Instituto Juan March
de Estudios e Investigaciones

5

CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Structure of the Major Histocompatibility complex

Organized by
A. Arnaiz-Villena and P. Parham

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I N D E X

P R O G R A M M E

STRUCTURE OF THE MAJOR HISTOCOMPATIBILITY COMPLEX

Monday, September 21st

MHC GENES
Chairperson: F.M. Brodsky.

J. Trowsdale - *The MHC as a gene cluster.*

A. Arnaiz-Villena - *Haplotype distribution and allelic diversity of the primate DRB6 locus.*

R.D. Campbell - *Organization and function of novel genes and their protein products in the MHC Class III region.*

L. Flaherty - *Immunogenetics of the Q and T regions of the murine MHC.*

MHC STRUCTURE
Chairperson: A. McMichael.

E.J. Collins - *Histocompatibility antigen structure.*

P. Travers - *Conformational change in MHC Class II molecules.*

M. Edidin - *Clustering of Class I HLA molecules in liposome and cell membranes.*

ANTIGEN PROCESSING PRESENTATION AND T CELL FUNCTION
Chairperson: J. Klein.

F.M. Brodsky - *Intracellular transport pathways for antigen presentation by Class I and Class II histocompatibility molecules.*

P. Cresswell - *Characterization of the MHC Class II processing defect in T2 cells.*

T.H. Hansen - *Role of peptide ligand in the folding, intracellular transport and surface expression of Class I MHC molecules.*

F. Lévy - *Transport of peptide across the membrane of the endoplasmic reticulum.*

G.J.Hämmerling - *Antigen presentation by MHC Class I in the absence of MHC encoded proteasome subunits.*

Tuesday, September 22nd

ANTIGEN PROCESSING PRESENTATION AND T CELL FUNCTION (cont.)

R.Germain - *Regulation of MHC Class II intracellular transport, folding, and expression by invariant chain and peptide.*

A. McMichael - *Epitopes presented by HLA B8.*

J.A. López de Castro - *Recurrent T cell crossreactivity between HLA-B27 and HLA-DR2.*

DISEASE
Chairperson: L. Flaherty.

P. Stastny - *Disease associations with HLA genes in different ethnic groups.*

H. Erlich - *HLA Class II sequence polymorphism and disease susceptibility.*

F. Garrido - *MHC antigens in tumors. Mechanisms of alteration and biological implications.*

PHYLOGENY
Chairperson: G.J. Hämmerling.

J. Klein - *Different modes of MHC evolution in the primates.*

P. Parham - *Class I MHC genes in primates.*

R.E. Bontrop - *Evolutionary conservation of MHC-peptide-TCR interactions in primates.*

D.I. Watkins - *New HLA-B variants in South American Amerindians suggest that the MHC Class I loci can evolve rapidly.*

OPEN QUESTIONS

INTRODUCTION

A. Arnaiz-Villena

Introduction

This book is a collection of summaries of presentations at the "Structure of the Major Histocompatibility Complex" meeting held in Madrid on 21st-22nd September 1992.

The idea of such a meeting was put forward by Andrés Gonzalez to some Spanish scientists doing research on the HLA field, and I decided to undertake what finally was a pleasant and easy task by starting contacts with Peter Parham, both at Granada and Strasbourg MHC meetings during 1990. Finally, Peter and myself called to active MHC scientists and decided to entitle the meeting "MHC structure", although other topics (all related to both MHC genes and proteins structure) were going to be dealt with. Peter and myself decided that formal presentations would be the best meeting format for good communication , since the small size of the meeting and the very good facilities of Juan March Foundation would make an easy person to person contact. The response to our call was very good and I wish to thank all the speakers, who made room in their busy schedules to come to Madrid and made possible the scientific success of the meeting. I must express my gratitude to Andrés Gonzalez for his intelligent and efficient management; without his personal effort, this and other brilliant biology meetings would have not been possible.

The rapid discovering of new HLA genes, the flood of new DNA sequences of HLA alleles, the advances on class I and class II molecules intracellular metabolism and crystallography and the important applications of this knowledge to understanding molecular anthropology, evolution and the obscure HLA/disease relationship allowed us to make a program covering not only structural topics, but also antigen presentation and disease association, and evolution.

It is clear that the best studied human genetic system is the HLA complex; however one must say that it deserves such an interest. This HLA analysis provided a significant aid in dealing with clinical problems: choosing donor and receptor in solid organs and bone marrow transplantation, solving paternity problems and helping in diagnosis, prognosis and management of some associated diseases (ankylosing spondylitis, insulin dependent diabetes mellitus, rheumatoid arthritis, etc...).

In Immunology, the HLA antigens together with the clonotypic T cell receptor complex, are one of the most interesting structures, since many antigens are in contact with both to elicit an appropriate (or inappropriate) immune response. Common medical problems, like autoimmunity and secondary immunodeficiencies may be relieved by knowing in detail the structures of both molecular complexes, antigen presentation pathways and T cell signalling.

In evolution, the comparative stories of the different regions of the MHC, give us a relatively overt picture of which may be the forces driving MHC allele generation, that are different in different MHC subregions; this may also give us an idea of the specific function of each group of alleles, and even of each allele.

In the study of human populations' origins and relationships, the extreme HLA polymorphism is helping in getting very informative neighbouring homology trees (see last workshop results). For example, Spanish, Basque and Sardinian populations cluster together according to HLA-B and DR allelism homology and are closer to African populations than to other Caucasoids. This confirms our own analysis (Arnaiz Villena et al. Human Genetics 58:344-348;1981 and unpublished results) concluding that Basques, Sardinians and largely Iberians are related to paleoNorth African populations, whose remnants are today's North African white isolates, like Berbers and Kabyles.

Unfortunately, the HLA and disease insidious relationship has not been clarified and new approaches may be necessary. Particularly, mouse models should not be transposed to humans. A good example are diabetic NOD mice, where at least six genes outside the MHC are working in diabetes susceptibility. Human insulin dependent diabetes mellitus (IDDM) seems to be genetically caused mainly by genes inside de MHC, since the level of disease concordance between monozygotic twins and between HLA identical sib does not differ significantly; thus, it is not acceptable the NOD mouse model as human IDDM counterpart.

Antonio Arnaiz Villena

MHC GENES

The MHC as a gene cluster.

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The human MHC spans about 4 Mbp. So far a number of the 75 or so MHC genes that have been characterised appear to share related functions in antigen processing and presentation, indicating that they may be co-evolving as a gene cluster. In the class II region for example, in addition to classIIα and β chain genes there are three new classes of genes: 1. Genes without any obvious function in the immune system. The *RING3* gene, for example, between *HLA-DNA* and *-DOB*, is highly related to a *Drosophila* developmental gene, *female sterile homeotic*. 2. Novel class II related genes. The *DMA* and *DMB* genes probably form a α/β heterodimer, like class II α and β but their sequences are only weakly related to classII. They appear to be evolutionary precursors to class I and II. 3. Genes involved in antigen processing. Two sets of interesting genes have been found, centromeric of *HLA-DOB*. *TAP1* and *TAP2* are related to 'ABC' transporter genes and form a heterodimer which is a candidate for transporting peptides into the endoplasmic reticulum for assembly with class I heavy chain. Closely associated with the *TAP* genes, *LMP2* and *7* are related to proteasomes and may be involved in producing peptides to feed the transporters. The functions of these genes are being investigated along with their polymorphism and relationship to diseases associated with the MHC.

