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CDw78 Defines MHC Class II-Peptide Complexes That Require Ii Chain-Dependent Lysosomal Trafficking, Not Localization to a Specific Tetraspanin Membrane Microdomain

Neil J. Poloson,* Lisa K. Denzin,† and Paul A. Roche‡*

MHC class II molecules (MHC-II) associate with detergent-resistant membrane microdomains, termed lipid rafts, which affects the function of these molecules during Ag presentation to CD4+ T cells. Recently, it has been proposed that MHC-II also associates with another type of membrane microdomain, termed tetraspan microdomains. These microdomains are defined by association of molecules to a family of proteins that contain four-transmembrane regions, called tetraspanins. It has been suggested that MHC-II associated with tetraspanins are selectively identified by a mAb to a MHC-II determinant, CDw78. In this report, we have re-examined this issue of CDw78 expression and MHC-II-association with tetraspanins in human dendritic cells, a variety of human B cell lines, and MHC-II-expressing HeLa cells. We find no correlation between the expression of CDw78 and the expression of tetraspanins CD81, CD82, CD53, CD9, and CD37. Furthermore, we find that the relative amount of tetraspanins bound to CDw78-reactive MHC-II is indistinguishable from the amount bound to peptide-loaded MHC-II. We found that expression of CDw78 required coexpression of MHC-II together with its chaperone Ii chain. In addition, analysis of a panel of MHC-II-expressing B cell lines revealed that different alleles of HLA-DR express different amounts of CDw78 reactivity. We conclude that CDw78 defines a conformation of MHC-II bound to peptides that are acquired through trafficking to lysosomal Ag-processing compartments and not MHC-II-associated with tetraspanins.

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Abbreviations used in this paper: MHC-II, MHC class II; pMHC-II, peptide-loaded MHC-II; DC, dendritic cell; IF, immunoprecipitate; siRNA, small interfering RNA; Ii, invariant chain; cRPMI, complete RPMI; cDMEM, complete DMEM.
epitope on MHC-II), was specific for MHC-II associated with tetraspanin proteins on APCs (6). MHC-II isolated using the CD78 mAb FN-1 displayed a very restricted peptide repertoire, which, depending on the HLA-DR allele examined, was predominately the class II-associated II chain peptide (CLIP) or peptides derived from endogenous HLA-A2 (6). The evidence for the relationship between CD78 expression and MHC-II tetraspanin association was 2-fold: 1) MHC-II isolated using anti-CD78 mAb were disproportionately associated with tetraspanin molecules as compared with the bulk of MHC-II in the cell; and 2) CD78 expression was abolished by saponin, a detergent that has been shown to disrupt tetraspanin-tetraspanin interactions (6, 26).

Given our longstanding interest in mechanisms of MHC-II association with membrane microdomains, we have re-examined the relationship between CD78 expression and the association of MHC-II with tetraspanins. We have found that CD78 is expressed on all MHC-II7 B cell lines examined, on human monocyte-derived dendritic cells (DCs), and even on heterologous cells expressing MHC-II. In stark contrast to the previous reports of Kropshofer et al. (6), we found that CD78 does not uniquely identify tetraspanin-associated MHC-II. Furthermore, we find that the CD78 epitope is heavily influenced by expression of Ii but does not correlate with expression of MHC-II-CLIP complexes on the cell surface. Thus, CD78 expression is not indicative of MHC-II-tetraspanin associations, and therefore, our study calls into question data using CD78 expression as an indicator of MHC-II-tetraspanin interactions (6, 26).

**Materials and Methods**

**Cell lines and plasmids**

The human B cell lines JY, .45, Pala, Raji, WI-L2, and T1 were maintained in RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 10 mM HEPES, and 50 μg/ml gentamicin (crPMI). The T2 transfectants T2.DR4, T2.DR4.DM, T2.DR11, and T2Dr11.DM have been described previously (27). T2.DR1, T2.DR3, and T2.DR.3.DM were gifts from Dr. P. Cresswell (Yale University, New Haven, CT). BJAB transfectants were maintained in IMEM containing 0.5 μg/ml puromycin. HeLa cells were maintained as a semiconfluent monolayer in dmEM. HeLa-CIITA was a gift from P. Cresswell and maintained as a semiconfluent monolayer in dmEM containing 2 μg/ml puromycin. PBMCs were obtained from the National Institutes of Health Blood Bank and differentiated into DCs in the presence of GM-CSF and IL-4 as described by Segal and colleagues (28). At day 6, DCs were recovered, analyzed for purity, and incubated with 1 μg/ml LPS for 18 h to induce DC maturation.

