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Engagement of B Cell Receptor Regulates the Invariant Chain-Dependent MHC Class II Presentation Pathway¹

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Frédérique Forquet,* Lee Leserman,^{2*} and Jean Davoust

The intracellular sites in which Ags delivered by the B cell receptor (BCR) are degraded and loaded onto class II molecules remain poorly defined. To address this issue, we generated wild-type and invariant chain (Ii)-deficient H-2^k mice bearing BCR specific for hen egg lysozyme. Our results show that, 1) unlike Ags taken up from the fluid phase, Ii is required for presentation of hen egg lysozyme internalized through the BCR in a manner independent of the peptide analyzed; 2) BCR ligation induces intracellular accumulation of MHC class II molecules only in Ii-positive B cells; and 3) these class II molecules reach intracellular compartments where BCR targets exogenous Ag. No differences in expression of adhesion and costimulatory molecules or in the presentation of soluble peptides were detectable between Ii-positive and -negative B cells. Therefore, the BCR delivers its ligand to compartments containing MHC class II-Ii complexes and bypasses the Ii-independent presentation pathway. The linked roles of Ag internalization and B cell activation of the BCR leads to potent Ii-dependent presentation in splenic B cells. *The Journal of Immunology*, 1999, 162: 2495–2502.

Recognition by CD4⁺ T cells of protein Ags requires their presentation in association with MHC class II molecules expressed by APCs. Newly synthesized α - and β -chains of MHC class II heterodimers associate with the invariant chain (Ii)³ in the endoplasmic reticulum and are transported to endocytic compartment(s) where they meet antigenic peptides derived from exogenous proteins (1–6). The peptide loading process is facilitated by the presence of H2-M molecules involved in the dissociation of Ii fragments from the class II peptide binding groove (7–9). The intracellular route(s) followed by class II-Ii complexes from the trans-Golgi network to the endocytic pathway remain(s) incompletely characterized, although direct targeting to early endosomes, and indirect recycling via the plasma membrane, have been proposed (2, 6, 10–14). A di-leucine targeting motif present in the cytoplasmic tail of Ii plays a pivotal role in the control of the movements of class II-Ii complexes through the endocytic pathway (reviewed in Ref. 15). Ii is a major regulator of the trafficking of newly synthesized class II molecules among different intracellular compartments. Ii expression influences class II association with

peptides derived from protein cores (16). In B cells, newly synthesized class II molecules are targeted by Ii to intracellular loading compartments (reviewed in Refs. 15 and 17).

In addition to newly synthesized class II molecules, an alternative Ii-independent pathway of Ag presentation relying on recycled MHC class II molecules has been demonstrated (13, 14). Class II molecules expressed at the cell surface require a di-leucine motif present in the tail of the class II β -chain for entry into early endosomes (16). Cytoplasmic domain truncation mutants of MHC class II molecules fail to present a set of protein determinants and to be internalized in endosomes (13, 14). The antigenic determinants accessible to this second presentation pathway are thought to be produced in early endosomal compartments from endocytosed protein under conditions of mild proteolytic degradation and can be loaded onto recycled MHC class II molecules (13, 14, 16). This recycling pathway does not require either protein synthesis or the presence of Ii (18).

B cells generated from mice bearing a genetically disrupted Ii gene show a striking alteration in the intracellular transport and maturation of class II molecules (19–21). Ii requirement for class II dimerization differs between haplotypes (22). In the H-2^k haplotype, α - and β -chains efficiently assemble in the absence of Ii (22). However, in these mice, Ii-deficient B cells were able to present to T cells only peptides generated in early endocytic compartments that associate with recycled MHC class II molecules after Ag uptake by fluid phase endocytosis (19, 23). The stringency of Ii requirement for MHC class II peptide loading differs also in different cell types. We showed recently that Ii-deficient dendritic cells (DC) from H-2^k mice present a broad range of peptides generated from hen egg lysozyme (HEL), regardless of their position in the protein sequence (23). The heterogeneity observed for class II assembly and transport in different cell types may reflect physiologically important functional or developmental differences between them (23, 24).

B cells use the B cell receptor (BCR) to mediate efficient uptake and concentration of exogenous Ags. The BCR is a complex comprising membrane Ig molecules sheathed by noncovalently associated Ig α and Ig β molecules, which are responsible for Ag endocytosis and transmembrane signaling (25, 26). In B lymphoma

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³ Abbreviations used in this paper: Ii, invariant chain; HEL, hen egg lysozyme; DC, dendritic cell; BCR, B cell receptor; Tg, transgenic; mIg, membrane Ig.