HAPLOTYPE DISTRIBUTION AND ALLELIC DIVERSITY OF THE PRIMATE DRB6 LOCUS

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The allelic HLA-DRB6 gene has been described in DR1, DR2 and DR10 haplotypes and is probably the last DRB gene to be described in these haplotypes according to the obtained specific DRB RFLPs. The DRB6 evolutionary pattern and the finding of at least a partial transcript suggest that it may be expressed. On the other hand, DRB6 has been found in all Orangutans, Gorillas and Chimpanzees tested, also showing several copies per haplotype and a variety of alleles. These findings together with the fact that 100% of closest to human primates and only 30% of humans bear this gene are discussed and several implications are inferred to better explain the DRB evolutionary pathway.

1. Exon-2 nucleotide sequences, polymorphism and haplotype distribution of a new HLA-DRB gene: HLA-DRB6. **Corell A., Martín-Villa J.M., Morales P., de Juan M.D., Varela P., Vicario J.L., Martínez-Laso J. and Arnaiz-Villena A.**
Molecular Immunology 28: 533-543, 1991

2. Allelic diversity at the primate major histocompatibility complex DRB6 locus. **Corell A., Morales P., Varela P., Paz-Artal E., Martín-Villa J.M., Martínez-Laso J. and Arnaiz-Villena A.**
Immunogenetics 36: 33-38, 1992

Organization and function of novel genes and their protein products in the MHC class III region

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The human Major Histocompatibility Complex (MHC) situated on the short arm of chromosome 6 contains more than 80 genes in about 4000kb of DNA. The organisation of the genes has been determined by a combination of different techniques including pulsed field gel electrophoresis (PFGE), cosmid walking, and Yeast Artificial Chromosome (YAC) cloning. Genes encoding the polymorphic class I and class II MHC antigens are located in 2000kb and 850kb segments of DNA at the telomeric and centromeric ends of the MHC, respectively. The class I and class II multi-gene families are separated by ~1100kb of DNA which is generally termed the class III region. This segment of DNA contains genes that encode proteins of diverse function such as the complement proteins C2, Factor B, C4A and C4B, the microsomal enzyme steroid 21-hydroxylase, the cytokines TNF α and β and three members of the major heat shock protein HSP70 family. However, our detailed characterisation of cloned genomic DNA in the class III region extending ~900kb from HLA-B has led to the discovery of 25 novel genes that encode proteins of unknown function. Novel genes have also been found in the class II region. Two of these encode proteins thought to be involved in transporting peptide antigens from the cytoplasm to newly synthesised MHC class I molecules in the endoplasmic reticulum, while two others appear to encode proteins which are components of the proteosome, illustrating the strong possibility that other novel genes in the MHC could also encode functions influencing antigen presentation by MHC class I and class II molecules, and/or other aspects of the immune response. In this regard the identification of HSP70 genes in the class III region is particularly interesting as there are a number of potential roles for HSP70 in antigen processing and presentation. The members of the HSP70 protein family are known to be involved in the folding, unfolding and translocation of proteins within the cell, and have been shown to bind a variety of synthetic peptides. The role of peptide binding has been attributed to a 170 amino acid region adjacent to the ATPase domain. It has recently been proposed that the protein/peptide binding domain of the HSP70 proteins has a structure highly related to that of the HLA class I peptide binding domain. We have identified a polymorphic amino acid in the HSP70-Hom protein (residue 493) which lies within the HSP70 peptide-binding domain. In the structural model for this region proposed by Rippmann and co-workers (EMBO J., 1991, 10, 1053) amino acid 493 lies on one of the β-sheets which form the floor of the peptide-binding groove. The corresponding position in the HLA-A structure (residue 114) is highly polymorphic and lies in one of the six pockets of the peptide-binding groove which determine binding specificity. If the HSP70 structural model is correct a Met → Thr substitution at amino acid 493 could be associated with variation in the peptide-binding specificity of HSP70-Hom between haplotypes.

Nucleotide sequence data has been determined for cDNA and/or genomic clones for a large number of the novel class III region genes and the sequences of their predicted protein products have been derived.

One way of identifying the possible functions of these proteins is to compare their amino acid sequences with the sequences in the National Biomedical Research Foundation (NBRF) and SwissProt protein databases. In rare cases significant sequence similarity with a known protein over the entire length of a novel protein will suggest the function of the latter. More usually restricted segments of sequence similarity with other proteins might indicate properties of the novel protein, eg. metal-binding capabilities, but will not necessarily define its precise function. G7a encodes a 1265 amino acid protein which shows 48.3 % identity with the valyl tRNA synthetase of *Saccharomyces cerevisiae* over 1043 amino acids. In addition the G7a protein contains two short consensus sequences characteristic of class I tRNA synthetases and has a predicted molecular mass (~140.5 kD) close to that of other mammalian valyl tRNA synthetases. Taken together this is strong evidence that G7a encodes the human valyl tRNA synthetase. The protein products of the BAT2 and BAT3 genes are both proline rich and contain novel repetitive elements, but they do not appear to be members of any known protein family. However, the proteins encoded by some of the other novel class III region genes do show sequence similarity with known proteins over restricted regions. G1 encodes a 10 kDa protein which contains two potential "EF-hand" type calcium-binding domains in its N-terminal half. The expression of G1 appears to be restricted to T cells, monocytes and macrophages suggesting that it may have a role in the activation of these cell types. The 110 kDa protein product of the G9a gene has been shown to contain six contiguous copies of a 33 amino acid repeat first identified in the Notch protein of *Drosophila*. Repeats of this type are found in a large number of proteins and may have a role in protein-protein interactions within the cell. The predicted protein product of G13 shows significant sequence similarity with the leucine zipper family of transcription factors suggesting that it may have a role in the regulation of transcription. Partial sequence of the Opposite Strand Gene (OSG) has indicated that this gene encodes a protein which contains a number of repeats corresponding to the consensus for the 90 amino acid fibronectin type III repeat. The type III repeats in OSG are most similar to those in tenascin - an extracellular matrix glycoprotein.

In order to verify the existence of expressed protein products corresponding to the novel class III region genes it is necessary to raise antibodies against these proteins for use in Western blot analysis. The approach used in our laboratory has been to express all or part of the predicted protein product of a novel gene in *E. coli*. To acheive this, cDNA sequences are cloned into a pGEX expression vector and expressed *in vitro* as fusion proteins with the carboxy terminal 26 kDa of the glutathione S-transferase from *S. japonicum*. The whole fusion protein or the purified polypeptide corresponding to the gene of interest is then used as an antigen to raise polyclonal antibodies in rabbits. Antibodies have been raised against the protein products of G1 and G9a and in Western blot analysis these cross-react specifically with proteins of the expected size.

Immunogenetics of the *Q* and *T* regions of the murine MHC

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Class I genes are found in the *K*, *D*, *Q*, *T*, and *M* regions of the mouse major histocompatibility complex. While the exact number of class I genes encoded within the MHC varies between strains, the largest proportion are found in the *Q* and *T* regions. The structural similarity of *Q* and *T* region class I products to those of the *K* and *D* regions suggest that the *Q* and *T* region class I molecules may be able to present peptides to cells bearing the appropriate receptors. However, unlike their *K/D* counterparts, class I genes of the *Q* and *T* regions are relatively nonpolymorphic and display limited tissue distributions. The function of these genes is largely unknown. Recently, several pieces of information have indicated that some of these genes, especially ones in the *T* region, may be involved in antigen presentation or perhaps, thymic development. Moreover, genes influencing early embryonic development have been mapped in this vicinity.

