The Journal of Immunology

The Ia.2 Epitope Defines a Subset of Lipid Raft-Resident MHC Class II Molecules Crucial to Effective Antigen Presentation

This information is current as of March 22, 2019.

Kathleen Busman-Sahay, Elizabeth Sargent, Jonathan A. Harton and James R. Drake

Supplementary Material	http://www.jimmunol.org/content/suppl/2011/05/04/jimmunol.1100336.DC1
References	This article **cites 36 articles**, 20 of which you can access for free at: http://www.jimmunol.org/content/186/12/6710.full#ref-list-1

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision

- **No Triage!** Every submission reviewed by practicing scientists

- **Fast Publication!** 4 weeks from acceptance to publication

*average

Subscription	Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription
Permissions	Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html
Email Alerts	Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts

The Ia.2 Epitope Defines a Subset of Lipid Raft-Resident MHC Class II Molecules Crucial to Effective Antigen Presentation

Kathleen Busman-Sahay,[1] Elizabeth Sargent,[1] Jonathan A. Harton, and James R. Drake

Previous work established that binding of the 11-5.2 anti–I-A^k mAb, which recognizes the Ia.2 epitope on I-A^k class II molecules, elicits MHC class II signaling, whereas binding of two other anti–I-A^k mAbs that recognize the Ia.17 epitope fail to elicit signaling. Using a biochemical approach, we establish that the Ia.2 epitope recognized by the widely used 11-5.2 mAb defines a subset of cell surface I-A^k molecules predominantly found within membrane lipid rafts. Functional studies demonstrate that the Ia.2-bearing subset of I-A^k class II molecules is critically necessary for effective B cell–T cell interactions, especially at low Ag doses, a finding consistent with published studies on the role of raft-resident class II molecules in CD4 T cell activation. Interestingly, B cells expressing recombinant I-A^k class II molecules possessing a β-chain–tethered hen egg lysosome peptide lack the Ia.2 epitope and fail to partition into lipid rafts. Moreover, cells expressing Ia.2$^-$ tethered peptide–class II molecules are severely impaired in their ability to present both tethered peptide or peptide derived from exogenous Ag to CD4 T cells. These results establish the Ia.2 epitope as defining a lipid raft-resident MHC class II conformer vital to the initiation of MHC class II-restricted B cell–T cell interactions. *The Journal of Immunology*, 2011, 186: 6710–6717.

Major histocompatibility complex class II-restricted cognate interactions between Ag-specific B cells and CD4 Th cells are necessary for initiation and full development of a humoral immune response. It has long been appreciated that MHC class II molecules can adopt multiple conformations with distinct activities. Possibly the most well-known example is SDS-stable versus SDS-sensitive peptide–class II complexes (1). Another example is type A versus type B peptide–class II complexes that were identified and characterized by Lovitch and Unanue (2). The structural difference between types A and B peptide–class II complexes is unknown, so the complexes are functionally defined based on their ability to be recognized by types A or B reactive T cells. Nevertheless, studies established that type A peptide–class II complexes are formed in late endocytic compartments under the influence of the class II chaperone HLA-DM/H-2M (DM), whereas type B complexes are formed in early endocytic compartments (which lack abundant DM) and are actually destroyed upon interaction with the DM chaperone. Because of the DM dependence of type A complexes, the distinction between types A and B are most likely centered on differences in bound peptides.

Early serological studies of H-2 Ia determinants (now I-A and I-E) revealed the presence of multiple determinants restricted to the k haplotype, such as Ia.2, Ia.19, and Ia.17 (3). Later, mAbs were used to confirm Ia.2 and Ia.19 as private epitopes restricted to I-A^k class II molecules (3, 4). In 1981, Pierres et al. (5) reported that the binding of multiple anti-Ia.2 mAbs augments subsequent binding of other anti–I-A^k mAbs, an effect that the investigators speculated was due to an anti-Ia.2–induced shift in the structure of the I-A^k molecule. Later, the binding of anti-Ia.2 mAbs, such as 11-5.2, was found to be highly dependent on residues arginine-57 and glutamine-75 of the I-A^k α-chain (6, 7), residues that are physically located close to the peptide-binding groove of the molecule. Follow-up studies revealed that expression of an epitope closely related to Ia.2 (Ia.19) was dependent upon the presence of the appropriate MHC class II β-chain, further supporting the idea that Ia.2 and Ia.19 may be conformational epitopes (8). Subsequently, in 1992, Cosson and Bonifacino (9) demonstrated that mutation of residues within the transmembrane domain of the I-A^k α- and β-chain polypeptides resulted in loss of the Ia.2 epitope without loss of I-A^k expression. Taken together, these results suggested that the Ia.2 epitope is a conformational epitope.

Most recently, studies of MHC class II signaling in resting B cells revealed one such example of a functional distinction between I-A^k class II molecules based on conformational epitopes (10). Specifically, binding of the anti-Ia.2 mAb 11-5.2 was found to elicit Src family kinase-dependent intracellular Ca^{2+} signaling and B cell activation, whereas binding of multiple anti-Ia.17 mAbs failed to elicit such signaling. These data support the hypothesis that MHC class II molecules with a particular conformation (Ia.2) may represent a functionally distinct population. This article extends those findings and establishes that the Ia.2 epitope bound by the 11-5.2 mAb defines a subset of lipid raft-resident MHC class II conformers central to effective activation of CD4 T cells. When taken in context with the previous literature on the Ia.2 epitope, these results establish that the Ia.2 epitope defines a subset of

MHC class II conformers localized to lipid rafts that are central to both effective Ag presentation to CD4 T cells, as well as MHC class II-mediated B cell activation.

Materials and Methods

Cells

TA3 B cells (a hybridoma derived by fusion of the BALB/c-derived A20 lymphoma with splenocytes from an H-2^k mouse and expressing wild-type I-A^k class II) were grown, as previously reported (11). Hen egg lysosome (HEL)$_{46-61}$–I-A^k–specific h4Ly50.5 (Ly50) T cells (a gift of Dr. Bill Wade, Dartmouth Medical School) were grown in Dulbecco's modification of Eagle's medium 10% FBS, 1 mM Na$^+$ pyruvate, 2 mM L-glutamine, and 50 μM 2-ME. HEK293T cells stably transfected to express CIITA (a gift from Dr. Karen Duus, Albany Medical College) were grown in Dulbecco's modification of Eagle's medium 10% FBS, 1 mM Na$^+$ pyruvate, 2 mM L-glutamine, 50 μM 2-ME, and 5 mM L-histidinol. IIA1.6 cells (American Type Culture Collection, Manassas, VA) were grown in αMEM 5% FBS and 25 μM 2-ME. Splenic B cells from I-A^k–expressing B10.Br and MD4.B10.Br mice (expressing a transgenic HEL-specific IgM^a BCR) were prepared as previously reported (12, 13). Animal protocols were reviewed and approved by appropriate institutional review committees.

Flow cytometry

Cells were stained with 10-3.6–PE (#109908; BioLegend, San Diego, CA) and 11-5.2–FITC (#553536; BD Pharmingen, San Diego, CA) in HBSS 0.1% BSA, with a final wash containing 0.1 μg/ml propidium iodide (splenocytes were also stained with anti–CD19-PE-Cy7 [BD Pharmingen] but no propidium iodide). Some samples were stained with Aw3.18 (14), followed by anti–muIgG$_1$-FITC (BD Pharmingen #553443). Samples were analyzed either on a FACScan or FACScanto (BD Biosciences, San Jose, CA). Splenic B cells were analyzed by gating on CD19$^+$ cells.

Class II immunoprecipitation and Western blotting

Cells were lysed in RIPA buffer (10), and lysates were cleared with Protein G Sepharose (Pierce Biotechnology, Rockford, IL). Class II was immunoprecipitated with 11-5.2 (anti-Ia.2), 10-2.16 (anti-Ia.17), and M5/114 (anti-I-A^{b,d} and I-E^k) plus Protein G Sepharose. Class II was immunoprecipitated from first-round immunoprecipitation (IP) supernatants by the same approach. Washed immunoprecipitates were analyzed by reducing SDS-PAGE and Western blotted with rabbit anti–I-A (15), followed by goat anti-rabbit IgG-HRP (#401353; Calbiochem, San Diego, CA). Blots were developed with SuperSignal West Dura ECL substrate (Pierce Biotechnology), using Blue Sensitive film (Laboratory Product Sales, Rochester, NY). Similar results were obtained using rabbit anti-class II β-chain cytoplasmic-domain Ab.