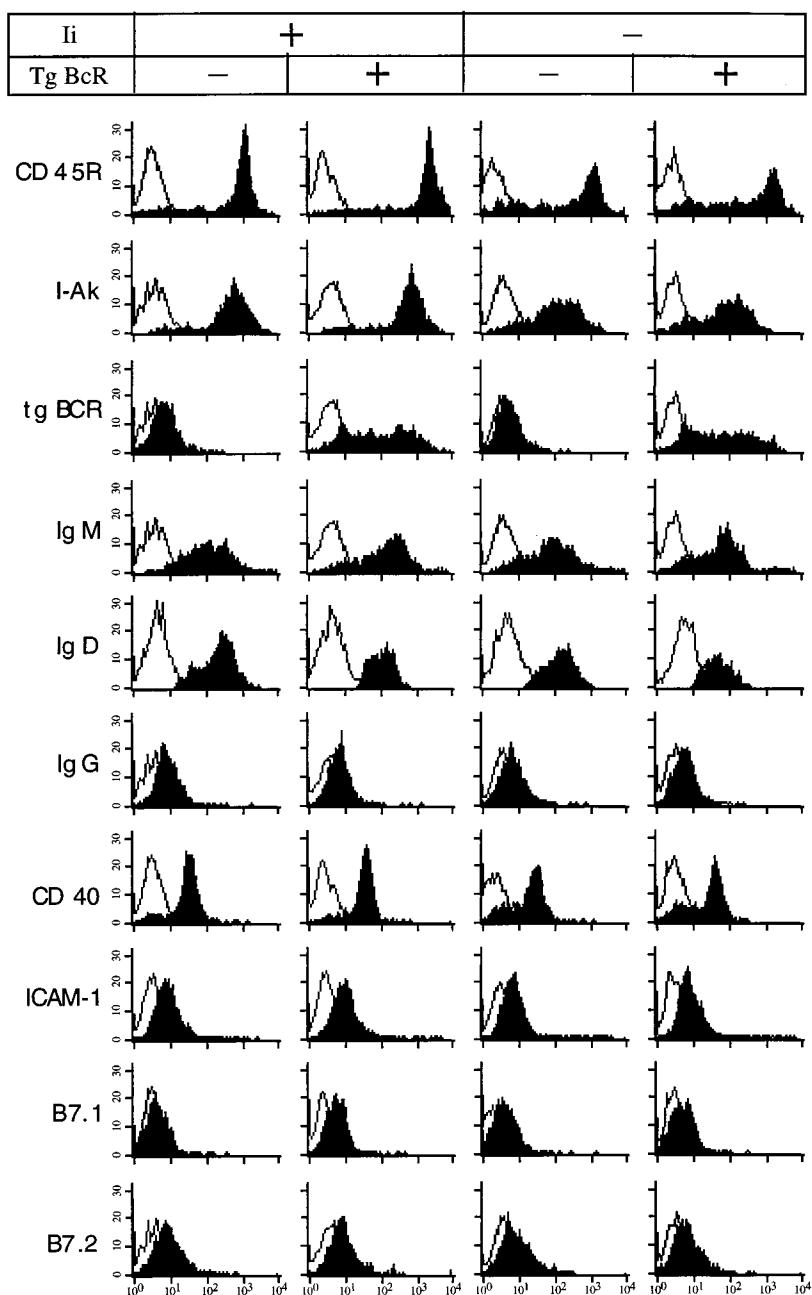


FIGURE 1. Phenotypic characterization of splenic B cells. Flow cytometric analysis of B cells derived from spleens of wild-type (Tg BCR⁻/Ii⁺), HEL-specific BCR Tg (Tg BCR⁺/Ii⁺), Ii-minus (Tg BCR⁻/Ii⁻), and HEL-specific BCR Tg Ii-minus (Tg BCR⁺/Ii⁻) mice. These four populations were analyzed for CD45R, I-A^k, Tg BCR, IgM, IgD, IgG, CD40, ICAM-1, B7.1, and B7.2 membrane expression (filled histograms). Control staining (open histograms) was performed using the second step reagent only.

cells, engagement of the BCR initiates a cascade of signaling events (26) that induce intracellular accumulation of MHC class II-Ii complexes (27, 28) and up-regulates costimulatory molecules. These events contribute to the ability of B cells to present their cognate Ags at extremely low concentrations (29–33). Most attempts to address the role of Ii in Ag presentation have relied on cells that were allowed to internalize Ag by fluid phase endocytosis. However, B cells are unlikely to present such nonspecifically internalized Ag in a physiological context.

To address the role of Ii in presentation of Ag internalized through the BCR, we crossed transgenic mice expressing an HEL-specific Ig molecule in their BCR complex with mice in which the Ii chain had been genetically deleted. We evaluated by confocal microscopy the relationship between BCR engagement and Ag compartmentalization. We also evaluated effects on Ag recognition by T cells. We wished in particular to determine the effect of BCR targeting on the presentation of three HEL-derived peptides

that are classically defined as “Ii-dependent” or “Ii-independent” (18). As expected, BCR targeting was found to increase the efficiency of all HEL-derived peptides by orders of magnitude. However, we found that BCR ligands are uniquely presented by the Ii-dependent Ag presentation pathway regardless of the peptide analyzed. This is consistent with our observation that BCR ligands are delivered into compartments in which the accumulation of MHC class II molecules is induced or markedly increased by engagement of the BCR.

Materials and Methods

Mice

B10BR mice (H-2^k) bearing a genetically disrupted Ii gene (Ii^{-/-}) were generously provided by D. Mathis and C. Benoist (Strasbourg, France) (19). The absence of Ii was tested by both Southern and Western blot analysis as described (23). CBA/J mice (H-2^k) bearing a transgenic BCR (IgM and IgD) (Tg BCR^{+/+}) specific for HEL were previously described

