Instituto Juan March
de Estudios e Investigaciones

8

CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

The Diversity of the Immunoglobulin Superfamily

Organized by

A. N. Barclay and J. Vives

A. N. Barclay
H. G. Boman
I. D. Campbell
C. Chothia
F. Díaz de Espada
I. Faye
L. García Alonso
P. R. Kolatkar
B. Malissen
C. Milstein
R. Paolini
P. Parham
R. J. Poljak
J. V. Ravetch
J. Salzer
N. E. Simister
J. Trinick
J. Vives
B. Westermark
W. Zimmermann

Instituto Juan March
de Estudios e Investigaciones

CENTRO DE REUNIONES INTERNACIONALES
SOBRE BIOLOGÍA

8

Workshop on
The Diversity of the Immunoglobulin Superfamily

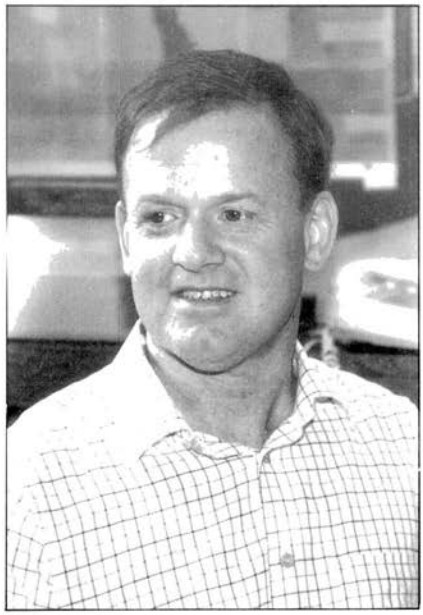
Organized by

A. N. Barclay and J. Vives

A. N. Barclay
H. G. Boman
I. D. Campbell
C. Chothia
F. Díaz de Espada
I. Faye
L. García Alonso
P. R. Kolatkar
B. Malissen
C. Milstein
R. Paolini
P. Parham
R. J. Poljak
J. V. Ravetch
J. Salzer
N. E. Simister
J. Trinick
J. Vives
B. Westermark
W. Zimmermann

The lectures summarized in this publication were presented by their authors at a workshop held on the 26th through the 28th of October, 1992, at the Instituto Juan March.

Depósito legal: M-40.452/1992
I.S.B.N.: 84-7919-507-8
Impresión: Ediciones Peninsular. Tomelloso, 37. 28026 Madrid.



Professor Alan F. Williams FRS

This meeting was dedicated to Alan Williams who organised the scientific programme with Jordi Vives but tragically died of cancer in April 1992.

Alan Williams first proposed the concept that proteins related to immunoglobulins in evolution might have a more general role than solely in antigen recognition. Subsequent events have amply proven this concept, which is now known as the immunoglobulin superfamily, as illustrated by the topic of this meeting.

I N D E X

P R O G R A M M E

THE DIVERSITY OF THE IMMUNOGLOBULIN SUPERFAMILY

MONDAY, October 26

Opening

Basic Concepts

A.N. Barclay - *The Superfamily Concept; Analysis of Recent Structures with an Ig-fold.*

I.D. Campbell - *Protein Modules in Cell Adhesion and Signalling.*

General Discussion

Concepts in Recognition

C. Chothia - *Structural Repertoires and Immunoglobulin Diversity.*

P. Parham - *Immunoglobulin Domains of the Class I MHC Molecule.*

R.J. Poljak - *Structure and Thermodynamics in an Antigen-Antibody Reaction.*

Fc Receptors and MHC-type Antigens

J.V. Ravetch - *The Role of the Ig Receptor Superfamily in Innate Immunity.*

N.E. Simister - *Immunoglobulin G-Fc Receptors that Resemble Class I MHC Antigens.*

F. Díaz de Espada - *Structure and Function of the CD1 Molecules.*

C.Y. Yu - *Molecular Genetic Analysis of CD1 Genes.*

C. Milstein - *The Expression of CD1 Genes.*

TUESDAY, October 27

Neural Biology, Muscle Proteins and Invertebrate Immunity

L. García Alonso - *Genetic Analysis of Fasciclin II Function During Neuronal Development in Drosophila.*

J. Salzer - *Members of the IgSF Mediate the Reciprocal Cell Interactions of Myelination.*

J. Trinick - *Titin and Related Proteins in Muscle.*

H.G. Boman - *From Insect Immunity to Mammalian Peptide Antibiotics.*

I. Faye - *The Immune Response in Insects Involves the Ig-like Molecule Hemolin and an Immunoresponsive Transcription Factor Related to NF-κB in Mammals.*

IgSF Molecules in Signal Transduction

B. Westermark - *Platelet-Derived Growth Factor and its Receptors.*

R. Paolini - *The High Affinity IgE Receptor (FcεRI): a Large Signalling Complex.*

B. Malissen - *Functional and Evolutionary Relationships Existing between the Transducing Subunits of the Antigen Receptors.*

J. Vives - *CDw50: A new Member of IgSF?.*

WEDNESDAY, October 28

IgSF Molecules in Disease Situations

W. Zimmermann - *The Carcinoembryonic Antigen Family: Structure, Function and Clinical Importance.*

P.R. Kolatkar - *Structure of a Human Rhinovirus Complexed with its Receptor Molecule.*

Short Oral Presentations

P.C. Driscoll
Ch. Somoza
K.L. Hilyard
M.H. Brown
R. Zamoyska (Abstract 2)
B. de Andrés
J. Alberola-Ila
Y. Kumagai

Discussion on Evolution of the IgSF.

Closing Session.

INTRODUCTION

A. N. Barclay
J. Vives

THE DIVERSITY OF THE IMMUNOGLOBULIN SUPERFAMILY

The amino acid sequences of immunoglobulins (Ig) were of particular interest because this large family of proteins was so heterogeneous that an antibody could be produced to virtually any new antigen. The structure of immunoglobulins revealed that they were built up of two types of domains, a constant C-domain and a variable V-domain that as it name suggests contained the heterogeneity necessary for antigen recognition. It was therefore surprising when first the beta 2 microglobulin protein that forms part of the major histocompatibility antigen and later the Thy-1 antigen, showed sequence similarity to this highly specialised group of proteins. The Thy-1 antigen was of particular interest as unlike the MHC antigens it has no role in immunity. Although the Thy-1 antigen is expressed on immature lymphocytes in mice and rats, its expression at very high levels in brain is conserved in chicken, mice, rats and man. The sequence of Thy-1 led Alan Williams to propose that it had a role in interactions at the cell surface and more importantly predicted that other cell surface proteins involved in cell surface interactions would have sequence similarities to immunoglobulins. This was indisputably proven by events and this group of proteins became known as the Immunoglobulin Superfamily. The immunoglobulin domain forms a characteristic structure known as the Ig-fold which consists of two sheets of beta stranded sequences that are usually linked by a disulphide bridge. Alan Williams suggested that the Ig-fold might be particularly suited to being recognized by forming a versatile backbone that was stable to proteolysis. Thus different structures could be to be displayed by variation of the loops between the sheets (as in antibodies) or in the accessible regions of the beta sheets.

The rapid increase in the number of amino sequences available in the 1980's proved the predictions so that about 40% of the leucocyte surface proteins that have been sequenced contain one or more Ig-like sequences. In recent years a number of 3-D structures have been determined for Ig superfamily domains showing the presence of an Ig-fold as predicted from sequence analsysis. About 100 proteins have been assigned to the Ig superfamily and a strong argument for all these to contain domains with Ig folds can be made. In the first examples of the Ig superfamily, Ig-like domains were not found mixed with other domain types but now there are many examples of this. A very common association is with the fibronectin type III domain which has recently been shown to have a similar folding pattern although no sequence similarity to Ig domains.

Ig superfamily members are found in diverse tissues and species. For instance in addition to those involved in antigen recognition, some are receptors for soluble proteins and viruses; many are involved in interactions between neural cells. The immunoglobulins themselves are only found in vertebrates but many other Ig superfamily members are found earlier in evolution. For example there are many examples in the nervous system of insects and one also involved in insect defense

against pathogens. Until recently all the members of the Ig superfamily had been found either as the extracellular parts of membrane proteins (e.g. Thy-1) or secreted proteins. However a new group of the Ig superfamily has recently been described in muscle such as the twitchin molecule in the worm *C. elegans* and some other very large muscle molecules such as titin that contain tens of Ig superfamily domains and are involved in muscle function.

This meeting is the first specifically on the subject of the Ig superfamily and the programme reflects the wide range of biology in which members of the Ig superfamily are involved.

Basic Concepts

The Superfamily Concept; Analysis of Recent Structures with an Ig-Fold

A. Neil Barclay,
MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK

The amino acid sequences of leucocyte surface proteins usually show similarities to sequences in other proteins. The term superfamily is used to describe sequences that show less than 50% identity and members of a superfamily are predicted to have similar structures and mediate similar types of interactions. About 20 different superfamilies have been identified at the surface of leucocytes and the Immunoglobulin superfamily (IgSF) is the largest of these [1]. IgSF domains are predicted in about 40% of the 150 polypeptides that have been sequenced at the surface of leucocytes. I will discuss the recent determination of the structures of IgSF domains by NMR and X-ray crystallography and their implications for the superfamily concept. These will be discussed in relation to other superfamilies that also appear to have the same type of fold as in the IgSF but with no significant sequence similarity.

RERERENCE

[1] Barclay, A.N., Beyers, A.D., Birkeland, M.L., Brown, M.H., Davis, S.J., Somoza, C. and Williams A.F. (1992) The Leucocyte Antigen FactsBoook. Academic Press, London.

Protein modules in cell adhesion and signalling

Iain D. Campbell, Department of Biochemistry, South Parks Road, University of Oxford, Oxford OX1 3QU

It is now clear that most extracellular proteins involved in cell adhesion and signal transduction are constructed from modular units like the immunoglobulin domain. Other examples of modules include epidermal growth factor (G), the complement protein (C) and the fibronectin type III (F3). NMR now offers an alternative way of determining protein structure and we have used this method to solve the structure of a variety of modules and module pairs. Our studies include an example of the immunoglobulin superfamily[1] as well as examples of G[2,3], C[4] and F3[5,6]. It is also becoming clear that modularity extends to intracellular domains. A good example is the *src* homology type II domain whose structure has also been determined recently[7]. These structural studies give insight into the ways in which protein modules are used by biological systems.

1. Structure of domain 1 of rat T lymphocyte CD2 antigen. Driscoll, P.C., Cyster, J.G., Campbell, I.D., and Williams, A.F. (1991) *Nature* **353**, 762-765.
2. The three-dimensional structure of the first EGF-like module of human factor IX: comparison with EGF and TGF-α. Baron, M., Norman, D.G., Harvey, T.S., Handford, P.A., Mayhew, M., Tse, A.G.D., Brownlee, G.G., and Campbell, I.D.(1992) *Protein Science* **1**, 81-90.
3. Human epidermal growth factor: high resolution solution structure and comparison with human TGF-α. Hommel, U., Harvey,T.S., Driscoll, P.C. and Campbell, I.D. (1992) *J.Mol.Biol.* **227**, 271-282.
4. Solution structure of the fifth repeat of factor H - a second example of the complement control protein module. Barlow, P.N., Norman, D.G., Steinkasserer, A., Horne, T.J., Pearce, J.M., Driscoll, P.C., Sim, R.B., and Campbell, I.D. (1992) *Biochemistry* **31**, 3626-3634.
5. ^{1}H NMR assignment and secondary structure of the cell adhesion type III module of fibronectin. Baron, M., Main, A.L., Driscoll, P.C., Boyd, J., and Campbell, I.D. (1992) *Biochemistry*. 31, 2068-2073.
6. Solution structure of the fibronectin type III module. Main, A.L., Harvey, T.S., Baron, M., Boyd, J. and Campbell, I.D. *Cell* **71** in press.
7. Structure of an SH2 domain of the p85α subunit of phosphatidylinositol-3-OH kinase. Booker, G.W., Breeze, A.L., Downing, A.K, Panayotou, G., Gout, I., Waterfield, M.D. and Campbell, I.D. (1992) *Nature* **358**, 684-687.

Concepts in Recognition

STRUCTURAL REPERTOIRES AND IMMUNOGLOBULIN DIVERSITY

Cyrus Chothia

MRC Cambridge Centre for Protein Engineering and
Laboratory of Molecular Biology
Hills Road, Cambridge CB2 2QH, England

Five years ago Arthur Lesk and I put forward a canonical structure model for the hypervariable regions of immunoglobulins. It stated that:

> (i) for at least five of the six hypervariable regions there is only a small repertoire of main chain conformations (canonical structures), and
> (ii) most of the canonical structures that exist in these regions are represented in the currently known structures and that their presence in an immunoglobulin of unknown structure can be predicted from its amino acid sequence.

The specificity and affinity of immunoglobulins being determined by:

> (i) sequence variations within the hypervariable regions that modulate the surfaces the canonical structures present to the antigens, and
> (ii) sequence variations within the hypervariable and framework regions that shift the canonical structures relative to each other by small but significant amounts.

The model was based on an analysis of the few immunoglobulins whose atomic structures were known at that time: four Fabs fragments and two Bence-Jones proteins. We now known the structure of more thatn 25 Fabs. These new structures have shown that the canonical structure model is essentially correct and have enabled us to refine and extend it.

The sequences of most of the human immunoglobulin gene segments are now known. The application of the canonical structure model to these sequences enables us to define in outline the structural repertoire of the gene segment products.

