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Beverly S. I. Strong and Emil R. Unanue

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Presentation of Type B Peptide–MHC Complexes from Hen Egg White Lysozyme by TLR Ligands and Type I IFNs Independent of H2-DM Regulation

Beverly S. I. Strong and Emil R. Unanue

In APCs, presentation by MHC II molecules of the chemically dominant peptide from the protein hen egg white lysozyme (HEL) generates different conformational isomers of the peptide–MHC II complexes (pMHC). Type B pMHCs are formed in early endosomes from exogenous peptides in the absence of H2-DM, whereas in contrast, type A pMHC complexes are formed from HEL protein in late vesicles after editing by H2-DM. Thus, H2-DM edits off the more unstable pMHC complexes, which are not presented from HEL. In this study, we show that type B pMHC complexes were presented from HEL protein only after stimulation of dendritic cells (DC) with TLR ligands or type I IFN. Type I IFN contributed to most TLR ligand-induced type B pMHC generation, as presentation decreased in DC lacking the receptor for type I IFNs (IFNAR1−/−). In contrast, presentation of type A pMHC from HEL and from peptide was minimally affected by TLR ligands. The relative effectiveness of CD8α2 DC or CD8α−/− DC in presenting type B pMHC complexes varied depending on the TLR ligand used. The mechanisms of generation of type B pMHC from HEL protein with TLR stimulation did not involve H2-DM or release of peptides. DC from H2-DM–deficient mice in the presence of TLR ligands presented type B pMHC. Such DC showed a slight enhancement of HEL catabolism, but peptide release was not evident. Thus, TLR ligands and type I IFN alter the pathways of presentation by MHC II molecules of DC such that type B pMHCs are generated from protein Ag.

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Harbor, ME). MyD88−/− mice and the type 1 IFNγ gene knockout (IFNAR1−/−) mice were backcrossed to the B10 BR background. mHEL mice (expressing membrane-bound HEL under the MHC II promoter) were previously described (35). H2-DM−/−deficient mice were also described previously (36).

Stimulants

Stimulants used were Ultra-pure LPS-EB from Escherichia coli 0111:B4 (InvivoGen, San Diego, CA), gardiquimod (InvivoGen), flagellin (InvivoGen), CpG-B: ODN 1826 (Integrated DNA Technologies, Coralville, IA), polyinosinic-polycytidylic acid (poly I:C) (Sigma-Aldrich, St. Louis, MO), zymosan A from Saccharomyces cerevisiae (Sigma-Aldrich), resiquimod (a kind gift from Dr. Marco Colonna, Washington University), rIFN-α, IFN-β (PBL InterferonSource, Piscataway, NJ), and IFN-γ (Kind gift from Dr. Robert Schreiber, Washington University). TLR ligands used in this study are listed in Table I.

Flow cytometry

For cell sorting, DC were stained with CD11c (N418; eBioscience, San Diego, CA; or BioLegend, San Diego, CA), CD86 (53-6-7; eBioscience), CD45RA (14-8; BD Pharmingen, San Diego, CA), Siglec H (440c; a kind gift from Dr. Marco Colonna), R220 (RA3-6B2; BD Pharmingen), and CD19 (1D3, BD Pharmingen). For intracellular staining, cells were fixed and permeabilized using a Cytofix/Cytoperm kit according to the manufacturer’s instructions (BD Pharmingen). Abs for intracellular staining were H2-DM (2E5A; BD Pharmingen), goat anti-rat–Cy5 (Zymed, Carlsbad, CA), and H-2Kk (36-7-5; BD Pharmingen). For analysis of DC, the following Abs were used: CD4 (BD Pharmingen), B220 (RA3-6B2; BD Pharmingen), CD86 (GL1; BD Pharmingen), I-Ak (40F; made in house), CD11c (N418; eBioscience, San Diego, CA; or BioLegend, San Diego, CA), and H2-DO Alexa 647 (Mags.Ob1; a kind gift from Dr. Lisa Denzin, Memorial Sloan-Kettering Cancer Center). Flow cytometry data were collected on a BD FACS LSR II or BD FACSCalibur (BD Biosciences) and analyzed using FlowJo software (Tree Star, Ashland, OR).

