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MHC class II antigen presentation and immunological abnormalities due to deficiency of MHC class II and its associated genes

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Abstract

Antigen presentation by Major Histocompatibility Complex (MHC) class II molecules plays an important role in controlling immunity and autoimmunity. Multiple co-factors including the invariant chain (Ii), HLA-DM and HLA-DO are involved in this process. While the role for Ii and DM has been well defined, the biological function of DO remains obscure. Our data indicate that DO inhibits presentation of endogenous self-antigens and that developmentally-regulated DO expression enables antigen presenting cells to preferentially present different sources of peptide antigens at different stages of development. Disruption of this regulatory mechanism can result in not only immunodeficiency but also autoimmunity. Despite the fact that deletion of each of the three genes in experimental animals is associated with profound immunological abnormalities, no corresponding human diseases have been reported. This discrepancy suggests the possibility that primary immunodeficiencies due genetic defect of Ii, DM and DO in humans are under diagnosed or diagnosed as “common variable immunodeficiency”, a category of immunodeficiency of heterogeneous or undefined etiology. Clinical tests for any of these potential genetic defects are not yet available. We propose the use of multi-color flow cytometry in conjunction with intracellular staining to detect expression of Ii, DM, DO in peripheral blood B cells as a convenient reliable screening test to identify individuals with defects in antigen presentation.

Keywords

MHC class II; antigen presentation; invariant chain; HLA-DO; HLA-DM; B lymphocytes; dendritic cells

Introduction

The immune system protects us from harmful microbial infections; and such protection results from the interplay between innate and acquired (adaptive) immunity, both of which involve differential recognition of infectious non-self from self. While innate immunity relies upon pattern-recognition receptors, such as Toll-like receptors (TLR), to broadly sense offensive signals (Medzhitov, 2001), adaptive immunity utilizes far more specific antigen receptors, expressed by B and T cells, to better discriminate various antigenic epitopes in order to achieve specific immunity and immunological memory. The generation of specific high-affinity B cell

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antigen receptors (BCR) involves somatic hyper-mutation and isotype switching of the immunoglobulin genes and is dependent on CD4 T cell help. Similarly, generation of potent CD8+ cytotoxic effector or memory T cells also requires participation of CD4 T cells. In addition, a special subset of CD4 T cells, namely the naturally arising Foxp3+ regulatory CD4 T cells (nTreg) are now known to play an essential role in preventing autoimmunity by suppressing potentially pathogenic autoreactive lymphocytes that are present at high frequency in a normal repertoire (Sakaguchi and Powrie, 2007). Therefore, to a great extent, CD4 T cells control immunity and autoimmunity. Both helper and regulatory functions of CD4 T cells involve antigen recognition through their antigen receptors (TCR). Unlike B-lymphocytes, CD4 T cells do not recognize native but only processed peptide antigens presented by antigen presenting cells (APC) in the context of the major histocompatibility complex class II (MHC-II). Since APCs present both self and non-self peptides, and recognition of peptide antigens can either activate or inactivate CD4 T cells, or induce Treg differentiation, depending on the phenotype of the APCs as well as the milieu where antigen presentation occurs, how antigens are presented, therefore, ultimately determines the outcome of an immune response. As discussed below, normal antigen presentation involves cooperation of multiple components of the class II antigen-processing pathway; and loss of function of any of the components, as demonstrated in gene-knockout animals, can give rise to severe immunodeficiency as well as autoimmunity. Herein, we review some major aspects of MHC-II antigen processing as well as the immunological abnormalities associated disrupted MHC-II antigen presentation due to loss of each of the major co-factors involved in the pathway.