The ideas that underlie the canonical structure model may be of use for understanding the evolution of the immunoglobulin superfamily.

AUTHOR: Prof. Peter Parham
Department of Cell Biology
STANFORD UNIVERSITY
USA

TITLE: IMMUNOGLOBULIN DOMAINS OF THE CLASS I MHC MOLECULE

Class I and II antigen presenting molecules consist of a peptide binding domain supported by a pair of immunoglobulin domains. The peptide binding domain interacts with the T cell receptor while one of the immunoglobulin domains interacts with the T cell co-receptor. For class I molecules the co-receptor is CD8. The binding site of this molecule, which is similar to that of antibody Fv, interacts with an acidic loop of the α_3 domain of the class I heavy chain. Although most polymorphic variation in class I heavy chains is focussed on the sites of interaction with antigenic peptides and T cell receptors there is some variation in the α_3 domain which has functional affects upon CD8 engagement. Previously we showed that A*6801 and A*6802 encode HLA-A locus molecules that do not bind CD8 in adhesion assays and present peptides to T cells in a CD8-independent fashion. These properties are due to substitution at position 245 in α_3. Recently we have identified an allele of the HLA-B locus - HLA-B48 - that also is substituted at position 245 and binds poorly to CD8 in in vitro adhesion assays. This allele is rare in many human populations but is common in American Indian populations and in some tribes is the most frequent HLA-B allele. Studies on the recognition of HLA-B48 by T cells are in progress. In contrast to α_3, the interactions of β_2-microglobulin (β_2-m), the second immunoglobulin-like domain of class I molecules, with CD8 or other proteins, are unknown. Site directed mutagenesis is being used to address this question.

References:

1. Salter et al., 1990. Nature. Vol 345, 41-46
2. Belich et al., 1992. Nature Vol 357, 326-329
3. Cerundolo et al., 1991. Proc. R. Soc. Lond. B 244. 169-177

Structure and thermodynamics in an antigen-antibody reaction.

T. N. Bhat, F. Schwarz, R. A. Mariuzza and R. J. Poljak. Immunologie Structurale, Institut Pasteur, 75015 Paris, France and CARB, Maryland Biotechnology Institute, University of Maryland, MD 20850, U.S.A.

The mouse monoclonal antibody D1.3 raised against hen egg-white lysozyme (HEL) has been the subject of extensive immunochemical and structural studies. Its Fv, consisting of the variable domains of the heavy and light polypeptide chains has been cloned and expressed in *Escherichia coli*, and the three-dimensional structures of the free FvD1.3 and the FvD1.3-HEL complex have been determined to 1.8 Å resolution, providing a detailed description of the free and antigen-bound antibody combining site.

Tightly bound water molecules represent an important part of the complex. Those bound to the free antibody combining site are conserved and additional water molecules bind to the complex, further bridging antigen and antibody. Thus, strict complementarity is not achieved by antibody residues alone but by a three dimensional network of solvent molecules, both buried and solvent-accessible, at and around the interface.

Three types of contacts are made by the antibody combining site: 1) by conformationally stable, bulky aromatic side-chains; 2) by polar amino acid side chains which change conformation to make hydrogen bonds to other side chains and to water molecules; and 3) by the conformationally stable main-chain and short side chains such as glycine and serine. The three types of contacts are made by a broad, nearly continuous band of aromatic residues across the combining site, and by isolated patches of hydrophilic residues and main chain atoms located at the sides of the combining site. The spatial segregation of these three types of contacting residues may favour formation of peripheral water networks as a consequence of the hydrophobic nature of the aromatic residues at the center and the more hydrophilic properties of residues at the periphery of the interface.

It is currently assumed that the decrease in conformational entropy due to the formation of a protein association complex might be compensated by an increase in the entropy of ordered water molecules displaced from the interface. However, we observed that formation of the complex leads to inclusion of additional buried water molecules in an interface pocket and to the formation of a network of ordered waters around the antigen-antibody interface. This indicates, at least for the tightly bound waters, a decrease rather than an increase in solvent entropy at the interface. Consequently, enthalpy of hydration must contribute to stability in the antigen-antibody complex. Indeed, the experimental determination of enthalpy and entropy by titration calorimetry shows that enthalpic effects drive the lysozyme-D1.3 reaction.

From the structure of the antibody combining site a kinetic binding model can be postulated in which three regions of the binding site play sequential roles: 1) the broad band of stable aromatic residues mediates the initial binding of the antigen; 2) main-chain N and O atoms permit new interactions with the antigen mediated in part by rearrangement of water molecules; 3) hydropilic side chains change their conformation to make contacts with the antigen and attract additional water molecules that reinforce binding.

Fc Receptors and MHC-type Antigens

THE ROLE OF THE Ig RECEPTOR SUPERFAMILY INNATE IMMUNITY

Jeffrey V. Ravetch, M.D., Ph.D.
Sloan-Kettering Institute

Receptors for the Fc domain of Ig couple humoral and cellular immune responses through the targetting of immune complexes to lymphoid and myeloid cells. Crosslinking of these receptors results in a diverse array of responses, ranging from phagocytosis and ADCC to modulation of B cell activation. The molecular basis for this functional diversity has been identified through the molecular characterization of this receptor family. Fc receptors for IgG, (FcγRs), are a structurally complex family of membrane glycoproteins, encoded by a clustered multigene family on human chromosome 1q22. FcγRs have homologous ligand binding domains and differ in their membrane spanning and intracellular domains. Cell type specific expression of these genes results in the selective distribution of these receptors, so that an IgG immune complex will evoke different cellular responses through its interaction with discrete FcγRs on different cell types. The cis acting elements responsible for macrophage and neutrophil restricted expression are being defined by in vivo and in vitro experiments.

Studies will be described on the reconstitution of functional FcγR expression in heterologous cells to dissect the functional domains involved in assembly and signal transduction. FcγRs have been found to interact with antigen receptors in a number of ways, ranging from the presence of common subunits for FcγRIII(CD16) and TCR/CD3 to the modulation of surface Ig-mediated activation of lymphocytes. The interactions between FcγRs and TCR/CD3 will be presented.

Immunoglobulin G-Fc Receptors that Resemble Class I MHC Antigens

Neil E. Simister
Rosenstiel Center for Basic Biomedical Research
and Biology Department,
Brandeis University, Waltham, MA , U.S.A.

Neonatal rats and mice acquire passive immunity by the uptake of maternal IgG from milk. IgG is transported through intestinal epithelial cells by receptors for the Fc region of IgG (Fc receptors, FcR). These receptors, called FcRn (neonatal), differ from the leukocyte FcγRs I, II & III but are strikingly similar to the class I antigens of the Major Histocompatibility Complex.

FcRn comprises a 12 KDa light chain and a 50 KDa heavy chain. The light chain is β2-microglobulin (β2m), which is also the small subunit of classical and non-classical class I MHC antigens and of CD1 antigens. β2m has a single Immunoglobulin C1-domain. The predicted amino acid sequences of FcRn heavy chains resemble those of class I MHC and CD1 antigen heavy chains. The extracellular part of these polypeptides are organized into three domains. α3, which lies closest to the membrane, is an immunoglobulin C1-domain. Domains α1 and α2 are not related to the Ig superfamily.

The heavy chains of mouse and rat FcRns are highly conserved (84% amino acid identity in α1, 88% in α2, 100% in α3, 91% in the transmembrane region and 98% in the cytoplasmic domain). Mouse FcRn heavy chain has 31% amino acid identity with H-2L^d in the exoplasmic region, and 25% with mouse CD1.1. The transmembrane and cytoplasmic domains are not significantly similar. FcRn heavy chain is encoded on chromosome 7 in the mouse, outside the MHC (on chromosome 17). The CD1s are also encoded outside the MHC. FcRn is as divergent from the CD1s as are classical and non-classical class I sequences, but has amino acid identities with CD1s that are not shared by class I MHC molecules. This may be because FcRn and the CD1s had a common ancestor after diverging from the MHC lineage, or it may represent the survival of residues lost to the rapidly diversifying MHC.

The functions of the classical class I MHC antigens and of FcRn both require interaction with other immunoglobulin superfamily molecules. Class I MHC antigens bind T cell receptors (Ig V-domains) through their α1 and α2 domains, and bind through α3 to CD8 (Ig V-domains), also on the T cell. The binding site for Fc (Ig C1-domains) within the exoplasmic region of FcRn has not yet been located. The binding of IgG is a simpler function than the concomitant recognition by HLA-A2, say, of processed antigen, T cell receptor and CD8. FcRn may therefore resemble an ancestral receptor for an immunoglobulin domain more closely than do class I MHC antigens.

Structure and function of the CD1 molecules.

Fernando Díaz de Espada. Department of Immunology. Clínica Puerta de Hierro. Madrid. Spain.

CD1 molecules were originally defined as a family of cell surface antigens with a restricted tissue expression. They share with ClassI MHC antigens a structure consisting of a heterodimer of an alpha chain associated with β_2-microglobulin, but they lack the extensive polymorphism associated to MHC-encoded molecules. The study of molecular genetics has revealed the existence of several closely linked genes which code for the different members of the family: 5 in humans (a-c), 2 in mice and 8 in rabbits. CD1 genes are basically similar to Class I MHC genes, with three predicted extracellular domains. CD1-alpha3 domains are highly conserved and show the greater homology to the corresponding Class I domains, although predicted secondary structure reveals similarities between CD1 and Class I alphal domains.

The tissue expression of CD1 molecules is very complex. Human CD1a-b-c are expressed by immature thymocytes and CD1c-d are present in some B lymphocytes, whereas epidermal Langerhans' cells and dendritic cells bear CD1a-c molecules. CD1d and the homologous molecules in the mouse are expressed by intestinal epithelial cells. Additionally, mouse CD1 is also found in the cytoplasm of hepatocytes. Mouse thymic cells express weakly CD1 (perhaps due to unspliced RNA), and its function could be fulfilled by the MHC-encoded T1 antigen.

The function of CD1 molecules is unknown. Although some anti-CD1 McAb can induce receptor-mediated endocytosis and transient elevations of intracellular Ca^{2+}, the physiological ligand -if any- for CD1 has not been found. Several CD4-CD8- cytolytic T cell clones have been shown to recognize either CD1a or CD1c molecules on the target cells. Moreover, some anti-CD1 McAb can block T cell responses to allogeneic Langerhans' cells, what suggests an MHC-like role of CD1 molecules in antigen presentation to T lymphocytes. The inverse correlation between the expression of CD1 and Class I molecules in the surface of cortical thymocytes and in Langerhans' cells indicates that both molecules can play similar roles. The binding of endogeneous peptides was studied in CD1 molecules isolated by affinity chromatography from human thymic cells. HPLC analysis of material eluted after acid treatment did not reveal any peak, which raises doubts about the capability of CD1 molecules to process and present antigenic peptides.

Recent progress in the molecular study of CD1. Calabi,F., C.A.G.Bilsland, C-Y.Yu, A.Bradbury, K.T.Belt, L.H.Martin and C.Milstein.1989.In "Leukocyte Typing IV" (Eds.Knapp,W. et al) Oxford University Press, Oxford 254-258.

CD1:From structure to function. Calabi,F., C-Y.Yu, C.A.G.Bilsland and C.Milstein. 1991.In "Immunogenetics of the Major Histocompatibility Complex" (Eds.Srivastava,R. et al) VCH Publishers, Cambridge, England 215-243.

Correlated expression of surface antigens in human thymocytes. Evidence of classI HLA modulation in thymic maturation. Cambón,F., M.Kroisler and F.Díaz-Espada.1988. Eur.J.Immunol. 18:153-159.

Possible mechanism of action of CD1a antigens. Hanau,D., D.A.Schmitt, T.Bieber, D.Schmitt and J-P.Cazenave.1990. J.Invest.Dermatol. 95:503-505.

Evolutionary origin and diversification of the Mammalian CD1 antigen genes. Hughes,A.L.1991. Mol.Biol.Evol. 8:185-201.

Molecular Genetic Analysis of CD1 Genes

C. Yung Yu[1,2], Charles D. Menachery[2], and Cesar Milstein[1]

(1) MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH UK
(2) The Children's Hospital Research Foundation, Columbus, Ohio 43205

CD1 antigens are members of the immunoglobulin superfamily. Molecular cloning of the CD1A cDNA led to the identification of five different CD1 genes in the human genome, named CD1A-CD1E. Determination of the DNA sequences for the five CD1 genes revealed intriguing information about the structural and evolutionary relationships of CD1 and MHC. The 3' untranslated regions of the CD1 genes are unique and gene specific. Long range mapping using pulse field gel electrophoresis and CD1 gene specific probes showed that the CD1 genes are physically linked in a 240 kb genomic region. Construction and screening of cosmid libraries established the presence of the CD1 complex of five CD1 genes in 190 kb of DNA, which mapped to the q22-23 region of the chromosome 1 instead of the chromosome 6 for the MHC. Interestingly, some other immunoglobulin superfamily genes such as those for IgG receptors FcγRI, FcγRII and FcγRIII are also located in the same chromosomal region as CD1. Southern blot analyses revealed a variation of CD1 gene number in mammals. There are 4 or 5 CD1 genes in the Rhesus monkey and pig. The rabbit. cattle, dog and sheep genomes each contain 8-12 CD1 genes. However, there are only two CD1 genes in the mouse or in the rat genomes. The two mouse genes are similar and belong to one of the two classes of CD1. To study the evolutionary relationship of the rodent CD1 genes, we have cloned one of the rat CD1 genes. Partial sequence analysis suggests that this rat CD1 is similar to the mouse CD1.1 gene.