DC isolation and Flt3 ligand treatment

DC were isolated from spleens of mice injected with 10 μg Flt3 ligand i.p. for 3 consecutive d. On day 8, spleens were harvested and digested with 0.14 U/ml Liberase Blendzyme 3 or 1.67 U/ml Liberase TL (Roche Applied Science, Indianapolis, IN) to make single-cell suspensions from which DC were isolated by CD11c magnetic beads (Miltenyi Biotec, Auburn, CA). Enriched DC were ≥95% pure as determined by flow cytometry. For sorting, DC isolated as above were stained for surface markers and resuspended in phenol-free DMEM (Invitrogen, Carlsbad, CA). Enriched DC were then stained with 0.1% PCS and 0.2% DAPI. Cells were counted using a BD FACSAria II (BD Biosciences). Conventional DC (cDC) were sorted as CD11chighCD45RA+ (B202) or CD11chiSiglec H B220−, cDC were further separated by CD8 expression. For analysis of H2-DM and H2-DO, cDC were further selected as CD19−.

Assays

For Ag presentation assays, 105 DC were incubated with HEL protein or the HEL (48–62) peptide (DGSTDYGILQINSRW) with or without stimulants for 18 h in 100 μl volume in V-bottom 96-well plates. Stimulant concentrations used were: 1 μg/ml zymosan A, 10 μg/ml poly I:C, 1 μg/ml LPS, 1 μg/ml gardiquimod, 6 μg/ml resiquimod, 1 μg ODN 1826 (CpG-B), 100 U/ml IFN-γ, 10 U/ml IFN-α, and 10 U/ml IFN-β. DC were washed three times with serum-free DMEM after incubation before adding 5 × 104 T cell hybridomas/well in a 200-μl volume; 24 h later, the release of IL-2 was measured in the culture fluid using CTLL as an indicator cell. Most experiments used two previously characterized T cell hybridomas: 11A10, which only reacts with type B pMHC, and 3A9, which recognizes type A pMHC. Sorted DC were pretreated with 1 μg/ml anti-IFNAR1 mAb MAR1-5A3 [a kind gift of Dr. Robert Schreiber (37)], or control anti-human IFN-γ receptor-1 GIR.208 Ab for 1 h at 37°C. Without washing, HEL or peptide was added with or without stimulants to the wells. The remaining assay followed the protocol described above.

Table I. TLR ligands used in this study

| TLR2/6 | TLR3 | TLR4 | TLR5 | TLR7 | TLR9 |
|--------|------|------|------|------|------|
| Agonist(s) | Zymosan A (also binds dendr1) | Poly (I:C) | LPS | Flagellin | Gardiquimod, resiquimod | ODN 1826 (CpG-B) |

incubated with or without stimulants for 18 h in 100 μl volume in 96-well V-bottom plates.

Peptide release assay

DC from CB.17 mice were incubated overnight with HEL with or without 1 μM CpG-B. Supernatants were collected and spun twice to remove cells and debris. Supernatants were serially diluted and added to parafomaldehyde-fixed DC from B10 BR mice. DC were fixed in 1% parafomaldehyde for 5 min, incubated with 0.2 M DL-Lysine, and washed extensively in media. T cells were added as described above.

HEL catabolism

DC were incubated for 18 h with 1 μM CpG-B (ODN1826) or without stimulation. Equal numbers of DC were incubated with 100 U/ml HEL for 2 h at room temperature with intermittent mixing. After the incubation, DC were washed and replated for indicated times. At each time point, TCA-soluble and -precipitable fractions were collected from the cells and the supernatant.

