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Expression of MHC Molecules in Neonates

1. INTRODUCTION

Whereas B cells recognize the epitopes on native antigens, T cells recognize only peptides derived from the processing of antigen by antigen-presenting cells (APCs) in association with major histocompatibility complex (MHC) molecules. Therefore, the expression of MHC molecules on APCs and the ability of APCs to process the antigens play a crucial role in the activation of T cells, which are the effectors of cell-mediated immunity (CMI).

In Chapter 9, we presented a multitude of evidence demonstrating that antigens can activate neonatal T cells. These results indicate that the low CMI response of neonatal T cells can not be attributed to T-cell immaturity but rather to Th2 dominance. Other possible explanations for the low CMI response in neonates include poor (or absent) expression of MHC molecules and/or poor capacity to process the antigens *(954)*.

2. NEONATAL EXPRESSION OF MHC CLASS I MOLECULES

MHC class I genes encode highly polymorphic molecules expressed on the surface of somatic cells that present short peptides mainly to CD8 T cells. The peptides are derived from endogenous proteins degraded in the cytosol by the multicatalytic proteasome lmp2 and lmp7 subunits and are transported into endoplasmic reticulum by TAP molecules, which deliver the peptides to class I molecules. In all vertebrate species, class I MHC molecules are heterodimers composed of a heavy chain noncovalently linked to β-2 microglobulin *(955)*.

Few studies have examined the expression and the density of class I molecules at the surface of somatic cells. The majority of studies on the expression of class I molecules on the surface of APCs have examined how APCs present class I-peptide complexes to CD8 T cells. In low vertebrate species, the expression of class I molecules during ontogeny has been thoroughly investigated in amphibians. Therefore, the expression of class Ia (the equivalent of mammalian class I) and class Ib (the equivalent of CD1) molecules was studied by immunofluorescence with alloantisera. Transcript levels of class I genes were

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also examined by polymerase chain reaction (PCR). Expression of lmp7, a component of proteasomes required for antigen processing, was also examined.

Immunofluorescence and immunoprecipitation studies detect class I molecules on Xenopus thymus epithelium but not in other tissues until the climax stage of the metamorphosis *(956)*. PCR studies of class I gene transcription indicate that the expression is not simultaneous in all tissues. Whereas class I transcripts are present in tadpole intestines, lungs, and gills, no class I transcripts can be detected in the thymus or spleen until after the metamorphic climax. The expression dramatically increases in all tissues after metamorphosis. In contrast, lmp7 was expressed at all stages of development *(957)*. Thus, in Xenopus, class I molecules are differentially expressed during ontogeny in concert with reorganization of many tissues at the metamorphosis stage.

In mice, class I molecules are expressed by inner cells at the late blastocyte stage but only at low levels on trophoblasts *(958)*. At d 12 of gestation, class I molecules appear in most embryonic tissues such as the gut, lung, limb bud, and heart; they appear in the kidney and gonads at d 15 of gestation *(959)*. The increased expression of class I molecules during embryonic life parallels the development of the immune system.

The expression of class I molecules on neonatal dendritic cells was studied by measuring their ability to prime cytotoxic T lymphocytes (CTL), which recognize peptides in association with class I molecules. Immature neonatal dendritic cells exhibited similar efficacy of uptake and processing of foreign antigen as their adult counterparts. In addition, dendritic cells from 7-d-old mice that were loaded with an L^d-restricted epitope 118 to 126 from the nucleoprotein of lympho choriomeningitis murine virus (LCMV) stimulated the activation of a CD8 T-cell hybridoma bearing a T-cell receptor (TCR) that is specific for this peptide. Furthermore, in vivo experiments demonstrated that neonatal dendritic cells were able to prime CD8 cells *(960)*. In these experiments, freshly purified neonatal $CD11c^+$ dendritic cells were pulsed with 118 to 126 peptide and then injected into adult syngeneic mice. Five days later, the CTL activity was measured. The magnitude of cytotoxic response in adult mice injected with neonatal peptide-pulsed dendritic cells was similar to that induced by the injection of adult dendritic cells *(961)*. These results demonstrate a high capacity of neonatal dendritic cells to induce a CTL response and strongly suggest that neonatal dendritic cells express sufficient class I molecules for efficient presentation of peptides to CD8 T cells.

