

MHC Class II-Associated Invariant Chain Isoforms Regulate Pulmonary Immune Responses¹

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Asthma, a chronic inflammatory disease of the lung, is characterized by reversible airway obstruction and airway hyperresponsiveness (AHR), and is associated with increased production of IgE and Th2-type cytokines (IL-4, IL-5, and IL-13). Development of inflammation within the asthmatic lung depends on MHC class II-restricted Ag presentation, leading to stimulation of CD4⁺ T cells and cytokine generation. Conventional MHC class II pathways require both MHC-associated invariant chain (Ii) and HLA-DM (H2-M in mice) chaperone activities, but alternative modes of Ag presentation may also promote *in vivo* immunity. In this study, we demonstrate that Ii^{-/-} and H2-M^{-/-} mice fail to develop lung inflammation or AHR following sensitization and challenge with OVA in a mouse model of allergic inflammation. To assess potentially distinct contributions by Ii chain isoforms to lung immunity, we also compared allergen-induced lung inflammation, eosinophilia, IgE production, and AHR in mice genetically altered to express either p31 Ii or p41 Ii isoform alone. Sole expression of either Ii isoform alone facilitates development of allergen-induced lung inflammation and eosinophilia. However, animals expressing only the p31 Ii isoform exhibit abrogated IgE and AHR responses as compared with p41 Ii mice in this model of allergen-induced lung inflammation, suggesting that realization of complete immunity within the lung requires expression of p41 Ii. These findings reveal a crucial role of Ii and H2-M in controlling the immune response within the lung, and suggest that p31 Ii and p41 Ii manifest nonredundant roles in development of immunity. *The Journal of Immunology*, 2003, 170: 1473–1480.

Major histocompatibility complex class II-associated invariant chain (Ii)³ and HLA-DM (H2-M in mice) are critical effectors of the MHC class II-restricted Ag presentation pathway. MHC class II α - and β -chains coassemble with a third protein, the Ii, within the endoplasmic reticulum of APCs (1–4). The Ii associates with MHC class II dimers within the peptide-binding groove and directs immature class II complexes to the endosomal pathway. Within acidic intracellular compartments, the Ii undergoes stepwise degradation to generate class II-associated Ii chain peptide (CLIP) (5–8). This CLIP fragment is subsequently released from the class II-binding groove in a peptide exchange reaction catalyzed by H2-M that promotes occupancy by diverse peptide ligands (9–11). The class II-peptide complexes then traffic to and are displayed on the cell surface of APC to be recognized by CD4⁺ T cells.

Mutant mice lacking expression of Ii or H2-M have been invaluable in elucidating the roles of these molecules in development of the immune response (12–17). The most profound phenotypes in Ii^{-/-} (and H2-M^{-/-}) animals are observed in mice that express

I-A^b class II, because these molecules are critically dependent on Ii for proper folding and intracellular trafficking (18). APC from I-A^b Ii^{-/-} mice show a marked decrease in cell surface MHC class II expression and, as a consequence, exhibit defective CD4⁺ T cell selection. Ii^{-/-} B cell maturation is also compromised, and recent data suggest that *in vivo* responses may be biased toward production of Th1-type cytokines (19–22). In contrast, H2-M^{-/-} APC display normal intracellular trafficking of MHC class II, but cannot effectively liberate CLIP from the Ag-binding groove. Increased levels of surface class II-CLIP complexes in these mice cause abnormal negative selection of CD4⁺ T cells.