Plasmids encoding full-length cDNA clones for HLA-DR1 α- and β-chains in the mammalian expression vector CMD8 (29) and the human p33 Ii cDNA in pcDNA3 (30) have been described. A cDNA encoding a truncation mutant lacking the amino-terminal 15 aa of human Ii (31) was subcloned into the Xbal site of CMD8.

**Antibodies**

Abs recognizing HLA-DR-α-chain complexes (L243; BD-Pharmingen), HLA-DR-CLIP (Cer.CLIP; BD Pharmingen), HLA-DM (BD Pharmingen), CD81 (Coulter), CD82 (Diaclone, for immunoblotting; clone 50F11 from S. Shaw (National Cancer Institute, Bethesda, MD) for flow cytometry), CD9 (BD Pharmingen for flow cytometry; Diaclone for immunoblotting), CD53 (Diaclone), CD37 (BioSource International), and B7-2 (BD Pharmingen) were obtained for use in this study. The Alexa-488-conjugated anti-HLA-DO Ab AB55 has been described previously (32). Abs recognizing CD1a, CD81, CD82, CD9, and CD53 were obtained from Southern Bio-technology Associates. mAb recognizing CD9, CD53, and CD82 (Diaclone) were biotinylated using EZ-link NHS biotin kit (Pierce) according to the manufacturer’s instructions and dialyzed before use. The FN-1 hybridoma was a gift from Dr. S. Funderud (Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway), and maintained in crPMI. mAb FN-1 was purified using protein G-Sepharose (Pierce) and dialyzed in PBS before use.

**Flow cytometry**

Cells were washed twice in staining buffer (HBSS containing 2% FBS) and adjusted to ~5 x 10^6 cells/ml for staining. Primary Abs were incubated with cells for 20 min on ice and washed twice. A specific secondary Ab or Alexa-546-conjugated streptavidin was then incubated for 20 min, washed thrice, and analyzed by flow cytometry. DC phenotyping was done by preincubating DCs with human IgG (20 μg/ml) for 20 min and then adding directly conjugated specific mAb for 20 min on ice. All cells were washed and analyzed as above. Cells were also analyzed by intracellular staining for HLA-DO. For this purpose, cells were fixed in PBC containing 2% paraformaldehyde, permeabilized (with 0.5% saponin), stained with an Alexa-488-conjugated HLA-DO mAb or an isotype control mAb in buffer containing 0.1% saponin, washed, and analyzed by flow cytometry as described above.

**Small interfering RNA (siRNA) knockdown and transfections**

Control siRNA and siRNA specific for CD82 were obtained from Ambion. siRNA for CD81 were designed as previously described (33) and purchased from Operon. Oligofectamine and Lipofectamine 2000 were obtained from Quigen. HeLa cells expressing CIITA were plated in 6-well plates and transfected using Oligofectamine per the manufacturer’s directions (100 nM siRNA/well). Forty-eight hours after transfection, cells were harvested and analyzed by flow cytometry and SDS-PAGE/immunoblotting. HeLa cell transfections were performed using Lipofectamine 2000 as described previously (34). Cells were analyzed 24–48 h after transfection by flow cytometry.