There is little information regarding the expression of class I molecules during human fetal development. There is a report that very small numbers of MHC class I-positive fetal cells can be detected by FACS analysis at 6 to 8 wk of gestation *(961)*. Immunostaining with antimonomorphic antibodies indicates that class I molecules have widespread reactivity with both epithelial and

hematopoietic cells during mid-trimester of gestation. The immunostaining results corroborate data from immunoprecipitation and PCR assays that indicate that HLA-A, -B, and -C class I proteins were not expressed in fetal livers, whereas nonclassical class I proteins such as HLA-F were expressed *(962)*. There is also compelling indirect evidence that MHC class I molecules are expressed in fetal life. First, maternal alloantibodies against the paternal class I antigens are detected in multiparous women. Such antibodies can be induced by the shed fetal class I molecules. Processing and presentation of peptides derived from fetal class I antigens would lead to activation of Th cells and production of antibodies against fetal MHC molecules *(963)*.

A second line of indirect evidence is the detection of adult-like CD8 T-cell responses in human fetuses with a congenital infection of *Trypanosoma cruzi*. The tremendous expansion of CD8 T cells was associated with major phenotypic changes, which were closely related to acquired effector functions by CD8 T cells, such as production of interferon (IFN)-γ and synthesis of high amounts of perforin *(964)*.

The *in utero* CD8 T-cell response of congenitally *T. cruzi*-infected newborns resembles the strong antigen-specific CD8 expansion observed in adults. This similarity indicates that this response results from the presence and presentation of antigens by class I molecules expressed in fetuses.

3. EXPRESSION OF MHC CLASS II MOLECULES DURING FETAL DEVELOPMENT IN NEONATAL ANTIGEN-PRESENTING CELLS

An MHC class II molecule is a heterodimer of two glycoproteins: α-chain of 34 kDa and β-chain of 29 kDa. Genes in the MHC locus encode both chains. Class II molecules are integral membrane proteins with intracellular and extracellular domains separated by a hydrophobic transmembrane fragment. The extracellular segment is composed of two domains. The outermost domain, encoded by α1 and β1 exons, has an immunoglobulin-like structure containing hypervariable residues that are responsible for allelic polymorphism and the binding of peptides. Class II molecules present the peptides that result from the processing of foreign antigen (reviewed in ref. *965*) and self-antigens (reviewed in ref. *966*) to CD4 T cells.

In contrast to class I molecules, which are expressed in all somatic cells, the class II molecules are constitutively expressed in professional APC, namely, macrophages, B cells, and dendritic cells. Figure 39 illustrates class II molecules on the surface of a rat macrophage detected with gold-labeled anti-class II antibodies. In certain conditions, other cell types, including myoblasts *(967)*, eosinophils *(55)*, epithelial cells of renal proximal tubule *(968)*, microglial cells *(969)*, astrocytes *(970),* and intestinal epithelial cells *(971)*, can express class II molecules and present peptides.