MHC-II antigen processing/presentation pathway

Professional APCs such as dendritic cells and B-lymphocytes constitutively express MHC-II, which are composed of $\alpha\beta$ heterodimers associated with self or antigenic peptides situated in their peptide-binding grooves. Some other cell types such as vascular endothelial cells, renal tubule cells or T cells can be induced to express MHC-II during infection through the action of IFN- γ the biological function of induced expression of MHC-II is not yet clear. A “master” transcription activator, CIITA, plays a major role in regulating the expression of MHC-II. CIITA activates transcription of not only the MHC-II structure genes, HLA-DR, DP and DQ, but also of the class II-associated genes including the invariant chain (Ii), HLA-DM and HLA-DO (Chang and Flavell, 1995; Nagarajan et al., 2002; Westerheide et al., 1997). Ii functions as a chaperon facilitating the assembly and transport of nascent class II molecules through the Golgi to the endosomal pathway (Bakke and Dobberstein, 1990). In the late endosome/lysosome, Ii is subjected to stepwise cleavage by proteases, leaving a short peptide, CLIP, in the peptide-binding groove of the MHC-II (Riese et al., 1996). Prior to transport to the plasma membrane, CLIP is removed and replaced by other peptides through the action of HLA-DM, an $\alpha\beta$ heterodimer encoded in the class II region of the MHC. DM not only replaces CLIP but also promotes peptide exchange between non-CLIP peptides, leading to formation of stable peptide-MHC-II complexes (Weber et al., 1996). In the absence of DM (or H2-M, the murine analog), peptide exchange is impaired; and as a result, almost all the class II molecules expressed on the cell surface are occupied by CLIP (Denzin and Cresswell, 1995) (Fung-Leung et al., 1996; Martin et al., 1996; Miyazaki et al., 1996). Therefore, DM is essential for antigen presentation. In B-lymphocytes, DM is found to be physically associated with HLA-DO, another nonpolymorphic $\alpha\beta$ heterodimer encoded in the class II region. DO must bind DM because free DO is unstable. In essence, DM functions as a chaperone required for the initial folding of DO, and it may also be required to maintain the DO protein in a stable conformation. In H2-M-knockout mice, no H2-O (murine DO) is detectable in B cells (Chen et al., 2006) because unstable or misfolded H2-O is degraded in the endoplasmic reticulum. The finding of DM in association with DO is unexpected because DO is found to be a strong inhibitor of DM function, both in vitro and in vivo by independent investigators (Denzin et al., 1997; Fallas et al., 2004; Liljedahl et al., 1998; van Ham et al., 2000). To accommodate this paradoxical

finding, it was once proposed that DO might specifically modify B cells' antigen presentation function so that B cells would only present specific antigens to T cells (Alfonso et al., 1999). This idea, though appealing, is not supported by subsequent studies (Alfonso et al., 2003a; Alfonso et al., 2003b) including our own showing that DO expression is highly regulated during B cell development and activation (Chen et al., 2002) and that its expression is actually not restricted to B-lymphocyte as previously reported but occurs also in primary dendritic cells (Chen et al., 2006; Hornell et al., 2006).

Expression of DO during B cell development and in dendritic cells

All developing B cells (dBC) including pro- and pre-B cells in human bone marrow express MHC-II despite the fact that MHC-II expression is not required for B cells development (Chen and Jensen, 2004; Cosgrove et al., 1991; Grusby et al., 1991; Madsen et al., 1999; Reith and Mach, 2001). As compared to mature B cells (mBC), however, dBCs present very little CLIP (Chen et al., 2002), suggesting that most CLIP have been removed from emerging MHC-II and replaced with other self-peptides before the class II complexes appear on the cell surface. The high levels of CLIP complexes on mBCs, on the other hand, implies that peptide exchange activity in mBCs is so slow that at a proportion of the MHC-II molecules do not even undergo a single round of peptide exchange before appearing on the cell surface. The attenuation in peptide-exchange in mBCs is not due to absence of DM, since a high level of DM protein is detected in mBC; rather, the reduction in peptide-exchange appears to result from expression of HLA-DO, which is identified only in mBCs and not in dBCs (Chen et al., 2002). Therefore, dBCs selectively express DM but not DO, and preferentially present non-CLIP self-peptides. In addition, dBCs express very little or no CD40 and costimulatory molecules, a characteristic of tolerogenic APCs. These findings, taken together, suggest that, by selectively expressing DM but not DO, d-BC are enabled to preferentially present endogenous self-peptide antigens, possibly as a mechanism to induce peripheral T-cell tolerance to self-components that are uniquely presented at this stage of the B cell development.