**Immunoprecipitation and immunoblotting**

Unless otherwise indicated, all cells were lysed at 10 x 10^6 cells/ml in a detergent solution of 1% BRJ-58 in 10 mM Tris and 150 mM NaCl (pH 7.4) containing protease inhibitors (50 μM PMSF, 0.1 μM Nα-p-tosyl-l-lysine chloromethyl ketone, 5 μM iodoacetamide, and 10 μg/ml aprotinin) (lysis buffer) for 1 h on ice. Cell lysates were cleared of nuclei by brief centrifugation at 13,000 g for 1 min and precleared using protein A-agarose beads (Sigma-Aldrich) for 1 h at 4°C. Preclared lysates were incubated overnight at 4°C with specific mAb bound to rabbit anti-mouse Ig coupled to protein A-agarose beads. Immunoprecipitates (IPs) were washed twice in cell lysis buffer, twice in 1/10 dilute lysis buffer (diluted into 10 mM Tris and 150 mM NaCl at pH 7.4), boiled in SDS-PAGE sample buffer under nonreducing conditions, separated by SDS-PAGE, and transferred to polyvinylidene difluoride membranes (34). Nonpecific protein binding sites on polyvinylidene difluoride membranes were blocked overnight in PBS containing 1% nonfat dry milk and 0.1% Tween 20. Immunoblots were probed with unconjugated primary Abs followed by H chain-specific, HRP-conjugated goat-anti-mouse Ig. When using biotinylated mAb, the primary biotinylated mAb was revealed using streptavidin-conjugated to HRP. Blots were developed by ECL using ECL reagent and developed using standard x-ray film (Kodak X-OMat) or with a Fuji-film charge-coupled device camera. Blots were quantitated using MultiGauge software (Fuji-film).

**Results**

**Expression of CD78 and tetraspanins on maturing DCs**

Tetraspanins have been proposed to organize MHC-II on the cell surface into specific domains, which display a specific epitope, CD78, that can be recognized by mAbs (6). During the course of DC maturation CD78 immunoreactivity increases, leading Kropshofer et al. (6) to propose that the association of MHC-II with tetraspanins increases during this process. To further clarify the relationship between CD78 expression and MHC-II-tetraspanin association in DCs, we examined the cell surface expression of CD78, MHC-II, and various tetraspanins on human monocyte-derived DCs. Immature DCs express high levels of MHC-II, CD81, CD82, CD9, and CD53, but only small amounts of CD78 (Fig. 1A). After activation with LPS, DCs up-regulate MHC-II, B7.2, MHC-II-CLIP complexes, and CD78 (Fig. 1A). Although CD78 expression increases dramatically during DC maturation, expression of individual tetraspanins are either unaltered or slightly diminished (Fig. 1A). These data demonstrate that CD78
up-regulation on the cell surface is not simply a consequence of increased tetraspanin expression on mature DCs.

To determine whether the increase in CDw78 expression was a consequence of increased association of MHC-II with tetraspans, we analyzed MHC-II and CDw78 IPs from mature and immature DCs using a mAb that recognizes peptide-loaded MHC-II (35) or the anti-CDw78 mAb FN-1. Although the absolute amount of MHC-II expressed on the cell surface increased in activated DCs, the relative amount of tetraspans bound per MHC-II in mature DCs actually decreased relative to that observed in immature DCs (Fig. 1). Most importantly, the amount of tetraspans bound to the MHC-II in the CDw78 IP appeared indistinguishable from the amount bound in the anti-pMHC-II IP (Fig. 1). Based on these data, we conclude that the increase in CDw78 expression during DC maturation is not due to an increase in MHC-II-tetraspanin complexes, and the anti-CDw78 Ab FN-1 does not exclusively recognize MHC-II-tetraspanin complexes in DCs.

CDw78 MHC-II is not enriched for tetraspanin binding

The original idea that CDw78 specifically recognizes a subset of MHC-II preferentially bound to tetraspans was based on immunoprecipitation studies from human B cell lines (6). Given our results using monocyte-derived DCs in which CDw78 reactive (FN-1) and anti-CDw78 mAb FN-1. Although the absolute amount of MHC-II expressed on the cell surface increased in activated DCs, the relative amount of tetraspans bound per MHC-II in mature DCs actually decreased relative to that observed in immature DCs (Fig. 1). Most importantly, the amount of tetraspans bound to the MHC-II in the CDw78 IP appeared indistinguishable from the amount bound in the anti-pMHC-II IP (Fig. 1B). Based on these data, we conclude that the increase in CDw78 epitope expression during DC maturation is not due to an increase in MHC-II-tetraspanin complexes, and the anti-CDw78 Ab FN-1 does not exclusively recognize MHC-II-tetraspanin complexes in DCs.