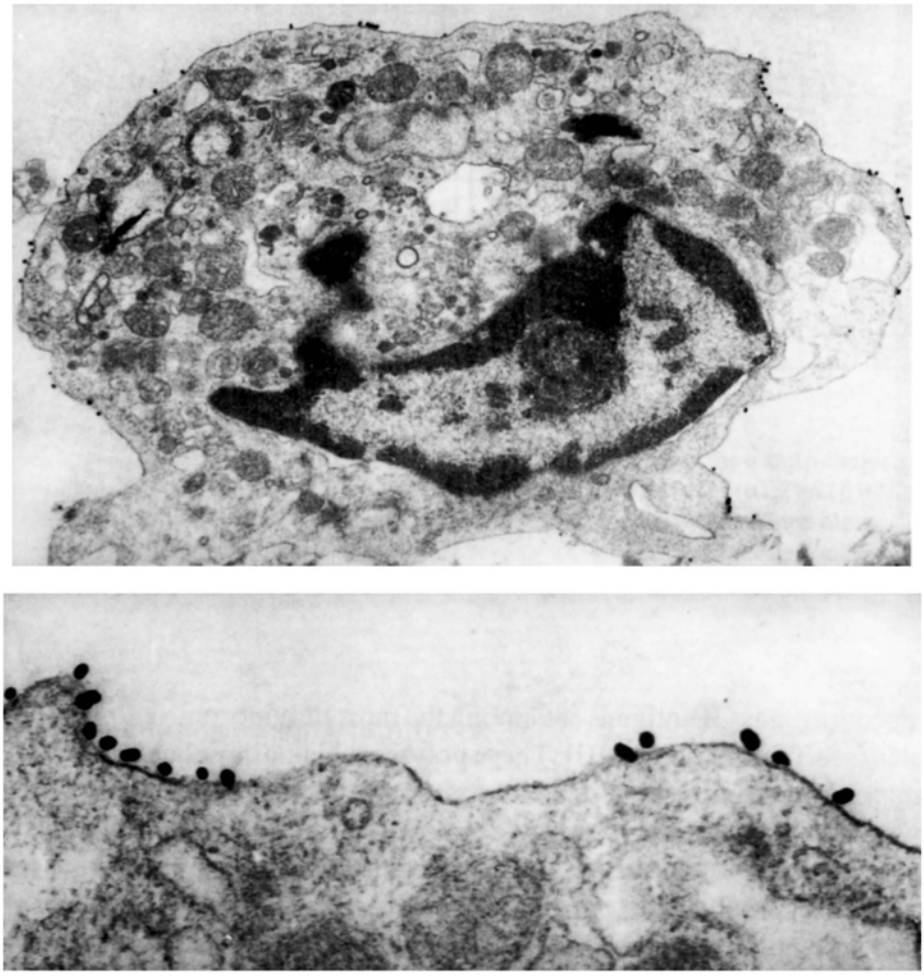

Fig. 39. Electron micrograph demonstrating the expression of MHC class II molecules on macrophages. Macrophages were incubated with gold-labeled anti-class II antibodies. Upper panel, gold particles appear as small black dots on the surface of an activated macrophage. Lower panel, higher magnification of a segment of the membrane. (From Bona C, Bonilla F. Textbook of Immunology. Harwood Academic Publ USA 1990, p. 225.)

There is a hierarchy in the ability of professional APCs to process and present antigens. In sum, the expression of class II molecules represents a potential important factor affecting the ability of APCs to generate peptides and present them to CD4 T cells.

3.1. Macrophages

Studies in mice demonstrate that both class II MHC expression and antigen presentation are defective in neonatal macrophages when compared to macrophages of adult mice *(972)*. Lu et al showed in various inbred murine strains that whereas 29% of peritoneal macrophages express class II molecules, only 2 to 9% of 7- to 10-d-old mice express class II antigens *(973)*. Low expression of class II antigens by neonatal macrophages correlated with an inability of those macrophages to stimulate Listeria-specific T lymphocytes when exposed to heat-killed Listeria. The macrophages obtained from 4-, 10-, and 15-d-old mice exhibited a reduction to 4.5, 8.4, and 11%, respectively, of the level of the proliferation of Listeria-specific T cells incubated with adult macrophages *(973)*. These results demonstrate that peritoneal macrophages from neonatal mice do not present antigen efficiently and that this is correlated with small numbers of macrophages bearing MHC class II molecules.

Similarly, rat neonatal alveolar macrophages fail to express class II antigens. It is well known that IFN-γ stimulation enhances the expression of class II molecules. The failure of rat neonatal alveolar macrophages was not related to weaker expression of IFN-γ-receptor but rather occurs at transcription level. The signaling events mediated by IFN-γ receptor in alveolar macrophages from 7-d-old rats showed a significant dose-dependent increase of interferon responsive factor (IRF)-1 and IRF-2 expression in response to IFN-γ stimulation. However, the expression of CITA, a transactivator of MHC class II and of invariant chain, was low or undetectable in neonatal alveolar macrophages stimulated with IFN-γ *(974)*.