Quantitative real-time PCR

Sorted cDC were incubated for 2, 6, or 18 h untreated or stimulated with TLR ligands or IFN-α. Total RNA was isolated using an RNAqueous-Micro kit according to the manufacturer’s instructions (Applied Biosystems). Forty nanograms cDNA were used per reaction for quantitative real-time PCR (qRT-PCR) analysis. qRT-PCR was performed using a Fast SYBR Green kit and ∆CT calculations on a StepOnePlus Instrument (Applied Biosystems). Primers used were the following: 18S forward (F), 5′-GTAACCCGTT-GAACCCCCATT-3′; 18S reverse (R), 5′-CCATCCAATCGTGATAGCCG-3′; IFN-β F, 5′-ATAAGACGTCCACGCCTCAAAAG-3′; IFN-β R, 5′-GTCCTCACCACCGTCTG-3′; DMα F, 5′-TAGGTCCTCAGGAGAC-3′; DMα R, 5′-AGTCGAAAGAGAGATTCG-3′; DOα F, 5′-CCCGAATGACCTCCTGAGTCCC-3′; DOα R, 5′-GTGTCGGCCCTTGATGCCC-3′; H2-Aα F, 5′-TCCAGGCGACGAGCTTGTATGTT-3′; and H2-Aβ R, 5′-GGGGCCTGGAATCCTCAGGG-3′. 18S was used as the qRT-PCR standard.

Results

TLR ligands induce type B presentation from HEL

To assess the ability of TLR ligands (Table I) to affect type B pMHC presentation, a culture system was set up involving the culture of DC with HEL in the presence of the stimulants for 18 h, after which the stimuli were removed, and the extent of presentation was assayed by adding the indicator T cell hybridomas. In agreement with the basic definition of type B presentation, unstimulated DC presented type B pMHC only at low levels of HEL (0.01 μM), only affecting type B presentation to 3A9, a T cell that recognizes type A pMHC, only at low levels of HEL (0.01 μM) (Fig. 1B). IFN-γ did not induce type B presentation from HEL (Fig. 1A). The induced type B presentation by TLR ligands was reproducible, testing three different type B T cell hybridomas recognizing the HEL 48–61 epitope (data not shown).

Induced type B pMHC presentation by MyD88-deficient DC followed the expected pattern, with TLR7 and -9 ligands being...
completely eliminated, TLR3 ligands being minimally affected, and TLR4 ligands only partially reduced, reflecting the degree to which each TLR depends on MyD88 as a signaling adapter (Fig. 2) (38).

Role of DC subsets in induced presentation

Whether a subset of DC was responsible for the presentation of type B epitopes from HEL was examined. Plasmacytoid DC comprised ∼10% of the total DC population (identified as CD11cint/low Siglec H+B220+), but were not essential for induced presentation as sorted cDC, identified as CD11c+ Siglec H B220− presented type B pMHC from HEL with TLR stimulation (data not shown.) To examine the role of the cDC subsets, sorted CD8α+ and CD8α2 DC were incubated with HEL protein with or without CpG as in previous experiments. CD8α+ DC strongly presented type B pMHC from HEL upon CpG stimulation (Fig. 3B), but not in the absence of stimuli. CD8α2 DC, however, had some degree of presentation of type B pMHC in the absence of stimulation at high HEL doses: it increased upon CpG stimulation, but not as strongly.

FIGURE 1. TLR ligand-induced presentation of type B pMHC from HEL. Presentation by DC incubated with HEL (A, B) or HEL:48–61 peptide (C, D) to type B 11A10 (A, C) or type A 3A9 (B, D) T cell hybridomas in the presence or absence of TLR ligands. Representative of at least four independent experiments. Error bars represent SD.