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Effect of siRNA knockdown of CD81 and CD82 on CDw78 expression

To examine the relationship between CDw78 expression and tetraspanin levels on a variety of MHC-II+ cells, we compared various B cell lines for both CDw78 and tetraspan expression by FACS. All B cell lines examined were CDw78+ but differed widely in the expression of certain tetraspans. The tetraspanin CD9 was not detectable on several lines, and thus this tetraspanin is not strictly required for CDw78 expression (data not shown). We
also investigated tetratraspin and CDw78 expression on HeLa cells stably expressing the CIITA. HeLa-CIITA cells express MHC-II as well as the MHC-II accessory proteins HLA-DM and Ii (data not shown). HeLa-CIITA cells are MHC-II and CDw78 but expresses minimal levels of the tetratraspins CD37 and CD53 at the cell surface, demonstrating that these tetratraspins are also not absolutely required for CDw78 expression (Fig. 3A).

CD81 and CD82 are abundant tetratraspins in APCs and were expressed (to varying extents) on all CDw78 cell lines examined. To investigate whether CD81 and CD82 regulate CDw78 expression, we used siRNA to knockdown expression of CD81 and CD82 both individually and simultaneously in HeLa cells expressing CIITA (Fig. 3B). siRNA directed against either of these molecules resulted in 70–90% reduction in their expression as measured by flow cytometry or immunoblot analysis (Fig. 3B and data not shown). By contrast, knockdown of CD81 and CD82 had no effect on expression of MHC-II, CDw78, or class II-CLIP expression in these cells (Fig. 3B). These results, combined with those above, demonstrate that expression of these tetratraspins does not play a significant role in the formation of the CDw78 epitope.

HLA-DR allelic differences result in altered CDw78 expression

CDw78-associated MHC-II possesses a restricted set of peptides as compared with the entire pool of peptides eluted from MHC-II (2, 6). Our analysis of CDw78 expression in human DCs revealed donor-specific expression of CDw78 that did not correlate with the degree of spontaneous DC activation (data not shown). Furthermore, we found wide variations in CDw78 expression on different EBV-transformed B cell lines, suggesting that expression of CDw78 could be allele dependent. To examine this possibility in a well-controlled system, we examined CDw78 expression on a variety of MHC-II-transfected T2 cells. T2 is a mutant lymphoblastoid cell line lacking the entire class II region of the MHC (37), and thus T2-transfectants represents an ideal system to examine the requirement for MHC genes in CDw78 expression. As expected, T2 cells do not express MHC-II and are thus CDw78− (data not shown). We found no correlation between expression of total MHC-II and CDw78 in T2 cells expressing HLA-DR1, -DR3, -DR4, or -DR11 (Fig. 4, A and B). Since all MHC-II transfectants showed some level of CDw78 expression, these results demonstrate that CDw78 is not strictly HLA-DR allele specific. However, the data also clearly show that specific MHC-II alleles can influence CDw78 levels in B cells. Since T2 cells lack the peptide editor HLA-DM and the DM-regulator HLA-DO, this data suggest that other MHC-II-related genes are not absolutely required for CDw78 expression. Since the repertoire of peptides bound to MHC-II varies depending on the allele expressed (38, 39), these data suggest that expression of CDw78 may be more related to the presence of specific peptides bound to MHC-II than to the binding of MHC-II to tetratraspins.

HLA-DM and HLA-DO do not influence CDw78 levels

The repertoire of peptides bound to MHC-II on B cells can be influenced by the peptide editor HLA-DM and the HLA-DM regulator HLA-DO (40–42). To directly address the question whether modulation of R peptide-editor function in B cells influence CDw78 expression, we analyzed a panel of HLA-DM and HLA-DO B cell transfectants. Examination of T2 cells expressing HLA-DR3, -DR4, or -DR11 alone or together with HLA-DO revealed that expression of HLA-DM dramatically diminished surface CLIP levels but had absolutely no effect on CDw78 expression (Fig. 5A and data not shown).

