in Thy-1 and are particularly seen if Thy-1 sequences are compared with the family of variable region sequences. From this a predominant sequence can be derived by taking the most commonly occurring residue at each equivalent position along the chain (Kabat *et al.*, 1979). Around Cys-19, Thy-1 has the sequence -Leu-Arg-Leu-Asp-Cys(19)-Arg-. In V_L and V_H domains the predominant sequences in the homologous regions are -Val-Thr-Ile-Ser-Cys-Arg- and Leu-Arg-Leu-Ser-Cys-Ala-. At Cys-85 the Thy-1 sequence is -Asp(79)-Glu-Gly-Asp-Tyr-Met-Cys(85)-Glu-Leu-. In both V_H and V_L domains an aspartic residue is highly conserved at a position homologous to Asp-79 of Thy-1 and at Gly-77 of Thy-1 the V domains almost always have either alanine (commonly) or glycine. The similarities between Thy-1 and V domains in this region are seen in the New V_L domain (Table 1) which has the sequence -Asp-Glu-Ala-Asp-Tyr-Tyr-Cys-Gln-. Compared to Thy-1 this shows five identities and two conservative substitutions (Gly:Ala, Glu:Gln) out of eight residues.

Discussion

The above evidence strongly suggests that there are structural and sequence homologies between

Table 2. *Sequence comparisons between assigned β-strands of Thy-1, Ig and β_2-microglobulin*

Above the diagonal are shown the average minimum base changes per residue for comparisons involving the 35 residues which have been assigned to the β-strands A B E C F G of Thy-1, Ig and β_2-microglobulin (β_2-μ) (see Table 1 and the text). To complete strand C of the constant Ig domains the next residue towards the *C*-terminus was aligned with the last residue in Thy-1 strand C. In strand G of V-regions the last residue was omitted in the comparisons. Below the diagonal are the percentage probabilities that the minimum base change scores are due to chance association of sequences. These values were read from a table in Moore & Goodman (1977).

	Thy-1	V_L	V_H	C_L	C_H1	C_H2	C_H3	β_2-μ
Thy-1		1.31	1.34	1.03	0.97	0.94	1.17	1.20
V_L	>10		0.94	1.09	1.14	1.26	1.26	1.37
V_H	>10	0.01		1.20	1.23	1.31	1.26	1.23
C_L	0.1	0.1	5		0.69	0.83	0.83	1.11
C_H1	0.01	1	5	<0.01		0.71	0.77	1.14
C_H2	0.01	10	>10	<0.01	<0.01		0.94	1.14
C_H3	1	10	10	<0.01	<0.01	0.01		1.06
β_2-μ	5	>10	5	1	1	1	0.1	

Thy-1 antigen and Ig domains. The most probable explanation for these similarities is that Thy-1 and Ig domains have evolved from a common ancestral gene. Given this, the question arises as to whether this implies any functional relationship. In considering this, the known functions of Ig are taken into account along with two puzzles concerning the Thy-1 glycoprotein (reviewed in Campbell *et al.*, 1981). Why is Thy-1 antigen found in large amounts on membranes of some apparently unrelated cell types yet not at all on other cells? Why is its tissue distribution not conserved in evolution?

Functions of Ig and postulates for Thy-1 functions

There are two aspects to Ig function; the recognition of antigen by the combining site made up of the V regions and the subsequent interaction with effector systems via the constant region domains. Thy-1 antigen shows no sequence variation and thus is unlikely to have a function analogous to antigen binding. However, the effector functions of Ig (Dorrington & Painter, 1977) may provide a realistic model for Thy-1. Interacting systems based on Ig constant domains include: (i) interaction of IgM and some subclasses of IgG with complement [in the classical pathway C1q binds to the C_H2 domain of IgG (Colomb & Porter, 1975) and C4 to Fd (Campbell *et al.*, 1980)]; (ii) binding of IgG to Fc receptors in the placenta (McNabb *et al.*, 1976) or neonatal gut (Rodewald, 1980); (iii) binding of IgG to a monocyte receptor, probably via the C_H3 domain (Barnett Foster *et al.*, 1980); (iv) binding of IgE to a mast cell cell receptor (Metzger, 1978); (v) binding of IgA to secretory component of intestinal epithelial cells (Poger & Lamm, 1974); (vi) recognition of the C_H3 domain of IgG by killer cells which are antibody-dependent (MacLennan *et al.*, 1974).