THE EXPRESSION OF CD1 GENES

C. Milstein and A. Woolfson

There are five CD1 genes in humans, and the protein products of four of them have been identified (1-3). Transfections of genomic fragments of the human CD1 genes have yielded a number of mouse cell lines which express in their surface a single human CD1 molecule (3,4). These cells will be available to future International Workshops of Human Leucocyte Differentiation Antigens.

More recently, we have prepared transfectants of CD1a, in which the leader, $\alpha 1$, $\alpha 2$ and $\alpha 3$ exons were fused together, while the $\alpha 3$-intron-transmembrane exon-intron-cytoplasmic 3'UT genomic structure was preserved. In one transfectant, a chain termination codon was introduced at the beginning of the TM domain in order to derive a secreted form of CD1a. When the supernatants of this construct were compared with the homologue not containing the termination triplet, we found that the amount of CD1a secreted was similar in both cases. We have analysed the splicing patterns of both types of constructs, and found that the wild type derived construct (no chain termination mutations) gives a more complex pattern than the site-directed mutant. Essentially, three forms were obtained, namely the normal spliced pattern (presumably the membrane form), an abnormally spliced form which excluded a large segment of the TM exon, and a totally unspliced form. In order to identify which of the latter two was the secreted form, we purified the CD1a secreted form, and subjected it to amino acid sequence studies. We found that the secreted form in fact originated from the unspliced form, which contains a chain termination signal in frame with $\alpha 3$, and extending it by 18 residues. The results suggest that the splicing pattern of CD1a is rather leaky, and this is supported by the diversity of mRNA sizes and abnormal splicing patterns which we have previously reported (5,6).

References

1. Milstein, C., Calabi, F., Jarvis, J.M., Kefford, R., Martin, L. & Migone, N. CDI: A family of MHC-related genes which do not map in chromosone 6. *In: Leucocyte Typing III,* pp. 882-889; Eds. A.J. McMichael *et al.* (Oxford University Press) (1987).

2. Bilsland, C.A.G. & Milstein, C. The identification of the β2-microglobulin binding antigen encoded by the human *CD1D* gene. *Eur. J. Immunol.* **21**, 71-78 (1991).

3. Martin, L.H., Calabi, F., Lefebvre, F.A., Bilsland, C.A.G. and Milstein, C. Structure and expression of the human thymocyte antigens CDla, CDlb, and CDlc. *Proc. Natl. Acad. Sci. USA,* **84,** 9189-9193 (1987).

4. Bilsland, C.A.G., Pannell, R., Gilmore, D.J. & Milstein, C. Analysis of the CD1 panel on CD1 stable transfectants. *In: Leucocyte Typing IV,* pp. 26-261; Eds. W. Knapp *et al.* (Oxford University Press, Oxford) (1989).

5. Bradbury, A., Belt, K.T., Neri, T.M., Milstein, C. & Calabi, F. Mouse CDl is distinct from and co-exists with TL in the same thymus. *EMBO J.* **7,** 3081-3086 (1988).

6. Calabi, F., Bilsland, C.A.G., Yu, C.-Y., Bradbury, A., Belt, K.T., Martin, L.H. & Milstein, C. Recent progress in the molecular study of CDI. *In: Leucocyte Typing IV,* pp. 254-258; Eds. W. Knapp *et al.* (Oxford University Press, Oxford) (1989).

Neural Biology, Muscle Proteins and Invertebrate Immunity

GENETIC ANALYSIS OF FASCICLIN II FUNCTION DURING NEURONAL DEVELOPMENT IN DROSOPHILA Luis Garcia Alonso, Gabriele Grenningloh, and Corey S. Goodman, Howard Hughes Medical Institute, Department of Molecular and Cell Biology, 519 LSA, University of California, Berkeley, CA 94720 USA

We are interested in understanding the molecular mechanisms by which neuronal growth cones find their way toward, and ultimately recognize, their correct targets during development. To address these issues, we use molecular genetic approaches in the fruitfly *Drosophila*.

During neuronal development, growth cones are able to distinguish one particular axon bundle, or fascicle, out of an array of many. To identify axonal recognition molecules, an immunological approach was used to identify and subsequently clone the genes encoding five different surface glycoproteins (fasciclin I, fasciclin II, fasciclin III, fasciclin IV, and neuroglian) which are dynamically expressed on subsets of axon pathways (for review, see Grenningloh et al., 1990). Three of these proteins (fasciclin II, fasciclin III, and neuroglian) are members of the immunoglobulin (Ig) superfamily; all three can function as homophilic cell adhesion molecules using in vitro aggregation assays. Fasciclin II shares a common ancestor with NCAM; the extracellular domain of both proteins consists of five C2-type Ig domains, followed by two fibronectin type III domains.

Fasciclin II was initially characterized and cloned in grasshopper where it is expressed during embryogenesis on the surface of a subset of axon pathways including the MP1 fascicle (Harrelson and Goodman, 1988). It was then cloned and characterized in Drosophila where it is also expressed on the axons in the MP1 pathway (Grenningloh et al., 1991). cDNA sequence analysis indicates that the gene generates multiple forms of the protein, including a PI-linked and two different transmembrane forms, by alternative splicing.

In the grasshopper embryo, in antibody block experiments (Harrelson and Goodman, 1988), the MP1 growth cone does not fasciculate as normal with the MP1 axon in the next posterior segment. In the Drosophila embryo, at a gross level of analysis, the overall structure of the CNS in *fas II* mutant embryos (Grenningloh et al., 1991) develops in a relatively normal way: the general shape of the commissures, longitudinal connectives, and peripheral nerve roots looks normal, as does the general pattern of axon outgrowth and fasciculation. However, the MP1 and vMP2 growth cones do not selectively fasciculate with pCC, as shown by EM reconstructions of *fas II* mutant embryos (R. Fetter and C.S. Goodman, unpublished results). In experiments in both organisms, the absence of fasciclin II appears to perturb the patterns of axon-axon recognition (selective fasciculation), suggesting that fasciclin II functions as an axonal recognition molecule.

fas II null mutant alleles lead to lethality; *fas II* hypomorphic (partial loss of function) mutant alleles are viable and reveal phenotypes in epidermal sensory structures including the ocelli (simple eyes) and bristles. We have established a highly sensitive genetic assay to look for other genes that appear to encode products that interact with the *fas II* gene. Adult individuals homozygous for the hypomorphic allele *fas II*^{e76} (with ~10% of normal protein levels) show a very weak phenotype. This same allele when combined with a null allele (*fas II*^{eB112}) generates a stronger phenotype. Thus, the observation that a reduction in the amount of fas II protein from 10% to 5% causes a strong increase in phenotype indicates the existence of a threshold for fas II function. Based on this *fas II*^{e76} adult hypomorphic phenotype, we are inducing and isolating other mutations that act as dominant enhancers. In this screen, we mobilize P[*lacZ*] transposons to generate heterozygous mutations in a *fas II*^{e76} mutant background. We then isolate and characterize those new mutations that are able to enhance the *fas II*^{e76} phenotype. At the same time, we have also been testing the ability of mutations in known signal transduction systems to act as dominant enhancers of this fas II hypomorphic allele. For example, one such gene that acts as a strong dominant enhancer of *fas II* is the *abl tyrosine kinase*.

Our long term goal is to dissect the fas II pathway to its component upstream and downstream functions, to molecularly characterize these genes, and to study their genetic interactions. In this way, we hope to understand the mechanisms whereby fasciclin II functions during neuronal development.

Members of the IgSF mediate the reciprocal cell interactions of myelination

James Salzer

The development and continued integrity of the nervous system depends on complex and reciprocal interactions between its various cellular elements. A striking example is found in the interactions of axons with myelinating glial cells, i.e. Schwann cells in the peripheral nervous system (PNS) and oligodendrocytes in the central nervous system (CNS). Contact with the axon regulates the proliferation of these glial cells and determines their state of differentiation, most dramatically their elaboration and maintenance of a myelin sheath. This has been most clearly established in the PNS, where an individual Schwann cell can either ensheath multiple smaller axons (i.e. in unmyelinated nerves) or, alternatively, establish a one to one relationship with, and form myelin around, selected nerve fibers (typically axons with diameters greater than one micron). The glial cell, in turn, influences both the topographic segregation of axonal membrane components (notably channel proteins) and the diameter of the underlying axonal segment. This intimate functional relationship between these two cell types is reflected in the highly regular apposition of their respective plasma membranes, which are separated by an extracellular space of 120-140 Angstroms.

Several abundant neural IgSF members have been found to mediate different stages of these axonal-glial interactions. During the initial interactions of the Schwann cell with the axon for example, L1 and NCAM are expressed at high levels on both cells. L1 appears to be particularly critical for these intial interactions, being required for the spreading along and engulfment of nerve fibers by the Schwann cell. Once myelination begins, NCAM and L1 are significantly downregulated whereas expression of the myelin-associated glycoprotein (MAG) by the Schwann cell is dramatically upregulated. Recent studies suggest that MAG is critical for the initial segregation of those fibers destined to be myelinated (away from the smaller fibers that will remain ensheathed). When formation of the myelin sheath is complete, MAG is present in the first turn of the glial cell adjacent to the axon, presumably binding to an axonal receptor which has not yet been identified. The expression of P0 is also substantially upregulated during myelination. P0 is comprised of a single Ig like domain, and in contrast to MAG, is present throughout the compact myelin where it is believed to promote the compaction of the myelin membranes.

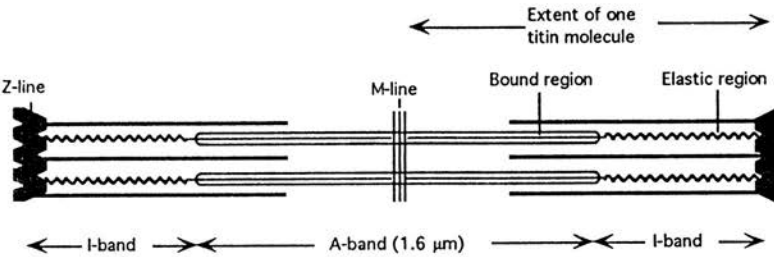
We have performed detailed structural studies on MAG to clarify its role during the intial events of myelination. Two isoforms of MAG, designated large MAG (L-MAG) and small MAG (S-MAG), have been identified. Both proteins have an identical, large extracellular segment, a single transmembrane segment, but differ in their carboxy terminal, cytoplasmic sequences, suggesting that L- and S-MAG have distinct interactions with cytoplasmic components. L-MAG is expressed during the active phase of myelination whereas S-MAG is expressed as the sheath matures. The extracellular segment contains five Ig like domains, with an initial V like domain and four C2 like domains. Structural studies of MAG, have further shown that its first and second domains are linked via an unusual interdomain disulfide linkage. This unusual domain structure is likely to be shared by several other IgSF members including CD22, CD33, and the Schwann cell myelin protein (SMP).

Finally, we have sought to identify cell surface proteins on axons that may be responsible for their differential ensheathment fates by Schwann cells. To this end we have biochemically characterized a variety of primary neurons by surface labeling techniques. We identified striking differences in the expression of GPI anchored proteins on the surface of these axons. Characterization of the major GPI anchored proteins expressed on these neurons indicates that they are all members of the IgSF and include F3, NCAM, OBCAM, CNTFR (alpha subunit), and Thy 1. These proteins are differentially expressed on different fiber types. Initial studies suggest that the CNTFR and the GPI anchored form of NCAM are preferentially expressed on myelinated fibers. We are actively investigating their potential involvement in the axonal-glial interactions of myelination at this time.

Titin and related proteins in muscle

John Trinick
Department of Veterinary Medicine, Bristol University,
Langford, Bristol, BS18 7DY, UK.

Titin is the largest polypeptide yet described (~3,000 kDa) and an abundant protein of striated muscle. Its molecules are approximately 1 μ m long and span between the M- and Z-lines in the sarcomere.



The I-band sections of titin make elastic connections between the ends of thick filaments and the Z-line. These form a third type of sarcomere filament additional to thick and thin filaments. The connections centre thick filaments between neighbouring Z-lines and are the main route of mechanical continuity through relaxed muscle fibres. Elastic behaviour in this region of titin is evidenced by the movement of epitopes away from both M- and Z-lines as sarcomere length is increased. Also, the central position of the A-band is lost after selective radiation scission of titin. By contrast, titin epitopes in the A-band are fixed with respect to the M-line, which suggests that this region of the molecule is an integral part of the thick filament. A possible function here is to act as a template or "protein-ruler" to ensure exact assembly of myosin and other thick filament components. Electron microscopy of titin molecules reveals beaded substructure suggestive of domains containing ~100 amino acid residues. cDNAs encoding ~10,000 residues identified by titin-specific antibodies consist almost entirely of repetition of two types of motif, termed class I and II, each with ~100 residues. Class I is similar to type III fibronectin and class II to C-2 immunoglobulins, thus both motifs probably form independent domains. The whole molecule may contain ~300 such domains. Throughout much of the A-band there is also a longer range sequence periodicity, termed the super-repeat, consisting of ~1100 residues arranged as $(I\text{-}I\text{-}I\text{-}II\text{-}I\text{-}I\text{-}I\text{-}II\text{-}I\text{-}I\text{-}II)_n$. Local sequences at comparable positions in different super-repeats are more similar than between domains of the same class within a super-repeat. The super-repeat probably spans 43 nm in situ, which is the repeat distance of the helix that describes the arrangement of myosin in the thick filament. Titin is one of an emerging group of intracellular muscle proteins composed of class I and II domains arranged in variety of patterns. A feature uniting this group is that they are all likely to interact with myosin. Some of the group appear to have a purely structural role but others are also enzymes. Smooth muscle myosin light chain kinase (smMLCK, M_r 108 kDa) has three class II and one class I domains in addition to its catalytic sequence. Titin and twitchin, which is a related ~800 kDa protein from C. elegans muscle, also have sequences similar to the catalytic domain, but the in vivo substrates of these are not yet known.

