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Dissecting MHC Class II Export, B Cell Maturation, and DM Stability Defects in Invariant Chain Mutant Mice

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Invariant (Ii) chain loss causes defective class II export, B cell maturation, and reduced DM stability. In this study, we compare Ii chain and class II mutant mouse phenotypes to dissect these disturbances. The present results demonstrate that ER retention of αβ complexes, and not β-chain aggregates, disrupts B cell development. In contrast, we fail to detect class II aggregates in Ii chain mutant thymi. Ii chain loss in NOD mice leads to defective class II export and formation of αβ aggregates, but in this background, downstream signals are misregulated and mature B cells develop normally. Finally, Ii chain mutant strains all display reduced levels of DM, but mice expressing either p31 or p41 alone, and class II single chain mutants, are indistinguishable from wild type. We conclude that Ii chain contributions as a DM chaperone are independent of its role during class II export. This Ii chain/DM partnership favors class II peptide loading via conventional pathway(s). The Journal of Immunology, 2004, 173: 3271–3280.

Diverse peptide/MHC class II complexes on the surface of specialized APCs guide CD4 T cell responses toward foreign pathogens and promote selection of a useful TCR repertoire (1). Class II peptide acquisition via the conventional exogenous pathway depends on essential contributions by specific chaperones, namely the invariant (Ii)3 chain and DM, required at distinct stages during maturation and export (2–5). Our current view of the class II peptide loading pathway has been shaped by studies of mice lacking Ii chain and/or DM functions (6–11). Both mutations disrupt class II maturation, Ag presentation, and CD4 T cell development, but functional analysis in the context of different MHC haplotypes reveals varying degrees of penetrance. Thus, as a general rule, b haplotype mice display severe disturbances, whereas d and k haplotype mice exhibit relatively mild phenotypes (7, 12–17). For example, in the exceptional case of Aβ molecules, Ii chain loss disrupts subunit assembly, but d and k haplotype mutants efficiently produce class II dimers (12). DM mutant strains also display allele- and isotype-specific defects (14, 16, 17).

Ii chain and MHC class II genes are encoded on separate chromosomes, and these molecules are structurally unrelated. Tissue-specific constitutive expression by selected cell types, namely B cells, dendritic cells, macrophages, and thymic epithelial cells, is tightly regulated. The class II enhanceosome complexes bound to common regulatory elements upstream of MHC class II, Ii chain, and DM genes recruit coactivators, promote chromatin remodeling, and correctly position the basal machinery responsible for transcription initiation (18–20). MHC class II, Ii chain, and DM transcripts are coordinately induced in response to inflammatory cytokines such as IFN-γ and TNF-α. Surface display of class II/peptide complexes is also controlled by posttranslational mechanisms, such as modulation of proteases responsible for Ii chain degradation and Ag processing (21–23). Fine tuning of class II, Ii chain, and DM expression ratios undoubtedly governs selection of peptide ligands and is therefore predicted to influence immune responses toward infectious agents and susceptibility to autoimmune diseases.

The preserved Ii chain found coassembled with polymorphic class II subunits, prevents irreversible misfolding of the subunits, protects the nascent empty groove, and promotes release from resident chaperones such as GRP94/BiP and ERP72/calnexin responsible for endoplasmic reticulum (ER) quality control (24–26). Via targeting signals in its cytoplasmic domain, the Ii chain also directs selective class II export to an endosomal compartment(s) where exposure to acidic pH and proteolytic enzymes leads to Ii chain degradation and Ag capture (27–29). The nonconventional class II product DM also acts inside endocytic compartment(s) to promote dissociation of a relatively short proteolytic product of Ii chain, corresponding to the so-called CLIP region, in exchange for tightly bound peptide ligand(s) (30–32). DM also binds empty class II and functions as a peptide editor, serving to increase the overall affinities of peptide/class II complexes (33–37). As for conventional class II, DM also contains its own endosomal targeting signal located in the β-chain cytoplasmic tail, but previous studies also suggest that DM transport is mediated via an association with the Ii chain (38–40).