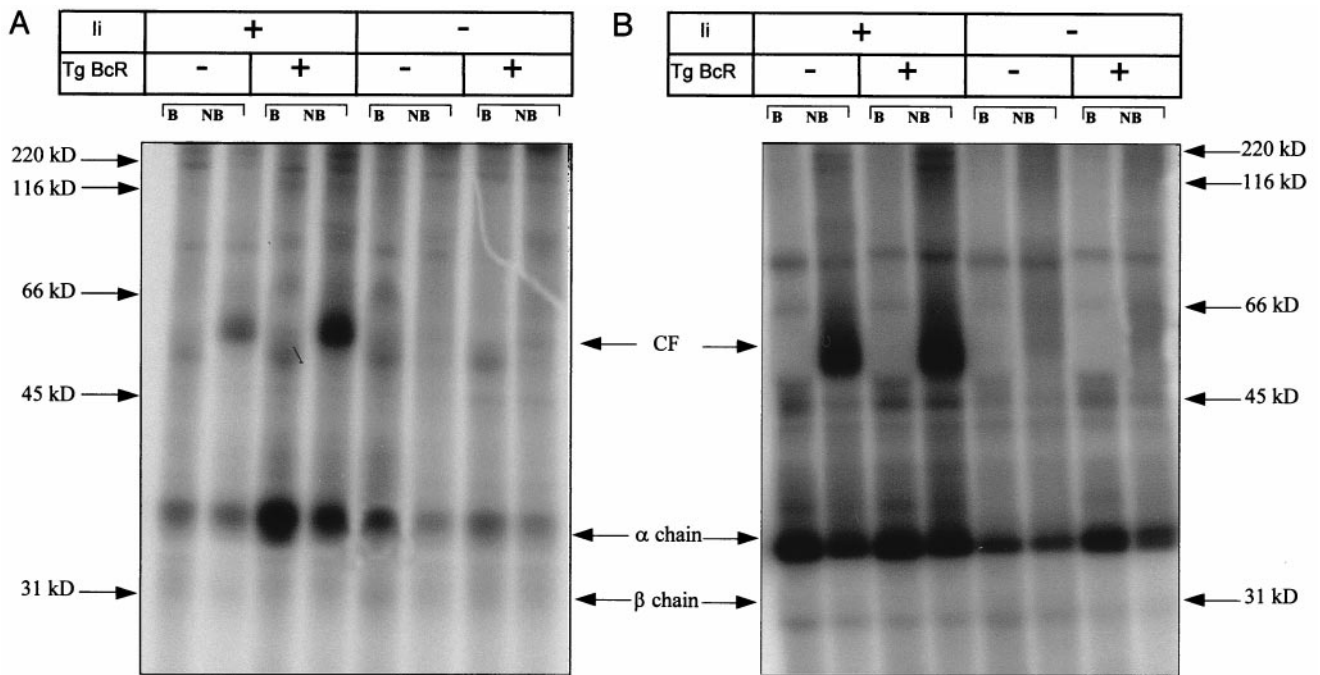


FIGURE 2. MHC class II lactoperoxidase-catalyzed surface iodination and SDS-PAGE analysis. Class II dimers were immunoprecipitated using the 10.2.16 mAb after surface iodination of splenic B cells derived from littermate mice expressing all possible phenotypes: (Tg BCR⁺/Ii⁺), (Tg BCR⁺/Ii⁻), (Tg BCR⁻/Ii⁻), and (Tg BCR⁻/Ii⁺). Before ¹²⁵I labeling, splenic B cells were incubated over night (B) or not (A) with 1 μ M HEL. Iodinated compact forms (CF) migrating as 55-kDa heterodimers were revealed in non boiled (NB) samples whereas α - and β -chains were detected in both NB and boiled (B) samples.

(34, 35). The F₁ generation between Ii^{-/-} mice and Tg BCR^{+/-} mice, Tg BCR^{+/-}/Ii^{+/-}, was back-crossed with the Ii^{-/-} mice. F₂ Tg BCR⁻/Ii⁺, Tg BCR⁺/Ii⁺, Tg BCR⁻/Ii⁻, and Tg BCR⁺/Ii⁻ littermates were used in all experiments. Mice were routinely characterized for Ii and Tg expression by Southern and Western blot and PCR (see below).

Southern blots, Western blots, and PCR.

To monitor Ii gene disruption, 21-day-old mice were routinely tested by Southern blot as described (19). Ii protein expression was also monitored by Western blot using an anti-Ii cytoplasmic domain Ab (23, 27). The expression of the BCR specific for HEL protein was monitored by PCR. Tail DNA aliquots (2 μ g) were amplified using two specific primers (final concentration, 2 pM) (5'-GCG ACT CCA TCA CCA GCG AT-3' and 5'-ACC ACA GAC CAG CAG GCA GA-3') in the following conditions: 5 min of denaturation (94°C), 30 cycles of amplification (30 s at 94°C, 30 s at 64°C, and 30 s at 72°C) and a cycle of elongation (5 min at 72°C). The products of PCR were analyzed on a 2% agarose gel. The presence of the transgene resulted in the amplification of a single fragment of 430 bp.

Media and cells

RPMI 1640 or DMEM medium (Sigma, St. Louis, MO) were supplemented with 5 mM L-glutamine, 100 IU/ml penicillin, 100 μ g/ml streptomycin (all from Life Technologies, Paisley, Scotland), 50 μ M β -mercaptoethanol, and 10% heat-inactivated FCS (BioMedia, Walkersville, MD). The isolation of B splenocytes was essentially performed as reported (23). T lymphocytes were depleted from spleen cells by Ab- and complement-mediated lysis (anti-Thy1, anti-CD4, anti-CD8). B cell enrichment varied between 85–95%, depending on the preparation as detected using the anti-CD45R mAb. The I-A^k-restricted T cell hybridomas (3A9, 3B11, and 2B6) were generously provided by L. Adorini (Milan, Italy) and cultured as described (18, 23, 36, 37). The I-A^k-restricted 3A9, 3B11, and 2B6 T cells are specific for the 46–61, 34–45, and 25–43 HEL peptides, respectively (18, 36). IL-2-dependent CTLL-2 cells (American Type Culture Collection (ATCC), Manassas, VA) were cultured in complete DMEM supplemented with 10 U/ml IL-2.