Two Ii isoforms, p31 Ii and p41 Ii, arise from alternatively spliced mRNAs (23–25). The p41 isoform contains an additional 64 aa, encoded by exon 6b, inserted near the C terminus of Ii. The p31 Ii is the predominant species expressed in B cells, but expression of p41 Ii is enhanced in dendritic cells (DC) and macrophages, key APC found within the lung. Both isoforms promote class II maturation, surface expression, Ag presentation, and CD4⁺ T cell selection (26–29). However, p41 Ii, via its extra cysteine-rich thyroglobulin-like repeat, has the ability to modulate the proteolytic environment within the endocytic compartments and in extracellular fluids (30–32). Previous studies suggest p41 Ii has enhanced Ag presentation activities (33, 34). Moreover, recent work has shown that p41 Ii animals manifest a more severe course of myelin oligodendrocyte glycoprotein (MOG)-induced experimental allergic encephalomyelitis, as compared with p31 Ii mice (35). Also, APC from double-mutant H2-M-deficient/p41 transgenic mice exhibit enhanced peptide-loading abilities as compared with H2-M-deficient/p31 transgenic animals (36). Thus, p31 Ii and p41 Ii may possess both overlapping and distinct functions related to class II maturation and Ag presentation.

Ii and H2-M requirements have been analyzed in several animal models (20, 35, 37, 38). Ii^{-/-} and H2-M^{-/-} mice fail to develop experimental allergic encephalomyelitis when immunized with either MOG or the dominant MOG peptide Ag, p35–55. Ii^{-/-} mice develop normal delayed-type hypersensitivity (Th1 type), but have

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³ Abbreviations used in this paper: Ii, invariant chain; AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; CLIP, class II-associated Ii chain peptide; DC, dendritic cell; MOG, myelin oligodendrocyte glycoprotein; Penh, enhanced pause; WT, wild type.

an attenuated Th2-type pulmonary inflammatory response within the lung. H2-M^{-/-}, but not Ii^{-/-}, animals exhibit an increased susceptibility to leishmaniasis. Also, H2-M^{-/-} animals display marked attenuation of T cell-dependent humoral responses to distinct Ags. Despite these previous studies, the exact influence of Ii and H2-M, and of the p31 Ii and p41 Ii isoforms, on a Th2-dependent, allergen-induced mouse model of pulmonary inflammation, IgE production, and airway hyperresponsiveness (AHR) has not been extensively investigated.

OVA-induced pulmonary inflammation is a Th2-type inflammatory response characterized by high IgE titers, eosinophilic pulmonary infiltrate, increased levels of IL-4 and IL-13 within the lung, and AHR to the cholinergic agonist methacholine (39). Importantly, this inflammatory response is dependent on class II-dependent Ag presentation by DC, as mice devoid of CD11c⁺ APC do not develop lung inflammation or AHR in this model (40). Class II pathways in DC are regulated by a variety of inflammatory mediators, and alternative Ii (and H2-M)-independent modes of peptide capture have been extensively documented. In this study, we test Ii^{-/-} and H2-M^{-/-} mice for their ability to develop allergen-induced lung inflammation and AHR. Both these class II chaperones are required. We also compare lung inflammation and eosinophilia in mice selectively expressing the p31 Ii or p41 Ii isoform alone. The p31 Ii mice, but not p41 Ii animals, demonstrate decreased IgE and AHR, suggesting that realization of complete immunity within the lung requires expression of p41 Ii. These data reveal a crucial role of Ii and H2-M in controlling the immune response within the lung, and suggest that p31 Ii and p41 Ii manifest nonredundant roles in immunity.

Materials and Methods

Mice

Mice genetically deficient in Ii, H2-M, p31 Ii, and p41 Ii have been well characterized in previous studies (15, 17, 29, 41). Each group of mice was backcrossed onto C57BL/6 background for at least five generations. C57BL/6 wild-type (WT) control mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All animals were maintained at the animal facilities of Harvard Medical School in compliance with institutional guidelines and used at 8–10 wk of age.

RNase protection assay and Western blot analysis

Riboprobe vectors suitable for analysis of alternatively spliced Ii mRNAs have been previously described (42). Briefly, pGEMII41 contains a 277-bp PstI-BglII fragment from a p41 Ii cDNA comprising exon 6 (92 nt) and exon 6b (185 nt) sequences. Plasmids digested with EcoRI were transcribed using T7 polymerase to yield the 321-nt pGEMII41 probe. Total cellular RNA (10 µg) was hybridized overnight with the ³²P-labeled riboprobe. Samples were subsequently digested with RNase A (40 µg/ml) and RNase T1 (2 µg/ml) for 60 min at 30°C, treated with SDS and proteinase K, extracted twice with phenol/chloroform, ethanol precipitated, redissolved in buffer containing 80% formamide, and analyzed by electrophoresis in a 6% polyacrylamide denaturing gel.