Unlike allele-specific contributions made by Ii chain during class II subunit assembly, B cell developmental defects have been universally observed in Ii chain-deficient strains regardless of MHC haplotype (15, 41, 42). Selective loss of mature B cell subsets has also been reported in class II α- but not β-chain mutants (42–45), and in high copy number transgenic strains expressing unpaired or mispaired β-chains (46–48). In contrast, B cells develop normally in mice carrying a large deletion spanning the entire class II locus (49). Collectively, these findings support the idea that toxic β-chain aggregates disrupt B cell differentiation and/or survival (50). In contrast, gain of function experiments demonstrate that N-terminal Ii chain fragment(s) can rescue mature B cells (51, 52). A quite different model suggested by these studies...
is that Ii chain, via its transmembrane plus cytoplasmic domain, provides essential signals necessary for B cell development (52, 53). The fact that this portion of Ii chain also binds class II and potentially influences early folding events (54) has made it difficult to assess the functional significance of these observations.

Remarkable progress has been made toward understanding ER stress responses induced by unfolded proteins in eukaryotic cells (55–57), but B cell signals downstream of class II and Ii chain remain mysterious. Previous experiments demonstrate that empty class II in the absence of Ii chain or peptide occupancy becomes irreversibly aggregated and is rapidly degraded (8, 26, 58–61). Perhaps as for other incomplete receptors, unfolded class II subunits are exported to the cytoplasm and targeted for destruction by ubiquitin/proteasome pathways (62). Recent evidence supports this suggestion (63). In contrast, newly assembled class II dimers expressed by d and h haplotype Ii chain mutants seem to be properly folded (12). Immature class II in the absence of Ii chain fails to escape ER quality control, but the fate of these empty class II molecules has yet to be determined.

To further explore the possible relationship(s) between misfolded class II and B cell defects, here we characterized assembly intermediates in Ii chain and class II single chain mutants. To our surprise, we found that class II targeted alleles created by insertion of a drug selection cassette produce substantial amounts of truncated α- and β-chains via exon skipping. These and unpaired excess free α- and β-chains, as well as class II dimers lacking Ii chain, are not targeted for ER degradation. Rather, our experiments strongly suggest that ER retention of empty class II αβ complexes, and not terminally misfolded β-chain aggregates, disturbs B cell development. Unlike mutant phenotypes described to date, Ii chain functional loss in NOD mice has no noticeable effect on selection of mature B cell subsets. Therefore, it appears in this exceptional background that downstream signals necessary for B cell survival are constitutively up-regulated. Finally, we report that Ii chain-deficient strains, regardless of MHC haplotype, consistently express reduced levels of DM at steady state, whereas mice expressing either p31 or p41 alone and class II single chain mutants are indistinguishable from wild type. Ii chain function as a DM chaperone is thus independent of its role during class II export, and cannot be explained due to p41/cathepsin L (cat.) interactions. Rather, as for conventional class II, Ii chain associations may promote DM export and stability inside endocytic compartments. Coordinate regulation of Ii chain/DM activities potentially mediated by conserved NF-κB binding sites upstream of Ii chain and DMB genes (19, 64–67) provides a positive regulatory loop for selection of best fit peptides in professional APCs activated by inflammatory stimuli.