FIGURE 2. Dependence on MyD88−/− DC for TLR ligand-induced presentation of type B pMHC from HEL. Presentation to type B 11A10 (A, C) or type A 3A9 (B, D) T cell hybridomas by DC from wild-type (A, B) or MyD88−/− mice (C, D) incubated with HEL with or without TLR ligands. Error bars represent SD. Representative of three independent experiments.
as the CD8α+ DC (Fig. 3C). The ability of CD8α+ DC or CD8α− DC to present type B pMHC from HEL slightly differed, depending on the TLR ligands, except for gardiquimod, a ligand of TLR7/8 (Fig. 3D). Gardiquimod induced a small level of presentation only by CD8α− DC, which is not surprising as TLR7 is minimally expressed in CD8α+ DC (39). There was no significant difference in presentation of HEL peptide by the DCs (Fig. 3E). Of note is that the degree of presentation of HEL at high doses by the unstimulated CD8α− DC varied greatly from no response whatsoever to the small response found in Fig. 3B, reflecting most likely a degree of activation by environmental stimuli. In sum, these data indicate that both splenic DC subsets are capable of presenting type B pMHC from protein HEL in response to TLR stimulation.

Role of type I IFN in induced type B presentation

In addition to TLR ligands, rIFN-α and IFN-β induced type B pMHC presentation from HEL (Fig. 4A), but only affected type A presentation marginally at low levels of protein (Fig. 4B). Type I IFNs had minimal effects on presentation from peptide, as with TLR ligands, only affecting type B pMHC presentation at low peptide doses (0.01 μM) (Fig. 4C, 4D).

qRT-PCR was used to assess expression of IFN-β by sorted cDC. Stimulation of cDC with CpG, poly (I:C), LPS, gardiquimod, and zymosan induced expression of IFN-β as indicated by increased mRNA levels after 2 h, decreasing by 6 h after stimulation (Fig. 4E).

To test the role of TLR-induced type I IFN on presentation, MAR1-5A3 Ab was used to block signaling through the type I IFNα/β receptor (37). DC were pretreated with control or MAR1-5A3 Ab before adding HEL with or without stimulants. MAR1-5A3 completely blocked IFN-β–induced type B presentation by CD8α+ DC (99.7% at half-maximum, Fig. 5A). It reduced CpG-induced presentation by 57% (at half-maximum) by CD8α+ DC. Blocking the IFNα/β receptor had little effect on presentation from peptide (data not shown) and followed a similar trend with CD8α− DC (Fig. 5B). Of note, MAR1-5A3 treatment reduced the level of type B presentation by unstimulated CD8α− DC, indicating that cytokine production may explain why CD8α− DC were able to present low levels of type B pMHC from protein in the absence of stimulation.

To more critically address the role of TLR-stimulated type I IFN in induced presentation, DC from IFNAR1-deficient mice were examined. Although rIFN-β and IFN-α had no effect on presentation by IFNAR1-deficient DC (Fig. 5C, 5D, Supplemental FIGURE 3. Presentation by sorted CD8α+ and CD8α− DC. A, Representative flow cytometry assay on sorted cells showing CD11c and CD8α expression on unsorted and sorted DC populations. Presentation to type B 11A10 T cell hybridoma by sorted CD8α+ (B) and CD8α− (C) DC incubated with HEL protein with or without CpG. Presentation to type B 11A10 T cell hybridoma by sorted DC incubated with HEL protein (D) or peptide (E) with or without TLR stimulants. Error bars represent SD. B and C are representative of 11 independent experiments. D and E are representative of four independent experiments.

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CpG-induced presentation was decreased by ∼60% in IFNAR1-deficient DC compared with wild-type DC (Fig. 5C, Supplemental Fig. 1A). The role of TLR-induced type I IFN was explored with the remaining TLR ligands shown to induce type B pMHC presentation from protein HEL using IFNAR1-deficient DC. poly (I:C)- and zymosan A-induced type B presentation were reduced by 25 and 36%, respectively, in IFNAR1-deficient DC (Fig. 5D, Supplemental Fig. 1A). Gardiquimod-induced presentation was completely inhibited, and LPS-induced presentation was unaffected. These data show that type I IFN signaling contributed differentially to each TLR pathway of induced type B pMHC presentation, augmented presentation through TLR3 and TLR9, was the sole mediator through TLR7, and had no effect on induction through TLR4. Of note, presentation from peptide and TLR7, and had no effect on induction through TLR4. Of note, presentation from peptide and type A pMHC presentation from protein was unaltered in IFNAR1-deficient DC (Supplemental Fig. 1B, 1C). Together, these results suggest that TLR and type I IFN signaling most likely initiate the same subcellular events and that TLR-induced type I IFN can amplify the signal, allowing for augmented type B pMHC presentation.