Antibodies

Hybridomas producing anti-mouse MHC class II-I-A^k molecule (10.2.16), anti-mouse Fc γ II/III receptor (2.4G2), anti-mouse ICAM-1, anti-mouse

Thy1 (J1J), anti-mouse CD4 (RL172Y), and anti-mouse CD8 (31 M) were all from ATCC. The 10.2.16 Ab precipitates compact and noncompact class II dimers. The hybridoma producing anti-mouse HEL peptide 46–61/I-A^k complex (C4H3) was kindly provided by R. Germain (National Institutes of Health, Bethesda, MD) (38). The rabbit polyclonal Abs against the cytoplasmic domain of Ii, I-A β -chains and H2-M β -chains, were a gift of N. Barois (Marseille, France) (27). Anti-CD45R, anti-B7.1, anti-B7.2, and anti-CD40 mAbs were all from PharMingen (San Diego, CA). The second step reagents and the anti-mouse IgM, IgD, and IgG were from Jackson Immunoresearch Laboratories (West Grove, PA). The anti-idiotypic mAb against the anti-HEL Tg BCR was previously described (35).

Surface iodination, immunoprecipitation and SDS-PAGE

B cells (10^7), incubated 16 h or not with 1 μ M HEL protein to induce class II peptide loading in Tg and non-Tg B cells, were labeled by lactoperoxidase-catalyzed iodination as described (19, 23). After extensive washing, cells were lysed in 0.5% Triton X-100. Class II molecules were immunoprecipitated with the 10.2.16 mAb. Before electrophoresis on an SDS-polyacrylamide gel (12.5%), the samples were either fully denatured at 95°C for 5 min or incubated for 30 min at room temperature in SDS sample buffer containing 5% 2-ME, to preserve the peptide-loaded compact forms of class II heterodimers (39).

Ag presentation

Ag presentation was performed by incubating, in 96-well flat-bottom plates, 7×10^4 3A9, 3B11, or 2B6 T hybridoma cells with 4×10^4 B cells, in the presence or in the absence of 10-fold serial dilution of the HEL protein or HEL peptide (46–61 peptide) (23). After 24 h, IL-2 secretion was assessed using thiazolyl blue (MTT) (Sigma) to evaluate the growth of the IL-2-dependent CTLL-2 line.

Immunofluorescence labeling

Cells were stained as previously described (40). The samples were analyzed using a FACScan instrument (Becton Dickinson, San Jose, CA). To monitor membrane Ig internalization after engagement in Tg BCR⁺/Ii⁺ and Tg BCR⁺/Ii⁻ B cells, cells were incubated either with 10 μ M HEL protein for 4 h or 16 h at 37°C or with an anti-mouse IgM Ab (10 μ g/ml) for 15 min at 4°C. After extensive washing, cells were either immediately processed for confocal analysis or incubated at 37°C for 1 h. To perform

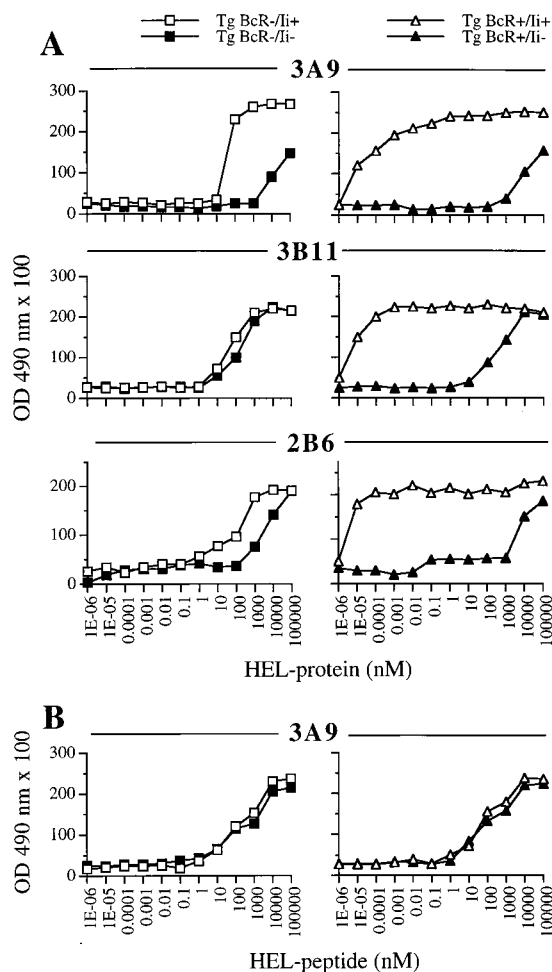


FIGURE 3. The efficient Ag presentation by HEL-specific BCR Tg B lymphocytes requires the expression of Ii. Tg BCR⁻/Ii⁺ (open squares) or Tg BCR⁻/Ii⁻ (filled squares) and Tg BCR⁺/Ii⁺ (open triangles) or Tg BCR⁺/Ii⁻ (filled triangles) B cells were incubated with serial dilutions of HEL and tested for their ability to stimulate the HEL 46–61 peptide-specific 3A9 T cell hybridoma or the HEL 34–45 and HEL 25–43 peptide-specific 3B11 and 2B6 T cell hybridomas (A). The four B cell populations were incubated with serial dilutions of HEL 46–61 synthetic peptide and tested for their ability to stimulate the 3A9 T cell hybridoma (B).

confocal microscopy analysis, cells were first attached to glass coverslips coated with poly-L-lysine (Sigma; 100 μ g/ml in distilled water for 1 h at room temperature) in medium devoid of FCS for 20 min at 4°C. Intracellular immunofluorescence was performed as previously described (41). The confocal laser scanning microscopy was conducted using a Leica TCS 4D instrument (Leica, Heidelberg, Germany) (23, 27).