To examine the role of HLA-DO in CDw78 expression we used BJAB HLA-DO transfectants. BJAB cells possessing very small amounts of HLA-DO express only small amounts of plasma membrane MHC-II-CLIP complexes yet these cells are CDw78− (Fig. 5B). Overexpression of HLA-DO in BJAB cells increased MHC-II-CLIP expression complexes on the cell surface, indicating that HLA-DO expression had inhibited HLA-DM-dependent CLIP editing in these cells. In agreement with our findings using HLA-DM transfectants, modulation of HLA-DM activity by overexpression of HLA-DO had no effect on surface expression of MHC-II or CDw78 (Fig. 5B). These data reveal that expression of MHC-II-CLIP is independent of cell surface expression of CDw78 and clearly demonstrate that HLA-DM and HLA-DO do not regulate expression of the CDw78 epitope.

To determine the extent to which CDw78-reactive MHC-II represents MHC-II-CLIP complexes, we performed immunodepletion/immunoblot experiments. Immunodepletion of Pala B cell lysates with CDw78 mAb removed ～90% of all Cer.CLIP-reactive MHC-II from the lysates (Fig. 5C), demonstrating that the CDw78-reactive mAb FN-1 recognized MHC-II-CLIP complexes. On the other hand, immunodepletion with the anti-CLIP mAb had little effect on the total amount of CDw78 reactivity in the lysate, demonstrating that only a small fraction of all CDw78-reactive MHC-II was present as MHC-II-CLIP. As anticipated, immunodepletion with the anti-pMHC-II mAb L243 removed essentially all CDw78-reactive MHC-II from the lysate (data not shown), a finding that is consistent with the known ability of L243 to recognize peptide-loaded MHC-II (35). These results demonstrate that while essentially all CDw78-reactive MHC-II is peptide loaded, only a subset of these molecules are bound to Ii-derived CLIP.

Ii chain is required for CDw78 expression

All cells that express CDw78 also express the MHC-II chaperone Ii (2, 6, 25, 43). Ii targets MHC-II into late endosomes/lysosomes...
and is cleaved to form the CLIP peptide, thus regulating the repertoire of peptides bound to class II (44). To examine the possibility that II expression is required for CDw78 expression, we used siRNA technology to knockdown II in CDw78−/H11001 cells. HeLa cells stably expressing the MHC-II transactivator CIITA express MHC-II as well as II and these cells are CDw78−. Introduction of II-specific siRNA into these cells reduced II expression by 80% as determined by flow cytometry and immunoblot analysis (Fig. 6A and data not shown). This reduction in II expression had no effect on expression of total MHC-II on the cell surface but did lead to a 65% reduction in CDw78 expression (Fig. 6B). The effect of II siRNA on CDw78 expression was specific, as control siRNA had no effect MHC-II, II, or CDw78 expression in HeLa-CIITA cells (Fig. 6A).

In a complimentary approach, we examined CDw78 expression in transiently transfected HeLa cells expressing MHC-II in the absence or presence of II. HeLa cells expressing MHC-II alone had minimal expression of CDw78 (~2.6% CDw78+ cells), and this was only observed on transfectants expressing the highest levels of MHC-II (Fig. 6A). Coexpression of MHC-II with II resulted in a 5-fold increase in the percentage of CDw78+ cells without affecting the absolute amount of MHC-II present on these cells (Fig. 6B). This increase correlated with the amount of cells expressing high levels of MHC-II and II chain (Fig. 6C and data not shown). These data, together with our siRNA studies, demonstrate that expression of II regulates CDw78 expression.

**FIGURE 3.** The tetraspanins CD81 and CD82 are not required for CDw78 expression. A, HeLa cells expressing CIITA were analyzed for their expression of pMHC-II (L243), CDw78 (FN-1), and the indicated tetraspanins. B, HeLa-CIITA cells were treated with control siRNA (solid line) or siRNA recognizing CD81 alone, CD82 alone, or both CD81 and CD82 together (bold line). Forty-eight hours after siRNA treatment, cells were harvested and analyzed for tetraspanin expression by flow cytometry. Staining using an isotype control mAb is indicated by a dotted line. The data shown are representative of at least three independent experiments.
While expression of Ii regulates CDw78 expression, FN-1 does not simply bind to either Ii-associated MHC-II or MHC-II-CLIP complexes. The dependence of CDw78 on Ii expression could be explained if CDw78 expression requires trafficking of MHC-II to lysosomal Ag processing compartments, process that is facilitated by MHC-II binding to Ii. To examine this question we coexpressed MHC-II with an Ii mutant lacking the critical di-leucine motif required for MHC-II-Ii endocytosis and transport to lysosomes (45). Expression of this Ii mutant has two consequences for the cell. First, since endocytosis of Ii is prevented, MHC-II-Ii complexes accumulate at the plasma membrane (45, 46). In addition, since sorting to lysosomes is inhibited, the expression of peptide-loaded MHC-II at the plasma membrane is reduced. Despite the presence of large amounts of MHC-II-Ii complexes at the cell surface in HeLa cells expressing MHC-II together with this trafficking mutant of Ii, CDw78 expression could not be detected, confirming our biochemical data showing that the CDw78 reactive mAb does not merely recognize intact surface MHC-II-Ii complexes (Fig. 6, A and C). These data, combined with our Ii knockdown and overexpression studies, suggest that Ii expression is required for efficient sorting MHC-II to lysosomal Ag-loading compartments where the CDw78 epitope is generated.

