EMBO reports

meeting report

MHC molecules lead many lives

Workshop on MHC Class I Molecules at the interface between Biology & Medicine

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The EMBO Molecular Medicine Workshop on MHC Class I Molecules at the interface between Biology & Medicine took place between 4 and 6 July 2008, in Porto, Portugal, and was organized by F. Arosa, S. Powis and G. Vamosi.

Keywords: MHC; receptors; NK cells; autoimmunity; cancer

EMBO *reports* (2009) **10,** 30–34. doi:10.1038/embor.2008.215

See Glossary for abbreviations used in this article.

Introduction

The states, partners and functions of MHC class I molecules were the subject of this European Molecular Biology Organization Molecular Medicine workshop. The organizers built on their experience with unexpected forms and associations of MHC class I molecules to put together a meeting that showed how much more there is to these molecules than their classical function of simply presenting peptides for recognition by T-cell receptors.

The many functions of MHC class I molecules

The abstract book of the meeting began with a historical perspective on the non-classical functions of MHC class I molecules and quoted several speculative comments, including a notable one made by Susumo Ohno: "It may be that many of the plasma membrane proteins concerned with organogenesis, growth and differentiation are indeed in association with class I MHC molecules"; the work leading up to this hypothesis is reviewed in Ohno (1977). This speculation and others along similar lines drove research in the 1970s and 1980s to associate MHC class I molecules—which could be detected using antibodies—with growth-hormone receptors (Edidin, 1988). This area of research dwindled after antigen presentation—an important MHC class I function—was defined by the work of Doherty & Zinkernagel (1975), and the MHC class I structure was solved (Bjorkman *et al*, 1987). The work on MHC class I molecules then focused on the peptide-binding groove, and the presenting surface formed by the peptide and helices of the groove, which yielded spectacular insights into this important function of MHC class I molecules. Consequently, the study of their lateral interactions in the plane of the plasma membrane was neglected by most laboratories. The small number of laboratories working on lateral interactions mostly mapped MHC I homo-oligomers and hetero-oligomers. Although this work indicated that free MHC I heavy chains were important for oligomer formation, there was little quantitative progress in this area until Santos and co-workers described the kinetics and signalling properties of MHC class I molecules that are not associated with β2m (also known as misfolded MHC class I molecules; Santos *et al*, 2004; Arosa *et al*, 2007). This gave a structural basis for the lateral associations described earlier, and indicated that there were binding sites on MHC class I molecules that were distinct from the peptide-presenting site. Another important area of immunology, NK-cell recognition of MHC class I molecules, also highlighted the existence of alternative binding sites for '*trans*' interactions. Members of the Ly49 family of NK-cell receptors were

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Submitted 8 September 2008; accepted 24 October 2008; published online 5 December 2008

shown to bind to the side of the MHC molecule using structural and mutational analyses (Tormo *et al*, 1999), in contrast to the binding of other NK-cell receptors, KIR and NKG2, which bind to the classical peptide-presenting region (Fan *et al*, 2001).

MHC partners, misfolding and disease

The meeting was introduced by P. Pontarotti (Marseille, France), who discussed the evolutionary genetics of the MHC based on the ideas of gene co-option and exon shuffling as the drivers of new functions for existing structures. These ideas about the evolution of the MHC suggest that conserved regions brought into new structures could retain their old (binding) functions. N. Bulleid (Manchester, UK) introduced the topic of MHC class I biosynthesis, describing a semi-permeable cell system with an intact endoplasmic reticulum that allowed him to address the timing and partners involved in the formation of disulphide bonds between MHC class I and endoplasmic reticulum molecules. He also emphasized the role of the endoplasmic reticulum redox environment and the importance of a transmembrane cysteine residue in associating nascent class I molecules with their appropriate partners. This talk connected to later talks that highlighted misfolding and aberrant disulphide-bond formation in disease. L. Boyle (Cambridge, UK) described a new chaperone that stabilizes empty forms of MHC I molecules, leading to an increase in their expression at the cell surface. As these empty molecules are important for lateral interactions of MHC class I molecules, their enhanced expression might modulate many cell functions.

