中山大學海洋資源學系博士班 2012 入學考筆試

(1) Use English to write yourself a recommendation letter in applying a faculty position in the Department of Marine Biotechnology and Resources. Max. 200 words (40%).

(2) In the early line of evolution, marine animals may develop defined major histocompatibility complex (MHC) that are inherited by the off-springs to further complex. Translate the human MHC functions that can be used as a reference for applying marine resources to human health (60%).

Translation: (60%)

Phagocytosis, the ingestion of particulate materials, such as bacteria, viruses, and remnants of dead cells, involves extensive remodeling of the actin-based cytoskeleton to accommodate the incoming particle. Although phagocytosis may be initiated by specific receptor-ligand interactions, these are not always required: Even latex particles can be ingested very efficiently by macrophages. In the process of opsonization, pathogens decorated by antibodies and certain complement components are targeted to macrophages and dendritic cells, recognized by cell-surface receptors for complement components or the Fe portion of immunoglobulins, and then phagocytosed (Figure 24-27). Macrophages and dendritic cells also express several types of nonspecific receptors (e.g., Toll-like receptors, scavenger receptors) that recognize molecular patterns on nonparticulate antigens; the bound antigen is internalized by receptor-mediated endocytosis. B cells, which are not phagocytic, also can acquire antigen by receptor-mediated endocytosis using their antigen-specific B-cell receptors (surface immunoglobulin) (Figure 24-28). Finally, cytosolic antigens may enter the class II MHC pathway via autophagy (see Figure 14-35). After formation of the autophagic cup, an autophagic vesicle is formed. These vesicles are of a size that can accommodate damaged organelles, and sizable quantities of cytoplasm may be encapsulated in the process. The resulting autophagosomes are destined to fuse with lysosomes, where the contents of the autophagosome then become available for digestion by lysosomal proteases.

Tagging Antigen for Destruction: Proteolysis is required to convert intact protein antigen into peptides of a size suitable for binding to Class II MHC molecules. Protein antigens are tagged for degradation by progressive unfolding, brought about by the drop in pH as proteins progress along the endocytic pathway. The pH of the extracellular environment is around pH 7.2, and that in early endosomes between pH 6.5 and 5.5; in late endosomes and lysosomes the pH may drop to pH 4.5. ATP-powered V-class proton pumps in the endosomal and lysosomal membranes are responsible for this acidification (see Figure 11-9). Proteins stable at neutral pH tend to unfold when exposed to extremes of pH, through rupture of hydrogen bonds and destabilization of salt bridges. Further, the environment in the endosomal/lysosoma l compartments is reducing, with lysosomes attaining a concentration of reducing equivalents in the millimolar range. Reduction of disulfide bonds that stabilize many extracellular proteins is catalyzed also by a thioreductase inducible by exposure to IFNγ. The combined action of low pH and reducing environment prepares the antigens for proteolysis.

Proteolysis: Degradation of proteins in the class II MHC pathway is carried out by a large set of lysosomal proteases, collectively referred to as cathepsins, which are either cysteine or aspartyl proteases. Other proteases, such as asparagine-specific endoprotease, also may contribute to proteolysis. A wide range of peptide fragments is produced, including some that can bind to class II MHC molecules. The lysosomal proteases operate optimally at the acidic pH within lysosomes. Consequently, agents that inhibit the activity of V-class proton pumps interfere with antigen processing, as do inhibitors of lysosomal proteases.

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This targeting is accomplished by means of a chaperone called the invariant chain, a type II membrane glycoprotein (see Figure 13-10). The invariant chain (Ii) plays a key role in the early stages of class II MHC biosynthesis by forming a trimeric structure onto which the class II MHC αβ heterodimers assemble. The final assembly product thus consists of nine polypeptides: $(\alpha\beta Ii)_3$. The interaction between Ii and the αβ heterodimer involves a stretch of Ii called the CLIP segment, which occupies the class II MHC peptide-binding cleft. Once the $(\alpha\beta Ii)_3$ complex is assembled, the complex enters the secretory pathway and is diverted to endosomes and lysosomes at the *trans-Golgi* network (see Figure 14-1). The signals responsible for this diversion are carried by the Ii cytoplasmic tail and do not obviously conform to the endosomal targeting or retrieval signals commonly found on lysosomal membrane proteins. Some of the $(\alpha\beta Ii)_3$ complexes are directed straight to the cell surface from which they may be internalized, but the vast majority end up in late endosomes.