**Materials and Methods**

**Animals**

C57BL/6 mice carrying a targeted disruption at the Aα locus (68) were previously described, and have been maintained by brother-sister matings. C57BL/6TacBR-(KO)β78N5g(B6.Ab) mice were purchased from TacTone Farms (Germantown, NY). The generation of Ii chain-deficient mice expressing three independent MHC haplotypes by backcrossing the targeted allele onto BALB/cAn (H-2b), B10.BR/SgSn (H-2d), or B.C-9, a strain congenic with C57BL/6 but expressing the Igβ allele of BALB/c, has been described (12). The congenic strains analyzed in the present report were established by intercross matings at the tenth backcross generation. BALB/cAn and B.C-9 Ii chain mutants are available from The Jackson Laboratory Induced Animal Resource (Bar Harbor, ME). To generate Ii chain NOD mutants, we crossed the targeted allele onto the NOD background. The PCR genotyping assay used to identify the mutant locus has been described (12). Intercross matings were set up at the ninth backcross generation. Linkage markers associated with Idd recessive loci were confirmed to be NOD-derived via microsatellite analysis by Charles River Laboratories Genetic Testing Services (Troy, NY). Mutants exclusively expressing p31 (69) or p41 (70) Ii chain isoforms have been described. The DMr-deficient mice (9) on a (129 × C57BL/6)F1 background that express the H-2d haplotype, DMe-deficient BALB/c (16), and h haplotype DM mutants (17) have been maintained by brother-sister matings. In all experiments, comparisons were made between age- and, whenever possible, sex-matched animals.

**Western blots**

Spleen cell suspensions were depleted of erythrocytes by ammonium chloride-Tris treatment and washed with PBS containing 2% FCS and antibiotics, and resuspended at 2 × 10^7 cells/ml. The cell pellets were lysed in buffer containing 1% Nonidet P-40, 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, 1 mM PMSF, and 10 μg/ml aprotinin. After incubation on ice for 20 min, extracts were cleared of nuclei and debris by centrifugation for 30 min at 15,000 rpm. Sample buffer was added to detergent extracts, and lysates were boiled for 5 min (B) or kept on ice (NB) before fractionation on 10 or 15% polyacrylamide gels. Proteins were transferred onto nitrocellulose membranes (catalogue BA83; Schleicher & Schuell, Dassel, Germany) for 2 h at 500 mA. Blots were rinsed in TBS-T then incubated overnight in TBS-T with 10% dry milk and 3% BSA followed by one rinse before the addition of primary Abs diluted in TBS-T containing 3% BSA and 5% calf serum. After a 60-min incubation, blots were extensively washed with TBS-T containing 0.1% BSA followed by a 30-min incubation in secondary Ab diluted in TBS-T containing 3% BSA. Preadsorbed HRP secondary Abs were donkey anti-rabbit Ig (cat. no. NA935; Amersham Biosciences, Arlington Heights, IL), goat anti-mouse Ig (cat. no. NA931V; Amersham Biosciences). Blots were washed with TBS-T and developed by chemiluminescence using ECL (catalogue RPN2106; Amersham Biosciences).

For thymic lysates, the whole thymus was depleted of thymocytes by gently squeezing the thymic lobes with forceps and rinsing them in PBS and resuspending the remaining capsule in sample buffer, sonicating it for 8 as described (71). Debris was cleared by centrifugation for 30 min at 15,000 rpm. Aliquots were fractionated by SDS/PAGE and transferred to nitrocellulose as above.

**Immunofluorescence analysis**

For single color surface staining analysis, spleen cell suspensions depleted of erythrocytes by ammonium chloride-Tris treatment were incubated on ice with saturating amounts of biotin-conjugated Abs followed by FITC-labeled avidin D. For double staining experiments analyzing B cell subsets, spleen or lymph node cells were incubated with PE-conjugated goat F(ab')2 anti-mouse IgM (cat. no. M31604; Caltag Laboratories, San Francisco, CA) as a pan-B cell marker used in combination with FITC-labeled Abs directed against the IgG FeR CD21 (cat. no. 01234D; BD Pharmingen, San Diego, CA) or surface IgD (cat. no. 02214D; BD Pharmingsen). Fluorescence was analyzed using a FACScan flow cytometer (BD Biosciences, Mountain View, CA) and the data displayed as cell number vs log fluorescence. Dead cells were eliminated from the analysis by appropriate gating.