The finding that DO is expressed selectively in mBCs but not d-BCs suggests that mBC may need DO to inhibit presentation of endogenous self-antigens in order to maximize their ability to present exogenous antigens. This change in antigen presentation might be important for two reasons. First, presentation of high levels of self-antigen by mBC might induce autoimmunity since mBCs have the capacity to activate pathogenic autoreactive T cells when presenting high levels of self-peptides (Seamons et al., 2006). Secondly, mBC need to efficiently present exogenous antigens in order to obtain optimal CD4 T cell help. How can DO, an inhibitor of DM, selectively inhibit presentation of endogenous self-peptide antigen but promote presentation of exogenous antigens? Observations made in previous studies suggest that the binding of DO to DM is temporally orchestrated such that DO only bind to DM in the early aspect of the endosomal pathway, and in the late aspect of the pathway, DODM complexes dissociate, resulting in degradation of free DO due to its intrinsic instability, and generation of free DM. These observations include i) only about half of the DM molecules in B cells are associated with DO (Chen et al., 2002; Kropshofer et al., 1998), ii) the DO to DM ratio is high in the early but low in the late aspect of the endocytic pathway (Gondre-Lewis et al., 2001), and iii) an increase in DO synthesis in DO transgenic mice is associated with both increased generation of DODM complexes as well as free DM (Fallas et al., 2004). Thus, DO might only inhibit DM function in the early aspect of the endosomal pathway, eliminating or slowing down the loading of endogenous self-peptides onto nascent emerging MHC-II. As a result, formation of stable class II complexes with endogenous high-affinity self-peptides is prevented prior to transport to the late endosomal compartments where antigens internalized through the B cell antigen receptor (BCR) are enriched. In late endosomal compartments, free DM is generated, which then can actively catalyze loading of exogenous peptides onto the emerging MHC-II that remain accessible for peptide exchange due to the lack of DM function in the early

components of the endocytic pathway. Through this mechanism, as an inhibitor of DM, DO may enhance presentation of exogenous antigens.

Similar observations have been made regarding DO expression in dendritic cells (DC). While early studies indicated that DC did not express H2-O based on immunofluorescence microscopy with frozen spleen sections, we and others have unequivocally identified DO expression in DC by flow cytometry in conjunction with intracellular staining and immunoblotting (Chen et al., 2006; Hornell et al., 2006). Failure to detect DO expression in the early studies might be due to the fact that the frequency of DCs in lymphoid organs is too low for detection by immunofluorescence microscopy. Flow cytometry, however, can be used to quantify DO expression in DC on single cell basis. Interestingly, similar to the observed differences in DO expression in mBC and dBC, only mature immunogenic conventional (myeloid) DC (mDC) but not tolerogenic immature plasmacytoid DC (pDC) express H2-O, and pDCs only express H2-M, suggesting that H2-O expression in mDC and absence of expression in pDC may serve a similar function as in mBC and dBCs.

The immunological abnormalities associated with deficiency of MHC-II and its associated co-factors

The importance of each of component of the class II pathway is further illustrated in gene-knockout mice. To be familiar to the phenotype of these animals might be important since none of corresponding genetic deficiencies in humans has yet been recognized, except for the bare lymphocyte syndrome.

i) MHC-II deficiency

MHC-II-deficient mice have been generated by targeted deletion of either the class II structural genes or the CIITA gene (Chan et al., 1993; Chang et al., 1996; Clausen et al., 1998; Cosgrove et al., 1991; Grusby et al., 1991; Madsen et al., 1999); and in these mice, CD4 T cell development is severely impaired. As a result, both cellular and humoral immunity are defective. Although B-lymphocytes develop normally and are capable of terminal differentiation to plasma cells, they fail to generate germinal center responses for the production of high affinity antigen-specific IgG antibodies. As described below, the phenotype of MHC-II-knockout mice is very similar to that of the human MHC-II deficiency known as bare lymphocyte syndrome type II (BLS).

ii) Invariant chain deficiency

Since Ii plays an essential role in the assembly and transport of nascent MHC-II, lack of Ii in Ii-knockout mice severely disrupts MHC-II synthesis. As a result, the Ii^{-/-} APCs express a very low level of MHC-II (Bikoff et al., 1993; Viville et al., 1993). APCs present protein antigens poorly, but have enhanced ability to present peptide antigens, which bind directly to cell surface MHC-II molecules. The number of CD4⁺ T cells is reduced, and so is the size of CD4 T cell repertoire (Wong and Rudensky, 1996). While the ability of Ii-deficient animals to respond to bacterial infection has not yet been determined, their Th-2 immunity is compromised (Topilski et al., 2002). The mice also show impaired ability to mount cellular and humoral immunity against viral infection (Battegay et al., 1996).