As we saw for class I MHC molecules and their CD8 coreceptor, the CD4 co-receptor recognizes conserved features on class II MHC molecules. Any mature T cell that bears the CD4 co-receptor uses class II MHC molecules for antigen recognition.

Antigen Presentation Is the Process by Which Protein Fragments Are Complexed with MHC Products and Posted to the Cell Surface

The process by which foreign materials enter the immune system is the key step that determines the eventual outcome of a response. A successful adaptive immune response, which includes the production of antibodies and the generation of helper and cytotoxic T cells, cannot unfold without the involvement of professional antigen-presenting cells. Professional antigen-presenting cells include dendritic cells and macrophages, both bone marrow-derived, and B cells. It is these cells that acquire antigen, process it, and then display it in a form that can be recognized by T cells. The pathway by which antigen is converted into a form suitable for T-cell recognition is referred to as *antigen processing and presentation.*

The class I MHC pathway focuses predominantly on presentation of proteins synthesized by the cell itself, and the class II MHC pathway is centered on materials acquired from outside the antigen-presenting cell. This distinction, however, is by no means absolute. Together, the class I and class II pathways of antigen processing and presentation sample all of the compartments that need to be surveyed for the presence of pathogens.

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MHC Molecules Bind Peptide Antigens and Interact with the T-Cell Receptor

Both class I and class II MHC molecules are highly *polymorphic;* that is, many allelic variants exist among individuals of the same species. Both classes of MHC molecules also are structurally similar in many respects as are their interactions with peptides and the T-cell receptor (Figure 24-23).

Class I MHC Molecules

Class I MHC molecules belong to the Ig superfamily and consist of two polypeptides. The larger subunit is a type I membrane glycoprotein (see Figure 13-10) encoded by the MHC. The smaller [32-microglobulin subunit is not encoded by the MHC and corresponds in structure to a free Ig domain. Originally purified from human leukocytes by digestion with pa pain, which releases the extracellular portion of the class I MHC molecules in intact form, these proteins are now produced by recombinant DNA technology procedures and have become important tools for the detection of antigen-specific T cells.

Implicit in the notion that MHC molecules are the targets of graft rejection is their structural variation, attributable to inherited variation (genetic polymorphism): If a recipient rejects a graft, the recipient's immune system must be capable of distinguishing unique features of the donor MHC molecules present on the graft. In fact, the genes encoded by the MHC are among the most polymorphic currently known, with over 2000 distinct allelic products identified in humans. The class I MHC molecules in humans are encoded by the HLA-A, HLAB and HLA-C loci (see Figure 24-21). Each unique allele is designated by a numerical suffix for that particular locus (e.g., HLA-A2 and HLA-A28 represent two distinct HLA-A allelic products). In the mouse, the class I MHC molecules are encoded by the H-2K and H-20 loci. A superscript is used to denote the identity of the allelic products (e.g., H-2Kb and H-2Kk represent two allelic variants of the H-2K locus product).

The three-dimensional structure of class I MHC molecules reveals two membrane-proximal Ig-like domains. These domains support an eight-stranded -pleated sheet topped by two α helices. Jointly the β sheet and the helices create a cleft, closed at both ends, in which a peptide binds (see Figure 24-23a). The mode of peptide-binding by a class I MHC molecule requires a peptide of rather fixed length, usually 8 to 10 amino acids, so that the ends of the peptide can be tucked into pockets that accommodate the charged amino and carboxyl groups at the termini. Further, the peptide is anchored into the peptide-binding cleft by means of a small number of amino acid side chains, each of which is accommodated by a pocket in the MHC molecule that neatly fits that particular amino acid residue (Figure 24-24a). On average, two such "specificity pockets" must be filled correctly to allow stable peptide binding. One of these pockets often involves the very C-terminal residue; a second specificity pocket accommodates a residue more central to the peptide.