In all these interactions a soluble or cell-borne receptor is involved in binding to one or a complex of Ig domains presented in solution or at a cell surface. Thus one can consider the domains of the different Ig classes and their receptors as constituting a moderately large set of ligand–receptor systems, each of which leads to a different effect as a result of antigen binding.

On the basis of this and with other features of Thy-1 in mind it is suggested that:

(1) There is a set of molecules (of which Thy-1 is a member) related to the Ig domain which mediate cell interactions in a manner analogous to the set of domains mediating the Fc functions of Ig. For each Ig-like molecule in the set there would be a specific receptor, again analogous to the specific receptors for Fc's of various Ig classes.

(2) The cell interactions concerned are not uniquely concerned with the immune system but are the basic interactions which lead to tissue formation by cells.

(3) The molecules mediating tissue interactions are not necessarily tissue-specific. The same ligand–receptor pair can be used in different tissues providing confusion of specificities cannot arise, e.g. if the tissues are anatomically distinct.

(4) The only function of the ligand–receptor pairs is to mediate recognition, with the consequences of recognition being due to the differentiated state of the cells. Any one pair from the set of ligand–receptor pairs would potentially be suitable, provided the correct ligand and receptor were expressed on the appropriate cells at the right time.

(5) The involvement of Ig-related structures in tissue interactions is more primitive than their involvement in the immune system and the immune functions evolved from the sets of molecules mediating tissue interactions.

Point (1) derives directly from the Ig effector functions and point (2) is suggested because Thy-1

cannot have an irreplaceable role in immunity since it is absent from human lymphoid cells (Dalchau & Fabre, 1979).

Point (3) attempts to deal with the fact that the expression of Thy-1 on unrelated cells does not fit with any known correlate with the different cell types. In cell interactions which are responsible for tissue formation, more than one receptor–ligand pair would be needed if there were to be specificity. However, it would not be essential for each tissue to have unique molecules for cell interactions, and it seems possible that the same ligand–receptor system could be used in different tissues. Cell-surface molecules showing odd patterns of tissue distribution are commonly found; for example, Ia antigens (Hämmerling *et al.*, 1975) and W3/13 and M.R.C. OX 2 antigens of rat thymocytes (Williams *et al.*, 1977; McMaster & Williams, 1979). These molecules may all be important in determining cell-surface properties essential for cell interactions.

Point (4) is suggested to try to explain why the pattern of expression of Thy-1 antigen is not conserved in evolution. If the Fc analogy is examined, it can be seen that the Fc domains of Ig function only to provide information for triggering another functional system in which the Ig plays no role. For example, IgE triggers anaphylactic reactions only because the mast cell has an IgE receptor and antigen binding can trigger cross-linking of the receptors (Metzger, 1978). The IgE probably plays no functional role in the subsequent events, and presumably any other class would have worked equally well in triggering mast cells had the receptor system evolved differently. A system of this type may be open to variation in evolution and the expression of H chain classes and L chain types is not strongly conserved (for review, see Spiegelberg, 1974; Nisonoff *et al.*, 1975). Perhaps the species variation in expression of Thy-1 antigen occurs because in the evolution of species different ligand–receptor pairs from a moderately large set are used in different tissues for the same basic phenomena of cell interactions.

Point (5) suggests that the primitive function of the Ig-domain was to act as a ligand and thus is not related to the antigen-binding function of antibodies. This latter function is structurally and genetically complicated and the use of Ig domains as a molecule for information seems a simpler starting point.

These ideas can be tested. Further structural studies on cell-surface molecules of a variety of tissues will reveal whether other Ig-related molecules exist. Receptors for glycoproteins like Thy-1 antigen can be sought by using the purified molecules in binding studies. A search for a homologue of Thy-1 antigen in neuronal cells of invertebrates may establish whether or not this structure is more primitive in evolution than immunoglobulin, which has been found only in vertebrates (Nisonoff *et al.*, 1975).

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