iii) H2-M deficiency

The APCs in H2-M^{-/-} mice express a normal level of cell surface MHC-II. However, the ability of H2-M^{-/-} APCs to present either protein or peptide antigen is severely compromised. Notably, the number of CD4 T cells H2-M^{-/-} mice is not remarkably reduced, indicating that presentation of a single peptide, CLIP, by thymic epithelial cells can effectively mediate

positive selection of a CD4 T cell repertoire with broad TCR specificities. Nevertheless, the CD4⁺ T cell repertoire of H2-DM-deficient mice, as compared to the wild type, appears different (Singh and Van Kaer, 1999), with the CD4 T cells from H2-M^{-/-} being highly reactive towards various self peptide epitopes presented by H2-M⁺ APCs (Surh et al., 1997). The antibody response in H2-M^{-/-} is slow, diminished in magnitude and composed primarily of low-affinity IgM Abs (Alfonso et al., 2001), although the severity of immunodeficiency varies with MHC haplotype (Koonce et al., 2003). The dependence on MHC haplotype can be attributed to the variable affinity of different MHC-II molecules for CLIP. Molecules with low affinity for CLIP can spontaneously release CLIP, provided an opportunity to (inefficiently) bind and present antigenic peptides.

iv) HLA-DO deficiency

As discussed above, the biological function of DO remains obscure. Our finding that DO is selectively expressed in the mature but not immature APCs and that its expression is associated with an attenuation in the presentation of self-peptides lead us to hypothesize that DO enhances the ability of mature APCs to present exogenous antigen by limiting presentation of endogenous self-peptides. To test this hypothesis, we have studied H2-O knockout mice for evidence of immunodeficiency and autoimmunity. Our preliminary results (data not shown) demonstrate that the H2-O deficient mice have impaired ability to generate humoral immune responses to various protein antigens. The antibody responses are slow and reduced in magnitude, affecting all Ig classes and subclasses. While the CD4 T cells from H2-O^{-/-} mice respond normally to in vitro TCR-crosslinking, H2-O^{-/-} APCs present exogenous antigens poorly, suggesting that the immunodeficiency identified in H2-O^{-/-} mice is due to impaired antigen presentation rather than an intrinsic T cell abnormality. Compared to wild-type APC, the level of CLIP presented on H2-O^{-/-} APC is lower, suggesting that these cells present more non-CLIP self-peptides than wild type APCs. Despite the impaired ability to generate antibody response against foreign antigens, the H2-O^{-/-} mice, as they grow older, spontaneously generate high titers of IgG antinuclear autoantibodies in their sera, which include anti-double-stranded-DNA antibodies. The development of autoimmunity is associated with an increase in the frequency of CD44^{hi} activated CD4 T cells in the spleen. Therefore, it appears that immunity in H2-O deficiency is deviated in focus from foreign to self, resulting in the development of autoimmunity at the expense of protective immunity against foreign antigens. Our preliminary results thus support the hypothesis deficiency of H2-O promotes presentation of endogenous self-antigens.

Human diseases associated with primary class II deficiency

i) MHC-II deficiency – bare lymphocyte syndrome

The critical role of MHC-II-mediated antigen presentation in immunity is best illustrated in the human disease, bare lymphocyte syndrome type II (BLS), an autosomal recessive disorder formally named as MHC-II deficiency by the World Health Organization. BLS is manifested as severe combined immunodeficiency (scid) affecting both cellular and humoral immunity (Reith and Mach, 2001) (Herve et al., 2007). The patients usually present at neonatal life with recurrent overwhelming bacterial, viral and fungal infections, which often lead to death before 4 years of age (Klein et al., 1993). The lack of MHC-II expression in BLS results from homozygous mutations in the transcription factors that coordinately control the expression of the class II genes (Nekrep et al., 2003). Therefore the expression of all the class II genes is affected. BLS due to mutation of each of MHC-II structural genes has not been reported, suggesting that loss of expression of either DR, DP or DQ alone may not be sufficient to give rise to BLS phenotype. It is not yet clear, however, whether loss of each of the structure genes, though not manifested as BLS, is associated with a milder form of immunodeficiency such as the common variable immunodeficiency, as discussed below.