These findings suggest that low expression of class II molecules in neonatal macrophages may be related to low activation of transcription factors controlling the expression of class II molecules and the synthesis of the invariant chain. The invariant chain is important in class II processing. It binds to nascent class II heterodimers in endoplasmic reticulum and mediates the translocation of class II molecules to endosomes.

3.2. B Cells

The major function of B cells is to synthesize antibodies. In addition, B cells have the capacity to take up antigens, process them, and present the peptides, in association with class II molecules, to CD4 T cells. There are three major mechanisms by which B cells internalize antigen. The first mechanism consists of fluid-phase pinocytosis. The antigen engulfed via fluid pinocytosis are localized within endocytic vacuoles, which fuse with lysosomes. The degradation of antigen takes place in low-density endosomes and lysosomal-dense compartments. The generation of peptides from antigen that are internalized

via fluid pinocytosis is slow. It takes about 2 h for the peptides to be expressed on the membrane in association with class II molecules (reviewed in ref.*966*). The process of presentation of antigen taken up by fluid pinocytosis is enhanced by heat shock, which leads to the induction of Hsp70 protein. B cells incubated several hours at 42°C were able to present peptides from purified class II antigen more efficiently than B cells maintained at 37°C *(975)*. This may result from enhanced binding of peptides to MHC molecules mediated by Hsp70, which is a chaperone protein. Alternatively, the heat shock may affect the assembly of the processed Ag-class II complex rather than the quantity of peptide available for class II binding.

The second mechanism of the internalization of antigen within B cells occurs via immune complexes. This is a nonspecific mechanism mediated by the Fc and complement receptors (CRs). Both FcγR and CR2 are expressed by normal B cells. The formation of immune complexes promotes activation of the C classical pathway, which consists of fixation of C3dg onto antibody that interacted with the antigen, and the internalization of complex via CR2. The processing of antigen internalized as immune complexes via FCγR and CR2 leads to peptide-MHC complexes on the cell surface within 15 min after internalization. Thus, this pathway is more efficient than fluid-phase pinocytosis. The binding of immune complexes to both FcR and CR2 favors the expression of CD80, which is a costimulatory molecule required for T-cell activation *(976,977)*.

The third mechanism for antigen internalization is antigen-specific because the internalization is mediated by the B-cell receptor (BCR). The transmembrane region of the BCR is involved in both the internalization process and the intracellular trafficking of BCR-antigen complex *(978)*. The processing of antigen internalized via the BCR and fluid pinocytosis may be different, because the two antigen presentation pathways are differentially inhibited by emitin, a protein inhibitor, and by brefaldin A, which blocks protein export to endosomes from the endoplasmic reticulum. These observations suggest that the internalization of antigen by these different mechanisms targets the antigen to different cellular compartments with the result that they are then processed differently and presented with different efficacy.

Study of the expression of class II molecules on murine fetal cells from 16-d-old embryos showed that the B-cell progenitors, pre-B cells, and immature B cells lack MHC class II expression as assessed by flow cytometry and PCR *(979)*. This is in sharp contrast with pre-B cells from the bone marrow of adult mice, in which approx 90% express class II molecules *(980)*. Cell surface expression of class II molecules was measurable at birth and increases rapidly, reaching the level of adult by d 10 *(981)*. However, acquisition of the ability to process the antigen occurs later—at 18 d in the case of antigens internalized by fluid-phase pinocytosis and at 28 d in the case of antigens internalized via the BCR *(982)*.

The expression of class II molecules in neonatal B cells is increased by culturing the cells in presence of anti-IgM antibodies and interleukin (IL)-4 but not by anti-IgM antibodies alone *(983)*.