The *Q* region has a variable number of class I genes. Ten are known for the *b* haplotype. At least seven of these genes are transcriptionally active--*Q2*, *Q4*, *Q6*, *Q7*, *Q8*, *Q9*, *Q10*. At least four of these genes have been shown to produce polypeptides. *Q7* and *Q9* are responsible for the expression of most of the Qa-2 antigens detectable on the cell surface. They produce cell surface molecules which are anchored to the membrane by phosphatidylinositol. The *Q6* and *Q8* genes may also contribute to the level of Qa-2 antigen expressed on adult cells. Recent transfection experiments involving the *Q8* gene have shown that *Q8* can be expressed as a cell surface molecule. Moreover, its expression responded to gamma interferon with increased levels of mRNA expression. Cell surface expression of a *Q8* product could also be detected by flow cytometric analysis with a Qa-2-specific monoclonal antibody. However, in contrast to *Q7* and *Q9*, the *Q8* protein product was not sensitive to cleavage by phosphatidylinositol-phospholipase C.

The *T* region of the murine MHC also has a variable number of class I genes. Because this region is less well defined than the *Q* region, the exact number of class I genes in any one strain is still unclear. The cell surface products of several of these genes have been defined. These include the TL (Thymus-leukemia) molecule, Qa-1, and *T22*. The TL molecule may be the most unique of the class I molecules both in sequence and tissue distribution.

Because of various hints concerning the functional significance of these class I genes, we have been investigating their tissue-specific expression during embryonic development. In particular, we have studied the RNA expression of most of the Q-region genes in the time period from 11 days gestation until birth. Most of these genes show the same pattern of expression; however, one Q-region gene shows a very unique pattern. This pattern is not only developmentally specific but also tissue-specific. The characteristics of this gene suggest that it may have functional importance in antigenic presentation.

MHC STRUCTURE

HISTOCOMPATIBILITY ANTIGEN STRUCTURE

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The structures of three human class I histocompatibility antigens, HLA-A2, HLA-Aw68, and HLA-B27, have been determined by X-ray crystallography. In two cases, HLA-B27 and HLA-Aw68, the averaged image of a collection of bound endogenous peptides can now be interpreted at 2.1Å and 1.9Å, respectively. In one case, HLA-B27, the collection is dominated by 9-mers with an arginine at peptide position 2. In the other case, HLA-Aw68, bound peptide appears to be held at their N and C-termini just as in HLA-B27 (by a network of hydrogen bonds to conserved MHC residues), but the center of the bound peptides appear to take many alternate pathways and be at different lengths by bulging out in the middle. The structure of a single influenza virus peptide, Np 91-99 bound to HLA-Aw68, has also been determined. It shows the same genera mode of binding as described above. That structure defines in atomic detail the antigenic surface constructed of MHC atoms and viral peptide atoms that is recognized by T-cell receptors responding to an influenza virus infection.

A comparison of these data with structured data on the human class II histocompatibility molecule, HLA-DR1, will be presented.

Conformational change in MHC class II molecules

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The structure of MHC molecules, as determined by X-ray crystallography, is the structure of the mature, peptide associated form of the molecule found at the cell surface. However, recent studies have shown that this mature conformation is dependent on the binding of peptide; peptide-free MHC molecules have a more open conformation. In the case of MHC class II molecules, the mature, peptide associated conformation has been termed "compact" while the open conformation is termed "floppy". The first step in the binding of a peptide to an MHC molecule is therefore the interaction of the peptide with the open or "floppy" conformer, with a subsequent folding event yielding the final, "compact" conformation. It should be noted that the terms "compact" and "floppy" are not synonymous with peptide-associated and peptide-free, while all "compact" molecules have bound peptide, some "floppy" molecules may also have bound peptide but may not have undergone the transition to the "compact" state.

The conformational mobility of MHC molecules is clearly an important feature of their function. Our goal is to understand the what conformational changes take place in the MHC class II molecule and how this is related to peptide binding.

While it is predicted that the MHC class II α and β chains will have similar three dimensional structures, one striking difference is the presence of a conserved disulphide bridge in class II β1 domains (and the homologous class I α2 domains) and the lack of a similar disulphide in the class II (and class I) α1 domains. To ask whether the lack of an α chain disulphide bridge allows a degree of segmental mobility necessary for the function of the class II molecule, we have introduced disulphide bridges into the DR α chain and assayed the folding and function of these mutants. While it is clear that some disulphide mutants have a profound defect in peptide binding and presentation, these are also associated with abnormal folding and are likely to result from conformational distortions resulting from the introduction of the cysteine residues required to form the disulphide bridge. Other disulphide bridged mutants show neither

conformational distortions nor obvious defects in function, suggesting that segmental mobility in the class II α chains is not an important component of the conformational change in class II molecules.

Point mutants in the α helical region of the DRβ chain, originally introduced to map the "footprint" of the T cell receptor, have given some idea of the nature of the conformational changes that occur in the class II molecule. These mutants fail to adopt the "compact" conformation and are only found in the "floppy" form. One of these mutants, an alanine to glycine change at position 73, is particularly informative and indicates that part of the β chain α helix is unfolded in the "floppy" state. Such partial helix formation has been seen in folding intermediates of other proteins. Other mutants point to interactions between the short α helix and the floor of the groove as being important in stabilising the class II molecule. We therefore propose a model for the binding of peptide in which the peptide stabilises an interaction with a short α helical segment in the β chain, thus allowing the complete folding of the long α helix. While not providing a complete description of the folding process, our results begin to give a picture of the dynamic behaviour of the class II molecule.

Clustering of Class I HLA Molecules in Liposome and Cell Membranes

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We are interested in the lateral organization of class I MHC molecules in membranes and the implications of this organization for antigen presentation. We study lateral organization of human class I molecules in synthetic lipid bilayers, liposome membranes and in intact cell plasma membranes. Data from both liposomes and cells show that a substantial fraction of class I MHC molecules is self-associated, clustered.

Purified HLA-A2 molecules self-associate, cluster, when reconstituted into dimyristoylphosphatidylcholine, DMPC or azolectin liposomes at low surface concentrations (Lipid:protein 4,000:1). Clusters are reported by three different biophysical parameters: the lateral diffusion coefficent of fluorescein-conjugated HLA-A2 molecules, the extent of fluorescence resonance energy transfer between Fluorescein-HLA-A2 and Rhodamine-HLA-A2 molecules, and the rotational diffusion coefficient of Erythrosin-HLA-A2 molecules. A comparison of the lateral and rotational diffusion coefficients suggests that in DMPC liposomes the clusters consist of 5-7 molecules.

The clusters of HLA molecules appear to increase in size with time (days) after reconstitution. Size increases (reduction in lateral diffusion coefficient) are not detected if liposomes are stored in solution containing 12 uM beta-2 microglobulin. Once formed, clusters are at least partly dispersed by incubating HLA/liposomes in 2 uM beta-2 microglobulin for 1-4 hours. Adddition of peptides known to be bound by HLA-A2 does not affect clustering and does not enhance the effect of beta-2 microglobulin.

Resonance energy techniques also detect clusters of class I HLA molecules in the surface membranes of activated normal B- and T-cells, on B-lymphoblasts (JY, LCL721 and IM-9) and on a transformed fibroblast line VA-2. There is no clustering of class I HLA molecules detectable on resting B- or T-cells on CEM T-lymphoblasts or on normal skin fibroblasts. These data correlate perfectly with the expression of beta-2-microglobulin-free class I heavy chains on the cell surface. The HLA clusters may include one or more molecules lacking beta-2-microglobulin, but they are detectable with conformation-sensitive mAb such as W6/32.

Exogenous beta-2 microglobulin appears to dissociate class I HLA clusters in cell membranes as it does in liposomes. We are presently investigating the effects of this dissociation on antigen presentation by target cells.