For Western analysis, tissues were sonicated in sample buffer and centrifuged to remove insoluble material. Tissue extracts were boiled and 50 µg/lane analyzed by 10% SDS-PAGE. Proteins were transferred onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) for 2 h at 500 mA. Blots were extensively washed with TBST containing 0.1% BSA and incubated with secondary Ab conjugated to HRP in TBST containing 3% BSA for 30 min. Blots were washed with TBST and developed by chemiluminescence using ECL (Amersham, Arlington Heights, IL).

Protocol for allergen sensitization and challenge

Mice were sensitized and challenged with OVA, as previously described (39). Briefly, animals were sensitized by i.p. injection of 10 µg of chicken OVA (Sigma-Aldrich, St. Louis, MO) and 2 mg of AL(OH)₃ (OVA/alum) (Sigma-Aldrich) on days 0 and 7. On days 14–20, mice received aerosil-

ized OVA challenge with 6% OVA for 25 min/day. OVA was dissolved in 0.5× PBS. Vehicle-treated animals received 2 mg alum in 0.5× PBS on days 0 and 7, and were nebulized with 0.5× PBS on days 14–20. An ultrasonic nebulizer (model 5000; DeVilbiss, Somerset, PA) was used for nebulizations into a chamber.

Determination of AHR

Twenty-four hours following the last aerosol challenge, AHR was assessed using whole body plethysmography (Buxco Electronics, Birmingham, U.K.) (43, 44). Mice were placed in individual chambers. Mice were first exposed to aerosolized vehicle (0.5× PBS) for 3 min. Readings were then averaged over the subsequent 5 min beginning after termination of the aerosol treatment. Mice were subsequently exposed to nebulized methacholine at 100 mg/ml for 3 min. Readings were then averaged over the subsequent 7 min beginning at the end of the methacholine challenge. The whole body plethysmography system measures changes in box pressure during expiration and inspiration, peak expiratory and peak inspiratory pressures (PEP and PIP, respectively), inspiratory time (Ti), expiratory