The diagnostic tests for BLS involve measurement of the expression of MHC-II (including DR, DP and DQ) at the protein and RNA level (Reith and Mach, 2001). Bone marrow transplantation (BMT) constitutes the mainstay curative therapy for BLS, which successfully restores functional CD4 T cells and the immune repertoire (Klein et al., 1995). The success of BMT raises a question regarding the mechanism of CD4 T cell development in the BSL recipient, since bone marrow-derived cells in mice are thought to be incapable of mediating positive selection of CD4⁺ T cells in the thymus (Markowitz et al., 1993; Martinic et al., 2006). A possible answer is provided by the finding that human (but not mouse) developing thymocytes and activated CD4 T cells express CIITA and MHC-II (Marinova et al., 2001; Park et al., 1992). Furthermore, transgenic expression of MHC-II selectively in mouse T-lineage cells or thymocytes can induce development of a functional CD4 T cell repertoire in the transgenic mice (Choi et al., 2005; Li et al., 2005). These results suggest that MHC-II-expressing thymocytes might have a capacity to mediate positive selection of CD4 T cells in the BLS patients post BMT.

Ii, DM or DO deficiency in humans

Despite the profound immunological abnormalities associated with deletion of the Ii, DM or DO genes in knockout mice, no human diseases have been reported resulting from defects in these genes (Fischer, 2004) for reasons not yet clear. One possibility is that mutations involving these genes are so rare that it has not been reported. The expected frequency of homozygous mutation for each of the genes, however, should be similar to that of other common autosomal-recessive disorders, if the heterozygous are phenotype-free. It is very unlikely that the homozygous mutants in humans are not associated with any immunological abnormalities since all the three genes are highly conserved and serve an important role in regulating MHC-II antigen presentation. Another possibility is that the affected individuals are diagnosed as “common variable immunodeficiency” (CVID), the most common primary immunodeficiency in humans. As an entity of primary immunodeficiency, CVID actually represents a group of diseases of heterogeneous etiology, including those that lack definitive genetic causes (Notarangelo et al., 2004). Although over the last few years, genetic defect involving ICOS, TACI, BAFF-R and CD19 have been identified in some cases of CVID (Salzer and Grimbacher, 2006), the underlying genetic or immunological basis for the disease with most patients remains unknown. Clinically, CVID is manifested as hypogammaglobulinemia, presenting with recurrent bacterial infection, associated in some patients with autoimmune phenomena. The hypogammaglobulinemia results primarily from impaired production of IgGs and/or IgA, but not IgM. Consistently, germinal center response and memory B cells generation are impaired in many patients (Wehr et al., 2007).

The features of the immunological abnormalities in CVID are consistent with impairment in T cell-B cell interactions. Thus it is possible that in some patients abnormalities in MHC-II antigen presentation due to various genetic defects involving Ii, DM or DO genes could account for the phenotype. Indeed, such a possibility cannot be ruled out until expression of each of these genes in CVID patients is examined. However, no clinical tests to screen for Ii, DM, or DO deficiency are currently available. We have developed a flow cytometry test to detect expression of DM, DO and Ii in B cells (figure 1) (Chen et al., 2002). Combining cell surface and intracellular staining with specific monoclonal antibodies, flow cytometric assays can unequivocally identify expression of DM, DO and Ii in peripheral blood B cells, and this could be readily adopted into the clinical laboratory setting as convenient screen for Ii, DM, or DO deficiency in patients with CVID.

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Figure 1. Identification of expression of HLA-DR, CLIP, DM, DO and Ii by flow cytometry
Whole blood cells are first stained on surface with anti-CD19 (a B cell marker) or CD14 (monocytic marker) plus HLA-DR and CLIP. The cells are then fixed, permeabilized and stained intracellularly for DM and DO, followed by flow cytometry analysis that unequivocally reveals the expression DR, CLIP, DM and DO in CD19+ B cells (A–). The monocytes also express DR (E) at a slightly lower level as compared to B cells, but the relative level of CLIP is much lower (F) than on B cells (B). This is because monocytes only express DM but not DO (not shown). The CLIP level on B cells may vary with DR-haplotypes, with some haplotypes such as DR401 associated with very low level of CLIP. If no CLIP are detectable on B cells, it then becomes necessary to check for Ii expression intracellularly to rule out Ii deficiency. Ii expression in mouse B cells can be revealed by antibody IN-1 (G). Several anti-human Ii antibodies are available including PIN-1 and CD74. We are testing these antibodies for identification of Ii in human B cells.