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ANTIGEN PROCESSING
PRESENTATION
AND T CELL FUNCTION

Intracellular transport pathways for antigen presentation by Class I and Class II histocompatibility molecules

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Peptides bound to class I histocompatibility molecules are recognized by T cell receptors during the development of an anti-viral immune response. T cells respond to peptides derived from cytoplasmic viral proteins as well as viral membrane proteins, indicating that a pathway exists for the transport of proteins or peptides from the cytosol into the compartment(s) where the MHC class I molecules assemble. To investigate this pathway, we have developed an *in vitro* assay for the transport of peptides into microsomal vesicles. We have observed transport of peptides derived from antigenic HIV gag and influenza B nucleoprotein sequences, but transport of a third randomly selected peptide was not detected, suggesting specificity of the transport process. We were not able to demonstrate ATP-dependence of this peptide transport process using apyrase and an ATPase inhibitor. This result was unexpected in light of the recent identification of MHC-linked genes with homology to "ATP-binding cassette" transporters, that have been proposed to mediate peptide transport. Further experiments analyzing the import of peptides into microsomes will be discussed.

Class II molecules can present peptides from endocytosed antigen as well as endogenous antigen. To determine whether intracellular trafficking and the associated invariant chain could be responsible for the versatility of peptide binding displayed by Class II histocompatibility molecules, their intracellular location was mapped by immunoelectron microscopy. Endocytic compartments were labeled by uptake of a variety of ligands, including internalized immunoglobulin, representing the pathway of antigen uptake. Class II molecules associated with invariant chain, *en route* to the cell surface were observed to intersect both early and late endocytic compartments in the antigen uptake pathway and the transferrin receptor uptake pathway. Proteolytic enzymes Cathepsin B and Cathepsin D colocalized in these compartments, along with mature Class II molecules, but higher levels of protease were detected following uptake of immunoglobulin compared to uptake of transferrin receptor. Since very little mature Class II is internalized by these cells, the endosomal mature Class II is probably generated by dissociation of invariant chain, which requires activity of proteases Cathepsin B and D. The presence of mature Class II molecules in both early and late endocytic compartments indicates that dissociation of invariant chain could occur throughout the endocytic pathway. This maturation is most likely accompanied by peptide binding, as associated invariant chain obscures the peptide binding site, suggesting a mechanism by which Class II molecules can bind peptides generated in several different endocytic compartments. The late endocytic compartments are more degradative so most antigenic fragments are likely to be generated and bound in late endocytic compartments. Furthermore, it is expected that most Class II molecules will mature (dissociate from the invariant chain) late in the endocytic pathway. Class I molecules were not observed in early or late endocytic compartments, suggesting that a trafficking pathway specific to invariant chain-associated Class II molecules contributes to their antigen presenting function.

Co-localization of Molecules Involved in Antigen Processing and Presentation in an Early Endocytic Compartment. L.E. Guaglliardi, B. Koppelman, J.S. Blum, M.S. Marks, P. Cresswell, and F.M. Brodsky. *Nature* 343, 133-139 (1990).

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Antigen Processing and Presentation; Close Encounters in the Endocytic Pathway. F.M. Brodsky. *Trends in Cell Biol.*2, 109-115 (1992).

Characterization of the MHC Class II Processing Defect in T2 Cells

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T2 is a cell line with a large homozygous deletion of the class II region of the HLA complex resulting in a well-characterized defect in class I-restricted antigen processing. Using transfectants of T2 expressing HLA-DR3 and I-A^k we have shown that the cell line is also defective in class II-restricted antigen processing. Class II molecules purified from T2 transfectants, and from similar mutants isolated by Pious and co-workers, lack the stability in SDS characteristic of those isolated from wild-type cells, leading to the suggestion that they might be devoid of associated peptides. We have now shown that a substantial proportion of HLA-DR3 αβ dimers isolated from T2 transfected with HLA-DR3 are associated with a nested set of invariant chain-derived peptides. These peptides can be replaced in vitro with known DR3-associated epitopic peptides to generate SDS-stable HLA-DR3 αβ-dimers with very high efficiency. The implications of these findings for understanding the nature of the class II-processing defect in T2 will be discussed.

Role of Peptide Ligand in the Folding, Intracellular Transport and Surface Expression of Class I MHC Molecules

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Present dogma suggest that nascent class I molecules bind peptides in the endoplasmic reticulum and that this initial interaction promotes their de novo folding. Furthermore, stability of class I structure and intracellular transport is thought to depend upon peptide occupancy. Thus it is attractive to hypothesize that peptide is locked into nascent class I molecules for functional presentation of only intracellular peptide ligands. In spite of several studies that support this dogma, apparent inconsistencies have also been noted. To critically assess the role of peptide in de novo class I folding, intracellular transport and cell surface expression, we have compared the alternative antigenic forms of the L^d class I molecule of the mouse. Whereas, folded L^d molecules are detected by any of a number of conformation-dependent mAbs, non-folded L^d molecules are uniquely detected by mAb 64-3-7. The define precise markers for class I folding we have mapped relevant serological epitopes, using site-specific mutants. The folding of nascent class I molecules was monitored in cell lysates using wild-type L^d molecules as well as variant L^d molecules. Intracellular transport of folded versus non-folded class I molecules was compared in pulse-chase experiments. Surface interaction of peptide with class I was monitored directly, using labeled-peptides or indirectly, using peptide-induced stability of class I turnover. Our findings support the model that peptide must interact with nascent class I molecules at a critical time to induce folding. Surprisingly, we found several examples of misfolded mouse and human class I molecules that are transported and stably expressed at the cell surface. The functional consequences of these findings will be discussed.

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Transport of peptide across the membrane of the endoplasmic reticulum.

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Most nucleated cells are subjected to immune surveillance by T lymphocytes. The T cell receptor recognizes peptides presented by MHC class I antigens at the surface of the target cells. Correctly assembled heterodimeric MHC class I proteins are transported through the cell to the surface and serve as carrier for cellular peptides. During infection, viral peptides will be associated with MHC class I antigens and recognized by T lymphocytes, resulting in the elimination of the infected cells.

Translation of HLA-B27 mRNA in vitro, in the presence of human microsomal membranes, mimickimg the endoplasmic reticulum (ER), and peptide specific for HLA-B27 (NP384-394 of influenza A virus), resulted in the formation of the trimeric complex (HLA-B27 heavy chain/β2-microglobulin/peptide). By using a biotinylated version of the peptide, direct binding of the peptide to HLA-B27 antigens could be demonstrated. The assembly process required ATP. However, the translocation of peptide across the ER membrane did not require ATP, as binding of biotinylated peptide to BiP, an ER luminal protein, occurred after ATP depletion. Proteinase K treatment of the microsomes did not block peptide translocation. Thus ATP was required in the lumen of the ER for efficient assembly to occur. The microsomal membranes were found not to be leaky for small molecules, as equilibrium of the Ca-potential over the microsomal membrane drastically reduced the recovery of assembled HLA-B27 antigens upon addition of the specific peptide.

Microsomes prepared from Raji and T1 cells showed similar levels of assembly, whereas assembly in T2 microsomes was 10-fold lower. This difference remained after the addition of exogenous peptide. The inefficient assembly in T2 microsomes was not due to impaired peptide translocation across the ER membrane, as no difference in peptide binding to BiP was found when compared with microsomes from T1 cells. Instead the defect seemed to reside in the lumen of the ER.

These results have challenged the current view on how peptides are thought to cross the ER membrane. A model which could reconcile the results of the different experimental systems will be presented.