**Discussion**

To date, MHC-II have been described as being constitutively associated with two types of membrane microdomains, namely lipid
rafts and tetraspanin-containing microdomains. Despite the controversy regarding the relationship between cholesterol dependent detergent-insoluble membranes and lipid rafts in intact cells (47), a number of groups have found that the association of MHC-II with plasma membrane lipid rafts is important for the ability of APCs to stimulate CD4+ T cells (3–5, 48). By contrast, there is only limited data regarding the importance of MHC-II association with tetraspanins and/or tetraspanin microdomains. MHC-II have been shown by many groups to bind to individual tetraspanin family members (6, 12–19). Recently Kropshofer and colleagues (2, 6) have reported that a MHC-II epitope, termed CDw78, specifically recognizes MHC-II associated with tetraspanins. Most importantly, these authors reported that CDw78 reactivity is functionally important, since a CDw78+ B cells were capable of presenting OspA peptide to Ag-specific T cells whereas CDw78− B cells were not (6). Precise identification of the CDw78 epitope is therefore critical as CDw78 reactivity is the basis for all subsequent studies examining the importance of MHC-II-tetraspanin interactions in Ag presentation.

In this study, we have reinvestigated the issue of CDw78 reactivity and MHC-II tetraspanin interactions and conclude that CDw78 does not uniquely recognize tetraspanin-associated MHC-II. The relative amount of tetraspanins bound to MHC-II in CDw78 IPs is identical to the amount present in an IP using a mAb that simply recognizes pMHC-II complexes. However, there is indeed a dramatic reduction in the relative amount of tetraspanins bound to MHC-II isolated using mAbs DA6.147, an Ab that recognizes MHC-II-peptide complexes, MHC-II complexes, and free MHC-II α-chains. Hammond et al. (12) have shown previously that the tetraspanin CD82 only binds to the li-free (presumably peptide-loaded) MHC-II. Since Kropshofer and colleagues (6) compared the amount of tetraspanins bound to MHC-II in an anti-CDw78 IP to the amount bound MHC-II using a mixture of mAbs that recognize the total pool of MHC-II, it is likely that their initial study underestimated the amount of tetraspanins associated solely with pMHC-II complexes.

Additional evidence given for CDw78 defining MHC-II present in tetraspan microdomains came from studies using the saponin (6). Saponin has been shown to disrupt tetraspan-tetraspan interactions (26) and Kropshofer et al. (6) found that saponin-treatment of B cells inhibited CDw78 immunoreactivity. Curiously, in the study by Kropshofer et al. (6) m saponin was added to cells that had been previously fixed with paraformaldehyde, making it difficult to understand how saponin could disrupt chemically cross-linked proteins. Nevertheless, we repeated these experiments as described but found little effect of saponin on CDw78 immunoreactivity (N. J. Poloso, unpublished observations). Additionally, these investigators showed that saponin treatment prevented CDw78+ B cells from presenting OspA-peptide to Ag-specific T cells (6). However, it is not clear that saponin-treatment B cells are able to stimulate any T cells, even those that recognize pMHC-II complexes, which are not reactive with CDw78 mAb (49).