Results

Phenotypic and biochemical characterization of Tg BCR⁺/Ii⁻ B cells

To define the role of Ii during the presentation of BCR-targeted Ags, we derived B cells from the spleens of Ii-positive and Ii-negative H-2^k B10BR \times CBA/J mice expressing or not a Tg BCR specific for HEL (34, 35). Regardless of Ii and Tg BCR expression, splenic B cells expressed high levels of membrane CD45R molecule (Fig. 1), and virtually all Tg B cells expressed high levels of the HEL-specific BCR (Fig. 1). The absence of Ii also did not compromise membrane expression of Igs IgM or IgD, or adhesion and costimulatory molecules (ICAM-1, B7.1, B7.2, and CD40). In contrast to cells derived from the H-2^b haplotype animals (42), H-2^k Ii^{-/-} B cells efficiently reached the mature stage, as revealed

by the membrane coexpression of IgM and IgD (Fig. 1). MHC class II surface expression was as expected reduced in Ii^{-/-} mice independent of Tg BCR expression (Fig. 1).

In the absence of Ii, the number of class II molecules available for Ag presentation is reduced. This is due to the inability of class II dimers to leave the endoplasmic reticulum to reach peptide loading compartments. A fraction of “empty” molecules in fact reach the plasma membrane and are identified by their relatively unstable conformation in SDS at 20°C (39). We labeled cell surface MHC class II molecules by lactoperoxidase-catalyzed iodination (19) and monitored the SDS stability of MHC class II I-A^k molecules expressed at the plasma membrane of B cells under the four conditions of Tg BCR and Ii expression (Fig. 2). To reveal peptide-stabilized “compact” membrane-dimers, the SDS extracts were run after boiling or not. Regardless of BCR expression, MHC class II molecules were unable to reach the B cell plasma membrane in a stable “compact” conformation in the absence of Ii (Fig. 2A). Furthermore, although B cell incubation for 16 h in the presence of HEL slightly increased the level of stable MHC class II dimers in Ii-positive B cells, this treatment did not induce compact class II molecules at the surface of Ii-negative cells, even when they expressed the HEL-specific BCR (Fig. 2B).

The BCR targets Ag for Ii-dependent presentation

To analyze the Ii dependence of class II-restricted presentation of HEL taken up by fluid phase or with the BCR, we used a panel of T cell hybridomas. The I-A^k-restricted 3A9 T cell hybridoma is specific for the 46–61 HEL peptide (36), which mainly associates with newly synthesized I-A^k molecules. 3B11 and 2B6 T cell hybridomas recognize respectively the 34–45 and 25–43 HEL peptides that bind to recycling class II molecules independently of Ii (18).

Tg BCR⁻/Ii⁺ splenic B cells presented HEL protein at μ M concentrations, consistent with fluid phase uptake, to the 3A9, 3B11, and 2B6 T cell hybridomas (Fig. 3A, open squares). The absence of Ii compromised the presentation of the 46–61 HEL-derived peptide to the 3A9 Ii-dependent T cell hybridoma but not the presentation of the HEL-derived 34–45 and 25–43 peptides to the 3B11 and the 2B6 T cells in Tg BCR⁻/Ii⁻ B cells (Ref. 19; and Fig. 3A, filled squares). This is consistent with the proposition that 3A9 T cell activation requires an intact Ii to target nascent class II molecules to MHC class II intracellular loading compartments, while 3B11 and 2B6 T cell activation can be elicited by recycled class II molecules in the absence of Ii (18).

The Tg BCR in Ii-positive B cells targeted HEL protein for internalization by B cells and reduced by a factor of 10,000 the amount of Ag necessary for stimulation of all three hybridomas (Fig. 3A, open triangles). Surprisingly, Ii-deficient B cells expressing an HEL-specific BCR failed to efficiently present either the Ii-dependent 46–61 HEL determinants or the Ii-independent 34–45 and 25–43 HEL determinants (Fig. 3A, filled triangles). The efficiency of presentation of the 46–61 HEL synthetic peptide was similar in all B cell types considered (Fig. 3B), even though the number of MHC molecules was reduced on Ii-deficient cells (Figs. 1 and 2) and was not increased by incubation with HEL protein (Fig. 2B).

MHC class II molecules require Ii to meet internalized BCRs

The engagement of the BCR leads to a redistribution of newly synthesized MHC class II molecules (27, 28). We used confocal microscopy to determine whether the absence of Ag presentation in Ii-deficient Tg B cells was associated with an Ii-dependent regulation of class II transport in response to BCR ligation. Fig. 4 shows the distribution of the HEL-specific BCR, H2-M, and class