(1) Whiting, A., Wardale, J., Trinick, J. Does Titin Regulate The Length Of Muscle Thick Filaments? J. Mol. Biol. 1989, 205, 263-268.
(2) Benian, G. M., Kif, f. J. E., Neckelmann, N., Moermann, D. G., Waterston, R. H. Sequence of an unusually large protein implicated in regulation of myosin activity in C. elegans. Nature 1989, 342, 45-50.
(3) Einheber, S., Fischman, D. A. Isolation and Characterization of a cDNA Clone Encoding Avian Skeletal-Muscle C-Protein - An Intracellular Member of the Immunoglobulin Superfamily. Proc. Nat. Acad. Sci. U. S. A. 1990, 87, 2157-2161.
(4) Labeit, S., Barlow, D. P., Gautel, M., Gibson, T., Holt, J., Hsieh, C. L., Francke, U., Leonard, K., Wardale, J., Whiting, A., Trinick, J. A Regular Pattern Of 2 Types Of 100-Residue Motif In The Sequence Of Titin. Nature 1990, 345, 273-276.
(5) Trinick, J. Elastic filaments and giant proteins in muscle. Curr. Opin. Cell. Biol. 1991, 3, 112-119.

From Insect Immunity to Mammalian Peptide Antibiotics

Hans G Boman
Department of Microbiology, Stockholm University,
S-106 91 Stockholm, Sweden.

My work on insect immunity started with Drosophila as a model system and already 20 years ago we could demonstrate that flies vaccinated by live non-pathogenic bacteria became resistant to an infection with a pathogen that killed all control flies. However, it was difficult to do biochemistry on the flies and we therefore turned to pupae of the giant silk moth, *Hyalophora cecropia.* Here we found an inducible cell-free immunity that eventually was traced to a lysozyme and two new families of antibacterial proteins, cecropins and attacins. In addition we found an inducible immune protein, hemolin, composed of four C2 domains (to be discussed by Ingrid Faye). Here I will concentrate on cecropins and other peptide antibiotics of animal oregin. Insect cecropins are 35-39 residues and have strongly basic N-terminals and hydrophobic C-terminals. They have later been found in many other insects, including Drosophila. Cecropins A and B act on a number of gram negative and gram positive bacteria, while the D-form has a narrow spectra. Cecropins have a specificity for bacterial membranes and they do not act on eukaryotic cells. They can also form voltage dependant anion channels in artificial membranes. Insect cecropins are made as preproproteins of 62-62 residues and their processing was worked out with synthetic precursors and partly purified enzymes. In Cecropia each of the three cecropins is encoded by a single-copy gene with a single intron and a conserved splice site. The cecropin cluster in Cecropia spans 20 kb, in part because two of the introns contain transposable elements. One of the elements resembles Mariner in Drosophila. The genetics is worked out also for Drosophila cecropins (by Hultmarks group).

The cecropin structures were early confirmed by solid-phase synthesis (collaboration with David Andreu and Bruce Merrifield). The synthetic program was then extended to include novel antibacterial peptides with domains from different natural peptides. So far the smallest active peptide was 15 residues. Natural peptides with only D amino acids were synthesized and found to be equally potent as the L-forms, a result that rules out any protein receptors on the bacterial surface.

The mammalian small intestine is known to be a rich source for physiologically active peptides and there are obvious reason to expect it to contain also peptide antibiotics. Three years ago we isolated a 31 residue peptide with convincing homology to the insect cecropins (collaboration with Mutt's group). This peptide, cecropin P1, was active chiefly against gram negative bacteria, including four coli strain pathogenic for piglets. We have continued and identified a 39 residue intestinal peptide with about 49 % Pro and 26 % Arg. This peptide, named PR-39, shows no homology to other proline rich peptides so far isolated. PR-39 is highly active against several gram negative bacteria and against Bacillus megaterium and Staphylococcus pyogenes.

The advantages of peptides as inducible immune substances will be discussed.

Two reviews:

1. Boman, H. G. et al: Cell-free immunity in Cecropia. A model system for antibacterial proteins. Eur. J. Biochem. 201: 23-31, 1991.

2. Boman, H. G.: Antibacterial Peptides: Key Components Needed in Immunity. Cell 65: 205-207, 1991.

The immune response in insects involves the Ig-like molecule hemolin and an immuno-responsive transcription factor related to NF-κB in mammals.

INGRID FAYE, Department of Microbiology, Stockholm University, S-106 91 Stockholm

Insect proteins which are synthesized and released into the hemolymph after bacterial challenge, have been designated immune proteins. They include, i) potent bactericidal or bacteriolytic peptides ii) proteins which are bacteriostatic in their action and, iii) recognition molecules, like lectins and Ig-like proteins (1).

Molecular cloning and sequence comparisons of several immune protein genes reveal upstream sequences that are homologous to the consensus binding site of the nuclear factor κB (NF- κB), found in mammals to direct the regulation of genes involved in inflammatory and immune responses (2). The functional importance of the κB motifs in the insect defense system was possible to demonstrate by transient expression of a reporter gene in a *Drosophila* hemocyte cell line (3). Furthermore, a κB-binding factor that only appears in insects after injection with bacteria or bacterial products, was isolated from pupae of *Hyalophora cecropia* and named Cecropia Immunoresponsive Factor (CIF). CIF is a dimer of two 65 kD subunits and shares many characteristics with NF-κB (4).

Hemolin is the immune protein that belongs to the Ig-superfamily and was first isolated from *H.cecropia*. It is present at low levels in the hemolymph, but is strongly induced by bacteria. It binds as a complex with two other hemolymph proteins on bacterial surfaces (5). The binding of this protein complex to *E.coli* cells is depending on Ca^{2+}-ions and on the sugar moity of the LPS core. Hemolin is a soluble protein which could constitute a primordial Ig-molecule involved in recognition of foreignness or the opsonization of bacteria before fagocytosis. As shown in *Manduca sexta* (6), hemolin also binds to hemocytes, thereby creating a possible signalling link for induction of immune protein synthesis.The *Manduca* hemolin also was shown to inhibit hemocyte aggregation. Structural analysis shows that hemolin is closely related to cell adhesion molecules both at protein and gene level(5, 7).

The fact that related molecules have been found in the immune system of insects and mammals both at the level of recognition, as effector molecules, and now also at the level transcriptional regulation, opens new possibilities for the study of basic immune mechanisms that have been kept through evolution.

1. Faye, I., and Hultmark, D. The insect immune proteins and the organization and regulation of their genes. In "Parasites and Pathogens of Insects." (N. Beckage, S. N. Thompson and B. Federici, ed.), Academic Press, in press.
2. Baeuerle, P. A. (1991). The inducible transcription activator NF-κB: regulation by distinct protein subunits. Biochim. Biophys Acta 1072, 63-80.
3. Engström, Y., Kadalayil L., Sun, C.-S., Samakovlis, C., Hultmark, D. and Faye, I. κB-like motifs regulate the induction of immune genes in Drosophila blood cells. Submitted to Nature
4. Sun, S.-C., and Faye, I. (1992b). Affinity purification and characterization of CIF, an insect immunoresponsive factor with NF-κB-like properties. Comp. Biochem. Physiol.103B, 225-233
5. Ladendorff, M. R., and Kanost, M. R. (1991). Bacteria induced protein P4 from Manduca sexta: A member of the immunoglobulin superfamily, which can inhibit hemocyte aggregation. Arch. Insect Biochem. Physiol. 15, 285-300.
6. Sun, S.-C., Lindström, I., Boman, H. G., Faye, I., and Schmidt, O. (1990). Hemolin: An insect immune protein belonging to the immunoglobulin superfamily. Science 250, 1729-1732.
7. Lindström Dinnetz, I., Sun, S.-C., Faye, I. The induction, regulation and organization of the hemolin gene. in manuscript

IgSF Molecules in Signal Transduction

PLATELET-DERIVED GROWTH FACTOR AND ITS RECEPTORS

Bengt Westermark and Carl-Henrik Heldin
Dept. of Pathology, University of Uppsala, and Ludwig Institute for Cancer Research, Uppsala, Sweden

The platelet-derived growth factor (PDGF) family consists of 30 kDa dimers of A and B polypeptide chains. The A and B chains are structurally related (~60% amino acid sequence similarity), with a perfect conservation of the location of the 8 cysteine residues. The 2nd and 4th cysteine residues in the PDGF chains participate in forming interchain disulfide bonds, such that the 2nd cysteine in one chain is linked to the 4th cysteine residue in the other.

The three isoforms of PDGF (PDGF-AA, -AB and -BB) bind with different affinities and specificites to two structurally related but distinct receptors; the PDGF α-receptor binds all three isoforms with high affinity whereas the β-receptor only binds PDGF-BB with high affinity. Each of the PDGF receptors consists of an extracellular portion of five immunoglobulin-like domains, a single transmembrane segment, and an intracellular part that contains a protein-tyrosine kinase. Other investigators have shown that phosphorylated tyrosine residues in the intracellular part of the ligand-activated receptor mediate the binding of substrate molecules, such as phosphatidylinositol 3´-kinase and GTPase activating protein.

Activation of the PDGF receptor´s protein tyrosine kinase is intimately related to receptor dimerization. Since PDGF is a dimeric protein one might envision that the molecule functions as a bivalent ligand; one subunit chain would then bind one receptor molecule. Recent studies, however, have shown that a monomeric, mutant form of the PDGF B-chain has agonist activity. It is therefore possible that monomeric PDGF B-chain induces a conformational change of the PDGF receptor, which promotes receptor dimerization. Alternatively, monomeric B-chains form dimers which are not stabilized by disulfide bonds.

The high affinity IgE receptor (FcεRI): a large signalling complex.

Rossella Paolini and Jean-Pierre Kinet.
Molecular Allergy and Immunology Section, NIAID, NIH, Rockville, MD USA.

The high affinity receptor for immunoglobulin E (FcεRI) is a multimeric complex composed of an IgE-binding α chain, a β chain and a homodimer of γ chains (1). It belongs to a class of multimeric receptors such as FcγRIII (CD16), the T cell receptor (TCR) and the B cell antigen receptor, all of which share homologous cytoplasmic amino-acid motifs responsible for triggering the intracellular signalling machinery (2,3).

One of the first events detected after engagement of these receptors is the activation of non-receptor tyrosine and serine/threonine kinases which leads to the phosphorylation of various substrates including the receptor β and γ subunits (4-6). The phosphorylation signal is very rapid, is restricted to activated receptors and is reversible upon receptor disengagement. Furthermore the phosphorylation/dephosphorylation of β and γ chains results in the coupling/uncoupling of the receptor with an undefined 125 K protein (7). The activated receptor complexes are also physically associated with tyrosine and serine/threonine kinases as part of a larger signalling complex. We also have found recently that receptor engagement results in the immediate ubiquitination of FcεRI β and γ chains. The ubiquitination is specific of activated receptors and is independent of receptor phosphorylation. Therefore, ubiquitination and phosphorylation generate a complex variety of activated receptor isoforms which may have different signalling properties.

1. Ravetch, J.V. & Kinet, J.-P. (1991) Annu. Rev. Immunol. 9, 457-492.
2. Reth, M. (1989) Nature 338, 383-384.
3. Cambier, J.C. (1992) Curr. Opinion Immunol. 4, 257-264.
4. Benhamou, M, Gutkind, J.S., Robbins, K.C. & Siraganian, R.P. (1990) Proc. Natl. Acad. Sci. USA 87, 5327-5330.
5. Connelly, P.A., Farrel, C.A., Merenda, J.M., Conklyn, M.J. & Showell,
6. Paolini, R., Jouvin, M.-H., & Kinet, J.-P. (1991) Nature 353, 855-858. H.J. (1991) Biochem. Biophys. Res. Commun. 177, 192-201.
7. Paolini, R., Numerof R., & Kinet, J.-P. (1992) Proc. Natl. Acad. Sci. USA in press.

FUNCTIONAL AND EVOLUTIONARY RELATIONSHIPS EXISTING BETWEEN THE TRANSDUCING SUBUNITS OF THE ANTIGEN RECEPTORS

B. Malissen, P. Beaufils, A. Gillet, M. Hermans, G. Ku, M. Malissen and A.M.K. Wegener.
Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, case 906, 13288 Marseille Cedex 9, France.

Recently, several groups, including ours, have shown that the cytoplasmic tails of most of the subunits found in antigen receptors (i.e. membrane immunoglobulins and T cell receptor) and some receptors for the constant domain of immunoblobulins (Fc receptors : FcεRI, FcγRIII...) involve a common sequence which consists of six, mostly conserved amino acids (D/E X_7 D/E X_2 Y X_2 L/I X_7 Y X_2 L/I, in the single amino acid code). Antibody-mediated oligomerization of a single copy of this sequence referred to as the "YLYL" motif suffices to initiate signals leading to protein tyrosine kinase activation, hydrolysis of phosphoinositides, calcium mobilization, degranulation of mast cells, initiation of cytolytic response and production of cytokines (e.g. IL-2) in T cells.

