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immunology today

Receptors, antibodies and disease

from John Newsom-Davis and Angela Vincent

Biochemists, endocrinologists, immunologists and neurologists share an interest in cell surface receptors, as was evident at a recent symposium held by the Ciba Foundation*. When these receptors are targets for auto-antibodies, endocrine or neurological disease can result. Attention focussed particularly on receptors (R) for thyroid stimulating hormone (TSH), insulin, acetylcholine (ACh), prolactin and β_2-adrenergic agonists.

The clinical importance of auto-antibodies to receptors, judged purely in numerical terms, varies considerably. Insulin-resistant diabetes (insulin-R antibody), for example, is extremely rare – less than 50 cases have been reported worldwide, to date. On the other hand Graves' disease (TSH-R antibody) and myasthenia gravis (ACh-R antibody) are moderately prevalent while asthma and other atopic disorders (β_2-adrenergic-R antibody) are very common. Direct implication of the relevant antibodies in the disease process seems well established for most of the disorders discussed, although some uncertainty exists about the role of the recently identified β_2-adrenergic-R antibody.

In asthma a reduction in lung tissue β_2 adrenergic receptors, as measured by agonist binding or β_2 responses, has been established for some time and antibodies specific for the β_2 receptor can be identified either by inhibition of agonist binding, by immunoprecipitation of solubilized

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* Receptors Antibodies and Disease (Ciba Symposium no. 90) was organized by Dr David Evered and chaired by Professor N. A. Mitchison. The proceedings will be published by Pitman Medical Ltd.

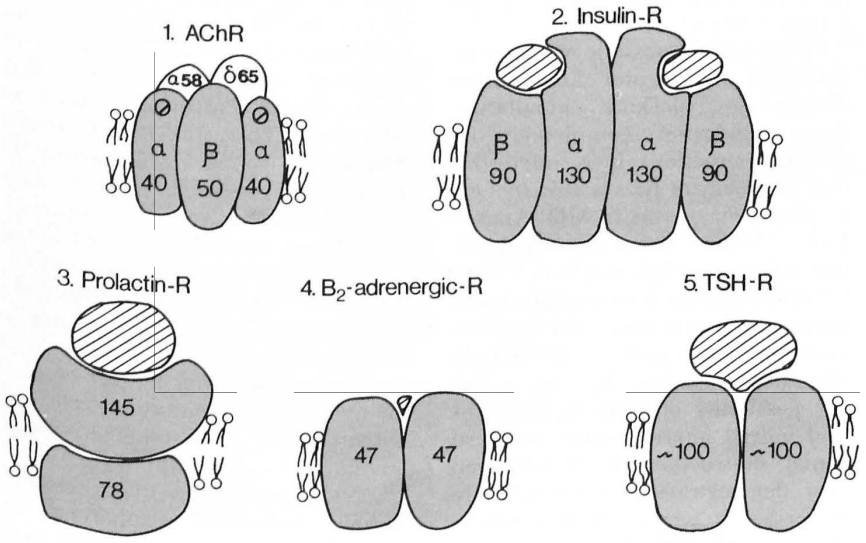

Fig. 1. Diagrammatic representation (roughly to scale) of proposed subunit structures of the principal receptors discussed, indicating the size and number of binding site(s) for the natural ligand (hatched). Receptors are ranked (1–5) according to the information available. Numbers denote the mol. wt.

Transplantation

Applied wisdom

from Elizabeth Simpson

The tradition of an annual 'Round Table Symposium on Applied Immunology' was carried into its 13th year in Axams, Austria, on 25–27 January.

On these occasions small numbers of clinicians and scientists meet to discuss new developments in research that have mutual interest.