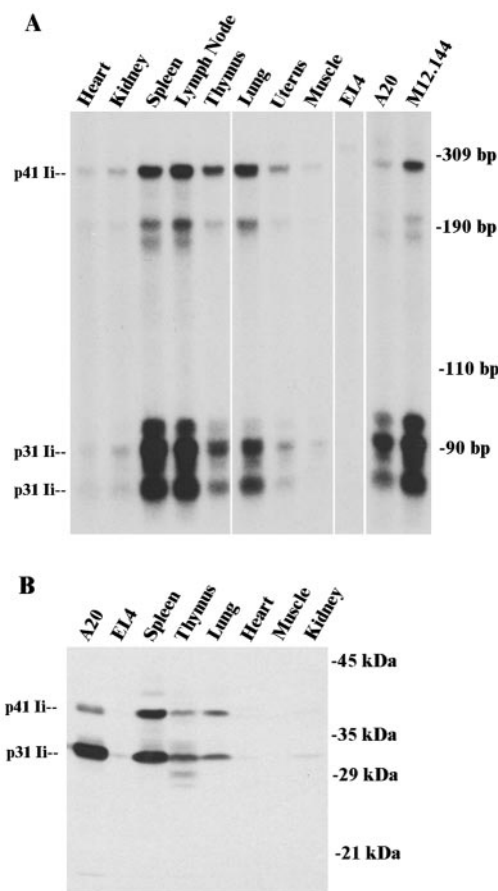


FIGURE 1. Tissue-specific expression of p31 Ii and p41 Ii. **A**, RNase protection analysis of p31 Ii and p41 Ii expression showing expression of the p41 Ii isoform in lung tissue. The pGEMII41 probe that distinguishes p31 Ii and p41 Ii transcripts was hybridized to total RNA (10 µg) extracted from the indicated tissue sources. The smaller p31 Ii product results from overdigestion at the short (5-nt) AT-rich sequence located at the 3' end of exon 6 (42). A20 and M12.144 are mouse B cell lines that exhibit high Ii expression, and EL4 is a murine T cell leukemia that does not express Ii. Numbers on right of gel indicate migration of base pair standards. Autoradiograph is representative experiment ($n = 5$). **B**, Western analysis of p31 Ii and p41 Ii protein expression in extracts derived from a variety of tissues and cell lines. p41 Ii protein is readily expressed in lung tissue. Tissues were sonicated in sample buffer, and 50 µg total protein/lane was analyzed by 10% SDS-PAGE. After transfer to nitrocellulose, the Ii isoforms were probed with mAb In1 and detected by HRP-conjugated secondary Ab, followed by chemiluminescence. Immunoblot is a representative experiment ($n = 3$).

time (T_e), and a relaxation time (T_r = time of the pressure decay to 36% of total box pressure during expiration), and generates a value termed enhanced pause ($Penh = PEP/PIP \times ((T_e - T_r)/T_r)$) that directly correlates with airway resistance. $Penh$ values following methacholine challenge were normalized to the $Penh$ values after exposure to $0.5\times$ PBS as a measurement of AHR.

Bronchoalveolar lavage (BAL) and histologic analysis

Following measurement of AHR, mice were anesthetized with ketamine/xylazine and sacrificed, and BAL was performed, as previously described (39). BAL cells were pelleted, and supernatant was stored at -80°C until analyzed. Slides for differential cell counts were prepared with Cytospin (Shandon, Pittsburgh, PA), and fixed and stained with Diff-Quik (American Scientific Products, McGaw Park, IL). Two counts of 100 cells were done for each sample.

For histopathologic assessment, lungs were inflated with 10% phosphate-buffered Formalin, embedded in paraffin, and sectioned. Lung sections were stained with H&E, and examined microscopically for evidence of inflammation.

Serum IgE

Following withdrawal by cardiac puncture, blood was spun at 13,000 rpm for 10 min. Serum total IgE levels were determined by ELISA, as previously reported (44). Anti-mouse IgE clone R35-72 (BD PharMingen) was used for capture, and anti-mouse Ig κ L chain clone 187.1 (BD PharMingen) conjugated to biotin was used for detection. Samples were normalized to a standard curve generated with IgE standard clone C38-2 (BD Phar-

Mingen). For OVA-specific IgE titers, an identical protocol was used with the substitution of biotinylated OVA in place of the detection mAb. OVA was biotinylated using sulfo-NHS-LC-biotin (Pierce, Rockford, IL), according to manufacturer's instructions.

Cytokine ELISA

Fluid concentrations of IL-13 and IFN- γ were measured by ELISA, according to the manufacturer's specifications (R&D Systems, Minneapolis, MN). Briefly, BAL fluid samples were aliquoted into duplicate wells of a 96-well plate precoated with Ab to IL-13 and IFN- γ , and assayed as per manufacturer's instructions. OD was measured at 450–540 nm. Cytokine concentrations were determined by comparison with known standards.

RT-PCR

Total cellular RNA from lungs was obtained using TRIzol reagent (Life Technologies, Grand Island, NY), as per the manufacturer's instructions. RNA samples were reverse transcribed, and gene-specific primers (Clontech, Palo Alto, CA) were used to amplify selected regions of each targeted cytokine. For each cytokine, the optimal numbers of cycles that will produce a quantity of cytokine product that is directly proportional to the quantity of input mRNA were determined experimentally. To verify that equal amounts of RNA were added in each RT-PCR, β -actin was used as an internal standard. Amplified PCR products were detected using ethidium bromide gel electrophoresis.