For cytoplasmic staining, spleen cell suspensions were treated with 10% formalin for 10 min at room temperature and extensively washed with PBS containing 0.1% saponin (Sigma-Aldrich, St. Louis, MO). In this case, incubations and all washing steps were conducted at room temperature in the presence of saponin (0.1%) as described (70). Primary Abs included rabbit chain-specific Abs kindly provided by R. Germain (National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD), rat monoclonal anti-H2M (2E5A) (11) kindly provided by L. Karlsson (R. W. Johnson Pharmaceutical Research Institute, San Diego, CA), and M5/114 (Aβ1β5/Eβ4), Y3P Aβ (α + β), 10-2-16 (Aββ), H11-32 (Aαα). Secondary reagents were FITC-conjugated goat F(ab')2 anti-mouse IgG (H + L) (cat. no. M31604; Caltag Laboratories), goat anti-rat IgG (H + L) (BD Pharmingsen, San Diego, CA) or goat anti-rabbit IgG (H + L) (cat. no. 4052-02; Southern Biotechnology Associates, Birmingham, AL). Fluorescence was analyzed using a FACScan flow cytometer (BD Biosciences) and the data was displayed as cell number vs log fluorescence.

**Results**

**ER retention of immature class II dimers lacking Ii chain**

Previous studies have described high m.w. class II aggregates produced by Ii chain-deficient splenocytes (8, 26, 60, 61). Do these complexes represent bona fide assembly intermediates or irreversibly misfolded class II subunits? Perhaps these are unfurled proteins upstream of ER stress responses. To explore underlying...
causes of B cell developmental defects and further characterize class II complexes expressed in the absence of Ii chain, freshly prepared spleen cell lysates were analyzed in Western blot experiments. Representative data are shown in Fig. 1. As expected, Ii chain-deficient splenocytes fail to express mature compact dimers. Rather, we readily detect class II complexes with discrete high m.w. mobilities. As judged by serological reactivity patterns, these species correspond to class II aggregates comprised of both α- and β-chains previously identified in pulse-chase experiments (8, 26, 60, 61). Conformationally dependent mAbs such as Y3P and 14-4-4 fail to detect any signal in the denatured samples, whereas Abs such as 10-2-16 and M5/114 (see Fig. 4) display partial cross reactivity toward free β-chains. To avoid bias introduced due to conformational influences, class II expression levels were also compared using chain-specific rabbit Abs. As shown in Fig. 1D, Ii chain mutant mice strongly express class II at levels equal to wild type, and as judged by characteristic gel mobilities, these immature complexes are retained in the ER. It also appears that k haplotype mutants express increased amounts of intracellular class II.

To evaluate this possibility and more accurately compare strain differences, class II expression was also examined via intracellular staining protocols. As above, we found Ii chain mutants strongly express class II at near wild-type levels (Fig. 2). Slightly decreased reactivity toward conformationally dependent Abs, such as M5/114 and Y3P, was observed in b haplotype mutants, but in striking contrast, FACS profiles reveal increased total class II observed in b haplotype mutants, intracellular staining profiles reveal increased levels of class II expressed by k haplotype mutants. These shifts were detectable with either chain-specific rabbit Abs or conformationally dependent mAbs and thus reflect increased total class II rather than a serological change. Isotype controls and secondary reagents alone gave no detectable staining.