Role of type I IFN on expression of costimulatory molecules

Because costimulatory molecule expression is a component of DC maturation, the contribution of type I IFN to expression of costimulatory molecules was examined to see if it mirrored the contribution to induced type B pMHC presentation. DC from B10.BR wild-type or IFNAR1−/− mice were analyzed for surface expression of MHC II and costimulatory molecules after 18 h in culture with or without stimulation. Although MHC II levels changed little after stimulation, CD40, CD80, and CD86 were all significantly increased on B10.BR DC after exposure to TLR ligands and IFN-β (Fig. 6, Supplemental Fig. 2). Costimulatory molecule expression after stimulation was lower on IFNAR1−/− DC than B10.BR DC for all stimulants tested, indicating that type I IFN signaling augmented upregulation of costimulatory molecule expression by all TLR ligands tested. As expected, increased surface expression of costimulatory molecules by IFN-β was completely dependent on type I IFN signaling, as IFNAR1−/− DC were unable to respond (Fig. 6F, 6G). Zymosan A-stimulated costimulatory molecule expression was minimally dependent on type I IFN signaling (Fig. 6D). Gardiquimod-stimulated costimulatory molecule expression was more significantly dependent on type I IFN signaling (Fig. 6B, 6E). Gardiquimod-stimulated costimulatory molecule expression was almost completely dependent on type I IFN signaling, mirroring the complete requirement for type I IFN signaling for induced type B pMHC presentation (Figs. 5D, 6D). The stimulation of costimulatory molecule expression by LPS was dependent partially on type I
IFN signaling (Fig. 6C), which was not the case for induced type B presentation (Fig. 5D, Supplemental Fig. 1A).

Role of H2-DM and HEL catabolism in induced presentation
Because H2-DM prevents type B pMHC from being generated (4), regulation of H2-DM was considered as an explanation for the effects of TLR ligands. The function of H2-DM can be modulated by H2-DO, so it was necessary to examine expression of both molecules. To determine if expression of H2-DM or H2-DO was altered by TLR ligand stimulation, sorted cDC were incubated for 2, 6, or 18 h with or without CpG-B, LPS, or IFN-β stimulation, and H2-DM, H2-DO, and H2-Aα (MHC II) were examined by qRT-PCR. Their expression increased 2 h after stimulation, dropping by 6 and 18 h (Fig. 7A). Protein levels of H2-DM and H2-DO were examined by intracellular staining after 18 h of stimulation. CpG-B did not significantly alter levels of H2-DM protein, but did modestly increase expression of H2-DO (Fig. 7B). LPS has previously been shown to have a similar effect on splenic DC, having no detectable effect on H2-DM protein levels; however, they observed a modest decrease in H2-DO protein levels (40).

To examine whether regulation of H2-DM in DC contributed to induced type B pMHC presentation, cDC from B10.BR wild-type or H2-DM–deficient mice were incubated with HEL protein with or without stimulants as previously described. Consistent with previous reports (36), presentation of the type A pMHC was decreased by ~10-fold in the H2-DM–deficient cDC, an indication that H2-DM is important for the assembly of HEL pMHC complexes. Importantly, H2-DM–deficient cDC presented type B pMHC from HEL protein in response to CpG and LPS, albeit at reduced levels compared with wild-type cDC. These data indicate that although regulation of H2-DM may have some contribution to TLR and type I IFN-induced type B presentation from HEL, it is not the key mechanism controlling the event.

Finally, we examined if CpG-B changed the rate of catabolism of [125I]-HEL. DC were incubated for 18 h alone or with CpG-B, then incubated with [125I]-HEL for 2 h at room temperature followed by 1-, 2-, 3-, or 4-h chases in media free of HEL. TCA-soluble and -precipitable fractions were collected from intracellular (Fig. 7G) and supernatant (Fig. 7H) fractions at each time point. CpG-B–activated DC showed slight acceleration of HEL catabolism compared with resting DC. We did not find evidence that CpG-B–treated DC released peptides that would contribute to the type B presentation. Supplemental Fig. 3 describes the experiment.