The evolutionary relationships existing between the receptor subunits harboring YLYL motifs is highlighted by the analysis of the exon-intron organization of the corresponding genes. Each individualized motifs straddles the boundary of two exons. Moreover, the nucleotide sequence of each motif is interrupted at the very same position by a class O intron. Therefore, the various gene fragments harboring these distinctive structural features probably derives from a common primordial building-block made of two exons, the product of which constituted a functional domain endowed with intracellular signaling properties. In the process of evolution, this primordial domain-encoding segment has undergone multiple rounds of duplication and transposition, and been subjected to triplication (ζ) or apposed, through exon-shuffling, to various Ig- (B29, mb-1, CD3-γ, -δ, and -ε) or non Ig-(FcεRIβ) like flanking domains. This probable evolutionary relationship may find some functional correspondence in that all the receptors built from these subunits have the capacity to activate signaling pathways operated by protein tyrosine kinases.

References

Anne-Marie K. Wegener, François Letourneur, Arnd Hoeveler, Thomas Brocker, Frédéric Luton and Bernard Malissen.
The T cell receptor/CD3 complex is composed of at least two autonomous transduction modules.
Cell 68 83-95, 1992.

Rick Klausner and Larry Samelson.
T cell antigen receptor-activation pathways : the tyrosine kinase connection.
Cell 64 875-878, 1991.

CDw50 : A NEW MEMBER OF IgSF?

Jordi Vives, Manel Juan, Agustí Miralles, Ramón Vilella and Jordi Yagüe.
Servei d'Immunologia. Hospital Clínic, Barcelona.

CDw50 is a leucocyte antigen defined in the last Workshop on Leukocyte Differentiation Antigens by MoAbs 101-1D2 and 140-11 (1), both of them produced in our laboratory. The CDw50 antigen is present on all cells of hematological lineage except platelets and erythrocytes. By immunological studies CDw50 was not found on any other tissues. Its cellular distribution is similar to LFA-1 (CD18), but its expression presents some discrepancies with LFA-1 on several cell lines. The SDS-polyacrylamide gel electrophoresis analysis of immune precipitates studied under reducing conditions, a band of about 125 kDa was obtained, whereas under non-reducing conditions CDw50 has an apparent molecular weight of about 105 kDa (2). This difference in the migration pattern indicates that CDw50 has some intrachain disulfide bonds. When cells were treated with neuraminidase or N-glycanase a reduction of its apparent molecular weight was observed. This reduction was not observed after treatment with O-glycanase. Moreover, no change in the immunofluorescence pattern was observed after treatment with PI-PLC. All these data point out that CDw50 is a highly glycosilated non-PI linkaged single chain surface molecule. About its possible role in signal transduction we have for the moment only indirect data on its phosphorylation pattern. CDw50 molecule constitutively is poorly or not at all phosphorylated but becomes heavily phosphorylated after treatment of the cells with phorbol ester (3). This phosphorylation is inhibited by inhibitors of protein-kinase C, indicating that most probably CDw50 is also involved in signal transduction.

CDw50 MoAbs do not alter the proliferation of PBMC stimulated either with PHA or other mitogens. However, CDw50 Moabs produced a significant inhibition when added at the beginning of the Mixed Leukocyte Cultures, indicating that CDw50 could play a role in the recognition stage. This functional characteristic, together with a cellular distribution similar to LFA-1 induce to assume that CDw50 is an adhesion molecule. This assumption is further confirmed by our preliminary data on the sequence of three peptide fragments of CDw50. These fragments were obtained by CnBr cleavage of immunoprecipitated CDw50 from PBMC. The resulting sequences showed a high homology with ICAM-1 (CD54), an adhesion molecule formed by five Ig-like domains (4). The fragments of CDw50 analyzed by microsequencing have m.w. of 30 kDa, 15.5 kDa and 25.5 kDa. The 15.5 kDa fragment showed an aminoacid sequence homology of 75% with the 2nd and 3rd fragments of ICAM-1.

The 30 kDa fragment showed an homology of 53.6% with the 4th domain of ICAM-1. The complete sequence of CDw50 will help to asses the precise relatioship between both molecules. Other fact that favours the inclusion of CDw50 in the group of the adhesion molecules consists in its increased expression on memory T cells (CD45RO). This increase has been described as a fairly common feature of several adhesion molecules (5). All the above mentioned data induce us to postulate as a working hypothesis that CDw50 is an adhesion molecule, member of the immunoglobulin superfamily playing a role in signal transduction.

1.- Leucocyte Typing IV, ed. Knapp W., et al., Oxford University Press, 667-670, 1989.

2.- Involvement of the CDw50 molecule in allrecognition: Vilella R., Milá J., Lozano F., Alberola-Ila J., Places L., Vives J., Tissue Antigens, 36, 203-210, 1990.

3.- Effect of protein kinase C activators on the phosphorylation and the surface expression of the CDw50 leukocyte antigen: Lozano F., Alberola-Ila J., Places L., Vives J., Eur.J.Biochem., 203, 321-326, 1992.

4.- Primary Structure of ICAM-1 Demonstrates Interaction between Members of the Immunoglobulin and Integrin Supergene Families: Staunton D.E., Martin S.D., Stratowa C., Dustin M.L., Springer T.A., Cell, 52, 925-933, 1988.

5.- Human Memory T Lymphocytes Express Increased Levels of Three cell adhesion molecules (LFA-3, CD2, and LFA-1) and Three Other molecules (UCHL1, CDw29, and Pgp-1) and Have enhanced IFN-γ Production: Sanders M.E., Makgoba M.W., Sharrow S.O., Stephany D., Springer T.A., Young H.A., Shaw S. J.Immunol., 140, 1401-1407, 1988.

IgSF Molecules in Disease Situations

The carcinoembryonic antigen family: Structure, function and clinical importance.

Wolfgang Zimmermann, Institute of Immunobiology, University of Freiburg, Germany

The gene encoding the widely used human tumor marker carcinoembryonic antigen (CEA) belongs to a large family of genes with 22 members, which can be divided into two subgroups. The CEA subgroup consists of 11 genes encoding immunologically closely related proteins such as nonspecific crossreacting antigen (NCA) and biliary glycoprotein (BGP). The 11 genes of the PSG subgroup code for the more distantly related pregnancy-specific glycoproteins (PSGs), an abundant class of placental proteins (1). All 22 genes are located in three subclusters on a 1.2 Mb segment of the long arm of chromosome 19 (q13.1-q13.2). The members of the CEA subgroup are arranged in two smaller clusters followed by the tightly linked PSG genes toward the telomere (2).

The gene family diverged quite rapidly during evolution in humans and rodents. Although made up of the same structural modules (IgV- and IgC-like domains), which show only marginal similarity in their primary structure, the combinations of these buildung blocks is quite different. In humans, CEA-related antigens are composed of one IgV-like domain and a variable number of IgC-like domains, whereas in rodents, for some antigens a variable number of IgV-like domains is predicted to exist (3). For some members multiple splice variants are found, which can lead to variation in the number of IgC-like domains or the type of the intracytoplasmatic domain of the encoded protein.

The various members of the CEA family exhibit a distinct expression pattern. *CEA* is expressed in normal colonic mucosa, in colorectal adenocarcinomas and in adenocarcinomas of other tissue origins, whereas most NCAs (NCA50/90, NCA95, CGM1, CGM6) and BGP are found on neutrophils. The NCA gene is often coexpressed with *CEA* in adenocarcinomas and there are indications that it might be overexpressed during tumor progression. The PSG genes are transcribed mainly in the fetal part of the placenta. In rat, during early placental development primarily the giant fetal trophoblast cells lining the maternal tissue express PSGs (1).

For most of the membrane-bound members of the CEA subgroup, homophilic and heterophilic cell adhesion properties have been reported *in vitro*. In colon, CEA-reated molecules have been suggested to be involved in the organization of the microvillar structure. Disturbances in the expression of CEA or related antigens in tumor cells might contribute to the malignant phenotype, as is assumed to be the case for the recently discovered recessive oncogene DCC, a presumed cell adhesion molecule (4). Recently, the highly glycosylated NCAs on neutrophils have been shown to present sialylated Le^x carbohydrate structures, which interact with E-selectin on endothelial cells thus allowing cell adhesion of granulocytes to blood vessels (5). As an additional function of CEA subgroup members, binding of enterobacteria via lectin molecules on type 1 fimbriae has been reported. This interaction might be important for the colonization by bacteria of the colonic mucosa as well as for the recognition of bacteria by granulocytes. The presumed mouse homologue was reported to represent the receptor of the mouse hepatitis virus (6). The function of PSGs is unknown. Based on their regional expression pattern on their inhibitory influence on certain immunological reactions, it is speculated that they might be involved in the protection of the allotypic fetus from the maternal immune system by specific immunosuppression.

The release of CEA from tumors into the serum is exploited for monitoring tumor regrowth and metastasis after operation. The presence of CEA on tumor cells can be utilized for immunolocalization of tumors and metastases with radiolabeled anti-CEA monoclonal antibodies. In the future various immunotherapeutic approaches are planned for targeted destruction of CEA-expressing tumor cells. Recently we have constructed transgenic mice, which express the human CEA both organ- and tissue-specifically. These animals will be useful in evaluating the different therapeutic approaches.

1. **Thompson JA, Grunert F, Zimmermann W.** The carcinoembryonic antigen gene family: Molecular biology and clinical perspectives. *J Clin Lab Analysis* **5:** 344-366, 1991.

2. **Thompson J, Zimmermann W, Osthus-Bugat P, Schleussner C, Eades-Perner A-M, Barnert S, von Kleist S, Willcocks T, Craig I, Tynan K, Olsen A, Mohrenweiser H.** Long range chromosomal mapping of the carcinoembryonic antigen (CEA) gene family cluster. *Genomics* **12:** 761-772, 1992.

3. **Rudert F, Saunders AM, Rebstock S, Thompson JA, Zimmermann W.** Characterization of murine carcinoembryonic antigen gene family members. *Mamm Genome* **3:** 262-273, 1992.

4. **Benchimol S, Fuks A, Jothy S, Beauchemin N, Shirota K, Stanners CP.** Carcinoembryonic antigen, a human tumor marker, functions as an intercellular adhesion molecule. *Cell* **57:** 327-334, 1989.

5. **Kuijpers TW, Hoogerwerf M, van der Laan L, Nagel G, van der Schoot E.** CD66 non-specific crossreacting antigens are involved in neutrophil adherence to cytokine-activated endothelial cells. *J Cell Biol* **in press:** , .

6. **Williams RK, Jiang G-S, Holmes KV.** Receptor for mouse hepatitis virus is a member of the carcinoembryonic antigen family of glycoproteins. *Proc Natl Acad Sci USA* **88:** 5533-5536, 1991.

STRUCTURE OF A HUMAN RHINOVIRUS COMPLEXED WITH ITS RECEPTOR MOLECULE

Michael G. Rossmann[1], Norman H. Olson[1], Prasanna R. Kolatkar[1], Marcos A. Oliveira1[1], R. Holland Cheng[1], Jeffrey M. Greve[2], Alan McClelland[2,3] and Timothy S. Baker[1]

[1]Department of Biological Sciences, Purdue University, West Lafayette, IN
[2]Institute for Molecular Biologicals, Miles Inc., 400 Morgan Lane, West Haven, CT
[3]Present address-Genetic Therapy Inc., 19 Firstfield Road, Gaithersburg, MD

Cryoelectron microscopy has been used to determine the first structure of a virus when complexed with its glycoprotein in cellular receptor (1). Human rhinovirus 16 (HRV16) complexed with the two amino-terminal, immunoglobulin-like domains of the intercellular adhesion molecule-1 (ICAM-1) shows that ICAM-1 binds into the 12 Å deep "canyon" on the surface of the virus. This is consistent with the prediction that the viral receptor attachment site lies in a cavity inaccessible to the host's antibodies (2). The atomic structures of HRV14 (3) and CD4 (4,5), homologous to HRV16 and ICAM-1, showed excellent correspondence with observed density, thus establishing the virus-receptor interactions.

1) Olson, N. H., P. R. Kolatkar, M. A. Oliveira, R. H. Cheng, J. M. Greve, A. McClelland, T. S. Baker, M. G. Rossmann. 1992. Structure of a human rhinovirus complexed with its receptor molecule. Proc. Natl. Acad. Sci. U.S. In press.
2) Rossmann, M. G. 1989. The canyon hypothesis. Hiding the host cell receptor attachment site on a viral surface from immune surveillance. J. Biol. Chem. 264:14587-14590.
3) Rossmann, M. G., E. Arnold, J. W. Erickson, E. A. Frankenberger, J. P. Griffith, H. J. Hecht, J. E. Johnson, G. Kamer, M. Luo, A. G. Mosser, R. R. Rueckert, B. Sherry, G. Vriend. 1985. Structure of a human common cold virus and functional relationship to other picornaviruses. Nature (London). 317:145-153.
4) Ryu, S. E., P. D. Kwong, A. Truneh, T. G. Porter, J. Arthos, M. Rosenberg, X. Dai, N. Xuong, R. Axel, R. W. Sweet, W. A. Hendrickson. 1990. Crystal structure of an HIV-binding recombinant fragment of human CD4. Nature (London). 348:419-426.
5) Wang, J., Y. Yan, T. P. J. Garrett, J. Liu, D. W. Rodgers, R. L. Garlick, G. E. Tarr, Y. Husain, E. L. Reinherz, S. C. Harrison. 1990. Atomic structure of a fragment of human CD4 containing two immunoglobulin-like domains. Nature (London), 348:411-418.