Collectively, Western blots and intracellular staining experiments demonstrate that high m.w. class II aggregates are not aberrantly misfolded targets for degradation, but rather represent a stable cohort of ER resident assembly intermediates.
Class II single chain mutants produce truncated α and β subunits, excess free α and β, but not higher order β-chain aggregates. The idea that β-chain aggregates are toxic to B cells comes in part from the observation that class II α- but not β-chain mutants display B cell maturation defects (42–45). To further explore possible relationships between class II export and B cell survival, we also analyzed assembly intermediates in class II single chain mutants. To our surprise, intracellular staining experiments demonstrate both mutant strains express class II α- and β-chains at near wild-type levels (Fig. 3A). This signal is not caused by nonspecific background staining because Western blots reveal specific band(s) corresponding to truncated α- and β-chains produced via exon skipping (Fig. 3B). Thus, both single chain mutant strains created via insertion of a drug selection cassette at a conserved site located inside exon 2 (43, 68, 72), efficiently generate in-frame fusion proteins comprised of the leader plus membrane proximal α2/β2 plus transmembrane plus cytoplasmic domains, as for recombinant chimeric class I products constructed in vitro (73). Analysis of nonboiled samples confirms the absence of subunit assembly (Fig. 3, C and D). In contrast to αβ aggregates strongly expressed by Ii chain-deficient splenocytes, under the same conditions class II α-chain mutants gave no evidence for self association of misfolded β-chains. We conclude that high m.w. β-chain aggregates per se are probably not responsible for B cell maturation defects.

**ER retention of class II aggregates by splenic B cells**

Ii chain-independent class II export, peptide acquisition, and Ag presentation have been described in k haplotype dendritic cells.

**FIGURE 3.** Class II mutant mice produce truncated α and β subunits, and excess free α- and β-chains, but not higher order class II aggregates. A, Saponin-treated spleen cells from wild-type (1), or class II-deficient strains targeted at the α- (2), or β- (3) chain locus were incubated with the indicated Abs followed by FITC-conjugated secondary reagents, and analyzed by FACS. Isotype controls and secondary reagents alone gave no detectable staining. B–D, Spleen cell lysates prepared from b haplotype strains as indicated were boiled and resolved on 15% gels (B) or were boiled (B) and run alongside lysates kept on ice (NB) on 10% (C and D) polyacrylamide gels under reducing conditions, subsequently transferred to nitrocellulose membranes, and the blots probed with class II-specific Abs as indicated. C and F indicate compact and floppy αβ dimers, respectively. The positions of in-frame fusion proteins comprised of the leader plus membrane proximal α2 or β2 domains plus transmembrane plus cytoplasmic regions are denoted by αf and βf, respectively.
We wondered whether formation of class II aggregates and the inability to escape ER quality control might be B cell specific. To test this possibility, we examined class II assembly intermediates expressed by Ii chain mutant thymi. Freshly prepared spleen and thymus lysates were analyzed side-by-side in Western blot experiments. Representative data are shown in Fig. 4. As expected, wild-type tissues produce mature compact dimers, and Ii chain-deficient splenocytes express class II aggregates. In contrast, under the same conditions, Ii chain mutant thymi fail to display higher order complexes. As for dendritic cells (13), we also observe here, in k haplotype mutant thymi, a substantial pool of mature compact dimers. Therefore, it appears that formation of class II aggregates and ER retention of assembly intermediates depends on B cell-specific cofactors.

Mature B cells develop normally in Ii chain-deficient NOD mice
The I-Ag7 molecule in NOD mice is strongly linked to diabetes susceptibility (74). Experiments to date fail to implicate exceptional Ii chain and/or DM requirements (75–78). To investigate allele-specific Ii chain contributions to class II export and B cell maturation, we decided to generate NOD mutants. We crossed the targeted allele onto the NOD background and set up intercross matings at the ninth backcross generation to establish the congenic strain analyzed in the present report. Linkage markers associated with Idd recessive loci were confirmed to be NOD-derived via microsatellite analysis, as described (79). As shown in Fig. 5A, Ii chain mutants display markedly reduced levels of surface class II. Western blots demonstrate class II aggregates (Fig. 5B). Ii chain loss disrupts selection of mature CD4 T cells (data not shown). Representation of B cell subsets was also examined. As a marker for mature B cells, we analyzed expression of CD23 the low affinity IgE FcR. Spleen and lymph node IgM cells were also compared for expression of surface IgD. Wild-type mature IgM B cells coexpress both IgD and CD23 surface markers. Ii chain-deficient mice described to date universally contain markedly increased percentages of immature B cells lacking surface IgD and CD23 coexpression and also display a striking reduction in the total number of IgM B cells in lymph node populations (15, 41, 42). In contrast, here as shown in Fig. 5C, Ii chain functional loss in NOD mice has no noticeable effect on selection of mature B cell subsets. Thus, in this exceptional background, signals downstream of Ii chain are misregulated and indeed B cell survival pathways appear to be constitutively activated.