HLA-B27 is strongly associated with the development of spondyloarthritis. P. Bowness (Oxford, UK) described the ability of HLA-B27 heavy chains to form stable homodimers lacking β2m, both *in vitro* and *in vivo*. HLA-B27 homodimers are present on the surface of the cells of patients with spondyloarthritis, as well as in B27 transgenic models of disease. Tetrameric complexes of HLA-B27 homodimers bind to NK receptors and related immunoreceptors on populations of lymphocytes, monocytes and NK cells from patients with spondyloarthritis. Patients with ankylosing spondylitis express increased numbers of one of these receptors, KIR3DL2, on their peripheral blood and joint (synovial) NK cells and CD4 T lymphocytes. HLA-B27 is also capable of misfolding during assembly in the endoplasmic reticulum, and R. Colbert (Cincinnati, OH, USA) presented data showing that upregulation of B27 expression by interferons exacerbates misfolding and the unfolded protein response. Additional stimulation with lipopolysaccharide results in the potent upregulation of pro-inflammatory cytokine production, including that of IL23p19. S. Powis (St Andrews, UK) showed that in B-cell lines, MHC class I is observed mostly in the fully folded, dimeric form within exosomes of 50–200 nm. The generation of mutant HLA molecules that lack a cysteine in the cytosolic tail and fail to dimerize provides a system in which to test the functional significance of dimerized HLA molecules in exosomes.

The themes of endoplasmic reticulum assembly, peptide loading and transport of MHC class I molecules were extended by several talks. J. Lopez de Castro (Madrid, Spain) discussed the crucial role of peptides in determining many of the molecular features of HLA-B27 and presented work showing similarities in peptide binding to HLA-B*1403, which has been described in sub-Saharan African patients with ankylosing spondylitis. By contrast, folding and other biological features did not correlate with disease association. X. Yu Rao (Utrecht, The Netherlands) used computer modelling to analyse the diversity of peptides that bind to HLA-A and compared them with those that bind to HLA-B; this raised the paradoxical point that although HLA-B molecules bind to fewer epitopes and with less affinity than HLA-A, the immundominant responses are often directed against peptides presented by HLA-B. S. Springer (Bremen, Germany) investigated the ability of defined peptides to induce the loading and transport of MHC class I molecules to the cell surface. These studies indicated that the carboxyl terminus of the peptide is crucial for endoplasmic reticulum quality control and plasma-membrane transport of the MHC class I–peptide complex, and suggested that it stabilizes a more compact MHC class I conformation. The viral proteins expressed by a host cell during infection undergo proteasomal processing in the cytosol, and the cleaved peptides are transported to the endoplasmic reticulum for loading onto MHC class I molecules. L. English (Montreal, Canada) showed that endogenous viral proteins can also be processed in lytic vacuoles, and that HSV-1 infection leads to an autophagic process that enhances vacuolar presentation and increases the efficiency of the immune response.

Glossary

β2m	β-2-microglobulin
B27	HLA-B27
CD	cluster of differentiation
CTL	cytotoxic T lymphocyte
EHD	Eps-15 homology domain
FcRn	neonatal Fc receptor
FIP2	Rab11-family interacting protein 2
FRET	fluorescence resonance energy transfer
H-2D^d	allele of the mouse class I molecule, H-2D
HER2+	human epidermal growth factor receptor 2 positive
HFE	haemochromatosis protein
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HSV-1	herpes simplex virus 1
ICAM1	intercellular adhesion molecule 1
IgG	immunoglobulin G
IL23p19	small subunit of the cytokine IL-23
KIR	killer cell immunoglobulin receptors
KIR3DL2	KIR allele
MAP	mitogen-activated protein kinase
MHC	major histocompatibility complex
NK	natural killer
PIR	paired immunoglobulin-like receptor
Qa-1b	non-classical class I MHC molecule
RAB	*ras* superfamily GTPase
TAP	transporter associated with antigen processing
TCR	T-cell receptor