In the opening session on cell-surface antigens H. Balner (Rijsvijk) discussed the *in-vivo* effect of monoclonal antibodies (MAbs) directed against T-cell subsets in Rhesus monkeys grafted with allogeneic skin. The MAbs defined human lymphocyte subsets that crossreact with the equivalent rhesus monkey cells. Antibodies against the helper/inducer subset (OKT4 equivalent) prolonged skin allograft survival whereas those directed against the cytotoxic/suppressor subset (OKT8 equivalent) not only failed to prolong survival but may have shortened it. These results are in line with recent reports that in mice and rats the T cells involved in initiating graft rejection are of the helper ($Ly1^+$) phenotype and not the cytotoxic/suppressor ($Ly1^+ 2^+$) phenotype. D. Arndt-Jovin (Göttingen) presented her use of sophisticated physical methods (fluorescence energy transfer, rotational diffusion and translational diffusion) to assess the density, proximity and mobility of mouse H-2 (K^k) antigens detected with monoclonal antibodies. The basic information obtained from these studies should allow direct examination of how H-2 antigens 'associate' with such extrinsic antigens as viruses and thus the necessary constraints for considering the altered self versus dual receptor hypothesis of T-cell receptors. P. Peterson (Uppsala) presented

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Guide for Authors

Although most of *Immunology Today's* contents are commissioned, unsolicited articles are welcome, preferably after consultation with the editorial office. Authors should be aware of the diversity of their readers' experience of immunology and should strive to be as widely understood as possible.

Review articles offer a synthesis of current knowledge in a field where rapid progress has been made. The text should not exceed 3000 words, 40 references and 6 figures/tables.

Compass articles are critical commentaries on one or several recent research papers that contain results of substantial importance. The text should not exceed 1000 words, 15 references and 2 figures/tables.

Rostrum articles offer hypotheses, statements of personal opinion, or speculation. The subject concerned need not be scientific. The text should not exceed 2000 words and should be supported by the minimum necessary number of references and figures.

Letters to the Editor offer comment on articles recently published in *Immunology Today* or other matters of concern to its readers. Authors should strive to be brief. Letters should be signed by all named authors.

Manuscripts should be submitted to the Editorial Office. Two copies are required, each with lettered artwork. For reference style, use a published article as a guide.

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The three-dimensional structure of antibodies

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Antibody molecules are glycoproteins which occur in vertebrate species. They recognize and bind an enormous variety of foreign substances (antigens) and subsequently trigger further defense mechanisms at the molecular or cellular level. Specific recognition requires surface structures complementary to the antigen and hence a huge variety of antibody molecules. In contrast the effector functions need identical interaction sites in all antibody molecules.

The determination of the primary structure of immunoglobulins[1-3] and the X-ray crystallographic studies of several antibody molecules and fragments[4,5,7,10,12-15] led to an advanced understanding of the way in which antibodies meet these opposing requirements.

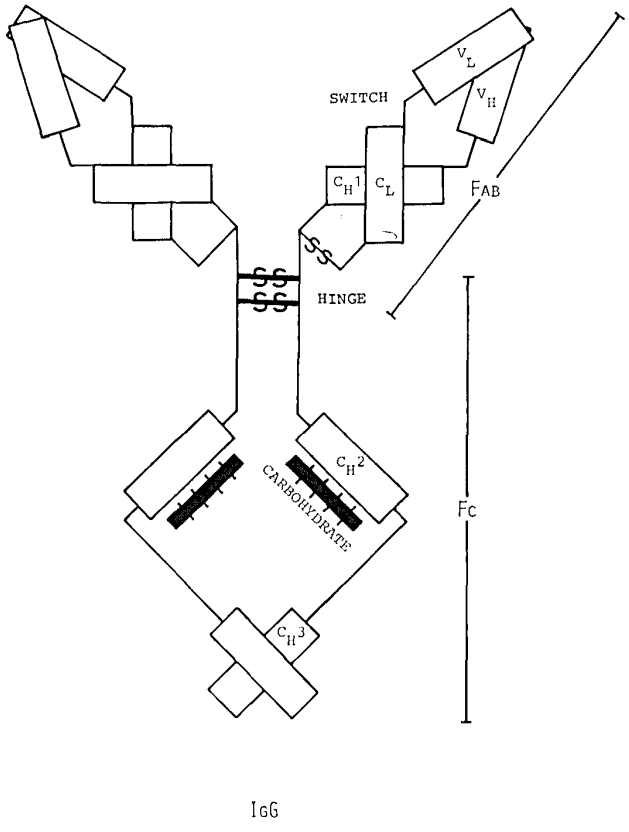

Fig. 1 Schematic representation of an IgG1 immunoglobulin molecule.
The arms of the Y-shaped molecule are formed by the Fab parts, the stem is made up by the Fc part. The light chains are linked to the heavy chains by a disulphide bridge close to the C-terminus. The two heavy chains are connected via two disulphide linkages in the hinge region.