POSTERS

TITLE:"MODULATION OF THE Fc-gamma R EXPRESSION ON MURINE EOSINOPHILS BY DIFFERENT CYTOKINES".

AUTHORS:B. de Andrés, V. del Pozo, B. Cárdaba, E. Martin, S. Gallardo, J. C. Fernandez, P. Palomino, C. Lahoz.

ADRESS:Fundación Jimenez Díaz. Immunology Dept. Avda. Reyes Católicos, 2. Madrid 28040. Spain.

ABSTRACT:Eosinophils have a great variety of surface receptors linked to different biologic processes. Among these receptors, the Fc-receptors are directly implicated in antibody-dependent cell-mediated cytotoxicity response with liberation of inflammatory mediators such as the leukotriene C_4 and generation of active oxygen species like the superoxide anion.

Using an experimental model of peritoneal eosinophilia, we have studied the heterogeneity of the low affinity Fc-gamma receptors expressed on murine eosinophils by the Northern blot technique. We have detected transcripts encoding the α-form of the Fc-gamma RIII, and transcripts encoding the ß-forms of the Fc-gamma RII. Expression of the low affinity Fc-gamma receptors (CD32 and CD16), can be modulated by different cytokines and growth factors on human eosinophils: rIFN induces and elevation of CD32 and CD16 membrane expression and GM-CSF induces and augmentation of the cytotoxicity mediated by the Fc-gamma receptors. First of all, we have studied which is the effect of GM-CSF (50U/ml), rIFN (500U/ml), IL-5 (500U/ml) and IL-4 (600U/ml) tested by flow cytometry technique using the mAb 2.4G2 (anti-Fc-gamma RII/III). Only GM-CSF and rIFN increase the mean fluorescence intensity (MFI). Northern blot results of eosinophils treated with these two cytokines from 3 hours of culture show an increase of the α transcript while the ß transcripts remain at the same level that the untreated cells. Our results suggest a transcriptional effect of GM-CSF and rIFN on the α-form of the Fc-gamma RIII on murine eosinophils.

Expression of IgSF domains as chimaeric molecules including domains 3 and 4 of CD4.

M.H. Brown, A.F. Williams and A.N. Barclay

There is a requirement for the production of soluble forms of T cell receptors and other IgSF members for which sequences are available. The soluble products provide a source of material for raising mAbs and for structural and functional studies. Recombinant proteins have been produced in CHO cells using a glutamine synthetase expression system developed by Celltech. TCR V domains were expressed at low levels as a chimaeric protein consisting of the V domain of the TCR and the hinge region of the CD8α chain. This system has the advantage that a mAb to the hinge region is used for both recognition and purification of the product. However, expression levels were less than 1 mg/l and these chimaeric products tended to aggregate (Classon, B.J., Brown, M.H., Garnett, D., Somoza, C., Barclay, A.N., Willis, A.C. and A.F. Williams, Int. Immunol.1992, 4, 215-225).
A construct encoding domains 3 and 4 of rat CD4 was expressed at 200mg/l,suggesting that this might be more effective than the CD8 hinge in production of chimaeric molecules. Chimaeric proteins including domains 3 and 4 of rat CD4 have been made for TCR V domains, mb-1 and the 2 IgSF domains of CD48. The recombinant proteins were monomeric and the TCR V domain to which there are mAbs, was antigenic. So far, immunization has failed produced new TCR mAbs. The CD48 chimaeric protein has been used to demonstrate a clear interaction with rat CD2 (A. van der Merwe et al., manuscript in preparation) and constructs are being made to study other ligand-receptor pairs.

NMR Studies of Domain 1 of the Rat T-cell Antigen CD2

Paul C. Driscoll, Arthur D. Crawford & Iain D. Campbell
Department of Biochemistry, Oxford University, Oxford OX1 3QU, UK.

Jason Cyster, Chamorro Somoza & Alan F. Williams
MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, Oxford University, Oxford OX1 3RE, UK.

CD2 (otherwise known as the T-cell erythrocyte receptor) is expressed on the surface of thymocytes and mature T-cells. CD2 is involved in the processes of adhesion of T-cells to antigen-presenting cells (via binding to the homologous protein LFA-3 (CD58) and the activation of the T-cell response (via a poorly understood mechanism which synergizes with the MHC/TCR/CD4). The domain organisation of LFA-3 and CD2 have been recognised as belonging to the immunoglobulin superfamily. However the absence of the consensus IgSF disulphide bond in the first domain of each protein combined with results from biophysical studies of solubilized human CD2 has lead to the alternate prediction of a novel non-Ig fold. The three-dimensional solution structure of domain 1 of rat CD2 has been determined by NMR spectroscopy. The 101 amino acid contruct including residues 1-99 of rat CD2 has been expressed in E. coli as a fusion protein with glutathione-S-transferase. The CD2 domain - recovered after cleavage of the fusion protein as an antigenically active protein - was investigated by NMR spectroscopy. Both uniform ^{15}N and fractional ^{13}C labelling of the *E. coli* cells have been applied to obtain protein suitable for a number of heteronuclear NMR experiments designed to investigate both the three-dimensional structure and backbone dynamics of the protein. The results reveal a polypeptide fold for the CD2 domain which is very similar to an immunoglobulin V-type domain - a β-sheet sandwich with two sheets made up from strands B, E and D, and strands A, G, F, C and C' respectively. The Ig consensus disulphide, which usually connects the two sheets between strands B and F, is replaced by a van der Waals contact between the sidechains of Ile-18 and Val-78. The D->E loop is much shorter and the B->C loop much longer compared to CD4 domain 1 and V_H and V_L domains from immunoglobulins. The structure is essentially identical to that found in the recent X-ray crystal structure of two domain rat CD2 obtained from CHO cells. The structure allows the interpretation of previously published random mutagenesis data for human CD2. In addition, a program of site directed mutagenesis of human CD2 has been instituted. Combined with homology modelling of the first domain of human CD2 using the rat CD2 structure, the results of this study show that residues on the major β-sheet surface (strands F, G, C and C') are implicated in binding to the ligand LFA-3. NMR studies are planned to investigate the interaction of rat CD2 domain 1 with the newly identified rodent CD2 ligand, CD48.

Structural analysis of sòluble T-cell receptor produced in *E.coli*

Katherine L. Hilyard, Jack L. Strominger
Department of Biochemistry, Harvard University.

An expression system has been developed for the production of soluble T-cell receptor (TCR) domains based on sequence homology with immunoglobulin (Ig) variable domains. We have produced Vα and Vß domains and the single chain Fv (scFv) fragment of a human TCR specific for HLA-A2/matrix peptide complex. The expression constructs were made with a six histidine fusion at the C-terminus of the polypeptide chain. The proteins were purified by metal-chelate chromatography in denaturing conditions and then refolded in a redox buffer system. Refolding efficiencies varied from 40% for Vα to 10% for the scFv, with final yields of 2mg/litre (Vα), 0.3mg/litre (Vß) and 0.5mg/litre (scFv). Analysis by gel filtration indicates that the Vα domain forms homodimers. Circular dichroism spectroscopy of Vα and scTCR showed the predicted ß-sheet secondary structure proposed from the sequence homology to Ig domains. Attempts are currently being made to determine if the refolded scFv recognises the appropriate MHC/peptide complex.

THE EXTENT AND LOCALIZATION OF THE BINDING SITE OF CD2 FOR LFA-3.

Chamorro Somoza*, Paul C. Driscoll¶ and Alan F. Williams*

*MRC Cellular Immunology Unit, Sir Willaim Dunn School of Pathology, University of Oxford, Oxford OX1 3RE ¶ Department of Biochemistry, University of Oxford, Oxford OX1 3QU

The CD2 cell surface glycoprotein is a member of the immunoglobulin superfamily (IgSF) whose extracellular region contains two Ig-like domains. CD2 is involved in the processes of T cell adhesion and signal transduction. The phenomenom of rosetting between human T lymphocytes and sheep and human erythrocytes is dependent on an specific interaction between the CD2 molecule its widely expressed ligand the LFA-3 molecule. CD2 interacts with LFA-3 through its amino-terminal domain.

In this study the binding site of human CD2 for its ligand LFA-3 has been determined by *in vitro* mutagenesis. The three-dimensional structure of domain 1 of rat CD2 was recently determined by NMR [1]. To identify the residues involved in the interaction of human CD2 with LFA-3 a series of 21 mutants were made, based in the rat CD2 structure. In those mutants out-pointing residues in both antiparallel β sheets of domain 1 of human CD2 were replaced with the equivalent rat CD2 residues. The results show that substitutions that abolish rosette formation with both human and sheep erytrocytes are localized in the β sheet containing the CC'C"F and G strands. The region of CD2 that interacts with LFA-3 is a wide and highly charged surface limited by the FG and CC" connecting loops in which the side chains of the solvent exposed contact residues protrude. In contrast mutations in the opposite β sheet dont affect ligand binding.

Finally we report that mutations in the ligand binding face of CD2 alter the binding of the OCH-217 monoclonal antibody (mAb) which reacts with an epitope localized in domain 2 of CD2. The expression of this epitope has been shown to increase after binding of mAb to other regions of CD2 and following T cell activation and has been proposed to result from a conformational change of CD2.

1. Driscoll, P.C., Cyster, J.G., Campbell, I.D. and Williams A.F. 1991. Nature 353, 762-5.

NEW APPROACHES TO THE IDIOTYPIC VACCINE AGAINST THE V3 LOOP OF THE HIV ENVELOPE PROTEIN. I. ARTIFICIAL CONSTRUCTION OF V3 LOOP VARIABILITY AT THE IMMUNOGLOBULIN HYPERVARIABLE REGIONS.

Yoshihiro KUMAGAI, Biomaterial Research Institute, 1 Taya-cho, Sakae-ku, Yokohama 244, Japan

The short peptides from the V3 loop domain of the HIV envelope protein is known to elicit the strong and potencial protective immune response. Neutralizing antibody epitopes, namely principally neutralizing determinants, as well as cytotoxic and helper T-cell epitopes have been mapped to this region.More than 500 V3 loop sequences are reported in the HIV data base. Based on the V3 loop sequences of the MN homology group, the variable constructs were artificially created and implanted into the hypervariable regions of mouse and/or human immunoglobulin VH/VL. The molecular grafts of V3 structure containing the conserved GPGRAFY sequence into the complementrity determining region 2 and 3 of mouse VH and VL induced the antibody and proliferating T-cell response in the mouse. However, the induction of immune response between humoral and cellular mechanism was different in terms of antigen doses and adjuvants. The constructed artificial immunoglobulins will be the new version of potencial idiotypic vaccines against HIV.The following points should be analyzed further: 1) the neutralizing activity of the induced antibody against HIV infection, 2) the possibility of cytotoxic killer cell response induction, 3) the recognition capacity of the grafted V3 structure to the cell surface tryptase molecules.

Abstract 1

Does CD8 dependence have anything to do with T cell receptor affinity?

Rose Zamoyska, Tim Ong & Gek Kwan-Lim
Division of Molecular Immunology, National Institutes of Medical Research, The Ridgeway, Mill Hill, London NW71AA

CD8+ class I restricted T cells have been classified as CD8 dependent or independent based on whether their stimulation or effector function is blocked by anti-CD8 antibody. A dependence on CD8 has been interpreted as a direct reflection of the affinity of the antigen dependent T cell receptor for its ligand. We have investigated how a panel of CD8+ CTL clones, typed by antibody blocking for CD8 dependence, behave when they are fused with BW and cease expressing the CD8 molecule. Three types of hybridomas were obtained: type A, from clones which were CD8 independent respond to antigen in the absence of CD8; type B, from clones which showed partial inhibition fall into two groups, responsive and non-responsive in the absence of CD8; and type C, from clones which were easily blocked by antibody, do not respond to antigen in the absence of CD8. In addition, to our surprise, we found a distinct hierarchy which correlated with CD8 dependence, in the ability of these hybridomas to respond to anti-CD3 antibody. Type A, CD8 independent hybridomas, respond very well, type B respond less well and type C, CD8 dependent hybridomas, respond relatively poorly to stimulation with anti-CD3 antibodies. Thus we feel that the primary distinction between CD8 dependent and independent cells is in the ease with which they can be stimulated to respond. CD8 independent cells seem to require low levels of receptor cross-linking which may be the T cell receptor alone. In contrast, CD8 dependent cells require higher levels of cross-linking which may need to include co-receptor molecules in addition to the T cell receptor, in order to signal efficiently. We are currently investigating whether these differences in responsiveness result from variations in levels of intracellular kinases.

We have retransfected CD8 into these hybridomas and find that in all cases anti-CD8 antibodies are able to inhibit IL-2 production to antigen. Given some of these cells respond perfectly well to antigen in the absence of CD8 it appears that CD8 antibodies can induce an inactivating signal. The same inhibition of response is observed with anti-CD4 antibodies if the cells are transfected with CD4 molecules, and this inhibition requires that the co-receptor molecules can interact with $p56^{lck}$. Thus antibodies to CD4 and CD8 co-receptors can inhibit responses by mechanisms other than sterically blocking interaction with antigen.