Class II-independent Ii chain functions as a DM chaperone equally shared by p31 and p41 isoforms
Recent experiments demonstrate Ii chain-deficient splenocytes express less DM at steady state in comparison with wild type (80). DM expression was partially rescued by treatment with cathepsin inhibitors but not lactacystin. It was suggested that Ii chain protects DM against lysosomal degradation, perhaps via selective p41/catL interactions (80). To further investigate allele- and isotype-specific Ii chain functional roles, we decided to compare DM expression levels in our various Ii chain mutant strains. As shown in Fig. 6A, intracellular staining experiments demonstrate decreased DM expression in Ii chain-deficient splenocytes regardless of MHC haplotype. We also found lactacystin fails to rescue DM expression (data not shown). DM expression levels in class II single chain

indicate compact and floppy αβ dimers, respectively. The positions of free α and β subunits or immature α' and β' chains readily recognized by rabbit chain-specific Abs (C) but showing limited reactivity toward conformationally dependent mAbs (A and B) are indicated.

FIGURE 4. Formation of class II αβ aggregates by splenic B cells but not thymic APC. Spleens or thymi from b or k haplotype strains as indicated were lysed and extracts analyzed via Western analysis. C and F indicate compact and floppy αβ dimers, respectively. The positions of free α and β subunits or immature α'- and β'-chains readily recognized by rabbit chain-specific Abs (C) but showing limited reactivity toward conformationally dependent mAbs (A and B) are indicated.
mutants (Fig. 6B) and mutant strains expressing either p31 or p41 alone (Fig. 6C) are indistinguishable from wild type. Collectively, these results demonstrate that Ii chain function as a DM chaperone is independent of its role during class II export, and cannot be explained due to p41/catL interactions. Rather as for conventional class II, Ii chain associations may promote DM subunit assembly, escape from ER quality control chaperones, and stability inside endocytic compartment(s). Results of our experiments dissecting MHC class II export, B cell maturation, and DM stability defects in various mutant mouse strains are summarized in Table I.

**Discussion**

Gene targeting experiments reported over a decade ago established that the conserved Ii chain acts as a specific chaperone governing class II assembly, transport, and peptide acquisition (6–8). The loss of function mutation also disrupts selection of mature B cell subsets (41). Is the underlying mechanism(s) responsible for these developmental defects dependent on Ii chain/class II associations? Because NF-κB/Rel family members control target gene expression in response to infection, inflammation, and stress stimuli, and play critical roles during B lymphocyte development (81–83), an attractive idea is that Ii chain cleavage triggers NF-κB pathways required for B cell survival (51–53). Exactly how N-terminal Ii chain sequences might regulate promiscuous components of the NF-κB signaling cascade remains ill defined. In addition, recent experiments demonstrate B cells develop normally in Ii chain/class II double mutants (84). Thus, it appears Ii chain expression is not essential for B cell maturation.
An alternative hypothesis is that misfolded β-chains are selectively toxic to B cells (50). Consistent with this possible scenario, class II α but not β single chain mutants display B cell maturation defects (42–45), and high copy number transgenic strains expressing excess β-chains also exhibit B cell abnormalities (46–48), whereas, in contrast, targeted deletion of both class II subunits rescues B cell development (49). On the other hand, Li chain mutants universally display B cell defects regardless of MHC haplotype (15, 42), and indeed Li chain loss in k haplotype mice disrupts B cell development (49). On the other hand, Li chain mutants display B cell defects regardless of MHC haplotype mice. To our surprise, we found that class II tar-
geted alleles created simply by insertion of a drug selection cassette into the locus produce substantial amounts of truncated α- and β-chains via exon skipping. These products comprised of the leader plus membrane proximal plus transmembrane plus cytoplasmic domains remain unassembled, and excess free α- and β-chains fail to form higher order aggregates. Near wild-type levels strongly suggest these free subunits expressed in isolation are not subjected to ER degradation.