Fig. 1 is a schematic drawing of an antibody molecule of class IgG1. It is composed of two identical heavy chains and two identical light chains with mol. wts of 50,000 and 25,000, respectively. Both types of polypeptide chain are folded into domains: the four domains of the heavy chain are VH, CH1, CH2, and CH3; the light chain consists of the two domains VL and CL. All domains except CH2 are arranged in pairs which are held together by non-covalent forces. Inter-chain disulfide bridges provide further stability.

Among antibody molecules of a given class and species, the V-domains differ considerably in amino acid sequence, whereas the C-domains have identical sequences. The V-domains are composed of about 110 amino acid residues at the N-terminal end of heavy and light chains. The VH-VL pair together forms the antigen binding site; different antibody specificities are the result of different amino acid sequences of the V-domains. The sequence variability in V-domains is most pronounced in a few hypervariable regions. On the other hand the framework residues are well conserved. The constant domains CH2 and CH3 are involved in effector functions such as complement activation and binding to receptors on certain cell types. There is significant homology between the amino acid sequences of all C-domains, and of the framework residues of V-domains.

Proteolytic cleavage at the hinge region yields stable and functional fragments: the antigen-binding fragment Fab, and the Fc fragment (Fc was the first antibody fragment obtained in crystalline form)[6].

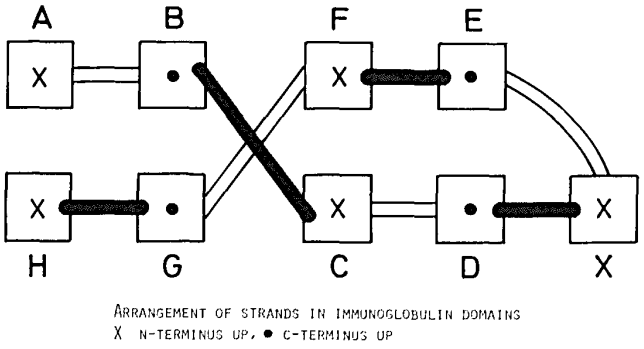

Fig. 2 Schematic drawing of the strand topology in a V-domain viewed parallel to the strands.
(×) and (●) indicate N- and C-terminal ends of the strands pointing towards the observer.

Besides IgG1, several other classes (IgM, IgA, IgD, IgE) and subclasses of immunoglobulins have been identified; the differences between these are located in the constant region of the heavy chain. The two types of light chain (kappa, lambda) can combine with heavy chains of any class.

Domain folding

The general folding pattern in all immunoglobulin domains is very similar. It is shown schematically in Fig. 2 for a V-domain. The folding is characterized by two pleated sheets connected by an internal disulphide bridge linking strands B and C. The two sheets cover a large number of hydrophobic amino acid side chains.

Despite that gross similarity there exist substantial differences when one compares V- and C-domains: C-domains lack strand X, strand D is very short (2–3 amino acids) and connected to strand E. In addition the length of the loop regions in C-domains is different from V-domains, thus changing the overall shape considerably.

VH and VL, on the other hand, show only minor differences when compared with each other (except in the hypervariable regions) as do CL, CH1 and CH3.

CH2 represents yet a third type of domain, differentiated from the other C-domains mainly by the branched carbohydrate chain linked to it. It will be discussed in more detail below.

Domain-domain interaction

Two kinds of domain interactions occur in immunoglobulins: lateral (or trans) interactions and longitudinal (or cis) interactions.

In lateral interactions immunoglobulin domains other than CH2 strongly associate to form modules VL–VH, CL–CH1, CH3–CH3. In V modules VH may be replaced by VL to form light chain V dimers as seen in the Bence-Jones protein fragments Rei or Au[7–9]. In Bence-Jones proteins, which are light chain dimers, one of the light chains simulates the Fab parts of the heavy chain, as described for Meg[10].