Abstract 2

T Cell Activation Results in Physical Modification of the Mouse CD8β Chain

Rose Zamoyska, Clio Mamalaki, Dimitris Kioussis and Lucia G. Casabó
Division of Molecular Immunology, National Institutes of Medical Research, The Ridgeway, Mill Hill, London NW71AA

The T lymphocyte glycoprotein, CD8, is an essential component of the response of class I MHC-restricted T cells to antigen. CD8 is expressed on the surface of class I restricted T cells as disulphide-bonded heterodimers and higher multimers of two distantly related polypeptides, α and β. In transfection studies expression of the CD8α polypeptide is generally sufficient to restore antigen responses and co-expression of CD8β polypeptides has either no effect or moderate enhancement. As the CD8β polypeptide does not get transported to the cell surface in the absence of the CD8α chain, it has not been possible to examine β function directly. Limited restoration of antigen responses has been observed by expression of CD8β-α hybrid molecules, suggesting the CD8β external domain may have some function, although the nature of this contribution could not be determined. CD8α homodimers can be stably expressed on the cell surface, as demonstrated by transfection studies and are found to occur naturally on NK and γδ T cells. However the strong preference for heterodimers on thymocytes and class I restricted T cells suggests that for these cells expression of CD8β is important.

We have found that the CD8β polypeptide changes physically during T cell maturation and activation by reversibly altering its sialic acid content. These changes occur specifically on β and not CD8α and are confined to the O-linked sugars. The O-linked sugars of the CD8β chain, as has been shown for the CD8α chain, are likely to be clustered in the connecting peptide region of the molecule. This region has extremely well conserved across species, 77% identity between mouse, rat and man, compared to 44% over the rest of the molecule. As this region is expected to adopt an extended structure, we predict that the change in charge which occurs on increasing sialylation will influence the physical structure of the CD8 complex. Such a change has implications for the interaction of CD8 with its ligand and possibly with other molecules on the T cell surface itself, which in turn suggests that the primary role of the CD8β chain may be regulatory.

List of Invited Speakers

Workshop on

THE DIVERSITY OF THE IMMUNOGLOBULIN SUPERFAMILY

List of Invited Speakers

A.N. Barclay — *MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, South Parks Road, OXford OX1 3RE (U.K.).*
Tel.: 44 865 27 55 98
Fax : 44 865 27 55 91

H. G. Boman — *Department of Microbiology, Stockholm University, S-106 91 Stockholm (Sweden).*
Tel.: 46 8 16 41 61
46 8 16 20 00
Fax : 46 8 612 95 52

I.D. Campbell — *Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU (U.K.)*
Tel.: 44 865 27 53 46
44 865 27 53 45
Fax : 44 865 27 52 53

C. Chothia — *MRC Cambridge Centre for Protein Engineering and Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH (U.K.).*
Tel.: 44 223 40 21 00
Fax : 44 223 40 21 40

F.Díaz de Espada — *Departamento de Inmunología, Clínica Puerta de Hierro, San Martín de Porres, 4, 28035 Madrid (Spain).*
Tel.: 34 1 316 30 40 Ext. 456
34 1 316 50 42 Ext. 456
Fax : 34 1 373 76 67

I. Faye — *Department of Microbiology, Stockholm University, S-106 91 Stockholm (Sweden).*
Tel.: 46 8 16 20 00
Fax : 46 8 612 95 52

L. García Alonso — *Department of Molecular and Cell Biology, Howard Hughes Medical Institute, Life Sciences Addition #519, University of California, Berkeley, CA. 94720 (USA).*
Tel.: 1 510 642 90 84
1 510 643 99 48
Fax : 1 510 643 55 48

P. R.Kolatkar — *Department of Biological Sciences, Purdue University, 1392 Lilly Hall of Life Sciences, West Lafayette, IN. 47907-1392 (USA).*
Tel.: 1 317 494 49 11
Fax : 1 317 494 08 76

B. Malissen	*Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille Cedex 9 (France).* *Tel.: 33 91 26 94 77* *Fax : 33 91 26 94 00*
C. Milstein	*MRC Medical Research Council, Laboratory of Molecular Biology, Protein and Nucleic Acid Chemistry Division, Hills Road, Cambridge CB2 2QH (U.K.).* *Tel.: 44 223 24 80 11* *Fax : 44 223 41 21 78*
R. Paolini	*Molecular Allergy and Immunology Section, National Institutes of Health, NIAID, Twinbrook II Bldg., 12441 Rockville, MD. 20852 (USA)* *Tel.: 1 301 402 17 56* *Fax : 1 301 40 20 993*
P. Parham	*Department of Cell Biology, Stanford University School of Medicine Ctr., Sherman Fairchild Bldg., Stanford, CA. 94305-5400 (USA).* *Tel.: 1 415 723 61 91* *Fax : 1 415 723 84 64*
R.J. Poljak	*Immunologie Structurale, Institut Pasteur, CNRS URA 359, 25 rue du Docteur Roux, 75724 Paris Cedex 15 (France).* *Tel.: 33 1 45 68 86 10* *Fax : 33 1 45 68 86 39*
J. V. Ravetch	*Laboratory of Biochemical Genetics, Sloan-Kettering Institute, 1275 York Avenue, New York, NY. 10021 (USA).* *Tel.: 1 212 639 86 71* *Fax : 1 212 639 28 61*
J. Salzer	*Department of Cell Biology, NYU Medical Center, 550 First Avenue, New York NY. 10016, (USA).* *Tel.: 1 212 263 53 58* *Fax : 1 212 263 81 39*
N.E. Simister	*Rosenstiel Center for Basic Biomedical Research and Biology Department, Brandeis University, Waltham, MA. 02254-9110 (USA).* *Tel.: 1 617 736 49 52* *Fax : 1 617 736 24 05*
J. Trinick	*Department of Veterinary Medicine, Bristol University, Churchill Building, Langford, Bristol BS18 7DY (U.K.).* *Tel.: 44 934 85 26 61* *Fax : 44 934 85 27 41*

J. Vives

Servicio de Inmunología, Hospital Clínico y Provincial de Barcelona, Villarroel, 170, 08036 Barcelona (Spain).
Tel.: 34 3 454 49 20
34 3 454 60 00 Ext. 2155
Fax : 34 3 451 80 38
34 3 451 52 72

B. Westermark

Department of Pathology, University of Uppsala, S-75185 Uppsala (Sweden).
Tel.: 46 18 66 30 00
46 18 66 38 00
Fax : 46 18 15 27 39

W. Zimmermann

Institute of Immunobiology, University of Freiburg, Stefan-Meier-Strasse 8, D-7800 Freiburg (Germany).
Tel.: 49 761 203 40 60
Fax : 49 761 203 40 16

List of Participants

Workshop on

THE DIVERSITY OF THE IMMUNOGLOBULIN SUPERFAMILY

List of Participants

J.P. Albar	*Laboratorio Fides S.A., Instituto Kabi de Biotecnología, Ctra. del Pardo, Km. 1, 28035 Madrid (Spain).* *Tel.: 34 1 373 10 17* *Fax : 34 1 373 95 62*
J. Alberola-Ila	*Department of Immunology, University of Washington, 1264 Health Sciences Bldg. SL-15, Seattle 98105 WA. (USA).* *Tel.: 1 206 685 20 28* *Fax : 1 206 543 08 58*
L. Alvarez	*Servicio de Inmunología, Clínica Puerta de Hierro, San Martín de Porres, 4, 28035 Madrid (Spain).* *Tel.: 34 1 316 23 40 Ext. 460* *Fax : 34 1 373 76 67*
B. de Andrés	*Servicio de Inmunología, Fundación Jiménez Díaz, Clínica Ntrª Srª de la Concepción, Avda. Reyes Católicos, 2, Ciudad Universitaria, 28040 Madrid (Spain).* *Tel.: 34 1 544 16 00* *34 1 544 17 00* *Fax : 34 1 549 47 64*
R. Bragado	*Servicio de Inmunología, Fundación Jiménez Díaz, Clínica Ntrª Srª de la Concepción, Avda Reyes Católicos, 2, Ciudad Universitaria, 28040 Madrid (Sapin).* *Tel.: 34 1 544 16 00* *34 1 544 17 00* *Fax : 34 1 549 47 64*
M.H. Brown	*MRC, Medical Research Council, Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE (U.K.).* *Tel.: 44 865 27 55 94* *Fax : 44 865 27 55 91*
O. de la Calle	*Servicio de Inmunología, Hospital de la Santa Cruz y San Pablo, San Antonio Mª Claret, 167, 08025 Barcelona (Spain).* *Tel.: 34 3 347 31 33* *Fax : 34 3 456 01 90*

P.C. Driscoll	*Department of Biochemistry, Oxford University, South Parks Road, Oxford OX1 3QU (U.K.).* *Tel.: 44 865 275 720* *Fax : 44 865 275 259*
J.M. Ferrer	*Padre Antonio Tauler, 4 - 5º - B, 07003 Palma de Mallorca (Spain).* *Tel.: 34 71 50 04 11*
A. García-Pardo	*Centro de Investigaciones Biológicas, C.S.I.C., Laboratorio de Inmunología, Velázquez, 144, 28006 Madrid (Spain).* *Tel.: 34 1 585 42 50* *Fax : 34 1 564 45 67*
A. Gaya	*Servicio de Inmunología, Hospital Clínico y Provincial de Barcelona, Villarroel, 170, 08036 Barcelona (Spain).* *Tel.: 34 3 454 49 20* *Fax : 34 3 451 80 38*
A. González-Fernández	*MRC, Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH (U.K.).* *Tel.: 44 223 24 80 11* *Fax : 44 223 41 21 78*
K.L. Hilyard	*Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA. 02138 (USA).* *Tel.: 1 617 495 56 14* *Fax : 1 617 496 83 51*
I. Illa	*Servicio de Neurología, Sección de Patología Neuromuscular, Hospital de la Stª. Cruz y San Pablo, Avda. San Antonio Mª Claret, 167, 08026 Barcelona (Spain).* *Tel.: 34 3 347 31 33* *Fax : 34 3 456 01 90*
Y. Kumagai	*Department of Immunology, Biomaterial Research Institute,1 Taya-cho, Sakae-ku, Yokohama 244 (Japan).* *Tel.: 81 45 851 92 72 or 92 73* *Fax : 81 45 851 92 70*
F. Lozano	*MRC, Medical Research Council, Laboratory of Molecular Biology Protein and Nucleic Acid Chemistry Division, Hills Road, Cambridge CB2 2QH (U.K.).* *Tel.: 44 223 40 22 43* *Fax : 44 223 41 21 78*

M. Ortiz de Landázuri

Sección de Inmunología, Hospital de la Princesa, Universidad Autónoma de Madrid, Diego de León, 62, 28006 Madrid (Spain).
Tel.: 34 1 402 33 47
Fax : 34 1 309 24 96

A. Plaza

Servicio de Inmunología, Clínica Puerta de Hierro, San Martín de Porres, 4, 28035 Madrid (Spain).
Tel.: 34 1 316 23 40 Ext. 460
Fax : 34 1 373 76 67

F. Sánchez-Madrid

Sección de Inmunología, Hospital de la Princesa, Universidad Autónoma de Madrid, Diego de León, 62, 28006 Madrid (Spain).
Tel.: 34 1 402 33 47
Fax : 34 1 309 24 96

B. Sánchez-Sánchez

Servicio de Inmunología, Hospital Universitario "Virgen del Rocio", Avda. Manuel Siurot, s/nº, 41013 Sevilla (Spain).
Tel.: 34 5 455 81 20
Fax : 34 5 455 81 19

Ch. Somoza

MRC, Medical Research Council, Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE (U.K.).
Tel.: 44 865 27 55 94
Fax : 44 865 27 55 91

J. Yagüe

Servicio de Inmunología, Hospital Clínico y Provincial de Barcelona, Villarroel, 170, 08036 Barcelona (Spain).
Tel.: 34 3 454 49 20
Fax : 34 3 451 80 38

C.Y. Yu

Department of Pediatrics, Division of Hematology/Oncology, The Ohio State University, Children's Hospital Research Foundation, 700 Children's Drive, Columbus, OH. 43205 (USA).
Tel.: 1 614 461 26 78
Fax : 1 614 460 70 88

R. Zamoyska

Division of Molecular Immunology, National Institutes of Medical Research, The Ridgeway, Mill Hill, London NW7 1AA (U.K.).
Tel.: 44 81 959 36 66 Ext. 2209
Fax : 44 81 913 85 31

A.G. Zapata

Departamento de Biología Celular, Facultad de Biología, Universidad Complutense de Madrid, Ciudad Universitaria, 28040 Madrid (Spain).
Tel.: 34 1 394 49 79
Fax : 34 1 394 60 79

Texts published in the
SERIE UNIVERSITARIA
by the
FUNDACIÓN JUAN MARCH
concerning workshops and courses organized within the
Plan for International Meetings on Biology (1989-1991)

246 **Workshop on Tolerance: Mechanisms and implications.**
Organized by P. Marrack and C. Martínez-A. Lectures by H. von Boehmer, J. W. Kappler, C. Martínez-A., H. Waldmann, N. Le Douarin, J. Sprent, P. Matzinger, R. H. Schwartz, M. Weigert, A. Coutinho, C. C. Goodnow, A. L. DeFranco and P. Marrack.

247 **Workshop on Pathogenesis-related Proteins in Plants.**
Organized by V. Conejero and L. C. Van Loon. Lectures by L. C. Van Loon, R. Fraser, J. F. Antoniw, M. Legrand, Y. Ohashi, F. Meins, T. Boller, V. Conejero, C. A. Ryan, D. F. Klessig, J. F. Bol, A. Leyva and F. García-Olmedo.