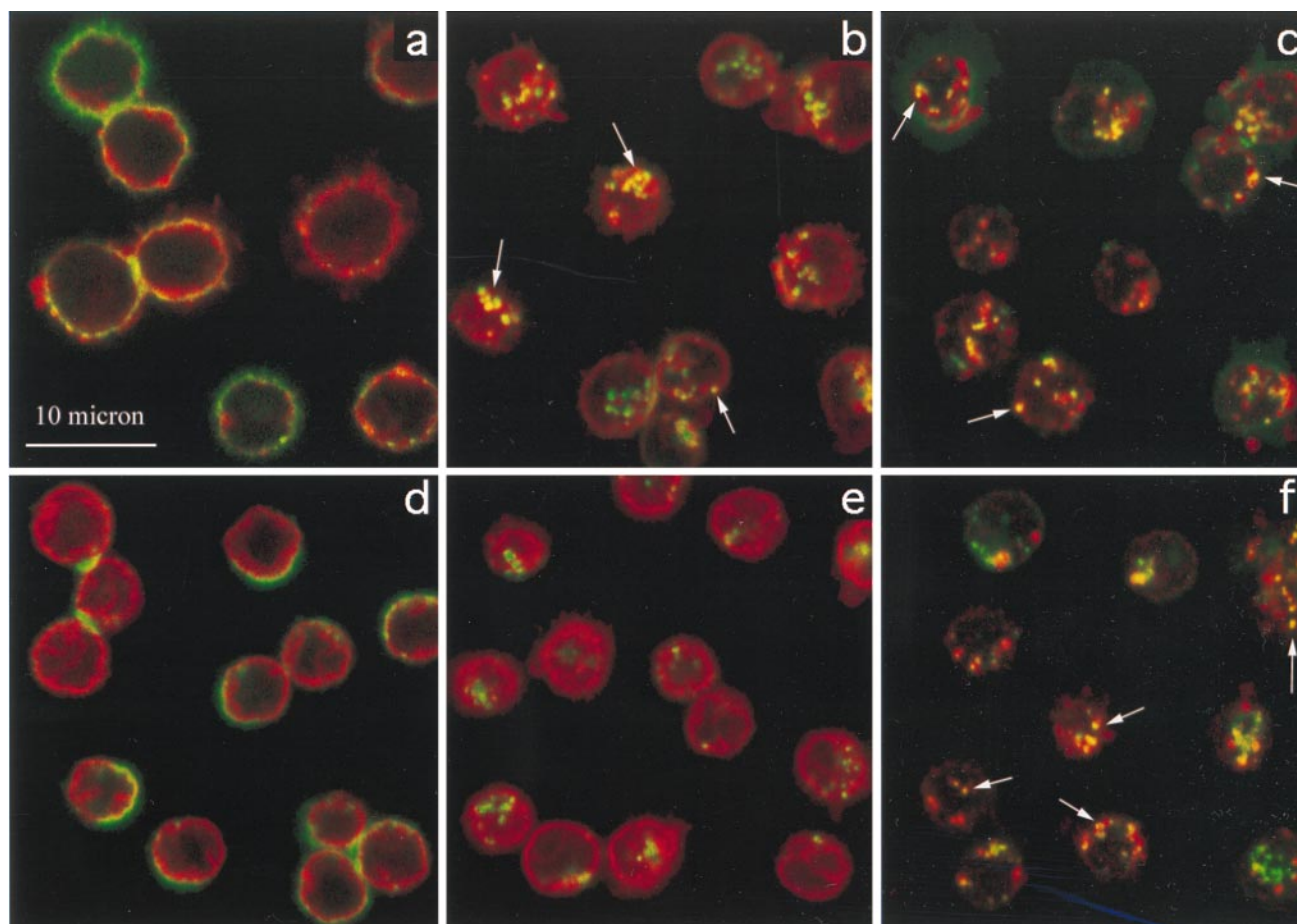


FIGURE 4. BCR engagement initiates the intracellular accumulation of class II molecules in Ii-positive splenic B cells. Tg BCR⁺/Ii⁺ (a-c) and Tg BCR⁺/Ii⁻ (d-f) B cells were analyzed by confocal microscopy after incubation with FITC-coupled goat anti-IgM for 1 h at 4°C (a and d) or for 1 h at 37°C (b, c, e, and f). The internalized FITC-labeled BCR ligands (b, c, e, and f, green and yellow color) are partially superimposed with intracellular I-A^k molecules (a, b, d, and e, red and yellow color) and H2-M molecules (c and f, red and yellow color).

II molecules in B cells before and after membrane Ig (mIg) engagement. In the absence of BCR engagement in Ii-positive cells, mIgs and MHC class II molecules were mainly located at the plasma membrane (Fig. 4a), while H2-M molecules were localized in internal vesicles (data not shown). Ig engagement resulted in a dramatic modification of the MHC class II intracellular distribution (Fig. 4b). mIg engagement by an anti-IgM Ab induced their time-dependent transport to intracytoplasmic vesicles. These sites correspond to the class II peptide loading compartments since they are partially positive for class II and H2-M molecules (Fig. 4, b and c, respectively).

In resting B cells, the absence of Ii did not compromise the level of mIg expression (Fig. 1, and Fig. 4, a and d). Some MHC class II molecules reached the plasma membrane while others were retained as immature glycoproteins in the endoplasmic reticulum (Ref. 21 and Fig. 4d). The confocal micrographs demonstrate that BCR engagement does not result in MHC class II colocalization with the BCR in intracellular compartments in the absence of Ii (Fig. 4e), despite the fact that BCR ligands colocalize with H-2 M molecules in internal vesicles in Ii-negative cells to the same extent that they did in Ii-positive cells (Fig. 4, c and f).