Translation: (60%)

During the course of an inflammatory response and in response to interferon , the three catalytically active β subunits (β1, β2, β5) of the proteasome can be replaced by three immune-specific subunits: βli, β2i and β5i. The βli, β2i and β5i subunits are encoded in the MHC. The net result of this replacement is the generation of the *immunoproteasome,* the output of which is matched to the requirements for peptide binding by class I MHC molecules. The immunoproteasome adjusts the average length of the peptides produced, as well as the sites at which cleavage occurs. Given the central role of the proteasome in the generation of peptides presented by class I MHC molecules, proteasome inhibitors potently interfere with antigen processing via the class I MHC pathway.

Delivery of Peptides to Class I Molecules: Protein synthesis, ubiquitin conjugation, and proteasomal proteolysis all occur in the cytoplasm, whereas peptide binding by class I MHC molecules occurs in the lumen of the endoplasmic reticulum (ER). Thus peptides must cross the ER membrane to gain access to class I molecules, a process mediated by the heterodimeric TAP complex, a member of the ABC superfamily of ATP-powered pumps (see Figure 11-14). The TAP complex binds peptides on the cytoplasmic face and, in a cycle that includes ATP binding and hydrolysis, peptides are translocated into the ER. The specificity of the TAP complex is such that it can transport only a subset of all cytosolic peptides, primarily those in the length range of 5-10 amino acids. The mouse TAP complex shows a pronounced preference for peptides that terminate in leucine, valine, isoleucine, or methionine residues, which match the binding preference of the class I MHC molecules served by the TAP complex. The genes encoding the TAP1 and TAP2 subunits composing the TAP complex are located in the MHC.

Binding of Peptides to Class I Molecules: Within the ER, newly synthesized class I MHC molecules are part of a multiprotein complex referred to as the peptide-loading complex. This complex includes two chaperones (calnexin and calreticulin) and the oxidoreductase Erp57. Another chaperone (tapasin) interacts with both the TAP complex and the class I MHC molecule about to receive peptide. The physical proximity of TAP and the class I MHC molecule is maintained by tapasin. Once peptide loading has occurred, a conformational change releases the loaded class I MHC molecule from the peptide-loading complex.

Display of Class I MHC-Peptide Complexes at the Cell Surface: Once peptide loading is complete, the class I MHC-peptide complex is released from the peptide-loading complex and enters the constitutive secretory pathway (see Figure 14-1). Class I MHC molecules, depending on the species and allelic identity, contain between one and three N-linked oligosaccharides, which receive extensive modifications in the Golgi complex. Transfer from the Golgi to the cell surface is rapid and completes the biosynthetic pathway of a class I MHC-peptide complex. The entire sequence of events in the class I pathway occurs constitutively in all nucleated cells, which express class I MHC molecules and the other required proteins or can be induced to do so.

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In this manner, a given MHC molecule can accommodate a large number of peptides of diverse sequence, as long as the "anchor" requirements are fulfilled.

The polymorphic residues that distinguish one allelic MHC molecule from another are mostly located in and around the peptide-binding cleft. These residues therefore determine the architecture of the peptide-binding pocket and hence the specificity of peptide binding. Further, these polymorphic residues affect the surface of the MHC molecule and hence the points of contact with the T-cell receptor. A T-cell receptor designed to interact with one particular class I MHC allele will therefore, as a rule, not interact with unrelated MHC molecules because of their different surface architecture (Figure 24-24b). The CD8 marker functions as a co-receptor, binding to conserved portions of the class I MHC molecules. The presence of CD8 thus "sets" the restriction specificity of any mature T cell that bears it.

Class II MHC Molecules

The two subunits (α and β) of class II MHC molecules are both type I membrane glycoproteins and belong to the Ig superfamily. The typical mammalian MHC contains several loci that encode class II MHC molecules (see Figure 24-21). Like the large subunit of class I molecules, both the α and β subunits of class II molecules show genetic polymorphism.

The basic three-dimensional design of class II MHC molecules resembles that of class I MHC molecules: Two membrane-proximal Ig-like domains support a peptide-binding portion composed of an eight-stranded β sheet and two α helices (see Figure 24-23b). For class II MHC molecules, the α and β subunits contribute equally to the construction of the peptide-binding cleft. This cleft is open at both ends and thus supports the binding of longer peptides that protrude from it. The mode of peptide binding involves pockets that accommodate specific peptide side chains, as well as contacts between side chains of the MHC molecule with main-chain atoms of the bound peptide. Class II MHC polymorph isms ma inly affect residues in and around the peptide binding cleft, so that the peptide-binding specificity will usually differ among different allelic products.