Analysis of MHC class II lipid raft partitioning

Lipid raft partitioning of I-A^k molecules was determined using a variation of our published protocol (16, 17). A total of 10^7 B cells was labeled with 11-5.2–biotin (btn) (#553534; BD Pharmingen), 10-3.6–btn (#109902; BioLegend), or cholera toxin B subunit (CTB)-btn (#C9972; Sigma) on ice, followed by streptavidin-HRP (15 min on ice, followed by 5 min at 37˚C). Cells were washed and lysed in 10 mM Tris (pH 7.5), 150 mM sodium chloride, 5 mM EDTA (TNE) 1% Triton X-100 (TX-100) at 10^8 viable cells/ml. Lysates were fractionated on a 5/32/37.5% sucrose step gradient using a TLS-55 rotor (4 h at 48,000 rpm). HRP activity was measured via a colorimetric assay (16, 17). Total class II was determined by SDS-PAGE and Western blot of equal volumes of gradient fractions, as detailed above.

MHC class II internalization

Internalization of 11-5.2–btn, 10-3.6–btn, and anti–IgM^b-btn (#553515; BD Pharmingen) was determined by flow cytometry, as previously reported (16), using streptavidin-Alexa Fluor 488 (#532354; Invitrogen) to detect surface mAb-btn. A similar approach was used to follow the internalization of 10-2.16, using anti–muIgG$_{2b}$-FITC (BD Pharmingen) as a probe.

Analysis of B cell–T cell conjugates

Formation of B cell–T cell conjugates was determined by a modification of our published protocol (13). Splenic B cells expressing type I (BCR-generated) or type II (fluid phase-generated) peptide–class II complexes were prepared as previously reported (13). B cells were labeled with anti–B220-PE (#553090; BD Pharmingen). Ly50 T cells were labeled with

anti–Thy1.2-FITC (#553014; BD Pharmingen). Labeled B and T cells were mixed at a 1:4 ratio and cosedimented by centrifugation for 3 min at 200 × g. After incubation for 20 min at 37˚C, samples were resuspended and incubated for an additional 5 min at 37˚C (to allow dissociation of unstable conjugates), and the level of B cell–T cell conjugation was determined by flow cytometry. Conjugate formation by non-HEL–pulsed B cells was consistently <5%. Anti-class II mAbs were bound to B cells on ice before the addition of T cells and were maintained throughout the remainder of the experiment.

Analysis of Ia.2 epitope expression by transfection

Aαk and Aβk proteins were expressed from the pcDNA3.1 and pcDNA3.1/Hygro vectors, respectively (Invitrogen, Carlsbad, CA). All mutagenesis was done using the QuikChange Site Directed Mutagenesis Kit (Agilent, Santa Clara, CA) and confirmed by sequencing. Cytoplasmic tail (CT)-deletion (ΔCT) constructs were generated by insertion of a stop codon following Aαk R202 and Aβk R221. The HEL-tethered Aβk and AβkΔCT constructs contain HEL$_{47-62}$ followed by an 8-aa glycine linker inserted between Aβk residues E4 and F7, similar to that done previously (18), resulting in the sequence E4-G-TDGSTDYGILQINSRW-GGGGG-GGG-SA-F7. Transient transfection into HEK293T-CIITA cells was done using FuGene HD (Roche, Mannheim, Germany) and 1 μg of each construct at a 2:7 DNA/FuGene ratio. Cells were analyzed 20–24 h post-transfection. Stable transfection of IIA1.6 cells was generated by electroporation with a GenePulser (Biorad, Hercules, CA) at 350 V/500 μF using 10 μg of each construct. Twenty-four hours after electroporation, cells were selected with 1.4 mg/ml G418 and 1 mg/ml hygromycin B (both from Mediatech, Manassas, VA) until cloning. Integration of the Aαk and Aβk constructs and derivatives was verified by RT-PCR of isolated mRNA.

Ag presentation

Clones of IIA1.6 cells expressing AαkΔCT/AβkΔCT and AαkΔCT/HEL-AβkΔCT, along with TA3 cells, were separately mixed with Ly50.5 T cells and doses of HEL from 0–100 μM. Following 20–24 h of incubation, supernatant was collected, and IL-2 levels were measured by Cytometric Bead Array (BD Biosciences).

Nycodenz density-gradient centrifugation

TA3 cells were homogenized with a ball-bearing homogenizer (0.2504" bore, 0.2493" ball), and cellular vesicles fractionated by Nycodenz density-gradient centrifugation, as previously published (19). Fractionated vesicles were lysed in 0.1% TX-100 and IP with either 10-2.16 (anti-Ia.17) or 11-5.2 (anti-Ia.2) plus protein G Sepharose. Immunoprecipitates were analyzed for MHC class II by Western blot, as detailed in Materials and Methods.

Invariant chain association

TA3 cells were lysed in RIPA buffer, and Ia.17$^+$ or Ia.2$^+$ I-A^k immunoprecipitates were lysed with 10-3.6 or 11-5.2, as detailed in Materials and Methods. Invariant chain (Ii) was detected by Western blot analysis of the immunoprecipitates with In-1 (anti-CD74; #555317; Pharmingen), followed by Gt anti-Rt IgG$_{2b}$-HRP (#PA1-84710; Thermo Scientific). Blots were developed with SuperSignal West Dura ECL substrate.

Results

The Ia.2 epitope marks a subset of I-A^k MHC class II molecules

Previous studies using resting B10.Br splenic B cells established that binding of the 11-5.2 anti–I-A^k mAb, which recognizes the Ia.2 epitope localized to Aαk (6, 7), elicits Src kinase-mediated intracellular Ca^{2+} signaling, whereas binding of the 10-2.16 or 10-3.6 anti–I-A^k mAb, which recognize the Ia.17 epitope localized to Aβk (20), fails to elicit such a response (10). To investigate the molecular mechanism behind this observation, the distribution of the Ia.2 and Ia.17 epitopes on the population of I-A^k class II molecules was determined.

Sequential IP of lysates from B10.Br splenic B cells (Fig. 1A) revealed that all I-A^k molecules that expressed the Ia.2 epitope also expressed the Ia.17 epitope, because no I-A^k could be detected in a second IP with anti-Ia.2 following an initial IP with anti-Ia.17. In contrast, some Ia.17$^+$ I-A^k molecules lacked the Ia.2 epitope, as indicated by the ability of anti-Ia.17 to immunoprecipitate I-A^k class II molecules from samples previously cleared
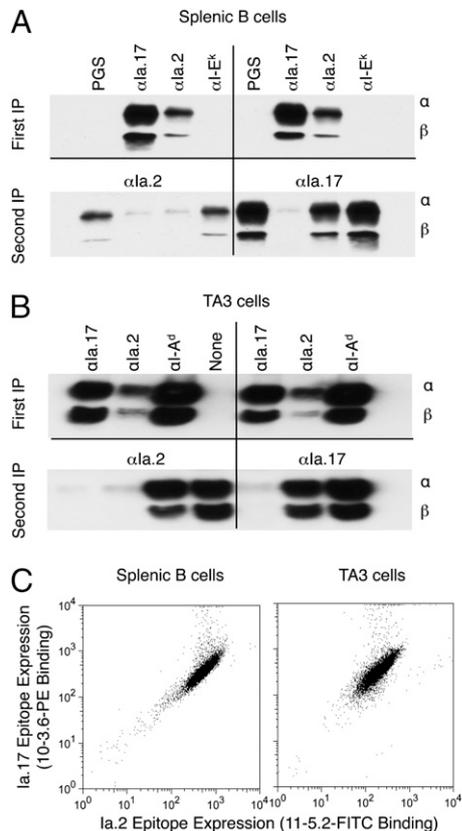
FIGURE 1. The signaling-competent anti-Ia.2 mAb 11-5.2 recognizes a subset of I-A^k class II molecules. *A*, B10.Br splenocyte lysates were immunoprecipitated (first IP) as indicated. Supernatants from the first IP were reprecipitated as indicated (second IP). Immunoprecipitates were analyzed for class II by Western blot with polyclonal anti–I-A Ab, which detects both class II α and β-chains (15). Western blot analysis of supernatants from anti-Ia.17 IP revealed a lack of residual class II molecules, establishing that the Ia.17 epitope marks all cell surface I-A^k molecules. Representative results from one of three independent experiments. *B*, TA3 B cell lysates were analyzed as described in *A*. Representative results from one of three independent experiments. *C*, B10.Br splenic B cells or TA3 B cells were stained with 11-5.2–FITC and 10-3.6–PE. Splenic B cells were also stained with anti–CD19-PE-Cy7. Shown is the level of 11-5.2 and 10-3.6 staining for CD19+ splenic B cells and total TA3 B cells from one of four independent experiments.