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Antigen presentation by MHC class I in the absence of MHC encoded proteasome subunits

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The MHC encodes not only the putative peptide transporters but also two subunits (LMP2 and LMP7) of the 20S proteasome. This observation raised the possibility that the 20S proteasome is involved in the generation of MHC class I binding peptides. Consistent with this assumption is the finding that some (but not all) of the proteasome subunits can be induced by IFN-γ (including LMP2 and LMP7) and that preliminary evidence suggests a similar tissue distribution for LMP7 and MHC class I. In vitro digestion of ovalbumin with purified proteasomes resulted in a peptide fraction which could bind to K^b positive EL4 cells and sensitize them for recognition by an ovalbumin specific and K^b restricted T hybridoma.

To analyze if the MHC encoded proteasome subunits are also essential for antigen presentation in vivo we utilized the T2 cells which have a large homozygous deletion of most of the MHC class II region including the ABC transporters and LMP2 and LMP7. The T2 cells were transfected with the rat transporters TAP1 and TAP2 (formerly mtp1 and mtp2). All transfections resulted in restoration of the surface expression of HLA-B5 and in a strong increase of HLA-A2 expression. The T2/TAP1+2 cells (but not T2) could be lysed by a CTL clone recognizing an A2 restricted minor H antigen. T2/TAP1+2 cells were also able after infection with influenza virus or VAC.M1 to present the matrix derived peptide to matrix specific and A2 restricted CTL clones and lines. However, the respective kill appeared to be somewhat lower than the lysis of infected T1 cells, but it is not yet clear if this difference is significant.

These data demonstrate that the MHC encoded proteasome subunits are not essential for MHC class I surface expression and antigen presentation. It is possible that this presentation is suboptimal and that a complete proteasome would be more efficient, perhaps by displaying higher proteolytic activity, by generating more accurately the optimal peptides, or by other mechanisms.

Regulation of MHC Class II Intracellular Transport, Folding, and Expression by Invariant Chain and Peptide

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The movement of MHC class II dimers from their site of initial assembly in the endoplasmic reticulum (ER) to the endosomal pathway and finally to the cell surface involves a complex interplay between the class II molecules and invariant chain (Ii). These interactions determine not only the physical movement of the class II molecules within the cell, but also the quality and quantity of acquired peptide and the location at which this acquisition takes place. We have used immunochemical and immunocytologic analyses to investigate various aspects of class II biology using normal hematopoietic cells spontaneously expressing class II and Ii, and also transfections models that permit alterations in the amount and structure of the participating proteins.

In vitro analysis using purified MHC class II molecules has shown that peptide plays an important role in the structure of the class II dimer [Sadegh-Nasseri, S. & Germain, R.N. Nature 353, 167-70 (1991)]. Low pH removal of pre-bound peptide from immunoaffinity purified class II molecules caused these dimers to lose their characteristic resistance to dissociation in SDS-PAGE buffers when analyzed without sample heating. Inclusion of appropriate peptide ligand in the low pH treatment buffer prior to neutralization led to preservation of the stable dimer structure, and use of labelled peptides showed that under these conditions, very rapid loading of class II ($t_{1/2}$ of 15 minutes) could be achieved. This was in contrast to the usual observed $t_{1/2}$ of several hours. These data support a model in which class II peptide binding involves a structural transition that might be facilitated at low pH. Ongoing studies aimed at evaluating the relationship between class II dimer structure and the kinetic properties of peptide-class II complexes [Sadegh-Nasseri, S. & McConnell, H.M. Nature 337, 274-6 (1989)], as well as the mechanisms by which low pH accelerates formation of long-lived complexes, will be discussed.

Similar transitions in structural stability can be observed in living cells when newly synthesized, Ii-associated class II dimers are compared to those present in the cell several hours after synthesis, or on the cell surface [Germain, R.N. & Hendrix, L.R. Nature 353, 134-9 (1991)]. This transition is inhibited by a variety of agents known to interfere with functional antigen presentation by class II molecules, such as chloroquine and leupeptin, and seems to reflect at a minimum the capacity of these agents to inhibit Ii degradation and dissociation from class II. Such Ii-associated class II does not show evidence of associated peptide, consistent with *in vitro* studies [Roche, P.A. & Cresswell, P. Nature 345, 615-8 (1990); Teyton, L., et al. Nature 348, 39-44 (1990)]. The proportion of class II molecules surviving for several hours in a cell or reaching the cell membrane is markedly altered by addition of antigen to the medium. The increase in immunoprecipitable and surface expressed material comes from inhibition of intracellular degradation. Recent work in an *in vitro* model of endosomal peptide loading has provided some insight into this effect. When pulse-labelled cell lysates containing $A\alpha^kA\beta^k$ -Ii complexes are exposed to low pH (5.0) for 15-30 minutes, most of the Ii dissociates. If no peptide is added, the class II becomes non-precipitable with a variety of antibodies, and can be found in sedimentable macroaggregates. If HEL 46-61 peptide is included during the low pH treatment, much of the otherwise lost class II now can be immunoprecipitated and appears as stable compact dimers upon SDS-PAGE. Thus, peptide binding prevents class II that is free of Ii from undergoing aggregation with itself and/or other proteins. We suggest that in the endosomal pathway, when Ii is cleaved and removed from class II, the exposed molecules have two possible fates: 1) to bind peptide, escape the endosomal compartment, and move to the cell surface for T cell recognition, or 2) to

aggregate if no peptide is bound, promoting endosomal retention and movement to lysosomes for degradation. This provides an intracellular editing mechanism that results in the selective surface expression of loaded class II dimers, a process analogous to the p88 retention of peptide-free class I molecules in the ER. This selection appears to be based on the change in protein structure resulting from peptide binding, which interferes with the aggregation properties of the class II molecules, as also observed in vitro by Stern and Wiley [Cell 68, 465-77 (1992)].

In the early post-synthetic compartment, Ii acts as a chaperone for class II [Layet, C. & Germain, R.N. Proc Natl Acad Sci U S A 88, 2346-50 (1991)], preventing such aggregation and association with endogenous ER chaperones that would lead to retention. This effect appears to involve the lumenal region of Ii and possibly the transmembrane region, but does not depend on the portion of the cytoplasmic tail that regulates movement to the endosomal pathway [Bakke, O. & Dobberstein, B. Cell 63, 707-16 (1990); Lotteau, V., et al. Nature 348, 600-5 (1990)]. In the endosomal pathway, Ii plays an active role in modifying movement through the early endocytic compartment. In transfected COS cells, Ii causes a marked enlargement of early (transferrin accessible) endosomes, and retards the movement of fluid phase and membrane material through this compartment *en route* to late endosomes. This function of Ii may be important in maximizing the likelihood that incoming antigen will be processed into available peptides in the presence of adequate amounts of available class II binding sites. Movement of class II to later compartments in the endocytic pathway is associated with cleavage of Ii and such cleavage is essential for movement of class II to the cell surface. Leupeptin inhibition of terminal Ii cleavage both prevents formation of the stable dimers and results in trapping of class II inside the cell, a phenomenon that does not occur in cells expressing class II without Ii or in the presence of Ii lacking the portion of the cytoplasmic tail necessary for endosomal targeting. Thus, class II movement within the endocytic pathway, and its acquisition of peptide during this movement, is regulated by post-translational changes in Ii, events that themselves may be actively regulated in different cell types such as Langerhans cells, possibly as a consequence of the action of cytokines.