MHC class I molecules and NK-cell responses

To avoid excessive killing of self and to maintain self tolerance, murine NK cells express differing stochastic combinations of five MHC class I-inhibitory receptors. As some NK cells do not express any inhibitory self receptors, self tolerance must be achieved through an 'education' process that curbs these potentially dangerous self-reactive NK cells. K. Karre (Stockholm, Sweden) discussed experiments in mice that express either single or multiple MHC class I alleles, and presented several models for the education process. Recent evidence has led to the proposition of a tuning 'rheostat model', in which cellular responsiveness to the activation threshold is continuously adjusted to achieve education in both bone marrow

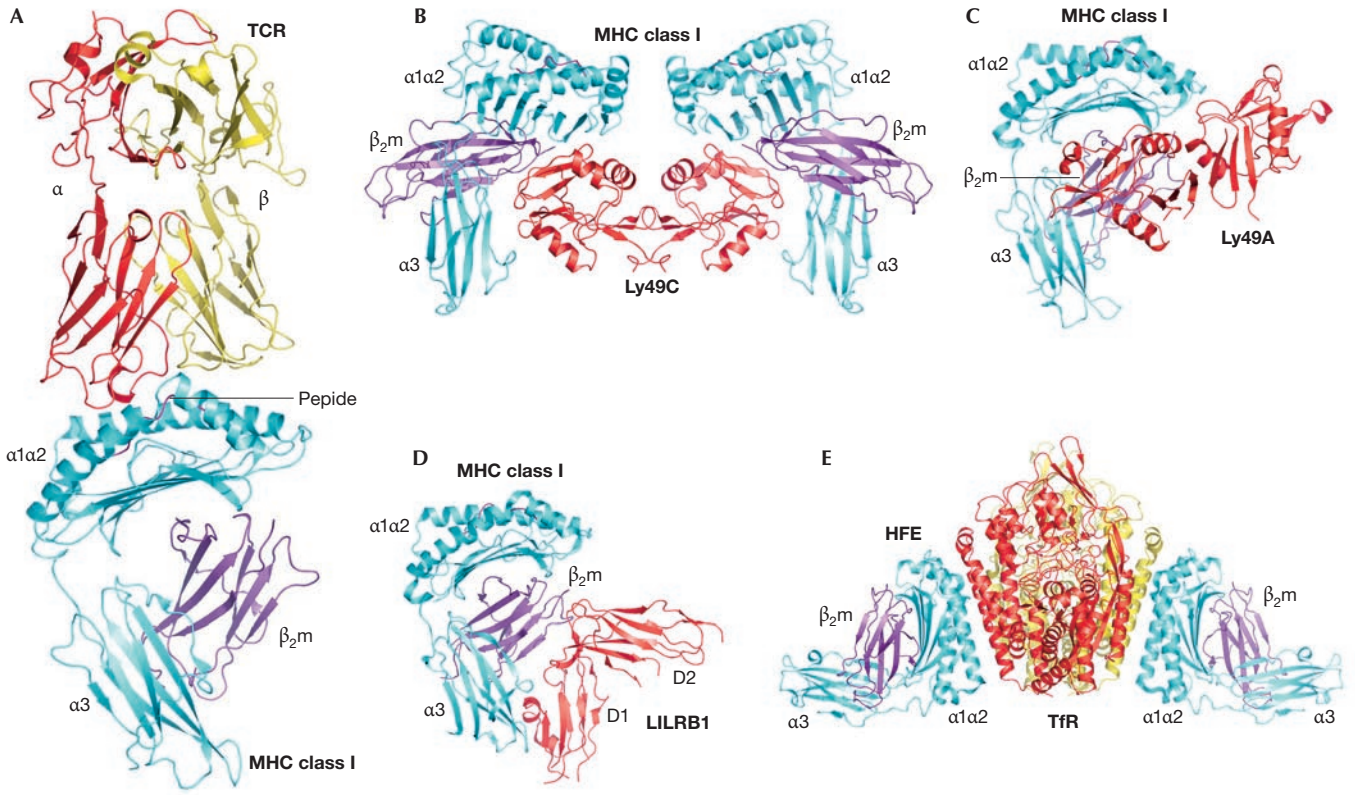

Fig 1 | MHC class I molecules have many partners that bind to various parts of the MHC molecule. Ribbon diagrams show the crystal structures of several MHC class I receptors bound to MHC class I ligands. The α1, α2 and α3 domains of the MHC class I heavy chain are shown in cyan, β2m is shown in purple and the MHC-bound peptide is shown in pink. Reprinted by permission from Macmillan Publishers Ltd: *Nature Reviews Immunology* (Held & Mariuzza, 2008), copyright 2008; see original article for definitions. MHC, major histocompatibility complex.

and the periphery. S. Khakoo (London, UK) discussed the importance of the KIR family of human NK-cell receptors, which have MHC class I-allele specificity, for protection and disease outcome in a range of pathologies, including chronic hepatitis C virus infection and HIV infection. Khakoo developed a complex pattern of KIR homozygosity and heterozygosity that, when considered in combination with the HLA C allotype, affected the outcome of infection. R. Mehr (Ramat-Gan, Israel) discussed her *in silico* models of the development of the repertoire of inhibitory receptors discussed by Karre. Mehr's model favours stochastic expression of many Ly49-inhibitory receptors, followed by selection among cells showing randomly generated receptor combinations. W. Held (Epalinges, Switzerland) identified new '*cis*' interactions (on the same membranes) between the NK Ly49A receptor and MHC class I molecules. If MHC class I molecules are removed from the NK cells, access to MHC class I molecules on opposing cell membranes in enhanced, suggesting that H-2D^d masks receptor binding