with anti-Ia.2 mAb. The finding that reprecipitation of these samples with anti-Ia.2 mAb did not bring down additional class II ruled out the possibility that subsaturating amounts of anti-Ia.2 was used for the initial IP. Similar results were obtained with the I-A^k–expressing TA3 B cell line (Fig. 1*B*).

To determine whether the Ia.2+/Ia.17+ and Ia.2−/Ia.17+ subsets of class II were expressed on different populations of B cells, both splenic B cells and TA3 cells were stained with anti–Ia.2-FITC and anti-Ia.17-PE and analyzed by flow cytometry (Fig. 1*C*). Both cell types stain uniformly positive for both Ia.2 and Ia.17, indicating that the Ia.2 epitope marks a subset of I-A^k class II molecules expressed by all B cells (Fig. 2*C*). This finding is consistent with previous ELISA-based analyses of the distribution of Ia.2 and Ia.17 epitopes on detergent-solubilized I-A^k (21), which led the investigators to suggest that the Ia.2 epitope is not present on all I-A^k molecules. Analysis of BCR activated splenic B cells revealed that activation upregulated the expression of both total (Ia.17+) and Ia.2+ class II to the same extent (Ia.17 upregulation: 2.08-fold ± 0.22; Ia.2 upregulation: 2.10-fold ± 0.06) and had no effect on the ability of the anti-Ia.2 mAb to elicit signaling
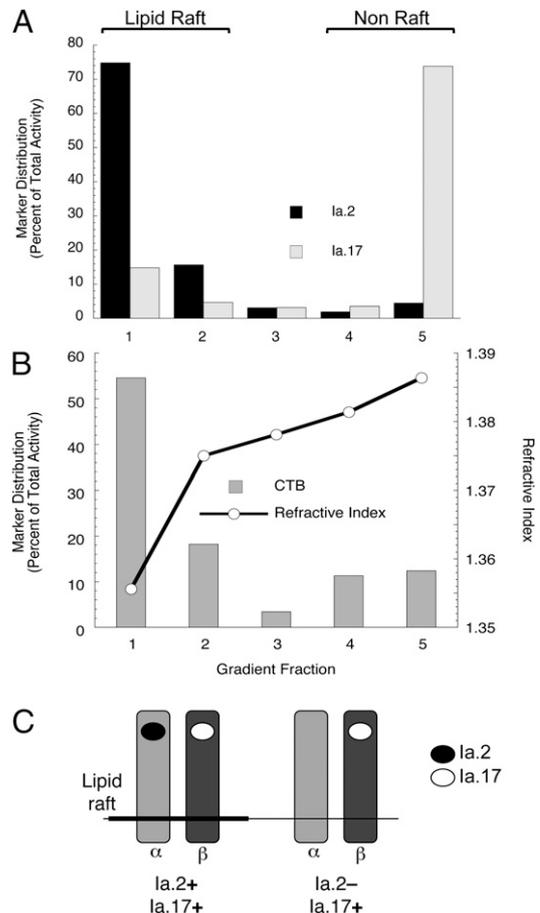

FIGURE 2. The Ia.2+ subset of I-A^k class II molecules exhibits increased partitioning into plasma membrane lipid rafts. *A*, Biotinylated anti-Ia.2 or anti-Ia.17 mAb was bound to TA3 B cells and then labeled with streptavidin-HRP. Cells were lysed in TNE 1% TX-100, lipid rafts were isolated by sucrose density-gradient centrifugation, and the distribution of the HRP-tagged anti-class II mAb was determined via a colorimetric assay (16, 17). Ab distribution is shown as the percentage of total activity detected in each fraction. Representative results from one of three independent experiments. The average level of anti-Ia.2 in lipid rafts (fractions 1 and 2) was 86.1 ± 7.8% (1 SD). The average level of anti-Ia.17 in lipid rafts was 18.0 ± 4.7% (1 SD). *B*, Parallel samples of TA3 B cells were labeled with CTB-HRP and analyzed as in *A*. The refractive index reflects the density profile of the sucrose gradient. Representative results from one of three independent experiments. *C*, Distribution of the Ia.2 and Ia.17 epitopes on the two subsets of I-A^k molecules. Black circle represents the Ia.2 epitope previously mapped to the I-A^k α-chain (6, 7). White circles represents the Ia.17 epitope previously mapped to the I-A^k β-chain (19).

(10). Thus, we restricted the subsequent analysis of Ia.2+ class II to resting B cells and I-A^k–bearing B cell lines.

The Ia.2+ class II subset is enriched in lipid rafts

Previous studies established a role for lipid rafts in MHC class II signaling in human monocytes (22) and class II-transfected sarcoma cells (23). To determine whether the previously reported signaling capacity of Ia.2+ I-A^k class II in B cells (10) is related to partitioning of this subset of class II molecules into lipid rafts, the raft localization of both the total (Ia.17+) and Ia.2+ populations of I-A^k class II molecules was determined (Fig. 2*A*). Consistent with previous reports (24), only 15–20% of total (Ia.17+) cell surface I-A^k is present in plasma membrane lipid rafts. In contrast, almost 90% of Ia.2+ class II is found in plasma membrane lipid rafts,

closely mirroring the distribution of CTB, which binds lipid raft-localized GM_1 glycolipid molecules (Fig. 2B).

Previous analysis of BCR function revealed that although lipid rafts play a critical role in BCR signaling, they represent inefficient internalization platforms (16, 17, 25). Therefore, to use an intact cell approach to further test the idea that the Ia.2 epitope marks a subset of lipid raft-restricted class II molecules, the kinetics of class II-mediated internalization of the anti-Ia.2 and anti-Ia.17 mAb was determined. Consistent with the extensive lipid raft partitioning of Ia.2$^+$ class II molecules (Fig. 2), internalization of the anti-Ia.2 mAb was minimal and occurred with relatively slow kinetics (Fig. 3), similar to the previously reported kinetics of lipid raft-mediated internalization of CTB (17) and GPI-linked BCR (25). In contrast, a significantly greater fraction of the anti-Ia.17 mAb (bound to all I-A^k molecules) was rapidly cleared from the cell surface, such that almost 40% of the bound anti-Ia.17 was internalized within 30 min (Fig. 3). Although it is possible that the lower level of anti-Ia.2 internalization is due, in part, to Ia.2$^+$ class II recycling back to the cell surface, the similarity between the kinetics of anti-Ia.2 internalization and those of lipid raft-bound CTB is fully consistent with the high-level lipid raft partitioning of the subset of Ia.2$^+$ class II molecules. Taken together, the results presented in Figs. 1–3 establish that although all I-A^k molecules bear the Ia.17 epitope, only a subset of signaling-competent lipid raft-localized I-A^k molecules possess the Ia.2 epitope recognized by the 11-5.2 mAb (Fig. 2C).