Taken as a whole, our studies indicate that class II molecules behave early after synthesis as improperly folded proteins requiring the action of invariant chain to protect them from the retention effects of ER chaperones. This interaction also markedly reduces the possibility of peptide acquisition in this early compartment. Class II-Ii complexes leave the ER and move through the Golgi to the trans-Golgi network, where a signal in the cytoplasmic tail of Ii results in deviation from the export route to the membrane and localization first in early endosomes. In this compartment, Ii affects the rate of transport, slowing down passage of the associated class II and also of incoming antigen to provide better opportunities for co-ordinate processing of the antigen and of Ii. Movement to later compartments is accompanied by cleavage of Ii, revealing the class II binding site. In a low pH environment, such 'naked' class II either captures peptide and undergoes a conformational change, or aggregates because of its failure to achieve a properly folded state. The peptide-loaded dimers, when free of the retention properties of the NH_2-terminal segment of Ii, move by an uncharacterized pathway to the cell membrane for presentation of peptide to T lymphocytes. This view of class II transport, folding, and expression emphasizes the role of conformation change in the class II molecule in regulating its movement in both the early and late post-synthetic compartments, and the reciprocal relationship between Ii and peptide in controlling the change in conformation required for class II expression at the cell surface.

Epitopes presented by HLA B8

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We have identified four peptide epitopes, three in HIV gag and one in influenza nucleoprotein (NP) that are presented to cytotoxic T lymphocytes (CTL) by HLA B8. Comparisons between these epitopes and three published B8 restricted peptides revealed a common sequence motif of:

1	3	5		8/9
x	x	R/K	x	R/K x x - L/I.

Nonamer or octamer peptides based on this motif were made and tested; all sensitised targets efficiently at low concentrations. In addition, HLA B8 was purified from C1R cells transfected with HLA B8. Peptides were eluted and purified by reverse phase HPLC and the pool was sequenced; strong lysine signals were found at positions three and five. HLA B8 was modelled from the coordinates of HLA B27 (kindly provided by Dean Madden and Don Wiley); the B pocket, which in HLA A2 and B27 takes the side chain of the second amino acid in the peptide, was occluded by phenylalanine 67 and there was negative charge in the D/E poskets from aspartate 156 and in the floor from aspartate 9. Therefore the structure of the B8 molecule was consistent with the anchor residues that we identified.

These findings have allowed us to re-examine the epitopes in HIV gag, presented by HLA B8, where possible escape mutations were identified (Phillips et al, Nature 354, 453-459, 1991). Those that were not recognised by any CTL had changes at position three, which suggests that binding to HLA B8 might be impaired. Another mutant was changed at position two; this only affected recognition by some CTL clones, implying that this mutation alters interaction with the T cell receptor.

Most HLA B8 positive individuals make a CTL response to the NP peptide 380-388, when challenged with influenza virus. However, one donor was found whose T cells did not respond, despite sharing an identical B8 molecule with a responder and making an HLA B2702 restricted anti-influenza CTL response. Cells from this donor could present added peptide NP 380-388 to HLA B8 restricted CTL, but the cells were not recognised when infected wth influenza virus. This result implied an antigen processing polymorphism that affected presentation or delivery of this epitope, derived from influenza NP. Presentation of an overlapping epitope presented by HLA B27 was also affected, but there was normal presentation by HLA B8 of an epitope in HIV gag p17.3. Thus different epitopes may be differently affected by this putative polymorphism in antigen processing.

RECURRENT T CELL CROSSREACTIVITY BETWEEN HLA-B27 AND HLA-DR2

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Fundación Jiménez Díaz and Consejo Superior de Investigaciones Científicas

HLA-B27$^-$ responder cells were stimulated in vitro whith B*2705$^+$ lymphoblastoid cell lines and alloreactive CTL clones were obtained by limiting dilution. Of the CD3$^+$ CD4$^-$ CD8$^+$ HLA-B27-specific CTL clones obtained, two of them, possessing the same TCR, crossreacted with HLA-DR2. The fine specificity of these CTL was established with HLA-B27 and HLA-DR2 subtypes. They recognized the B*2701 to B*2706 subtypes, but only DR2Dw2. Lysis of DR2$^+$ target cells was specifically inhibited by anti-CD3, anti-class II and anti-DR monoclonal antibodies, but not with an anti-CD8 antibody. The monoclonal nature of the crossreaction was established by the mutual inhibition of HLA-B27 and DR2Dw2 cells in cold target competition experiments.

A correlation between the fine specificity of these CTL clones and the amino acid sequences of HLA-B27 and HLA-DR2 subtypes revealed a shared structural motif between HLA-B27 and the DR2 B5*0101 chain, that could be responsible for the observed crossreaction. This motif was contributed for by several residues located in adjacent ß-strands, at the floor of the peptide binding site. The contribution of two of these residues, as well as other ß-pleated sheet residues, to HLA-B27 allorecognition by the crossreactive CTL clones was directly demonstrated with site-directed mutants. These results strongly suggest that the dual reactivity pattern reflects presentation of identical or structurally related peptides by HLA-B27 and HLA-DR2Dw2. As T-cell crossreactivity between HLA-B27 and HLA-DR2 was previously found in cells from an unrelated individual (Aparicio et al. J. Exp. Med. 165:428, 1987) the results reported here are likely to reflect an intrinsic property of HLA-B27, rather than the fortuitous finding of a rare clonal reaction pattern. We speculate on the potential implications of these results for the pathogeny of HLA-B27-associated spondyloarthropathies.

DISEASE

Disease associations with HLA genes in different ethnic groups.

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DNA typing has greatly facilitated the study of HLA alleles in samples shipped to our laboratory from distant locations while at the same time making it possible to determine the HLA class II alleles more completely and more accurately. We have recently tested samples of rheumatoid arthritis (RA) patients from Ashkenazi and Non-Ashkenazi Israeli Jews and from Orientals residing in the North of China, we have assayed specimens of African-American diabetes patients and studied patients with juvenile arthritis from Czechoslovakia. Our previous studies were performed in North American Caucasians living in Texas. The lessons learned from comparing HLA-associated susceptibility, and resistance factors for the same disease in different populations will be discussed. In the case of Israeli Jews, the prevalence of DRB1*0402, an allele not associated with susceptibility was found to explain the absence of association of DR4 with RA in this population. Interestingly, another allele, DRB1*0415 was found to be a strong risk factor for RA in Ashkenazi (RR=7) and Non-Ashkenazi (RR=10) Jews. This allele was associated with RA in Orientals. Comparison of juvenile arthritis patients in Texas and in Czechoslovakia showed interesting similarities and differences. In the course of our studies we have observed that one disease (Pauciarticular Juvenile Arthritis) can be associated with alleles encoded by multiple HLA loci (HLA-A, HLA-DR, HLA-DP). These associations are independent and not based on linkage disequilibrium, but appear to be synergistic in determining susceptibility. The role of HLA associated protection has been of considerable interest, in particular in insulin-dependent diabetes (DR2, DQw6) and in endemic Brazilian Pemphigus Foliaceus (DQw2). While resistance for development of IDDM associated with DRB1*1501 in Caucasians has been thought to map to the allele DQB1*0602, in Blacks the comparable haplotype (DRB1*1503, DQB1*0602) did not protect. We have sequenced the first domain of the Black DQB1*0602 and found it to be identical to that reported in Caucasians. Another topic to be discussed relates to mechanisms in HLA and disease associations. An attempt is being made to answer the question of whether the disease associated allele is also the main restriction determinant for the immune response that causes the disease. Data will be presented regarding an analysis of T cell clones specific for cedar pollen antigen, a common cause of allergy in Texas.