V modules associate in a different way than C modules do. In V modules HGCD faces (see Fig. 2) of the domains get into contact, in C modules the ABFE faces are involved.

A considerable loss of accessible surface area[11] is connected with contact formation of the immunoglobulin domains. It amounts to 1760 Å², 1923 Å² and 2180 Å² for VL–VH, CL–CH1 modules of IgG Kol[12,13] and the CH3–CH3 module of an human Fc fragment[14,15] respectively. In VL–VH association both framework residues and amino acids from hypervariable segments are involved. A comparison of V-domain amino acid sequences of different animal species shows that the contacting framework residues are highly conserved. Also the constant domain residues participating in lateral contact are either invariant or replaced by homologous residues in different immunoglobulin chains. This low degree of sequence variability for the residues important for lateral contact formation provides an explanation for the fact that different L-chains can associate with different H-chains to give intact immunoglobulins.

In addition to the extensive Van der Waals contacts, there exist a few trans hydrogen bonds, in which mainly polar side chain groups are involved. There are two salt linkages in Kol CL-CH1 contact: Glu 125 light chain – Lys 214 heavy chain, Glu 126 light chain – Lys 148 heavy chain, which have their analgon in CH3 – CH3 pairing: Glu 356 – Lys 439, Glu 357 – Lys 370.

CH2 is an exception, as it forms a single unit without lateral domain interactions (see Fig. 3)*. Instead it interacts with bound carbohydrate, which is attached to Asn 297. The CH2 residues that are involved in carbohydrate contact are, with a few exceptions, structurally in the same positions as the residues that form the CH3–CH3 contact (face ABFE in Fig. 2). This demonstrates that the carbohydrate in CH2 provides a substitute for the C–C contact and presumably helps to stabilize the CH2-domain. The branched carbohydrate forms a few hydrogen bonds with the CH2-domain, but the dominant interactions are hydrophobic in nature. The carbohydrate covers a hydrophobic patch of the protein made up of Phe 241, 243, Val 262, 264, Tyr 296, Thr 260, Arg 301, which would otherwise be exposed to the solvent. The loss of accessible surface area of one CH2 domain is 522 Å², which is only about half as much covered surface area as seen in CH3–CH3 contact (1080 Å²). This observation could explain the apparent 'softness' of those parts of the CH2-domain, as seen in the crystal structure[14,15], which are most remote from the CH3–CH2 interface.

The functional relevance of carbohydrate in antibodies is unclear. It might be involved in intracellular movements of the glycoproteins and in secretion[16–18]. It may well be that the origin of the altered functional properties of carbohydrate-free antibody variants is structural destabilization.

In contrast to the extensive lateral interactions, nonbonded longitudinal interactions along the heavy chain or light chain are much weaker or do not exist at all. However, they are interesting because conformational changes in antibodies affect those interactions.

Fig. 3, which represents the Fc part of an IgG1 molecule shows the CH2–CH3 interaction. With a loss in accessible surface area of 778 Å² this contact has roughly one third of the size of CH3–CH3 contact. The residues that participate in CH2–CH3 contact are highly conserved in all Ig classes, suggesting that this contact is likely to be found in IgG and IgA and as CH3–CH4 contact in IgE and IgM.

* Most readers will need a stereo viewer (commercially available) to see in three dimensions the structures shown in the paired diagrams on pages 162, 163 and 166.