in *trans*. Accordingly, a model has been proposed by which *cis* interactions sensitize the NK cells by sequestering the accessible receptors and limiting the inhibitory signals. The structures of several Ly49 receptors in complex with murine MHC class I molecules have been solved, and clearly show that the receptors bind to conserved residues at the 'side' of the MHC molecules, well away from the peptide-binding groove (Fig 1; Held & Mariuzza, 2008).

Biophysics of MHC class I molecules

The biophysics and cell biology of some cell-to-cell interactions that involve the recognition of MHC class I molecules were discussed by D. Davis (London, UK) and M. Edidin (Baltimore, MD, USA). Davis discussed the formation of nanotubes upon intercellular contacts. Although some nanotubes contain microtubules that are involved in vesicular transport, Davis found that T cells lack microtubules but are enriched in F-actin. Despite the heterogeneity of nanotube structure in various cell types, nanotubes seem to have important roles in facilitating oriented cell-to-cell secretion, as well as contributing to the spreading of HIV from infected to non-infected T cells. Davis presented compelling images of nanotubules that form in target/effector cell conjugates. Edidin showed that clustering of MHC class I molecules enhances their recognition by T-cell receptors. He also presented data suggesting that the transport of MHC I molecules to the surface might be stimulated on conjugation with an effector T cell.

The lateral organization of membrane proteins within the plane of the plasma membrane, and their distribution within various functional complexes, is crucial for transmembrane signalling and downstream events. S. Damjanovich (Debrecen, Hungary) highlighted the use of a range of biophysical techniques to probe the pairwise molecular proximity of interaction partners. FRET, atomic-force microscopy, confocal microscopy, and fluorescence and cross-correlation spectroscopy were among the methods used to analyse

the dynamic state of membrane protein patterns. One of the interactions described by the Damjanovich group, MHC I/ICAM1, has recently proved to be important in T-cell recognition of target MHC class I molecules (Segura *et al*, 2008). J. Matko (Budapest, Hungary) described a new mechanism for enhancing lateral interactions by concentrating the partner proteins in membrane lipid domains, as well as in actin-bound domains.