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HLA CLASS II SEQUENCE POLYMORPHISM AND DISEASE SUSCEPTIBILITY

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A variety of diseases, many but not all of them autoimmune, have been associated with DNA-defined alleles of the HLA class II loci. In principle, these associations could reflect either linkage disequilibrium of the HLA markers with other genes within the MHC or the polymorphic amino acid residues of the HLA molecules could contribute to disease susceptibility. Variation in the regulatory sequences, provided that it is in linkage disequilibrium with the coding sequence polymorphism detected by serologic and molecular methods, could conceivably also account for the observed HLA-disease associations. These explanations need not be mutually exclusive. Also, the strong linkage disequilibrium that characterizes the HLA region makes it difficult to identify which locus and allele (or combinations, see below) on a disease associated haplotype is responsible for the susceptibility. One pattern of disease association, namely the increase of DR3 and DR7 among celiac disease (CD) patients, supports the notion that the HLA molecules are directly involved in predisposition and do not simply serve as serve as genetic markers for other loci that confer susceptibility. The same DQ molecule (DQA1*0501/DQB1*0201) is encoded in cis by DR3 haplotypes and in trans by DR11/7 heterozygotes. The increased frequency of DR3 among CD patients and, among Southern European patients, the increased frequency of DR11 among DR7+ patients suggest that this particular DQ alpha-beta dimer, encoded either in cis or in trans is responsible for the observed pattern of DR associations with CD. In general, multiple alleles at an HLA locus, as well as multiple loci within the MHC can contribute to susceptibility. For example, pauciarticular juvenile rheumatoid arthritis is associated with the DPB1 allele, *0201, and with the DRB1 alleles, *0801 and *1101 (Ref. 1).

This lecture will focus on the HLA associations with insulin-dependent diabetes mellitus (IDDM) and, in particular, on the analysis, using PCR/ oligonucleotide probe typing methods, of Mexican-American families (Ref. 2). Previous work had shown that DR3 and DR4 haplotypes were increased (susceptible) and DR2 was decreased (protective) among Caucasian IDDM patients. In the Mexican-American population, which is ancestrally derived from Native Americans and Hispanic Caucasians, both DR3 and DR4 are increased and DR3/4 heterozygotes show the highest risk, as in Caucasians. Most previous studies of DR4 susceptibility have focused on the DQB1*0302 allele; however, the role of the DR4 -DRB1 subtypes in IDDM risk is revealed clearly in this population, in part due to the remarkable diversity (n=16)of DR4 DR/DQ haplotypes among Mexican-Americans. Virtually all the DR4+ patients carried the DQB1*0302 allele (as did most DR4+ controls), but distinctions between high and low risk DR4 haplotypes could be made on the basis of the DRB1 allele. For example, the DRB1*0405,DQB1*0302 and the DRB1*0402,DQB1*0302 haplotypes confer a high risk while the DRB1*0408,DQB1*0302 haplotype confers a low risk. DRB1*0405 and DRB1*0408 differ only at position 57 (Ser vs. Asp). (It should be noted that the presence of Ser-57 alone does not predict high IDDM risk. This study also showed (see below) that the presence of DQ-beta Asp-57 does not, as previously suggested, confer IDDM protection. For example, the DRB1*1402, DQB1*0301 haplotype, derived from Native Americans, is highly protective; the DRB1*1602,DQB1 haplotype, also frequent in Native Americans and absent in Europeans, is neutral although these two haplotypes encode the same (Asp-57) DQ alpha-beta dimer. These data suggest that the protection conferred by the DRB1*1402 haplotype cannot be encoded by the DQA1 and DQB1 loci. Based on these and previous observations, it appears likely that both DRB1 and DQB1 can contribute to susceptibility as well as resistance to IDDM. In this study of Mexican-Americans, those haplotypes associated with IDDM were of European origin (*DRB1*0301, DRB1*0402, DRB1*0405) while the most strongly protective haplotype (DRB1*1402) was of Native American origin.

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MHC ANTIGENS IN TUMORS. MECHANISMS OF ALTERATION AND BIOLOGICAL AMPLICATIONS.

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It is well established in experimental tumour systems that MHC class I and II alteration influences local tumour growth and metastasis. We have analyzed the H-2 typing of different clones of a chemically induced fibrosarcoma (GR9) and demostrated their heterogeneity and influence on local growth and metastatic capacity. In humans, a variable proportion of solid tumours completely losse MHC class I antigens (HLA, A,B,C). These tumours derive from epithelia that are positive for these molecules. In addition, locus or allelic losses have been described. We observed that an average of 15% of total losses can be detected in laryngeal, breast, colon and cervical carcinomas. In broncogenic carcinomas this figure reached 27%. When selective losses were added, the alterations in HLA expression increased from 5 to 10%. IEF analysis was used to define locus or allelic-specific losses on tumour cells, in comparison with EBV-transformed B-lymphocytes. With this method rhabdomyosarcoma and pancreatic tumour lines were analyzed. The association between low levels of HLA expression and tumour aggressivenes is variable depending of the origin of the tumour. In laryngeal, lung and breast carcinomas, HLA loss correlated with poor clinical course of the disease.

In most cases, mechanisms involved in malignant transformation are not directly tied to HLA expression, although some protooncogene activation systems have been associated with HLA expression. HLA under-expression correlated well with poorly differentated tumours of the lung, breast and larynx. The molecular defects underlying altered HLA class I expression were variable. Normal regulatory factors controlling HLA class I expression appeared to be affected, and a reduction in DNA-binding transcription factors was observed in HLA negative tumours. Altered HLA expression in HLA negative colon tumours was due to a different defect ie, the absence of β_2 microglobulin gene expression.

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PHYLOGENY

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DIFFERENT MODES OF MHC EVOLUTION IN THE PRIMATES

The human major histocompatibility complex *Mhc* is a chromosomal segment, approximately 4 Mb long, which at least 84 genes Some of these genes code for the class I and class II molecules, while the remaining genes code for complement components, cytochrome P450, tumor necrosis factor, and many other, unrelated proteins. We will demonstrate on three examples *DP*, *C4 -CYP21*, and *DRB)* that different regions of the *Mhc* have different evolutionary histories . The organization of the *DP* region, which in humans contains 4 genes, was established in the ancestral Anthropoidea, or earlier, and has not changed since. The duplication that generated the two *C4-CYP21* modules occurred in the ancestral Catarrhini, or earlier, but the region has been undergoing periodic homogenizations via unequal crossing-over, which make paralogous genes in the same species more similar to each other than orthologous genes of different species. The 8 or 9 genes of the *DRB* region were also generated in the ancestral Catarrhini, but the region has since been subject to frequent rearrangements, which generated various *DRB* haplotypes Not only the alleles, but in part also the haplotype polymorphism is evolving trans-specifically. The *DRB* region of the Platyrrhini has a different origin from that of the Catarrhini. The picture emerging from these studies is that of stability in some regions of the *Mhc* and tremendous evolutionary instability in other regions.

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Class I MHC Genes In Primates

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The structure and polymorphism of class I major histocompatibility complex genes in humans and other primates wil be compared. In humans there exists 3 classical class I genes: HLA-A,B, and C, three non-classical genes: HLA-E,F and G and various pseudogenes including HLA-H and J. Of these HLA-B and C are closely related as are HLA-A,G,H and J. Within this latter group of HLA-related loci, HLA-A is more closely related to HLA-H while HLA-J is closer to HLA-G.

In the great apes, the chimpanzees and gorillas, the structures of class I genes appears similar to those of human. Homologues of HLA-A,B and C have been found in both species and a homologue of HLA-F in the chimpanzee. In both human and chimpanzee, the F gene has an altered splicing pattern which results in the deletion of exon 7. Individual gorillas have also been shown to express a gene with similarities to both HLA-A or HLA-H. As yet,there is no information on the organization of the class I genes in apes.