We also evaluated Tg B cells incubated or not with their cognate ligand, HEL. Resting HEL-specific B cells displayed little or no intracellular class II molecules (Fig. 5a), and this pattern did not change after overnight incubation in medium alone (not shown). After 16 h of HEL incubation followed by fixation, internal class

II molecules were detectable and mainly colocalized with internalized BCR (arrows in Fig. 5, c and b, respectively). As in the case when we incubated cells with an anti-IgM Ab (Fig. 4), no colocalization of class II with the BCR was seen in Tg B cells lacking Ii when incubated with HEL (data not shown). To assess whether intracellular class II molecules were loaded with the 46–61 peptide, we used the C4H3 mAb, which recognizes HEL 46–61 in the context of I-A^k molecules. After 4 h or 16 h of HEL incubation, the internalized BCR partially colocalized with 46–61 peptide-loaded I-A^k molecules (arrows, Fig. 5, d and e, respectively). Faint background staining with the C4H3 Ab in cells incubated without HEL, apparently due to cross-reactive self peptides, has been reported (38) but was not seen by us in confocal microscopy in the absence of HEL (data not shown). This background staining was observed in FACS studies, but the binding of the C4H3 Ab was markedly increased by incubation with HEL (Fig. 5f). Thus, BCR engagement of cognate ligands induces the accumulation of class II molecules at sites where they are available for peptide loading in Ii-positive spleen cells.

Discussion

The mechanisms whereby exogenous Ags are internalized influences their processing and leads to the generation of peptide-MHC complexes that differ both quantitatively and qualitatively according to the receptor used. The T cell repertoire recruited during an