Regulation and loss of MHC class I molecules

Loss of HLA class I expression is common in many human cancers—for example, it occurs in approximately 40% of primary breast tumours—and is generally greater in metastases than in primary tumours. HLA class I expression defects correlate with a poor prognosis in laryngeal carcinoma and many other tumours. Understanding the molecular basis of these defects can identify instances in which specific individualized treatment can be initiated. S. Ferrone (Pittsburg, PA, USA) described an HER2+ squamous-cell carcinoma cell line, PCI-30, which is not usually recognized by HLA-A2-restricted HER2-specific CTL; however, recognition can be induced with γ-interferon, presumably because this raises the levels of MHC class I molecules on the cell surface. By contrast, in a second melanoma cell line, which was derived from a patient with disease recurrence and contained the β2m mutation C25W, recognition could be reconstituted only by the transfection of wild-type β2m, as native MHC class I molecules could not reach the cell surface in the absence of β2m.

The metabolism and shedding of MHC class I molecules was discussed from several viewpoints. S. Caplan (Omaha, NE, USA) discussed the role of the C-terminal EHD proteins in regulating the recycling of internalized MHC class I molecules back to the plasma membrane. EHD1 coordinates endocytic regulation with RAB family members through their effectors, rabenosyn 5 and RAB11-FIP2, and the EHD1 paralogue, EHD4, controls MHC class I trafficking at the early endosome. EHD proteins associate with and/or tubulate membranes through their C-terminal EH domain, and the recent nuclear magnetic resonance solution structure of the EHD1 EH domain—solved by the Caplan group—has provided new insight into the mode by which EHD proteins function. Several viral infections also downregulate host MHC class I expression as a way of avoiding host CTL-response priming and recognition of viral epitopes presented by MHC I molecules. P. Lehner (Cambridge, UK) elucidated the function of two ubiquitin E3 ligases produced by human herpesvirus 8. The K3 and K5 viral E3 ligases initiate the formation of Lys63-linked ubiquitin conjugates on MHC class I molecules that are essential for internalization and endolysosomal degradation. Various HLA molecules, including HLA-A, HLA-B, HLA-C and HLA-G, have been detected in soluble form in serum and body fluids. F. Puppo (Genoa, Italy) showed that soluble HLA molecules can trigger the apoptosis of $CD8^+$ T cells and NK cells. He speculated about the role of soluble HLA molecules in the immunomodulation of a range of physiological and disease states, including renal, heart and liver transplants, the stability of multiple sclerosis symptoms, fertility, allergies and cancer.

MHC class I molecules as signalling molecules

MHC class I molecules are not only involved in signalling to other cells but they are also capable of initiating intracellular signalling cascades that culminate in survival and/or cell proliferation decisions. E. Reed (Los Angeles, CA, USA) showed that HLA class I antibodies derived from patients who have rejected grafts can induce the proliferation of primary endothelial cells in a dose-dependent manner. Low levels of circulating antibodies lead to 'good survival' signals through the focal adhesion kinase-signalling pathway, whereas higher levels of antibodies enhance the expression of fibroblast growth factor on the plasma membrane and lead to proliferation by signalling through the MAP kinase pathway. These MHC class I signals are transduced in combination with integrin signalling, as antibody-initiated signalling and proliferation was blocked in endothelial cells depleted of β4 integrin by small-interfering RNA.

C. Shatz (Stanford, CA, USA) described new and unexpected functions of classical MHC class I molecules and their putative ligand, PIRB, in synaptic plasticity as the brain develops. During this remodelling, some synapses are strengthened and others regress. β2-m/TAP double-knockout mice show enhanced strengthening of synapses and preservation of the ones that normally regress. Similarly, knockout of PIR—an immunoglobulin-domain molecule previously shown to be expressed by B cells, mast cells and myeloid cells, and to be a ligand for several MHC class I molecules—increases synaptic plasticity. These notable results suggest that classical MHC class I molecules and their ligands might act both in the development of synaptic connections and in the remodelling of these connections in response to stimuli and experience (Fig 2).