Discussion
The present report adds not only to the understanding of presentation of type B pMHC conformers of HEL, but also to the general effects of TLR ligand and type I IFN on the MHC II processing and presentation pathway. In the current study, exposure of DC to TLR ligands and type I IFN changed HEL protein handling, allowing strong processing and presentation of type B pMHC complexes. Notably, in the absence of TLR or type I IFN stimulation, as reported before, DC did not present type B pMHC complexes from HEL unless at very high concentrations. Both major DC subsets participated in induced type B pMHC presentation of HEL, although the degree to which each participated varied depending on the inflammatory signal.

Type I IFN signaling was important for type B pMHC presentation initiated by CpG, poly (I:C), gardiquimod, and zymosan A, but not for LPS-initiated events. Type I IFN has been argued to be important for both cross-priming of CD8 T cells and direct

FIGURE 5. Role of type I IFN signaling in CpG-induced pMHC presentation from HEL. Presentation to type B 11A10 T cell hybridoma by sorted CD11c+CD8α+ (A) versus CD11c+CD8α− (B) cells pretreated with MAR1-5A3 (anti-IFNAR1) or control mouse IgG before adding Ag and stimulants. C, Presentation to type B 11A10 T cell hybridoma by sorted cDC from B10.BR or IFNAR1−/− mice incubated with HEL protein with or without stimulants. D, Presentation to 11A10 by cDC treated as in R. Results are shown for 10 μM HEL protein dose. Complete Ag titrations are shown in Supplemental Fig. 1. Error bars represent SD. All graphs are representative of two independent experiments.
priming of CD8 T cells (13, 34, 41) and for cross-presentation induced by CpG (35). In this study, with MHC II, we found a strong effect not on presentation of the conventional type A epitopes, but rather on the type B pMHCs. Induction of CD40 by LPS, poly (I:C), and CpG on GM-CSF bone marrow-derived DC has been shown to be at least partially dependent on type I IFN signaling (28–31). Similarly, the increase in CD40, CD80, and CD86 expression after stimulation with zymosan A, poly (I:C), LPS, and CpG was partially dependent on type I IFN signaling. Gardiquimod-induced expression of these costimulatory molecules absolutely required type I IFN signaling. In our experiments, we used T cell hybridomas that do not require costimulation, allowing examination exclusively of changes in levels of pMHC.

Previous studies showed effects of TLR ligands on the generation of class II pMHC (15–17) as well as on the modulation of costimulatory molecules (28–31). These findings have varied in the kind of assays the APC used for examination and the amounts and forms of ligands, so a general consensus has not emerged on the key mechanisms of action. An issue thus is to separate processing and generation of pMHC from other components of the presentation pathway. We and others (9, 10) have shown subsequently, comparing results with T cell hybridomas and primary T cells, that both resting and TLR-activated DC were capable of processing and presenting Ag about equally.