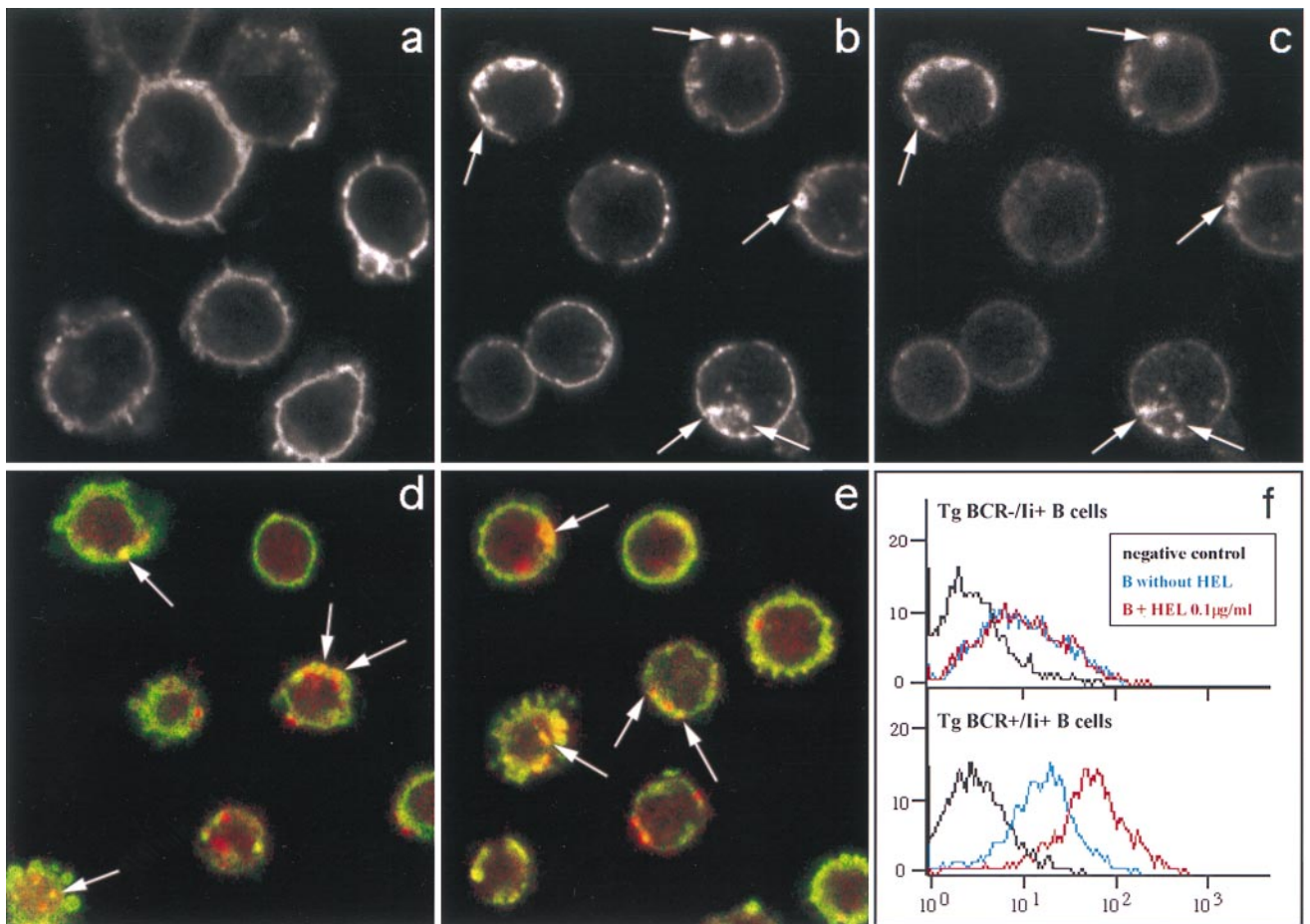


FIGURE 5. BCR-mediated HEL internalization triggers the accumulation of peptide-loaded intracellular class II molecules. Tg BCR⁺/Ii⁺ B lymphocytes were analyzed by confocal microscopy without HEL (*a*) or after engagement of the Tg BCR with 10 μ M HEL at 37°C for 16 h (*b* and *c*). Cells were labeled with rabbit anti-I- β molecules (*a* and *c*) or with anti-IgM Ab (*b*). Cells incubated with 10 μ M HEL at 37°C for 4 h (*d*) or for 16 h (*e*) were double labeled with the anti IgM Ab (*d* and *e*, green color) and the C4H3 mAb, which recognizes HEL 46–61 peptide-loaded I-A^k molecules (*d* and *e*, red color). The colocalization of internalized BCR and class II molecules is indicated (arrows in *b*–*e*). Panel *f* shows a FACS analysis of the surface expression of HEL 46–61/I-A^k complexes in Tg BCR⁻/Ii⁺ (*upper panel*) and Tg BCR⁺/Ii⁺ (*lower panel*) B cells after overnight incubation with (red line) or without (blue line) 0.1 μ g/ml of HEL. Control staining was performed using the second step reagent only (black line). The C4H3 mAb gives some background staining with surface I-A^k molecules not necessarily loaded with the HEL 46–61 peptide (38).

immune response can therefore vary according to Ag uptake and processing pathways (reviewed in Ref. 43). The molecular constraints underlying these phenomena are still poorly understood (43, 44). In the present study, we used naive B cells from BCR Tg mice expressing or not expressing Ii. Cells expressing this BCR presented the 34–45 and 25–43 “Ii-independent” and the 46–61 “Ii-dependent” HEL peptides very efficiently when Ii was present. However, a general presentation defect in Ii-negative cells was seen for all of these peptides, even when HEL was efficiently internalized by the same BCR. These data indicate that segregation of Ag internalized by Ig is very efficient and that association with class II molecules occurs in compartments containing newly synthesized, rather than recycling, class II molecules. The role of newly synthesized class II molecules is also emphasized by recent results from our laboratory showing that presentation of Ags targeted to the BCR requires protein synthesis (45, 46), regardless of whether the peptides concerned are classically “Ii-dependent” or “independent.”

There may be several explanations for this result. 1) The physical association between the Ag and this high affinity (47, 48) BCR involved in endocytosis delivers the Ag into a class II recycling compartment but interferes with the initial phases of the degradation pro-

cess, as already discussed (49–51). This would prevent the generation and association of peptides in early endosomal compartments with recycling MHC class II molecules. Ags bound to the BCR are also internalized more rapidly than Ags bound to other cell-surface molecules, which could limit their exposure to the proteolytic environment of early endosomes (52). In this situation, only newly synthesized MHC class II molecules transported by Ii would have the opportunity to interact with peptides generated in deeper endocytic compartments. 2) Ags internalized through the BCR could follow a route of internalization that bypasses early intracellular compartments where class II recycling occurs. Our results do not exclude either of these possibilities. The observation that BCR engagement by anti-Ig or specific Ag initiated an accumulation of MHC class II molecules in peptide loading compartments in a manner dependent on the expression of Ii provides a basis for augmented Ag presentation to T cells. These results confirm and extend to normal B cells our observations (27), as well as recent results of Siemasko et al. (28), that internalized Ig accumulates in compartments rich in newly synthesized class II molecules. We also show that this compartmentalization correlates with efficient Ag presentation to naive T cells.

It has been known for a long time that the quantitative advantage for Ag acquisition for BCR-mediated Ag uptake cannot account

for all of the gain in Ag presentation, since Ag targeted to molecules expressed at comparable levels were nonetheless much less well presented (53, 54). Despite equivalent internalization, as compared with membrane Ig containing heterologous cytoplasmic motifs from other expressed proteins such as LDL and MHC class I, BCR-mediated Ag uptake was shown to lead to an accelerated intracellular targeting and presentation in B cell transfectants (52). Evidence has been presented indicating that mutating a single amino acid in the transmembrane region of human IgM may inhibit the ability of cells expressing this molecule to present Ag, while leaving bulk Ag endocytosis and degradation intact (31). In addition, experiments using cells transfected with Fc receptor Ig α or Ig β chimeras have shown that the Ig α -chain directs Ag to a compartment containing newly synthesized class II molecules, while Ig β targets Ag to a population of recycling class II-containing vesicles (55).

B cells appear to have other means to differentially control BCR-mediated Ag presentation in a manner involving class II molecules. The H2-O molecule, which regulates the catalytic function of H2-M in releasing class II-associated invariant chain-derived peptides (CLIP) from class II molecules (56, 57), was shown to focus B cell presentation on Ags internalized by the BCR (58). Taken together, these data suggest that Ag internalization by the BCR is a tightly regulated process intimately associated with class II maturation. In contrast to B cells, DC capture and present Ags using a broader receptor repertoire (44). The fact that H2-O molecules are expressed in B cells but not in DC and the differential Ii requirement observed for Ag presentation by dendritic (23) and B cells reinforce the distinction between the strategies used by B cells and DC to load their class II molecules. B lymphocytes rely on newly synthesized molecules "escorted" by Ii, whereas, in DC, Ii is not required for I-A^k peptide presentation (23). The data presented here, in which we crossed the same Ii-negative H-2^k mice with BCR Tg mice, suggest that the expression of Ii is required both for the advantage in Ag presentation conferred by the presence of the Ag-specific BCR and also to promote the intracellular accumulation of MHC class II molecules in response to BCR ligation. The accumulation of class II molecules in compartments accessible to Ag endocytosed by the BCR could account for much of the gain in efficiency of the presentation to T cells of Ii-positive B cells specific Ags.

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