Addition of a class II-tethered peptide eliminates Ia.2 expression and class II lipid raft partitioning

Because the CT of class II was reported to be important for lipid raft-dependent class II signaling in sarcoma cells (23), the role of these domains in the expression of the Ia.2 epitope was investigated. Either wild-type or ΔCT mutants of Aαk and Aβk were expressed in CIITA-expressing HEK293T cells, and Ia.2 epitope expression was monitored by staining with anti–Ia.2-FITC and anti-Ia.17-PE. As shown by the results presented in Fig. 4A and 4B, deletion of the CT of both I-A^k chains had no impact on the level of Ia.2 epitope expression. Moreover, because HEK293T cells are not B cells (and therefore do not express CD79), these results establish that formation of the Ia.2 epitope is not dependent upon association of class II and CD79 (26). However, this does not
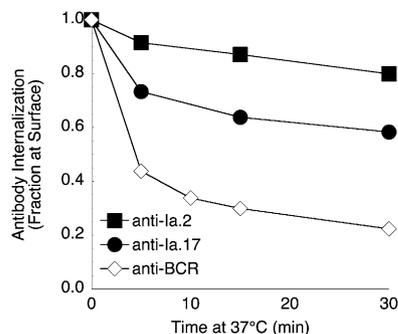

FIGURE 3. Ia.2-bearing class II molecules exhibit a diminished Ab-elicited clearance from the cell surface. Kinetics of internalization of class II-bound anti-Ia.2 and anti-Ia.17 mAb were analyzed as previously reported (16). anti-IgM^b-btn internalization ($n = 2$) was analyzed as a control. Shown is the average level of anti-Ia.2 and anti-Ia.17 mAb internalization ($\pm$ 1 SD, which is smaller than the icon) for four independent experiments (two experiments of 11-5.2 versus 10-2.16 and two experiments of 11-5.2 versus 10-3.6). The difference in the endocytosis of the anti-Ia.2 and anti-Ia.17 mAb was confirmed by following the internalization of 11-5.2–btn + streptavidin-HRP and 10-3.6–btn + streptavidin-HRP using a colorimetric assay that tracks both total and cell surface HRP (19) (data not shown).
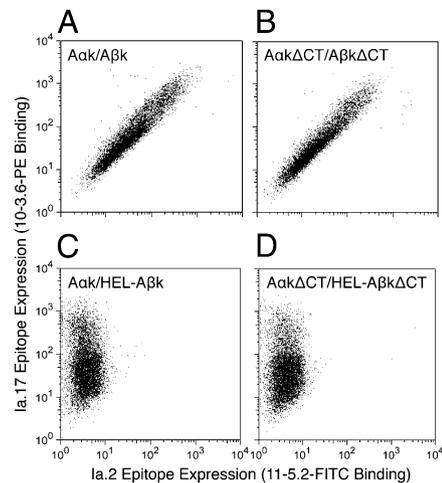

FIGURE 4. Addition of a class II-tethered peptide abolishes Ia.2 epitope expression. HEK293T embryonic fibroblasts stably expressing CIITA (CIITA-293T) were transiently transfected with full-length I-A^k (Aαk/Aβk) (A), ΔCT I-A^k (AαkΔCT/AβkΔCT) (B), full-length I-A^k with a tethered peptide (Aαk/HEL-Aβk) (C), or HEL–I-A^{k}ΔCT (AαkΔCT/HEL–AβkΔCT) (D). Twenty-four hours after transfection, cells were stained with 11-5.2–FITC and 10-3.6–PE and analyzed by flow cytometry. Representative results from one of five independent experiments.

rule out the possibility that lipid raft-localized Ia.2$^+$ class II molecules may exhibit a level of CD79 association distinct from that of total class II molecules.

Another way in which Ia.2 epitope acquisition could be controlled is during class II folding in the endoplasmic reticulum or peptide-loading compartment (i.e., MHC class II enriched compartment [MIIC]). Because Ii is critically involved in both of these aspects of class II biosynthesis, it is possible that Ii is controlling formation of the Ia.2 epitope. To assess the impact of Ii association on Ia.2 epitope acquisition, a previously reported I-A^k construct, with the immunodominant 47–62 peptide of HEL tethered to the N terminus of Aβk via a flexible linker, was expressed in CIITA-transfected HEK293T cells along with Aαk, and the cells were analyzed by staining and flow cytometry. The tethered peptide should negate the need for CLIP-dependent Ii–class II association and impede Ii-chaperoned class II maturation. (The tethered peptide was added to the ΔCT class II molecule to minimize class II internalization and subsequent cleavage and editing of the tethered peptide.) Interestingly, addition of the tethered HEL peptide [which can be detected bound to I-A^k at the cell surface (Fig. 5A)] abolishes Ia.2 epitope expression in both full-length (Fig. 4C) and ΔCT class II molecules (Fig. 4D). These results support the notion that Ii association supports generation of the Ia.2 epitope and demonstrates that there is essentially no contribution of the cytoplasmic domain of class II in Ia.2 epitope formation.

Two ways by which Ii may control Ia.2 epitope acquisition are by controlling the intracellular trafficking of nascent class II molecules or by controlling MHC class II protein folding. To address the potential role of class II trafficking in acquisition of the Ia.2 epitope, DM$^+$ MIIC Ag-processing compartments were isolated from TA3 B cells by Nycodenz density-gradient centrifugation (19), and the Ia.2 status of the MIIC-localized I-A^k was determined by IP/Western blot (Supplemental Fig. 1). The results established the presence of Ia.2$^+$ class II in DM$^+$ MIIC, demonstrating that Ia.2 forms within the biosynthetic pathway. Because work by Lovitch and Unanue (2) showed that DM essentially prevents the formation of type B peptide–class II complexes, these
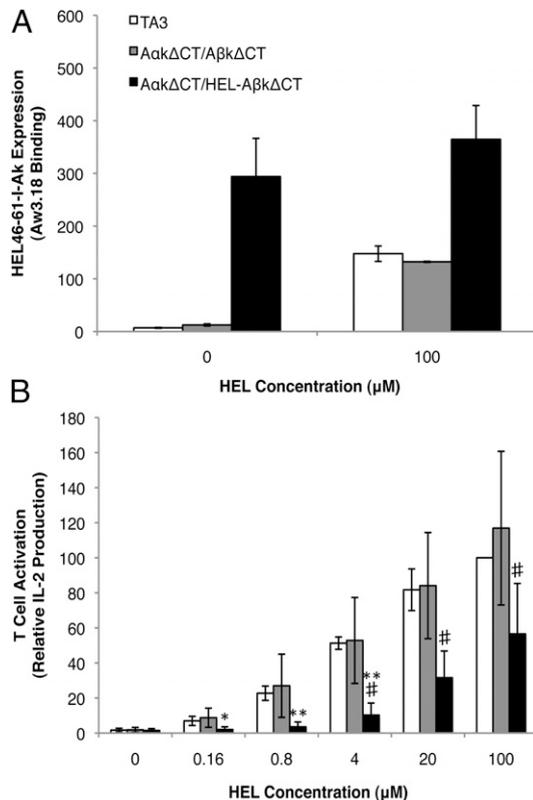
FIGURE 5. Ia.2$^-$ class II lacks the ability to stimulate CD4 T cells. *A*, IIA1.6 B cells stably expressing tailless I-A^k or HEL-tethered tailless I-A^k, along with TA3 B cells, were incubated overnight in media with or without 100 μM HEL protein. The cells were stained with the anti–HEL$_{47-62}$-I-A^k–specific Aw3.18 mAb, and the level of mAb binding was determined by flow cytometry. Average mean fluorescence intensity of Aw3.18 staining ± 1 SD was calculated from three independent experiments. Analysis of the Aw3.18 staining of HEL-pulsed versus non–HEL-pulsed IIA1.6 cells expressing HEL-tethered tailless I-A^k by a paired, two-tailed Student *t* test established a *p* value = 0.02. *B*, IIA1.6 B cells stably expressing I-A^k or HEL-tethered tailless I-A^k, along with TA3 B cells, were incubated overnight with the I-A^k–HEL$_{47-62}$–specific Ly50 T cell hybridoma and increasing doses of HEL protein. Supernatants were collected, and IL-2 levels (as a readout of T cell activation) were determined by cytometric bead array. Shown is the average IL-2 production (normalized to the amount of IL-2 produced by T cells activated by TA3 B cells pulsed with 100 μM HEL) ± 1 SD from three independent experiments. $^#p \leq 0.05$, HEL–I-A^k–ΔCT versus I-A^k–ΔCT; *$p \leq 0.05$; **$p \leq 0.01$, HEL–I-A^k–ΔCT versus TA3.

results also established that Ia.2$^+$ class II molecules are not equivalent to type B complexes as defined by Lovitch and Unanue.