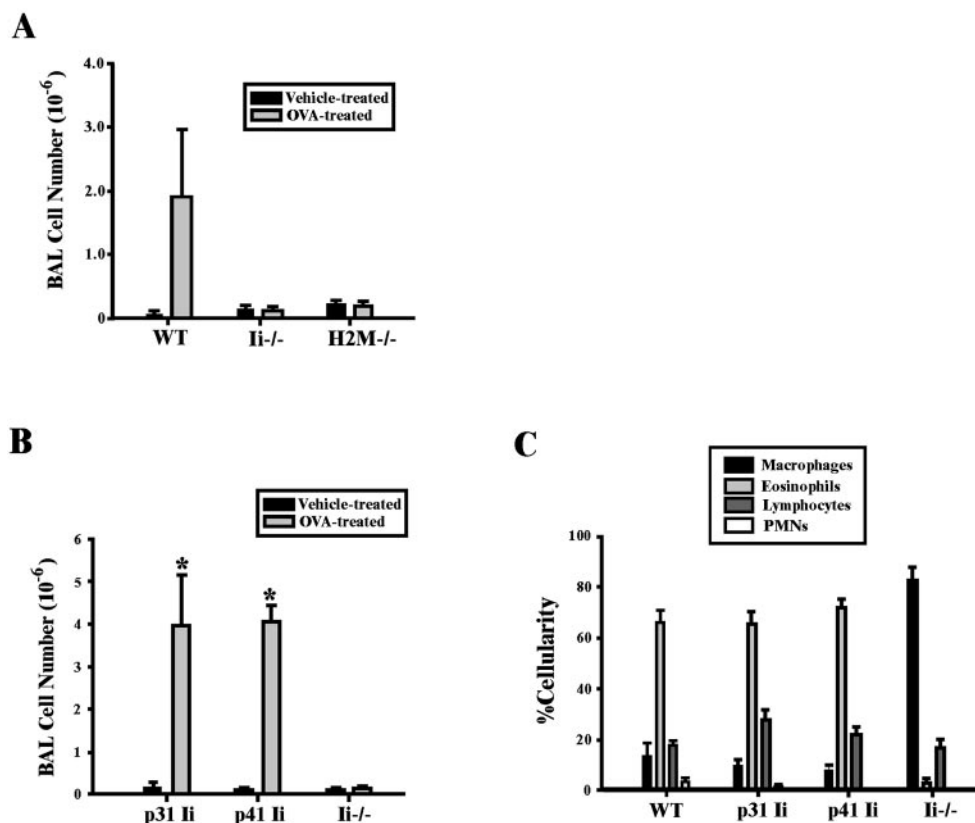


FIGURE 2. Expression of either p31 Ii or p41 Ii is sufficient for generation of airway inflammation and eosinophilia. *A*, Total cell numbers were determined from BAL fluid obtained from WT, Ii^{-/-}, and H2M^{-/-} mice treated with OVA or vehicle (sensitized with $0.5\times$ PBS/alum and nebulized with $0.5\times$ PBS). WT, but not Ii^{-/-} or H2M^{-/-} mice exhibit increased BAL fluid cellularity following treatment with OVA. Bars represent the mean $\pm$ SEM of cell number from BAL fluid ($n = 5$ for all OVA-treated groups, $n = 3$ for Ii^{-/-} vehicle-treated group, and $n = 2$ for WT and H2M^{-/-} vehicle-treated groups). *B*, Total BAL cell numbers were determined from p31 Ii, p41 Ii, and Ii^{-/-} mice treated with OVA or vehicle. Both Ii isoforms reconstitute allergen-induced lung inflammation, as determined by increased BAL fluid cellularity. Data represent the mean $\pm$ SEM of total cell number from BAL fluid ($n = 10$ for p31 Ii and p41 Ii OVA-treated groups, $n = 9$ for Ii^{-/-} OVA-treated animals, $n = 5$ for p31 Ii vehicle-treated group, and $n = 4$ for p41 Ii and Ii^{-/-} vehicle-treated groups). Results were pooled from two separate experiments. *C*, BAL cell differentials were determined from WT, p31 Ii, p41 Ii, and Ii^{-/-} animals following sensitization and challenge with OVA. Expression of either Ii isoform alone is sufficient for promoting pulmonary eosinophilic inflammation. Data represent the mean $\pm$ SEM percentage of cellularity ($n = 5$ for all groups). BAL differentials from all vehicle-treated animals consistently showed 99–100% macrophages (data not shown). *, $p < 0.002$ as compared with Ii^{-/-} OVA-treated animals. PMNs, polymorphonucleocytes.