A T-cell receptor designed to interact with a particular class II MHC molecule will not, as a rule, interact with a different allelic molecule, not only because of the difference in the peptide-binding specificity of the allelic molecules, but also because of the polymorphisms that affect the contact residues with the T-cell receptor. As discussed below, class II MHC molecules evolved to present peptides generated predominantly in endosomes and lysosomes. The interactions between a peptide and class II MHC molecule take place in these organelles, and class II MHC molecules are targeted specifically to those locations after their synthesis in the endoplasmic reticulum.

Translation: (60%)

In the absence of a virus infection, protein synthesis and proteolysis continuously generate a stream of peptides that are loaded onto class I MHC molecules. Healthy, normal cells therefore display on their surface a representative selection of peptides derived from host proteins. There may be several thousand distinct MHC-peptide combinations di splayed at the surface of a typical class I MHC positive cell. Developing T cells in the thymus calibrate their antigen-specific receptors on these sets of MHC-peptide complexes, and learn to recognize self-MHC products as the "restriction elements" on which they must henceforth rely for antigen recognition. At the same time, the display of self-peptides by self-MHC molecules enables the developing T cell to learn which peptide MHC combinations are self-derived and must therefore be ignored to avoid a self-destructive autoimmune reaction. It is not until a virus makes its appearance that virus-derived peptides begin to make a contribution to the display of peptide-MHC complexes. The overall efficiency of this pathway is such that approximately 4000 molecules of a given protein must be destroyed to generate a single MHC peptide complex carrying a peptide from that particular polypeptide.

An unusual mode of antigen presentation that is nonetheless crucial in the development of cytotoxic T cells is *cross-presentation.* This term refers to the acquisition by dendritic cells of apoptotic cell remnants, immune complexes, and possibly other forms of antigen by phagocytosis. By a pathway that has yet to be understood, these materials escape from phagosomal/endosomal compartments into the cytosol, where they are then handled according to the steps described above. Only dendritic cells are capable of cross-presentation, and so allow the loading of class I MHC molecules complexed with peptides that derive from cells other than the antigen-presenting cell itself.

Class II MHC Pathway Presents Antigens Delivered to the Endocytic Pathway

Although class I MHC and class II MHC molecules show a striking structural resemblance, the manner in which the two classes acquire peptide and their functions in immune recognition differ greatly. Whereas the primary function of class I MHC molecules is to guide CD8-bearing cytotoxic T cells to their target cells, class II MHC molecules serve to guide CD4-bearing helper T cells to the cells with which they interact, primarily professional antigen-presenting cells.

As noted previously, class II MHC molecules are expressed primarily by professional antigen-presenting cells: dendritic cells and macrophages, which are phagocytic, and B cells, which are not. Hence, the class II MHC pathway of antigen processing and presentation generally occurs only in these cells. The steps in this pathway are depicted in Figure 24-26 and described below:

Acquisition of Antigen: In the class II MHC pathway, antigen is acquired by pinocytosis, phagocytosis, or receptor-mediated endocytosis. Pinocytosis, which is rather nonspecific, involves the delivery, by a process of membrane invagination and fission, of a volume of extracellular fluid and the molecules dissolved therein.

Translation: (60%)

Delivery of Peptides to Class II Molecules: Recall that most class II MHC molecules synthesized in the endoplasmic reticulum are directed to late endosomes. Because the peptides generated by proteolysis reside in the same topological space as the class II MHC molecules themselves, the delivery of peptide to class II MHC molecules does not require traversal of a membrane. The sole requirement, therefore, is to allow peptides and class II MHC molecules to meet. This is accomplished in the course of biosynthesis by a sorting step that is dependent on the invariant chain (Ii) and ensures delivery of the class II $(\alpha\beta Ii)_3$ complex to endosomal compartments.