Li chain mutant mice display markedly reduced class II surface expression (6–8). In contrast here, intracellular staining protocols and Western blot experiments reveal total class II is expressed at near wild-type levels. Indeed, Li chain loss in k haplotype mice leads to increased class II retained inside the ER. As suggested previously (26, 60), these class II aggregates comprised of both chains possibly cross-linked via associations with ER chaperones, such as BiP and calnexin, represent bona fide assembly intermediates. In the absence of Li chain, we would argue that these higher order αβ complexes, and not misfolded β-chain aggregates, selectively signal ER stress responses in mature B cells. To reconcile this interpretation with the fact that class II α- but not β-chain mutants also display B cell defects (42–45), we propose that signals downstream of partially unfolded nascent β-chains alone closely resemble those triggered by incomplete class II complexes lacking Li chain. The specific components governing ER retention of these class II assembly intermediates probably also regulate B cell survival.

The dileucine targeting motif mapped to the class II β-chain cytoplasmic tail promotes recycling and presentation via nonconventional pathway(s) (85, 86). As for signals controlling Li chain transport (27–29), intracellular trafficking has been extensively analyzed in transfected cell lines. Early experiments suggested rather that endogenous Li chain trimers are retained inside the ER in the absence of class II associations (87), as expected if the quality control machinery distinguishes assembly intermediates vs structurally complete complexes. It thus appears in normal B cells that Li chain alone, unpaired β-chains, and class II αβ dimers in the absence of Li chain cannot escape ER quality control. The Li chain and class II β-chain cytoplasmic domains expressed in isolation probably bind common downstream effectors, but cooperative signals may be essential to create export competent oligomers under physiological conditions.

The possible overlap between BCR signaling and class II peptide-loading pathways has been intensely investigated. Surface class II appears late during B cell development coincident with the onset of surface IgD expression and is up-regulated as a consequence of BCR cross-linking (88, 89). It is also known that cross-linking of surface class II activates cAMP/protein kinase C, induces Ca2+ mobilization, and causes apoptotic cell death in resting B cells and dendritic cells (90–98). These activities mapped to the class II β-chain cytoplasmic tail have been extensively described.
in cell lines, but the physiological significance of these observations remains unclear because only subtle defects were observed in high copy number transgenic mice exclusively expressing truncated β-chains (99). The fact that CD4 T cell development and immune responses to conventional protein Ags, parasitic infections, and skin grafts were efficiently executed strongly argues that β-chain signals cannot be essential for class II functions. In contrast, in vitro Ag presentation capabilities were compromised, and class II molecules isolated from these mice display a distinct repertoire of self peptide ligands (99, 100). Representation of mature B cell subsets was not analyzed. It will be interesting to examine whether class II signals via the β-chain cytoplasmic tail influence B cell development. It was recently shown that class II binds components of the BCR, namely Ig-α/Ig-β (101). In addition to its role as a signaling module, Ig-α/Ig-β also acts as a chaperone to promote BCR transport from the ER to the cell surface (102, 103). It is tempting to speculate that Ig-α/Ig-β associations may also regulate class II export and/or recycling perhaps via a conserved mechanism(s).