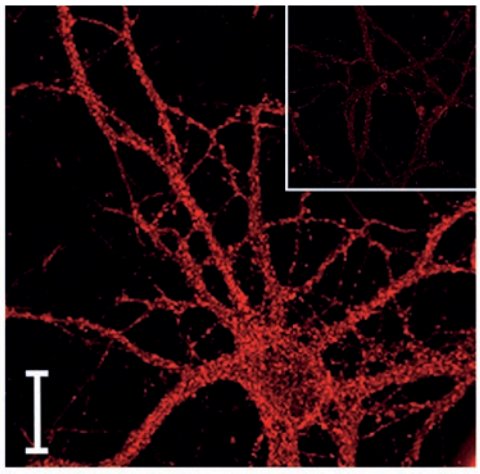

Fig 2 | MHC class I expression in hippocampal neurons. MHC class I was detected with the OX18 antibody and compared with an equimolar amount of control mouse IgG (inset). Immunoreactivity can be seen in the soma and dendrites. The scale bar represents 20 μm. Reproduced with permission from Goddard *et al*, 2007, copyright (2007) National Academy of Sciences, USA. IgG, immunoglobulin G; MHC, major histocompatibility complex; OX18, mouse anti-rat MHC monoclonal antibody.

Non-classical MHC class I molecules

There are many molecules that have evolved away from the classical MHC class I structure and belong to the so-called non-classic MHC class I molecules. The differences of these non-classical MHC I-like structures range from altered binding grooves that focus on particular conserved peptides (known as class 1b molecules) or binding grooves that are enlarged to accommodate the large hydrophobic tails of glycolipid antigens, to the loss of a binding groove

altogether. C. Clements (Victoria, Australia) compared the newly solved crystal structure of the mouse class 1b molecule Qa-1b with that of the human class 1b molecule HLA-E. Both molecules bind to a limited peptide repertoire, which notably is a peptide derived from common signal sequences cleaved from nascent proteins. Although the genes seem to have evolved independently and the proteins share only 71% sequence identity in the peptide-binding region, the structures of the two class 1b molecules are similar and this explains their common function. G. De Libero (Basel, Switzerland) focused on the CD1e molecules, which are expressed only in dendritic cells, and reported that CD1e interacts with the membrane of phagosomes to present large glycolipids for cleavage. Additional studies indicate that some TCRs discriminate between different sulphatides by the length of their fatty acid chain. F. Spinozzi (Perugia, Italy) presented data showing that CD1-restricted γ-δ T cells can recognize pollen-derived phospholipids in allergy, and that NK T-cell lines from the lungs of patients with asthma are particularly long lived.

The members of another group of non-classical MHC class I molecules are associated with β2-m but lack a binding groove. FcRn is a member of this group that is important for the passive transfer of maternal IgG to the fetus. FcRn also rescues IgG and albumin from degradation throughout life. I. Kacskovics (Budapest, Hungary) showed that overexpression of this receptor can prolong the circulatory half life of IgG; such manipulations have important implications for the quantities of antibodies that can be obtained from mammals for human therapeutic use. The HFE protein is another non-classical HLA molecule that is crucial for human health; mutations of this protein cause the iron storage-overload disease haemochromatosis. HFE is present only in mammals, and functions by laterally associating with the transferrin receptor to modulate its affinity for transferrin. M. De Sousa (Porto, Portugal) described the iron-overload phenotype of mice that are deficient in β2m, HFE and MHC class I. She showed that besides its effect on receptor-mediated transferrin uptake, HFE knockout also affects erythropoiesis. This discussion of the cell biology of HFE brought the meeting back to a fundamental problem in the field: the way in which classical MHC class I molecules also act to modulate the function of other surface receptors.

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