To further investigate the potential role of Ii in Ia.2 epitope formation, the association of Ii with both total (Ia.17$^+$) and Ia.2$^+$ class II was determined by IP and Western blotting (Supplemental Fig. 2). Consistent with the MIIC localization of some Ia.2$^+$ class II, Ii is associated with both Ia.17$^+$ and Ia.2$^+$ class II. Moreover, Ii p31 is the predominant form of Ii associated with both Ia.17$^+$ and Ia.2$^+$ class II. Consistent with the apparently identical Ii association and intracellular trafficking of both total and Ia.2$^+$ nascent class II, Western blot analysis of anti-Ia.17 and anti-Ia.2 immunoprecipitates also revealed the equivalent relative level of SDS-stable αβ dimers (1) in both samples (data not shown). Taken together, these results are consistent with formation of the Ia.2 epitope early in the biosynthetic pathway and indicate that if Ii directly controls acquisition of the Ia.2 epitope, it must be occurring by a mechanism other than a selective association of class II with the Ii p31 or p41 isoform. This idea is under investigation.
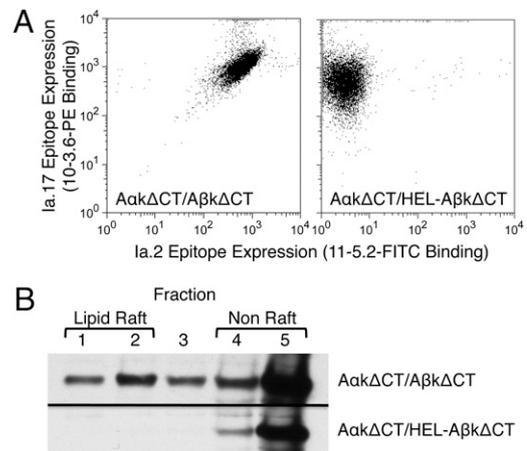

FIGURE 6. Addition of a class II-tethered peptide abolishes class II lipid raft localization. *A*, IIA1.6 B cells were stably transfected to express tailless I-A^k or tailless I-A^k possessing a tethered HEL peptide. Cells were stained with 11-5.2–FITC and 10-3.6–PE and analyzed by flow cytometry. *B*, Surface I-A^k class II of stably transfected IIA1.6 cells was tagged with 10-3.6–btn and streptavidin-HRP, and the cells were warmed to 37°C for 5 min. Cells were then lysed in TNE 1% TX-100, lipid rafts were isolated by sucrose density gradient centrifugation (16, 17), and the distribution of the btn-tagged anti-class II mAb was detected by probing a Western blot of the sucrose fractions with streptavidin-HRP. Representative results of one of three experiments.

Because the Ia.2$^+$ class II subset is normally found in lipid rafts, we next determined the impact of adding the β-chain–tethered HEL peptide on class II lipid raft partitioning. IIA1.6 B cells were stably transfected with either Ia.2$^+$ AαkΔCT/AβkΔCT (I-A^k–ΔCT) or Ia.2$^-$ AαkΔCT/HEL-AβkΔCT (HEL–I-A^k–ΔCT), and clones expressing similar levels of total I-A^k class II (Ia.17) were selected for analysis (Fig. 6*A*). When lipid rafts were isolated from transfectants where cell surface class II was tagged with anti–Ia.17-btn, it became apparent that although Ia.2$^+$ I-A^k–ΔCT exhibits a level of raft partitioning similar to wild-type I-A^k (Fig. 6*B*, compare with Fig. 2*A*), Ia.2$^-$ HEL–I-A^k–ΔCT was absent from plasma membrane lipid rafts (Fig. 6*B*). This altered plasma membrane distribution suggested that the Ia.2 epitope [defined by the 11-5.2 mAb that binds Aαk near the peptide-binding region of the molecule (6, 7)] marks a distinct conformational state of the I-A^k protein that has a higher affinity for lipid raft membrane domains.

The Ia.2$^+$ subset of class II is central to efficient T cell stimulation

Because lipid raft-resident peptide–class II complexes play a central role in CD4 T cell activation (27–29), the role of the Ia.2$^+$ subset of lipid raft-resident class II molecules in Ag presentation was investigated. Both I-A^k–ΔCT and HEL–I-A^k–ΔCT transfectants express levels of total I-A^k class II (Ia.17) similar to that of the endogenous wild type I-A^k class II molecules expressed by TA3 cells (Figs. 1*C*, 6*A*). Moreover, direct staining with the HEL$_{47-62}$–I-A^k–specific mAb Aw3.18 revealed that a significant amount of the HEL$_{47-62}$–I-A^k construct expressed by the HEL–I-A^k–ΔCT cells traffics to the cell surface with the tethered peptide bound in the class II peptide-binding groove (Fig. 5*A*, 0 μM HEL).

To test the Ag-presentation capabilities of all three types of I-A^k class II molecules (i.e., wild type, I-A^k–ΔCT and HEL–I-A^k–ΔCT), TA3 or I-A^k–transfected B cells were pulsed overnight with 100 μM HEL protein (a treatment that does not modulate expression of the Ia.2 epitope), and the resulting levels of HEL$_{47-62}$–I-A^k complexes were determined by staining with Aw3.18 (Fig.

5A). Both TA3 and I-A^k–ΔCT cells pulsed with 100 μM HEL protein exhibited a similar increase in expression of cell surface HEL$_{47-62}$–I-A^k complexes, demonstrating that both wild-type and ΔCT class II molecules are capable of being loaded with exogenous Ag-derived peptides. In addition, HEL-pulsed HEL–I-A^k–ΔCT cells exhibited a statistically significant increase in Aw3.18 binding (Fig. 5A), demonstrating that at least a fraction of the Ia.2–I-A^k molecules expressed by these cells can be loaded with exogenous Ag-derived peptides.

To test the relative T cell-stimulatory activity of Ia.2^+ and Ia.2–HEL$_{47-62}$–I-A^k peptide–class II complexes, the ability of all three B cell lines (pulsed with varying doses of HEL protein) to activate HEL$_{47-62}$–I-A^k–reactive Ly50 T cells was tested (Fig. 5B). For TA3 cells (expressing wild-type I-A^k) and I-A^k–ΔCT B cells, addition of increasing amounts of HEL protein resulted in a similar dose-dependent increase in T cell activation. In contrast, HEL–I-A^k–ΔCT B cells elicited significantly less T cell activation upon addition of exogenous Ag. Moreover, the HEL–I-A^k–ΔCT B cells also failed to activate Ly50 T cells in the absence of added HEL protein, although the cells expressed significant levels of tethered HEL$_{47-62}$–I-A^k complexes detectable with the Aw3.18 mAb (Fig. 5A). Although the lack of T cell stimulation by the HEL–I-A^k–ΔCT B cells could be due to a blocking effect of the tethered peptide, this is unlikely because the tethered peptide–class II complexes are recognized by the Aw3.18 mAb (Fig. 5A), which recognizes the same HEL$_{47-62}$–I-A^k complex as does the Ly50 TCR.