The present experiments reveal striking cell type-specific differences governing quality control of class II assembly and export. In contrast to splenic B cells, li chain-deficient thyms gave no evidence for production of high m.w. class II αβ aggregates. Moreover, in k haplotype thyms as for dendritic cells (13), li chain activities are not essential for class II export and peptide acquisition. Previous studies have shown that li chain extinguishes presentation of selected T cell epitopes (16, 104–106). li chain-independent pathway(s) may selectively promote presentation of endogenous self peptide ligands during thymic development. ER stress responses are induced by excessive traffic during viral infections. Under these conditions, up-regulated production of ER chaperones, such as BiP, coupled to the loss of li chain expression in dendritic cells, probably favors acquisition of nascent viral glycoproteins via endogenous secretory route(s).

As for mutant strains described to date, li chain-deficient NOD mice display defective class II export and reduced DM expression levels, and Western blot experiments clearly demonstrate ER retention of class II αβ aggregates. Remarkably in NOD mice, li chain loss fails to perturb B cell development. The tendency of NOD mice to develop autoimmune diabetes is a complex phenomenon and mapping studies have identified at least 20 susceptibility loci (74, 107). B cells are required for the initiation of insulin-dependent diabetes mellitus (78, 108), but NOD alleles conferring abnormal B cell selection have not been identified. In contrast, dendritic cells and macrophages from NOD mice display elevated levels of NF-κB activation (109–111). The structural basis of NF-κB signaling defect(s) has not been determined. NF-κB DNA-binding subunits (RelA, RelB, c-Rel, p50, and p52) as well as IκB proteins are potentially misregulated. It will be interesting to learn how NOD B cells escape negative selection in the absence of li chain and whether class II β-chain/NF-κB signaling defect(s) influence disease susceptibility.

The present experiments strengthen the idea that the conserved li chain also functions as a DM chaperone. As for conventional class II complexes, transient li chain/DM associations were previously described in pulse-chase experiments (38, 40). Recent studies demonstrate that li chain-deficient mice express less DM at steady state (80). Similarly, here we observe that li chain mutant strains regardless of MHC haplotype display reduced DM stability. In contrast, DM expression levels in class II single chain mutants and strains expressing either p31 or p41 alone are indistinguishable from wild type. Thus, we conclude li chain chaperone activities are independent of class II export and cannot be explained due to selective p41 modulation of proteolytic environment(s).

The simplest scenario is that li chain physically binds DM and stabilizes partially unfolded molecules. X-ray crystal studies reveal DM has a single deep pocket on its surface and, in contrast to conventional class II, its two extra disulfide bonds cause the membrane distal regions to be tightly packed together so that its peptide-binding groove is closed (112, 113). A strong argument can be made that li chain cannot possibly bind as for conventional class II via its CLIP segment. The li chain has also been shown to weakly interact with conventional class II transmembrane domains, and these contacts depend on detergent and isolation conditions (54, 114, 115). Interestingly, DM transmembrane/cyttoplasmic domains are known to contribute to its peptide editing functions (116). Perhaps li chain also associates with DM via its transmembrane and/or cytoplasmic segments. li chain/DM associations may cooperatively strengthen downstream signals governing export and/or recycling to the cell surface.

Another nonmutually exclusive possibility is that as for conventional class II, li chain associations may promote early folding of the nascent chains, subunit assembly, and/or escape from ER quality control chaperones. li chain partnerships might even create novel DM isoforms via alternative disulfide bonds having distinct half-lives and/or export characteristics. The conserved NF-κB binding sites upstream of li chain and DMB, but not conventional class II genes (19, 64–67), can independently modulate li chain/DM activities in discrete types of APCs. We suggest that class II-independent li chain/DM associations provide a positive regulatory loop to favor Ag presentation via the conventional exogenous route and promote selection of best-fit peptides upon exposure to viral infection and/or inflammatory stimuli.

Note added in proof. A recent paper by Serreze and coworkers (117) describes B cell selection defects in NOD mice. It will be interesting to learn more about possibly shared mediators governing these B cell signaling pathways.

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T cell function in mice lacking the p4i isoform of invariant chain. *Immunity* 3:385.