Peptide elution studies have shown that CDw78 almost exclusively recognizes MHC-II-containing peptides derived from endogenous II or the MHC-I protein HLA-A2 (6, 13). While a portion of CDw78-reactive MHC-II is bound to CLIP, we found no correlation between surface CDw78 reactivity and CLIP expression. Our analysis of CDw78 reactivity using a panel of MHC-II+ B cell transfectants did, however, reveal that CDw78 expression is strongly influenced by the MHC-II allele examined. While this could reflect preferential immunoreactivity with particular alleles, we favor the hypothesis that different MHC-II alleles show different CDw78 reactivity due to their ability to bind different amounts (or types) of self-peptides. For a given allele, overexpression of Ii enhanced CDw78 immunoreactivity, while inhibition of Ii expression (using siRNA) dramatically reduced CDw78 immunoreactivity without altering the amount of MHC-II on the plasma membrane. In addition, expression of an Ii lysosomal targeting mutant abrogated the Ii-dependent increase in CDw78 expression. Since expression of Ii regulates MHC-II trafficking into lysosomal Ag-processing and peptide-loading compartments (45), it is likely that the effect of Ii on CDw78 expression is therefore due to alterations in the repertoire of peptides bound to MHC-II.

While CDw78 requires expression of Ii and the anti-CDw78 mAb FN1 does indeed recognize MHC-II-CLIP complexes, we were surprised to find that altering CLIP levels on B cells does not affect CDw78 immunoreactivity. Furthermore, overexpression of the peptide editors HLA-DM and HLA-DO dramatically altered MHC-II-CLIP expression on the plasma membrane but had no effect on CDw78 expression. While both MHC-II-CLIP and CDw78 are up-regulated during DC maturation (our data and Ref. 13), it is possible that these two phenomena are unrelated. For example, the observed plasma membrane increase in MHC-II-CLIP is almost certainly due to the release of these complexes from intracellular Ag-processing compartments during DC maturation (50), whereas the increase in CDw78 could be due to enhanced self-Ag degradation and peptide loading onto MHC-II that is independent of MHC-II-CLIP expression.

Because MHC-II is indeed associated with tetraspanins in CDw78+ cells, we set out to examine the requirement for individual tetraspanin family member expression for CDw78 immunoreactivity. During the course of our studies, we identified professional APCs that were CDw78+ yet lacked expression of the tetraspanins CD9, CD37, and CD53, thereby demonstrating that these tetraspanins are not required for CDw78 immunoreactivity. MHC-II has been shown by many investigators to interact with the abundant tetraspanins CD81 and CD82 (6, 12, 14–16); however, siRNA-mediated reduction of both CD81 and CD82 had no effect on CDw78 expression. While MHC-II isolated with anti-CDw78 mAb certainly are bound to tetraspanins, we conclude that this does not indicate a requirement for any particular MHC-II-tetraspanin interaction for CDw78 reactivity. In agreement with this conclusion are recent data identifying MHC-II-tetraspanin complexes in monocytes that are CDw78+ (18). The authors speculate that these complexes are present in CDw78-independent, tetraspan-containing microdomains and go on to show that MHC-II-CD9 complexes are present in detergent-insoluble microdomains that behave like lipid rafts (18). These data, taken together with our biochemical studies showing that pMHC-II binds tetraspanins as well as CDw78-reactive MHC-II, strongly suggest that CDw78 does not “define” a domain in and of itself but instead defines a subset of pMHC-II bound to tetraspanins that reside in “conventional” lipid rafts.

If CDw78 immunoreactivity does not define pMHC-II bound to tetraspanins in tetraspan microdomains, then what does this epitope define? Given the very restricted repertoire of self-peptides eluded from anti-CDw78 IPs, it is likely that CDw78 mAb simply recognize a unique conformational epitope present on this subset of pMHC-II. Part of the difficulty in defining this epitope comes from the fact that different “CDw78-specific” mAbs have completely different patterns of immunoreactivity on APCs (24) (N. J. Poloso, unpublished observations). Our data showing that CDw78 reactivity does not uniquely recognize tetraspanin-associated MHC-II, we feel it would be more appropriate to examine the importance of MHC-II-tetraspanin interactions using RNA interference technology or genetically manipulated mice than to rely on CDw78 reactivity as an indicator of MHC-II-tetraspanin complexes.