To extend the analysis of T cell activation by Ia.2^+ class II to nontransformed B cells, the role of Ia.2^+ class II in Ag presentation by splenic B cells was investigated. Although immunologically relevant B cell Ag presentation occurs subsequent to the BCR-mediated processing of cognate Ag, B cells can also process and present noncognate Ag internalized via fluid-phase endocytosis. Moreover, previous studies established that, in splenic B cells, these two distinct pathways of Ag processing result in the formation of functionally distinct peptide–class II complexes that differ in their ability to elicit B cell activation (13). Specifically, BCR-mediated processing resulted in the formation of type I peptide–class II complexes, whereas fluid-phase Ag processing resulted in the formation of type II complexes. Therefore, the impact of anti-Ia.2 mAb on the formation of B cell–T cell conjugates via either type I or II peptide–class II complexes was determined [for these studies, anti-Ia.2 mAb was not used to block induction of T cell cytokine production because anti-Ia.2 binding elicits B cell activation (10), introducing a confounding variable into T cell-activation studies]. As shown by the results presented in Fig. 7, prebinding of anti-Ia.2 to the Ia.2^+ subset of class II molecules profoundly inhibited B cell–T cell conjugate formation via both types I and II HEL–I-A^k peptide–class II complexes. In contrast, binding of the anti-Ia.17 mAb, which binds all cell surface I-A^k, exhibited only a partial inhibitory effect, at best. These results establish that both BCR-mediated and fluid-phase Ag processing result in the formation of Ia.2^+ antigenic peptide–class II complexes and that these Ia.2^+ signaling-competent lipid raft resident cell surface peptide–class II complexes are critical to cognate B cell–T cell interactions.

Discussion

This study establishes that the Ia.2 epitope defines a conformational subset of lipid raft resident I-A^k class II molecules that are critically involved in MHC class II-restricted B cell–T cell interactions. These observations are consistent with previous studies establishing the critical role for lipid raft resident peptide–class II complexes in T cell activation, especially when Ag is present at
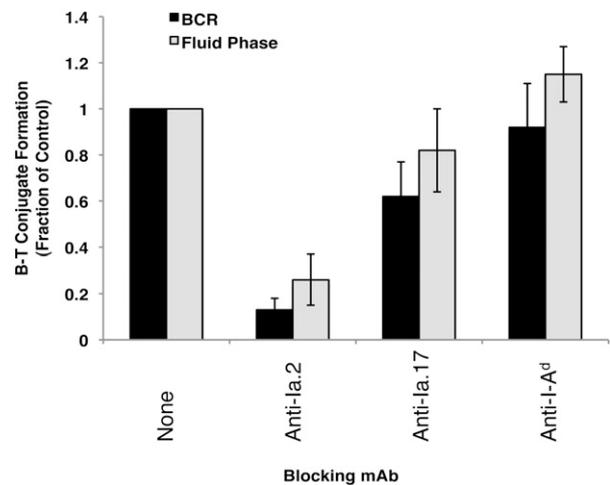

FIGURE 7. The 11-5.2 anti-Ia.2 mAb efficiently blocks B cell–T cell interactions via both BCR and fluid-phase–generated peptide–class II complexes. B cells expressing BCR or fluid-phase–generated peptide–class II complexes were labeled with anti-B220-PE and cocultured with anti–Thy-1.2–FITC–labeled HEL$_{47-62}$–I-A^k–specific Ly50 T cells, and the level of B cell–T cell conjugates was determined by flow cytometry. Ab inhibitors (used at saturating concentrations) were prebound to B cells and maintained throughout the assay. Bars indicate the average level of B cell–T cell conjugates ($n = 4$) formed under each condition (relative to the no inhibitor control) ± 1 SD. $p \leq 0.01$, for both types I and II complexes, 11-5.2 blocking versus no blocking mAb.

limiting concentrations (24, 27, 28, 30). More importantly, these data uncover an additional level of complexity in the structure and function of MHC class II that significantly impacts our understanding of MHC class II-restricted Ag processing and presentation.

There are two significant questions that arise from this study. One is the structural basis of the Ia.2 epitope. The other is the molecular explanation for the enhanced lipid raft partitioning of the Ia.2^+ class II molecules. Initially, the finding that addition of a class II-tethered peptide, which prevents CLIP-mediated class II–Ii interactions, blocked Ia.2 expression suggested that Ii-mediated delivery of class II to MIIC may drive acquisition of the Ia.2 epitope. However, subcellular fractionation studies suggested that this is not the case. Moreover, the finding that Ia.2^+ class II can be associated with Ii means that acquisition of the Ia.2 epitope occurs early in the biosynthetic pathway, possibly within the endoplasmic reticulum.

Because Ia.2^+ class II is found in lipid rafts, which have an ordered lipid structure distinct from the disordered lipid structure of the nonraft region of the membrane, it is more likely that the conformational differences between Ia.2^+ and Ia.2^- class II molecules involve the transmembrane domain of the molecule. Consistent with this scenario, it was reported that transmembrane domain mutants of the I-A^k class II molecule lose expression of the Ia.2 epitope while retaining the Ia.17 epitope (9). Furthermore, prior studies established a CLIP-independent role for the transmembrane domain of the Ii protein in class II–Ii interactions (31). Therefore, addition of the class II-tethered peptide may ablate Ia.2 epitope expression by altering class II–Ii transmembrane domain interactions in the endoplasmic reticulum (or other subcellular compartment), resulting in class II–Ii complexes incapable of adopting the Ia.2^+ conformation. This possibility is under investigation.

Functional studies were the first hint that Ia.2^+ class II represents a subset of class II molecules (10). Likewise, type A versus type B peptide–class II complexes were initially defined on a functional

basis (2). Although the precise structural differences between types A and B complexes remain unknown, it is well established that formation of type A complexes is DM dependent, whereas type B complexes seem to be destroyed upon interaction with the DM chaperone. Initially, the idea that Ia.2$^+$ class II molecules might correspond to either type A or B complexes was very attractive. However, both Ia.2$^+$ and Ia.2$^-$ class II molecules are found in DM-rich MIIC, an environment that would lead to the rapid destruction of type B complexes, which suggests that this is not the case. Nevertheless, a more subtle role for DM in the formation of Ia.2$^+$ versus Ia.2$^-$ peptide–class II complexes remains a topic of active investigation.

Another MHC class II conformational variant that impacts T cell activation is the formation of class II "dimer-of-dimers" (i.e., a dimer of MHC class II αβ dimers) (32). In 1999, Wade and colleagues (33) examined the effect of mutations that disrupt I-A^k dimer-of-dimers formation on T cell activation and anti–I-A^k mAb binding, including anti-Ia.2 and anti-Ia.17 mAbs. Their results demonstrated that although multiple class II mutants that inhibit I-A^k dimer-of-dimers formation significantly impacted T cell activation, these mutations failed to alter the ratio of anti-Ia.2/anti-Ia.17 mAb binding. Their results indicated that formation of the Ia.2 epitope is not dependent on the formation of I-A^k dimer-of-dimers. However, additional investigations are required to address related questions, such as whether Ia.2$^+$ versus Ia.2$^-$ class II complexes differ in their inherent ability to form class II dimer-of-dimers.

Another potential explanation for the unique structure and function of the Ia.2$^+$ subset of class II resides in the association of known MHC class II "accessory" proteins, such as the CD79 signaling subunit of the BCR (26), as well as multiple members of the tetraspan family of proteins [e.g., CD9 (22), CD20 (34), and CD82 (35)], which were shown to form lipid raft-like membrane domains (36). Because HEK293T cells lack CD79, the results presented in Fig. 4 rule out the likelihood that the Ia.2 epitope marks pre-existing class II–CD79 complexes. However, the potential role of one or more tetraspan proteins remains an open question, because multiple members of the tetraspan family of proteins are expressed in many cell types.

In summary, this study establishes that the Ia.2 epitope (recognized by the widely used 11-5.2 anti–I-A^k mAb) defines a conformational subset of 10–20% of cell surface I-A^k MHC class II molecules that exhibit a high level of partitioning into plasma membrane lipid rafts. This discovery highlights a previously unappreciated level of MHC class II structural heterogeneity and defines a new tool for the investigation of MHC class II biology. The results also establish that the Ia.2-bearing class II conformer found in lipid rafts is critically important for the formation of cognate B cell–T cell interactions and subsequent T cell activation. Future studies to define the mechanism of generation of the Ia.2 epitope will provide greater insight into MHC class II structure and function, as well as the molecular mechanisms that control membrane protein partitioning into distinct membrane microdomains.