In humans, alleles of the HLA-A locus form 5 families (A2/A3/A9/A10/A19) which derive from 2 lineages: one comprising the A2,A10 and A19 families, the other the A3 and A9 families. Chimpanzee A alleles all derive from the same lineage as A3 and A9, whereas gorilla A alleles derive from the other lineage. Although certain pairs of chimpanzee and human A alleles are very similar in sequence no class I allele that is identical in any two species has been found. Moreover, the differences always include functional residues of the peptide binding site. Thus, it seems that no single class I antigen-presenting specificity which was present in the common ancestor of humans and apes has survived unchanged to this day.

HLA-B alleles are less easily ordered into families than those of the HLA-A locus assortment of short polymorphic motifs in the 5' part. Comparison within the human population suggest HLA-B has been evolving faster than HLA-A and this conclusion also emerges from comparison between the species. Although the data available are still limited.

In the other great apes and old world monkeys, homologues to many of the human MHC class I loci are present. HLA-A and -B-like cDNAs have been cloned from the orangutan, gibbon and rhesus monkey. The isolation of 3 HLA-B-like cDNA from individual orangutan, gibbon and rhesus monkey has suggested this locus is duplicated in these species. The absence of HLA-C-like sequences from these species suggests alternatively that the 2 B-like loci might be orthologous to HLA-B and C. Additionally HLA-E and F homologues are also present in the rhesus monkey.

In non-human primate species that have been separated from humans for longer periods of time, the simple concordance between human and non-human primate loci breaks down. In the New World primate, the cotton top tamarin, the expresssed genes are more closely related to the HLA-G locus than they are to HLA-A,B or C loci. An HLA-F-like locus is also present in the cotton-top tamarin. Attempts to isolate HLA-A, -B or -C-like genes from genomic DNA have failed in the cotton top tamarin suggesting they may have been deleted during the evolution of the New World primates or were never present in this lineage.

Evolutionary conservation of *Mhc*-peptide-*Tcr* interactions in primates

R.E. Bontrop

Many major histocompatibility complex (*Mhc*) class II polymorphisms predate speciation and are inherited in a trans-species mode of evolution. However, *Mhc-DRB* sequences are generally species unique due to accumulation of genetic variation, mainly generated by point mutations or recombination like events. With regard to the biological function *Mhc* class II molecules are involved in the presentation of processed antigen segments (peptides) to T helper cells which may lead to antibody production. To execute this function, *Mhc* molecules are equiped with a peptide binding site. The T cell receptor (*Tcr*) on the T helper cell recognizes peptides only in the context of self-*Mhc* structures, a phenomenon known as *Mhc* restriction. To study the relevance of inter-species *Mhc* variation among *Mhc-DR* molecules in primates we have studied their capacity to bind certain antigen segments. Here we show that some *HLA-DRB1*03* like molecules in chimpanzees and rhesus monkeys can bind a certain epitope derived from the 65 kD heat shock protein of *Mycobacterium leprae*. Comparison of the *Mhc-DR* sequences involved, allowed to predict which shared amino acid residues are critical for peptide binding, but demonstrated also that some variable amino acid residues did not interfere with binding. These studies emphasize that some polymorphic contact residues (motifs) may have been conserved in evolution for more than 30 million years. Next to the binding assays, the antigen presentation capacity of these *HLA-DRB1*03* like molecules in various primate species was studied. It will be demonstrated that some *Mhc-DRB1*03* positive non-human primate cells can present the 65 kD heat shock derived peptide to *HLA-DRB1*03* restricted human T cells. Therefore, these studies demonstrate that not only the peptide binding capacity for some peptide *Mhc* combinations may have been conserved in evolution but also that these structures can be successfully recognized by T cells from another species despite some interspecies variations.

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New *HLA-B* variants in South American Amerindians suggest that the MHC class I loci can evolve rapidly.

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The gene products of the human MHC class I loci are highly polymorphic in most populations studied. Comparison of chimpanzee *HLA-A* homologues and human *HLA-A* molecules suggests that the MHC class I loci evolve slowly. Indeed, it has been suggested that chimpanzees and humans have inherited alleles that are essentially unchanged from alleles present in their common ancestor. Data from skin grafting and antibody studies in mice, however, would suggest that generation of new alleles can actually take place rather quickly. In an attempt to address this issue in humans we have analyzed the MHC class I molecules of two tribes of Amerindians in North and South America. In analogous studies, the MHC class I molecules of another three tribes of both North and South American Amerindians have also been examined.

The Amerindians of North and South America provide and important opportunity to study the effects of selection on MHC class I genes over a short period of time in a population with a limited number of MHC class I alleles. Evidence suggests that the Americas were colonized only 16,000-40,000 years ago by Paleo-Indians who crossed the Bering land bridge. Since serological analysis demonstrates that these Amerindians express only a small number of alleles at their *HLA-A, -B* and *-C* loci, it is likely that there were only a limited number of MHC class I alleles present in the founding population.

The Waorani are one of the most isolated of these South American tribes and are unlikely to have any admixture of European MHC class I alleles. This proto-agricultural tribe which now numbers approximately 600 individuals, appears to be highly inbred and quite distinct, both genetically and linguistically, from other South American tribes. cDNA cloning and sequencing revealed that four of the Waorani *HLA-B* alleles were new functional variants. The *HLA-A* and *-C* alleles of the Waorani, on the other hand, were unremarkable and the majority of these alleles had been previously described in other caucasian and oriental populations. Very similar data were obtained from an analysis of the MHC class I molecules of two tribes of Brazilian Amerindians. The *HLA-A* and *-C* alleles were familiar, whereas six of seven of the *HLA-B* alleles were novel. Surprisingly, only one of these novel Brazilian *HLA-B* alleles were found in the Waorani. Furthermore, all of the new South American *HLA-B* alleles could be accounted for by intralocus recombination between alleles present in the Amerindians.

To determine whether these new *HLA-B* variants arose in South America or whether they were actually rare oriental variants, the MHC class I alleles of two tribes of North American Amerindians were then examined. Both the Zuni and the Pima, like the Waorani, Kaingang and Guarani were derived from the founding Paleo-Indians. However, unlike the South American Amerindians no new *HLA-A, -B* or *-C* functional variants were isolated from these two tribes of North American Amerindians. Thus it is possible that some of the MHC class I diversity seen in the South American Amerindians might have taken place after the arrival of the Paleo-Indians in South America. This would imply that the MHC class I loci can evolve much more rapidly than was previously estimated.

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PERSPECTIVES

A. Arnaiz-Villena

Perspectives

The meeting was successful in discussing the most topic and interesting questions in MHC research.

It was established that class I antigens presentation pathway was not as clear as the class II one; new HLA class I crystals were shown with little variations to the previously described ones; the HLA and disease relationship examples were as obscure as ever, as far as mechanisms are concerned and some interesting evolutionary consequences from different MHC regions were put forward.

From the presented data and the respective discussions, it is deduced that the following MHC related questions should be addressed in the near future:

1.- To clarify the class I MHC antigens metabolism in relation to peptide presentation.

2.- To actually show that class II molecule crystals are concordant with the postulated theoretical models.

3.- To discover which are the mechanism of HLA and disease association, since little or no advance has been achieved in the last 20 years. Probably to reach this aim, an intermediate fundamental discovery or/and change of research strategy is needed.

4.- To further analyze the particular MHC allelic protein interaction with peptides.

5.- To exhaustively identify the endogenous peptides.

6.- To explain alloreactivity.

7.- To find a function to all new MHC gene products which are being discovered.

8.- To complete the MHC evolutive puzzle.

9.- To sort out at which level MHC compatibility in clinical transplantation must be done (natural policlonal antibodies, monoclonal antibodies, DNA alleles).

Antonio Arnaiz Villena

LIST OF INVITED SPEAKERS

Workshop on

STRUCTURE OF THE MAJOR HISTOCOMPATIBILITY COMPLEX

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