A cursory glance at the current evidence indicates that ability of neonatal B cells to process and present antigen is weaker than that of adult B cells. This was clearly shown in an experiment comparing the ability of young (3- to 28-d-old) and adult B cells to stimulate the proliferation of antigen-specific T cells *(984)*. The T-cell proliferation response using B cells from 3-d-old mice was less than 20% of the adult response, and adult-like presentation was not seen until the mice were 28 d *(984)*. It is still not clear whether the weaker capacity of neonatal B cells to present antigen is related to the fact that in immature B cells, class II molecules contain smaller amounts of peptides or that immature B cells do not develop class II transactivator CITA compared to adult B cells.

3.3. Dendritic Cells

Dendritic cells are the most potent class of professional APCs. In mice, dendritic cells are a heterogenous population characterized by two functionally different stages of differentiation. Immature dendritic cells are able to capture antigen but display poor capacity to stimulate naïve T cells. After the uptake of antigen and activation by microbial and inflammatory stimuli, the dendritic cells maturate, a process associated with upregulation of the expression of class II and costimulatory molecules and ability to stimulate the proliferation of T cells.

Dendritic cells isolated from 3-d-old mice exhibit a similar morphology as those isolated from the spleen of adult mice, but fail to express the dendritic cell-specific marker CD11c *(984)*. In contrast, dendritic cells from 7-d-old mice express CD11c and similar levels of MHC class II and CD40, CD80 and CD86 costimulatory molecules as adult dendritic cell *(960)*. The plasmocytoid CD11c and $CD8\alpha^+$ are completely absent at birth and they gradually appear between 3 to 21 d of age (2 to 3% in 3 to 7-d old, 11 to 16% in 14-d and 21-d-old) reaching the level of adult mice (25%) at d 28 *(984)*. Following in vitro stimulation with lipopolysaccharide (LPS), neonatal dendritic cells mature rapidly like the adult dendritic cells with marked increase in surface expression of class II, CD40, CD80 and CD86 molecules *(960)*.

Neonatal and adult immature dendritic cell take up antigen with similar efficacy as assessed by the internalization of FITC-dextran. This demonstrates the efficient endocytic capacity of neonatal dendritic cells *(960)*.

Study of antigen presentation by neonatal dendritic cell was investigated by measuring the ability to stimulate T cells specific for foreign or alloantigens. These studies showed that dendritic cell isolated from mice which were less than 28 d old were less effective in stimulating the proliferation of T cells *(984)*. Similarly, human dendritic cells from cord blood are less effective than

adult dendritic cells at supporting the proliferation of T cells in response to antigeneic or allogeneic stimulation. The mechanism responsible for poor stimulatory capacity of cord blood dendritic cells is unclear, however, it might result from reduced expression of class II molecules *(985)*.

Langerhans cells (LCs) represent a subset of dendritic cells located in the skin. LCs have been identified in fetal skin at d 19 of gestation by their expression of MHC class II molecules and cytological properties *(986)*. However, the Birbeck granules, a marker of maturation of LCs, are not detectable until d 4 postpartum *(987)*. Although LCs within neonatal epidermis from 3-d-old mice express MHC molecules at lower densities, they do not express the DEC205 molecule. DEC205 is first detected by d 7 after birth, and by age 14 d, both MHC class II and DEC205 expression are similar with adult skin. The expression of DEC205, which functions as an endocytic lectin-type receptor, correlates with antigen uptake of fluorescent haptens. Immaturity of neonatal LCs also correlated with a contact sensitivity response. Although the immune response of mice sensitized at age 14 d is not significantly different to that observed in adult mice, animals sensitized after birth or at age 7 d give significantly lower responses *(988)*.

These observations indicate that there is a sequential maturation of LCs after birth that is characterized by an initial expression of class II molecules, followed by occurrence of Bierbeck granules and the expression of DEC205 molecules, which correlates with a contact sensitivity response reaching the adult level by 14 d after birth. Taken together, these results suggest that there is a direct correlation between the expression of MHC class II molecules on neonatal APCs and their ability to function as efficient APCs. Neonatal dendritic cells rapidly acquire the phenotypic and functional properties of adult dendritic cells during postnatal life and are able initiate CMI responses.