Acknowledgments

Disclosures

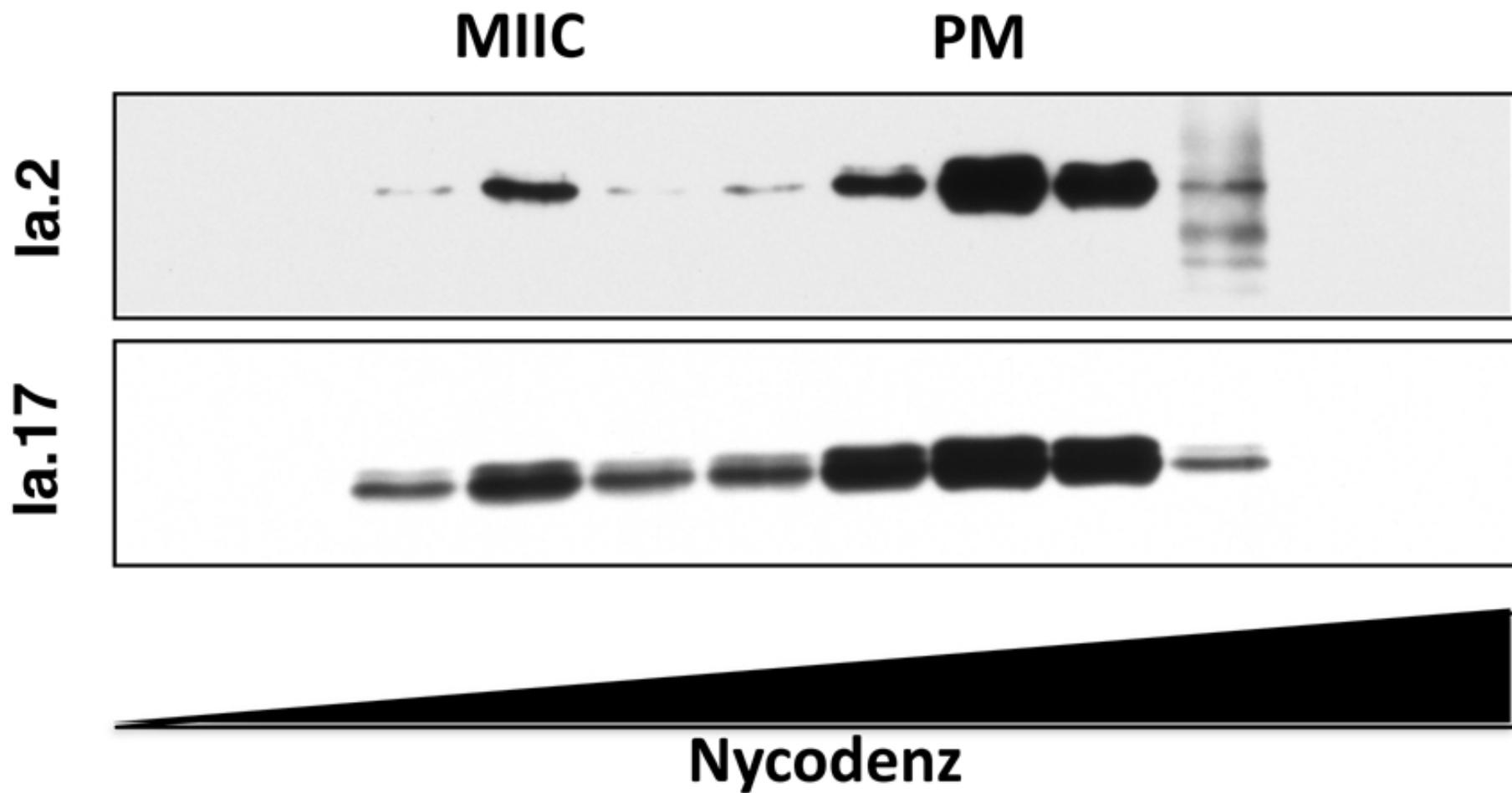The authors have no financial conflicts of interest.

References

1. Sadegh-Nasseri, S., L. J. Stern, D. C. Wiley, and R. N. Germain. 1994. MHC class II function preserved by low-affinity peptide interactions preceding stable binding. *Nature* 370: 647–650.
2. Lovitch, S. B., and E. R. Unanue. 2005. Conformational isomers of a peptide-class I major histocompatibility complex. *Immunol. Rev.* 207: 293–313.
3. Klein, J. 1979. The major histocompatibility complex of the mouse. *Science* 203: 516–521.
4. Oi, V. T., P. P. Jones, J. W. Goding, L. A. Herzenberg, and L. A. Herzenberg. 1978. Properties of monoclonal antibodies to mouse Ig allotypes, H-2, and Ia antigens. *Curr. Top. Microbiol. Immunol.* 81: 115–120.
5. Pierres, M., C. Devaux, M. Dosseto, and S. Marchetto. 1981. Clonal analysis of B- and T-cell responses to Ia antigens. I. Topology of epitope regions on I-Ak and I-Ek molecules analyzed with 35 monoclonal alloantibodies. *Immunogenetics* 14: 481–495.
6. Landais, D., H. Matthes, C. Benoist, and D. Mathis. 1985. A molecular basis for the Ia.2 and Ia.19 antigenic determinants. *Proc. Natl. Acad. Sci. USA* 82: 2930–2934.
7. Landias, D., B. N. Beck, J. M. Buerstedde, S. Degraw, D. Klein, N. Koch, D. Murphy, M. Pierres, T. Tada, K. Yamamoto, et al. 1986. The assignment of chain specificities for anti-Ia monoclonal antibodies using L cell transfectants. *J. Immunol.* 137: 3002–3005.
8. Braunstein, N. S., and R. N. Germain. 1987. Allele-specific control of Ia molecule surface expression and conformation: implications for a general model of Ia structure-function relationships. *Proc. Natl. Acad. Sci. USA* 84: 2921–2925.
9. Cosson, P., and J. S. Bonifacino. 1992. Role of transmembrane domain interactions in the assembly of class II MHC molecules. *Science* 258: 659–662.
10. Nashar, T. O., and J. R. Drake. 2006. Dynamics of MHC class II-activating signals in murine resting B cells. *J. Immunol.* 176: 827–838.
11. McGovern, E. M., A. E. Moquin, A. Caballero, and J. R. Drake. 2004. The effect of B cell receptor signaling on antigen endocytosis and processing. *Immunol. Invest.* 33: 143–156.
12. Gondré-Lewis, T. A., A. E. Moquin, and J. R. Drake. 2001. Prolonged antigen persistence within nonterminal late endocytic compartments of antigen-specific B lymphocytes. *J. Immunol.* 166: 6657–6664.
13. Nashar, T. O., and J. R. Drake. 2005. The pathway of antigen uptake and processing dictates MHC class II-mediated B cell survival and activation. *J. Immunol.* 174: 1306–1316.
14. Dadaglio, G., C. A. Nelson, M. B. Deck, S. J. Petzold, and E. R. Unanue. 1997. Characterization and quantitation of peptide-MHC complexes produced from hen egg lysozyme using a monoclonal antibody. *Immunity* 6: 727–738.
15. Amigorena, S., J. R. Drake, P. Webster, and I. Mellman. 1994. Transient accumulation of new class II MHC molecules in a novel endocytic compartment in B lymphocytes. *Nature* 369: 113–120.
16. Caballero, A., B. Katkere, X. Y. Wen, L. Drake, T. O. Nashar, and J. R. Drake. 2006. Functional and structural requirements for the internalization of distinct BCR-ligand complexes. *Eur. J. Immunol.* 36: 3131–3145.
17. Putnam, M. A., A. E. Moquin, M. Merrihew, C. Outcalt, E. Sorge, A. Caballero, T. A. Gondré-Lewis, and J. R. Drake. 2003. Lipid raft-independent B cell receptor-mediated antigen internalization and intracellular trafficking. *J. Immunol.* 170: 905–912.
18. Fremont, D. H., D. Monnaie, C. A. Nelson, W. A. Hendrickson, and E. R. Unanue. 1998. Crystal structure of I-Ak in complex with a dominant epitope of lysozyme. *Immunity* 8: 305–317.
19. Drake, J. R., T. A. Lewis, K. B. Condon, R. N. Mitchell, and P. Webster. 1999. Involvement of MIIC-like late endosomes in B cell receptor-mediated antigen processing in murine B cells. *J. Immunol.* 162: 1150–1155.
20. Rosloniec, E. F., D. Gay, and J. H. Freed. 1989. Epitopic analysis by anti-I-Ak monoclonal antibodies of I-Ak-restricted presentation of lysozyme peptides. *J. Immunol.* 142: 4176–4183.
21. Dubreuil, P. C., D. Z. Birnbaum, D. H. Caillol, and F. A. Lemonnier. 1982. Identification on I-Ak molecules of a functional site recognized by proliferating T-lymphocytes. *Immunogenetics* 16: 407–424.
22. Zilber, M. T., N. Setterblad, T. Vasselon, C. Doliger, D. Charron, N. Mooney, and C. Gelin. 2005. MHC class II/CD38/CD9: a lipid-raft-dependent signaling complex in human monocytes. *Blood* 106: 3074–3081.
23. Bécart, S., N. Setterblad, S. Ostrand-Rosenberg, S. J. Ono, D. Charron, and N. Mooney. 2003. Intracytoplasmic domains of MHC class II molecules are essential for lipid-raft-dependent signaling. *J. Cell Sci.* 116: 2565–2575.
24. Hiltbold, E. M., N. J. Poloso, and P. A. Roche. 2003. MHC class II-peptide complexes and APC lipid rafts accumulate at the immunological synapse. *J. Immunol.* 170: 1329–1338.
25. Mitchell, R. N., A. C. Shaw, Y. K. Weaver, P. Leder, and A. K. Abbas. 1991. Cytoplasmic tail deletion converts membrane immunoglobulin to a phosphatidylinositol-linked form lacking signaling and efficient antigen internalization functions. *J. Biol. Chem.* 266: 8856–8860.
26. Lang, P., J. C. Stolpa, B. A. Freiberg, F. Crawford, J. Kappler, A. Kupfer, and J. C. Cambier. 2001. TCR-induced transmembrane signaling by peptide/MHC class II via associated Ig-alpha/beta dimers. *Science* 291: 1537–1540.
27. Anderson, H. A., E. M. Hiltbold, and P. A. Roche. 2000. Concentration of MHC class II molecules in lipid rafts facilitates antigen presentation. *Nat. Immunol.* 1: 156–162.
28. Poloso, N. J., and P. A. Roche. 2004. Association of MHC class II-peptide complexes with plasma membrane lipid microdomains. *Curr. Opin. Immunol.* 16: 103–107.
29. Setterblad, N., S. Bécart, D. Charron, and N. Mooney. 2004. B cell lipid rafts regulate both peptide-dependent and peptide-independent APC-T cell interaction. *J. Immunol.* 173: 1876–1886.