Although this report does not identify the way by which the type B pMHC epitopes were presented, some issues are pointing to possible mechanisms of action. The initial data indicate that H2-DM, a logical molecule to examine, may not be key in the effects seen in this study with TLR ligands. Pointedly, H2-DM−/− deficient DC presented type B pMHC from HEL protein after TLR ligands. Although TLR stimulation regulated expression of H2-DO, which controls H2-DM activity, modulation of H2-DM function was ultimately not the key mechanism of type B presentation, as shown with the H2-DM gene knockout DC. We have ruled out the release of peptides from the treated APC, whereas the effects on catabolism by CpG were modest. The observation that TLR and type I IFN stimulation minimally increased type B pMHC presentation from peptide at low doses could be due to increased total MHC II expression and/or surface pMHC $t_1/2$. However, these effects were modest, whereas the effects on type B pMHC presentation from protein were robust; it is unlikely that they are the sole mechanism of induced type B pMHC presentation. We posit that presentation may be explained by changes in the dynamics of vesicular traffic, leading to a flow of peptides from late vesicles into endosomes lacking H2-DM. Such a mechanism of action is now the subject of ongoing examination. This could happen through changes in acidification of, or recruitment of proteases to, an early or recycling compartment. Interestingly,
FIGURE 7. Role of H2-DM regulation in TLR-induced type B pMHC presentation from HEL and accelerated catabolism in CpG-B–activated DC. A, qRT-PCR analysis of RNA isolated from sorted cDC (CD11c<sup>high</sup>SiglecH<sup>2</sup>CD19<sup>2</sup>) were incubated with stimulants for 2, 6, or 18 h. B, Intracellular flow cytometry analysis of H2-DM and H2-DO protein levels in sorted cDC after 18 h of stimulation. Presentation to type B 11A10 (A, E) or type A 3A9 (B, D) by sorted wild-type B10.BR (C, D) or H2-DM<sup>−/−</sup> cDC (E, F) incubated with HEL protein with or without stimulants. G and H, DC were incubated for 18 h with or without CpG-B, washed, incubated with [125I]-HEL for 2 h at room temperature, washed extensively, then chased for indicated times at 37˚C. TCA-Soluble and TCA-Precipitable fractions were collected from the intracellular (G) and supernatant (H) fractions. Data plotted as percentage of total cpm at each time point.
when bone marrow-derived DC were treated with CpG prior to Ag exposure, MHC II processing and presentation occurred in early and recycling compartments (14), a finding compatible with our hypothesis on how TLR ligands could promote presentation of type B pMHC of HEL.

A longstanding issue has been whether T cells recognizing type B epitopes from self-Ags could be involved in autoimmunity. This study indicates that TLR- and type I IFN-activated DC present type B pMHC from soluble HEL. It will be critical to determine whether type B pMHC from self-proteins are presented by TLR- and/or type I IFN-activated DC, allowing priming of autoreactive T cells in vivo. We have previously shown that naturally arising insulin-reactive T cells against type B pMHC can transfer diabetes in the NOD model (7).

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Disclosures

The authors have no financial conflicts of interest.

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Supplemental Figure 3

A

B

C

Antigen Added to Fixed DC
Supplemental Figure 1. Complete Results from Comparison of B10.BR and IFNAR1\(^{−/−}\) DC. (A) Presentation to type B 11A10 T cell hybridoma by sorted cDC from wild type B10.BR or IFNAR1\(^{−/−}\) mice incubated with HEL protein with or without stimulants. (B) Presentation to type A 3A9 T cell hybridoma by sorted cDC from B10.BR or IFNAR1\(^{−/−}\) mice incubated with HEL protein with or without stimulants. Bar graphs for 10 and 0.1 μM HEL protein doses shown. (C) As in A and B except cDC incubated with 10 μM HEL peptide 48-61. Error Bars represent SD. Data are representative of two independent experiments.

Supplemental Figure 2. Histograms from Role of Type I IFN Signaling in TLR-Induced Changes in Costimulatory Molecule Expression. DC from wild type B10.BR or IFNAR1\(^{−/−}\) mice were incubated alone or with stimulants for 18 hours. DC were stained for CD40, CD80, CD86, or I-A\(^{k}\) and analyzed by flow cytometry. Data gated on live CD11c\(^{\text{high}}\) SiglecH\(^{−}\) cells. Data are representative of two independent experiments.

Supplemental Figure 3. CpG Treatment of DC Does Not Induce Functional Peptide Release Leading to Antigen Presentation. (A, B) CB.17 DC were incubated overnight with 60 μM HEL with or without 1 μM CpG-B. Cell-free supernatants were serially diluted and added to fixed B10.BR DC and (A) 11A10 or (B) 3A9 T cell hybridomas were added to assess presentation. (C) 10 μM HEL or 48-62 peptide were added to fixed B10.BR DC with 11A10 or 3A9 T cell hybridomas.