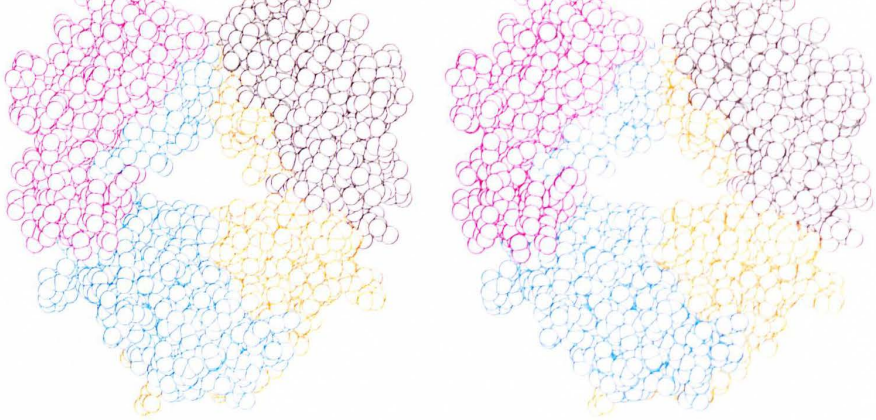

Fig. 3 Stereo drawing of a space filling model of human Fc-fragment.
The molecule is built from two identical polypeptide chains (chain 1, chain 2), and identical carbohydrate groups. Both halves are related by approximate diads.
red, black: CH2-domains of chain 1 and chain 2, respectively.
blue, orange: CH3-domains and carbohydrate of chain 1 and chain 2, respectively.

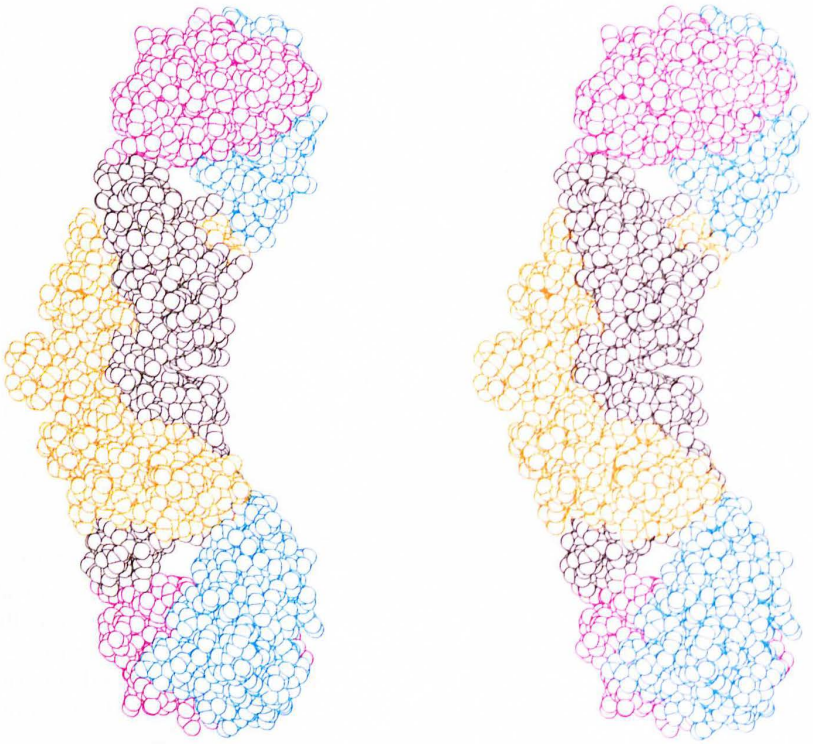

Fig. 4 IgG1 molecule Kol.
The Fab parts and the hinge segment are well ordered in the Kol crystals, the Fc part is disordered and not visible
red: VL-domains
black: CL-domains
blue: VH-domains
orange: CH1-domains and hinge segment

Fig. 5 Amino acid comparison of residues 98–119 (Eu numbering) of M603, New, Kol and Eu heavy chains. The underlined residues were left out in Fig. 6c.

	End of VH			D segment													
	98																
M603:	Cys	Ala	Arg	Asn	Tyr	Tyr	Gly	Ser	Thr	—	—	—	—	—	—	—	
New :	Cys	Ala	Arg	Asn	Leu	Ile	Ala	Gly	Cys	Ile	—	—	—	—	—	—	
Kol :	Cys	Ala	Arg	Asp	Gly	Gly	His	Gly	Phe	Cys	Ser	Ser	Ala	Ser	Cys	Phe	
Eu :	Cys	Ala	Gly	Gly	Tyr	Gly	Ile	Tyr	Ser	—	—	—	—	—	—	—	

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