30. Poloso, N. J., A. Muntasell, and P. A. Roche. 2004. MHC class II molecules traffic into lipid rafts during intracellular transport. *J. Immunol.* 173: 4539–4546.

31. Castellino, F., R. Han, and R. N. Germain. 2001. The transmembrane segment of invariant chain mediates binding to MHC class II molecules in a CLIP-independent manner. *Eur. J. Immunol.* 31: 841–850.

32. Schafer, P. H., S. K. Pierce, and T. S. Jardetzky. 1995. The structure of MHC class II: a role for dimer of dimers. *Semin. Immunol.* 7: 389–398.

33. Nydam, T., T. K. Wade, S. Yadati, J. L. Gabriel, B. G. Barisas, and W. F. Wade. 1998. Mutations in MHC class II dimer of dimers contact residues: effects on antigen presentation. *Int. Immunol.* 10: 1237–1249.

34. Szöllósi, J., V. Horejsí, L. Bene, P. Angelisová, and S. Damjanovich. 1996. Supramolecular complexes of MHC class I, MHC class II, CD20, and tetraspan molecules (CD53, CD81, and CD82) at the surface of a B cell line JY. *J. Immunol.* 157: 2939–2946.

35. Hammond, C., L. K. Denzin, M. Pan, J. M. Griffith, H. J. Geuze, and P. Cresswell. 1998. The tetraspan protein CD82 is a resident of MHC class II compartments where it associates with HLA-DR, -DM, and -DO molecules. *J. Immunol.* 161: 3282–3291.

36. Levy, S., and T. Shoham. 2005. The tetraspanin web modulates immune-signalling complexes. *Nat. Rev. Immunol.* 5: 136–148.

Supplementary Figure Legends:

SUPPLEMENTARY FIGURE 1. *Ia.2 Positive Class II in MIIC.* TA3 cells were homogenized and fractionated by Nycodenz density gradient centrifugation as previously reported (26). Gradient fractions were solublized and I-A^k class II immunoprecipitated with either anti-Ia.2 (11-5.2) or anti-Ia.17 (10-2.16). IP were analyzed for class II by western blot. The position of MIIC and plasma membrane (PM)-derived vesicles on the gradient is indicated. Shown are representative results from 1 of 3 independent experiments.

SUPPLEMENTARY FIGURE 2. *Association of Invariant Chain with Ia.2 Positive Class II.* TA3 cells were lysed and I-A^k class II IP with either anti-Ia.2 (11-5.2) or anti-Ia.17 (10-2.16) or protein G-Sepharose only (PGS). IP as well as whole cell lysate (WCL) were analyzed for invariant chain (Ii, CD74) by western blot. Shown are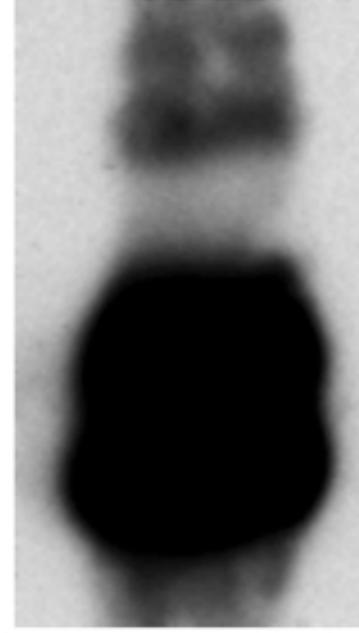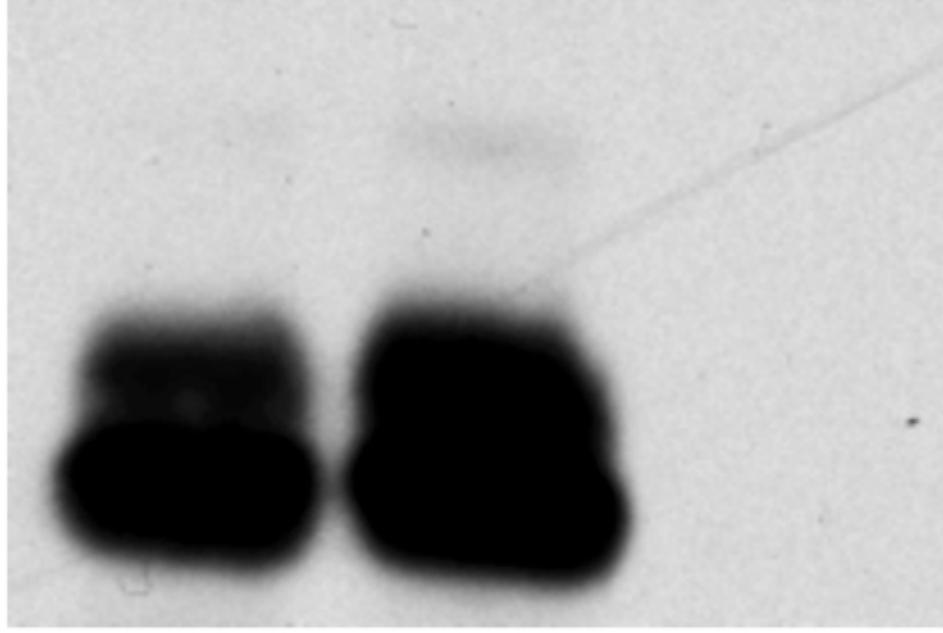 representative results from 1 of 3 independent experiments.