248 Beato, M.:
Course on DNA - Protein Interaction.

249 **Workshop on Molecular Diagnosis of Cancer.**
Organized by M. Perucho and P. García Barreno. Lectures by F. McCormick, A. Pellicer, J. L. Bos, M. Perucho, R. A. Weinberg, E. Harlow, E. R. Fearon, M. Schwab, F. W. Alt, R. Dalla Favera, P. E. Reddy, E. M. de Villiers, D. Slamon, I. B. Roninson, J. Groffen and M. Barbacid.

251 **Lecture Course on Approaches to Plant Development.**
Organized by P. Puigdoménech and T. Nelson. Lectures by I. Sussex, R. S. Poethig, M. Delseny, M. Freeling, S. C. de Vries, J. H. Rothman, J. Modolell, F. Salamini, M. A. Estelle, J. M. Martínez Zapater, A. Spena, P. J. J. Hooykaas, T. Nelson, P. Puigdoménech and M. Pagès.

252 **Curso Experimental de Electroforesis Bidimensional de Alta Resolución.**
Organizado por Juan F. Santarén. Seminarios por Julio E. Celis, James I. Garrels, Joël Vandekerckhove, Juan F. Santarén y Rosa Assiego.

253 **Workshop on Genome Expression and Pathogenesis of Plant RNA Viruses.**
Organized by F. García-Arenal and P. Palukaitis. Lectures by D. Baulcome, R. N. Beachy, G. Boccardo, J. Bol, G. Bruening, J. Burgyan, J. R. Díaz Ruiz, W. G. Dougherty, F. García-Arenal, W. L. Gerlach, A. L. Haenni, E. M. J. Jaspars, D. L. Nuss, P. Palukaitis, Y. Watanabe and M. Zaitlin.

254 **Advanced Course on Biochemistry and Genetics of Yeast.**
Organized by C. Gancedo, J. M. Gancedo, M. A. Delgado and I. L Calderón.

255 **Workshop on The Reference Points in Evolution.**
Organized by P. Alberch and G. A. Dover. Lectures by P. Alberch, P. Bateson, R. J. Britten, B. C. Clarke, S. Conway Morris, G. A. Dover, G. M. Edelman, R. Flavell, A. Fontdevila, A. García-Bellido, G. L. G. Miklos, C. Milstein, A. Moya, G. B. Müller, G. Oster, M. De Renzi, A. Seilacher, S. Stearns, E. S. Vrba, G. P Wagner, D. B. Wake and A. Wilson.

256 **Workshop on Chromatin Structure and Gene Expression.**
Organized by F. Azorín, M. Beato and A. A. Travers. Lectures by F. Azorín, M. Beato, H. Cedar, R. Chalkley, M. E. A. Churchill, D. Clark, C. Crane-Robinson, J. A. Dabán, S. C. R. Elgin, M. Grunstein, G. L. Hager, W. Hörz, T. Koller, U. K. Laemmli, E. Di Mauro, D. Rhodes, T. J. Richmond, A. Ruiz-Carrillo, R. T. Simpson, A. E. Sippel, J. M. Sogo, F. Thoma, A. A. Travers, J. Workman, O. Wrange and C. Wu.

257 **Lecture Course on Polyamines as modulators of Plant Development.**
Organized by A. W. Galston and A. F. Tiburcio. Lectures by N. Bagni, J. A. Creus, E. B. Dumbroff, H. E. Flores, A. W. Galston, J. Martin-Tanguy, D. Serafini-Fracassini, R. D. Slocum, T. A. Smith and A. F. Tiburcio.

258 **Workshop on Flower Development.**
Organized by H. Saedler, J. P. Beltrán and J. Paz Ares. Lectures by P. Albersheim, J. P. Beltrán, E. Coen, G. W. Haughn, J. Leemans, E. Lifschitz, C. Martin, J. M. Martínez-Zapater, E. M. Meyerowitz, J. Paz-Ares, H. Saedler, C. P. Scutt, H. Sommer, R. D. Thompson and K. Tran Thahn Van.

259 **Workshop on Transcription and Replication of Negative Strand RNA Viruses.**
Organized by D. Kolakofsky and J. Ortín. Lectures by A. K. Banerjee, M. A. Billeter, P. Collins, M. T. Franze-Fernández, A. J. Hay, A. Ishihama, D. Kolakofsky, R. M. Krug, J. A. Melero, S. A. Moyer, J. Ortín, P. Palese, R. G. Paterson, A. Portela, M. Schubert, D. F. Summers, N. Tordo and G. W. Wertz.

260 **Lecture Course Molecular Biology of the Rhizobium-Legume Symbiosis.**
Organized by T. Ruiz-Argüeso. Lectures by T. Bisseling, P. Boistard, J. A. Downie, D. W. Emerich, J. Kijne, J. Olivares, T. Ruiz-Argüeso, F. Sánchez and H. P. Spaink.

261 **Workshop The Regulation of Translation in Animal Virus-Infected Cells.**
Organized by N. Sonenberg and L. Carrasco. Lectures by V. Agol, R. Bablanian, L. Carrasco , M. J. Clemens, E. Ehrenfeld, D. Etchison, R. F. Garry, J. W. B. Hershey, A. G. Hovanessian, R. J. Jackson, M. G. Katze, M. B. Mathews, W. C. Merrick, D. J. Rowlands, P. Sarnow, R. J. Schneider, A. J. Shatkin, N. Sonenberg, H. O. Voorma and E. Wimmer.

263 **Lecture Course on the Polymerase Chain Reaction.**
Organized by M. Perucho and E. Martínez-Salas. Lectures by D. Gelfand, K. Hayashi, H. H. Kazazian, E. Martínez-Salas, M. Mc Clelland, K. B. Mullis, C. Oste, M. Perucho and J. Sninsky.

264 **Workshop on Yeast Transport and Energetics.**
Organized by A. Rodríguez-Navarro and R. Lagunas. Lectures by M. R. Chevallier, A. A. Eddy, Y. Eilam, G. F. Fuhrmann, A. Goffeau, M. Höfer, A. Kotyk, D. Kuschmitz, R. Lagunas, C. Leão, L. A. Okorokov, A. Peña, J. Ramos, A. Rodríguez-Navarro, W. A. Scheffers and J. M. Thevelein

265 **Workshop on Adhesion Receptors in the Immune System.**
Organized by T. A. Springer and F. Sánchez-Madrid. Lectures by S. J. Burakoff, A. L. Corbi-López, C. Figdor, B. Furie, J. C. Gutiérrez-Ramos, A. Hamann, N. Hogg, L. Lasky, R. R. Lobb, J. A. López de Castro, B. Malissen, P. Moingeon, K. Okumura, J. C. Paulson, F. Sánchez-Madrid, S. Shaw, T. A. Springer, T. F. Tedder and A. F. Williams.

266 **Workshop on Innovations on Proteases and their Inhibitors: Fundamental and Applied Aspects.**
Organized by F. X. Avilés. Lectures by T. L. Blundell, W. Bode, P. Carbonero, R. W.Carrell, C. S. Craik, T. E. Creighton, E. W. Davie, L. D. Fricker, H. Fritz, R. Huber, J. Kenny, H. Neurath, A. Puigserver, C. A. Ryan, J. J. Sánchez-Serrano, S. Shaltiel, R. L. Stevens, K. Suzuki, V. Turk, J. Vendrell and K. Wüthrich.

267 **Workshop on Role of Glycosyl-Phosphatidylinositol in Cell Signalling.**
Organized by J. M. Mato and J. Larner. Lectures by M. V. Chao, R. V. Farese, J. E. Feliu, G. N. Gaulton, H. U. Häring, C. Jacquemin, J. Larner, M. G. Low, M. Martín Lomas, J. M. Mato, E. Rodríguez-Boulan, G. Romero, G. Rougon, A. R. Saltiel, P. Strålfors and I. Varela-Nieto.

268 **Workshop on Salt Tolerance in Microorganisms and Plants: Physiological and Molecular Aspects.**
Organized by R. Serrano and J. A. Pintor-

Toro. Lectures by L. Adler, E. Blumwald, V. Conejero, W. Epstein, R. F. Gaber, P. M. Hasegawa, C. F. Higgins, C. J. Lamb, A. Läuchli, U. Lüttge, E. Padan, M. Pagès, U. Pick, J. A. Pintor-Toro, R. S. Quatrano, L. Reinhold, A. Rodríguez-Navarro, R. Serrano and R. G. Wyn Jones.

269 **Workshop on Neural Control of Movement in Vertebrates.**
Organized by R. Baker and J. M. Delgado-García. Lectures by C. Acuña, R. Baker, A. H. Bass, A. Berthoz, A. L. Bianchi, J. R. Bloedel, W. Buño, R. E. Burke, R. Caminiti, G. Cheron, J. M. Delgado-García, E. E. Fetz, R. Gallego, S. Grillner, D. Guitton, S. M. Highstein, F. Mora, F. J. Rubia Vila, Y. Shinoda, M. Steriade and P. L. Strick.

Texts published by the CENTRE FOR INTERNATIONAL MEETINGS ON BIOLOGY

1 **Workshop on What do Nociceptors Tell the Brain?**
Organized by C. Belmonte and F. Cerveró. Lectures by C. Belmonte, G. J. Bennet, J. N. Campbell, F. Cerveró, A. W. Duggan, J. Gallar, H. O. Handwerker, M. Koltzenburg, R. H. LaMotte, R. A. Meyer, J. Ochoa, E. R. Perl, H. P. Rang, P. W. Reeh, H. G. Schaible, R. F. Schmidt, J. Szolcsányi, E. Torebjörk and W. D. Willis Jr.

2 **Workshop on DNA Structure and Protein Recognition.**
Organized by A. Klug and J. A. Subirana. Lectures by F. Azorín, D. M. Crothers, R. E. Dickerson, M. D. Frank-Kamenetskii, C. W. Hilbers, R. Kaptein, D. Moras, D. Rhodes, W. Saenger, M. Salas, P. B. Sigler, L. Kohlstaedt, J. A. Subirana, D. Suck, A. Travers and J. C. Wang.

3 **Lecture Course on Palaeobiology: Preparing for the Twenty-First Century.**
Organized by F. Álvarez and S. Conway Morris. Lectures by F. Álvarez, S. Conway Morris, B. Runnegar, A. Seilacher and R. A. Spicer.

4 **Workshop on The Past and the Future of Zea Mays.**
Organized by B. Burr, L. Herrera-Estrella and P. Puigdoménech. Lectures by P. Arruda, J. L. Bennetzen, S. P. Briggs, B. Burr, J. Doebley, H. K. Dooner, M. Fromm, G. Gavazzi, C. Gigot, S. Hake, L. Herrera-Estrella, D. A. Hoisington, J. Kermicle, M. Motto, T. Nelson, G. Neuhaus, P. Puigdoménech, H. Saedler, V. Szabo and A. Viotti.

5 **Workshop on Structure of the Major Histocompatibility complex.**
Organized by A. Arnaiz-Villena and P. Parham. Lectures by A. Arnaiz-Villena, R. E. Bontrop, F. M. Brodsky, R. D. Campbell, E. J. Collins, P. Cresswell, M. Edidin, H. Erlich, L. Flaherty, F. Garrido, R. Germain, T. H. Hansen, G. J. Hämmerling, J. Klein, J. A. López de Castro, A. McMichael, P. Parham, P. Stastny, P. Travers and J. Trowsdale.

6 **Workshop on Behavioural Mechanisms in Evolutionary Perspective.**
Organized by P. Bateson and M. Gomendio. Lectures by J. R. Alberts, G. W. Barlow, P. Bateson, T. R. Birkhead, J. Carranza, C. ten Cate, F. Colmenares, N. B. Davies, R. I. M. Dunbar, J. A. Endler, M. Gomendio, T. Guilford, F. Huntingford, A. Kacelnik, J. Krebs, J. Maynard Smith, A. P. Møller, J. Moreno, G. A. Parker, T. Redondo, D. I. Rubenstein, M. J. Ryan, F. Trillmich and J. C. Wingfield.

7 **Workshop on Transcription Initiation in Prokaryotes.**
Organized by M. Salas and L. B. Rothman-Denes. Lectures by S. Adhya, H. Bujard, S. Busby, M. J. Chamberlin, R. H. Ebright, M. Espinosa, E. P. Geiduschek, R. L. Gourse, C. A. Gross, S. Kustu, J. Roberts, L. B. Rothman-Denes, M. Salas, R. Schleif, P. Stragier and W. C. Suh.

The Centre for International Meetings on Biology has been created within the *Instituto Juan March de Estudios e Investigaciones*, a private foundation which complements the work of the *Fundación Juan March* (established in 1955) as an entity specialized in scientific activities in general.

The Centre's initiatives stem from the Plan for International Meetings on Biology, supported by the *Fundación Juan March*. A total of 30 meetings and 3 Juan March Lecture Cycles, all dealing with a wide range of subjects of biological interest, were organized between 1989 and 1991 within the scope of this Plan.

The Centre endeavours to actively and sistematically promote cooperation among Spanish and foreign scientists working in the field of Biology, through the organization of Lecture and Experimental Courses, Workshops, Seminars, Symposia and the Juan March Lectures on Biology.

Instituto Juan March de Estudios e Investigaciones
Castelló, 77 • Teléfono 34 - 1 - 435 42 40 • Fax 576 34 20
28006 Madrid (España)

The lectures summarized in this publication were presented by their authors at a workshop held on the 26th through the 28th of October, 1992, at the Instituto Juan March.

All published articles are exact reproductions of author's text.

There is a limited edition of 400 copies of this volume, available free of charge.