Binding of Peptides to Class II Molecules: The $(\alpha\beta Ii)_3$ complex delivered to endosomal compartments is incapable of binding peptide because the peptide-binding cleft in the class II molecule is occupied by Ii. The same proteases that act on internalized antigens and degrade them into peptides act also on the $(\alpha\beta Ii)_3$ complex, resulting in removal of Ii with the exception of a small portion called the CLIP segment. Because it is firmly lodged in the class II MHC peptide-binding cleft, CLIP is resistant to proteolytic attack. The class II MHC molecules themselves are also resistant to unfolding and proteolytic attack under the conditions that prevail in the endocytic pathway. The CLIP segment is removed through interact ion of the CLIP complex with a chaperone, DM. Although the DM protein is MHC-encoded and structurally very similar to class II molecules, it is unable to bind peptides. Newly formed class II MHC-peptide complexes are themselves susceptible to further "editing" by DM, until the class II molecule acquires a peptide so stably bound that it cannot be dislodged by interaction of the complex with DM. The resulting class II MHC-peptide complexes are extremely stable, with estimated half lives in excess of 24 hours.

Display of Class II MHC -Peptide Complexes at the Cell Surface: The newly generated class II MHC-peptide complexes are localized mostly in late endosomal compartments, which include multivesicular endosomes (or bodies) (see Figure 14-33). Recruitment of the internal vesicles of the multivesicular bodies to the delimiting membrane expands their surface area; by a process of tubulation along tracks of microtubules, these compartments elongate and ultimately deliver class II MHC-peptide complexes to the surface by membrane fusion. These events are tightly regulated: Tubulation and delivery of class II MHC molecules to the surface are enhanced in dendritic cells and macrophages following their activation in response to signals, such as bacterial lipopolysaccharide (LPS), which is detected by their Toll-like receptors.

For professional antigen-presenting cells, the above steps are constitutive-happening all the time-but they can be modulated by exposure to microbial agents and cytokines.

Translation: (60%)

Antigen processing and presentation in both the class I and II pathways may be divided into six discrete steps that are useful in comparing the two pathways: (1) acquisition of antigen, (2) tagging the antigen for destruction, (3) proteolysis, (4) delivery of peptides to MHC molecules, (5) binding of peptide to a MHC molecule, and (6) di splay of the peptide-loaded MHC molecule on the cell surface. In the next two sections, we describe the molecular details of each pathway.

Class I MHC Pathway Presents Cytosolic Antigens

Figure 24-25 summarizes the six steps in the class I MHC pathway using a virus-infected cell as an example. The following discussion describes the events that occur during each step:

Acquisition of Antigen: In the case of a virus infection, acquisition of antigen is usually synonymous with the infected state. Viruses rely on host protein synthesis to generate the building blocks for new virions. This includes the synthesis of viral cytosolic proteins as well as membrane proteins. Protein synthesis, unlike DNA replication, is an error-prone process, with 10-30 percent of newly initiated polypeptide chains being terminated prematurely or suffering from errors (misincorporation of amino acids, frame shifts, improper or delayed folding). These mistakes in protein synthesis affect both the host cell's own proteins and those specified by viral genomes. Such error-containing proteins must be rapidly removed so as not to clog up the cytoplasm or engage partner proteins in nonproductive interactions. The rate of cytosolic proteolysis must be matched to the rate at which mistakes in protein synthesis occur. These rapidly degraded proteins are an important source of antigen peptides destined for presentation by class I MHC molecules. With the exception of cross-presentation (discussed below), the class I MHC pathway results in the formation of peptide-MHC complexes in which the peptides are derived from proteins synthesized by the class I MHC-bearing cell itself.

Tagging the Antigen for Destruction: For the most part, the ubiquitin conjugation system is responsible for tagging a protein for destruction (see Chapter 3, p. 88). Ubiquitin conjugation is tightly regulated.

Proteolysis: Ubiquitin-conjugated proteins are destroyed by proteasomal proteolysis. The proteasome is a highly processive protease that engages its substrates and, without the release of intermediates, yields final digestion products, peptides in the size range of 3-20 amino acids (see Figure 3-29).

During the course of an inflammatory response and in response to interferon γ, the three catalytically active β subunits ($\beta1$, $\beta2$, $\beta5$) of the proteasome can be replaced by three immune-specific subunits: βli, $\beta2$i and $\beta5$i. The βli, $\beta2$i and $\beta5$i subunits are encoded in the MHC.