Statistical analysis

Data analysis was performed using ANOVA analysis for the OVA-treated groups in each experiment, followed by the Bonferroni/Dunn test for multiple comparisons. Data are reported as means $\pm$ SEM. Statistical significance is defined by $p < 0.05$.

Results

p41 Ii is expressed in the lung

Resident APC within the lung and Ii expressed on APC play a critical role in immunosurveillance, both in protection against infection and generation of allergic inflammation. To compare expression of the p31 Ii and p41 Ii isoforms in the lung and other tissues, we conducted an RNase protection assay to examine mRNA expression, and Western analysis to investigate protein levels (Fig. 1) (42). As expected, spleen, lymph node, and thymus as well as the B cell lines A20 and M12.144 strongly express Ii mRNA and protein, in contrast to heart, kidney, uterus, muscle, and the T cell leukemia line EL4. Interestingly, the lung exhibits a relatively intense signal in both mRNA and protein quantities. When looking at p41 Ii mRNA expression alone, the lung exhibits levels similar to that observed for lymph nodes and spleen. These data are consistent with enhanced p41 Ii expression on resident macrophages and DC within the lung (45), and prompted us to investigate the role of Ii and its isoforms in lung immunity.

Expression of either p31 Ii or p41 Ii is sufficient for generation of lung inflammation and eosinophilia

To examine the role of Ii and its isoforms, as well as H2-M, on a Th2-dependent immune response, we used a mouse model of allergic inflammation within the lung. Sensitization and challenge

with OVA in this model induce a robust perivascular and peribronchiolar inflammatory response within the lung and marked BAL eosinophilia (39). This inflammatory response can be quantitated by examining the cellular content and differential within BAL fluid. A recent study has shown that allergen-treated Ii-deficient mice exhibit an attenuation in cellularity of the BAL fluid as compared with WT animals (20). Consistent with these findings, we found that our allergen-treated Ii^{-/-} mice exhibit a profound reduction in BAL cellular number and percentage of eosinophilia, consistent with an overall decrease in lung inflammation (Fig. 2, A and C). Similarly, allergen-treated H2-M^{-/-} mice also exhibit a marked attenuation in BAL cellular infiltration. Expression of either p31 Ii or p41 Ii isoform is sufficient to restore the inflammatory response, as measured by BAL cellular infiltration (Fig. 2B). BAL eosinophilia in the p31 and p41 Ii animals is comparable in magnitude, indicating no difference in the character of lung inflammation between the p31 and p41 Ii transgenic mice, and is similar to that observed with WT animals (Fig. 2C).

As shown in Fig. 3, alterations in lung histology are consistent with differences observed for BAL cellularity. WT animals exhibit a perivascular and peribronchiolar inflammatory cell permeation following OVA sensitization and challenge (Fig. 3). This infiltration is absent in the Ii^{-/-} and H2-M^{-/-} mutants, again reflecting the importance of class II-restricted Ag presentation in this allergic model. Expression of either Ii isoform reconstitutes the lung inflammatory response, with p31 Ii and p41 Ii animals exhibiting histologic abnormalities closely resembling those seen in WT animals. Thus, as assessed by both lung histology and BAL cellular content, OVA-induced lung inflammation requires expression of Ii and H2-M, and both Ii isoforms reconstitute the response.

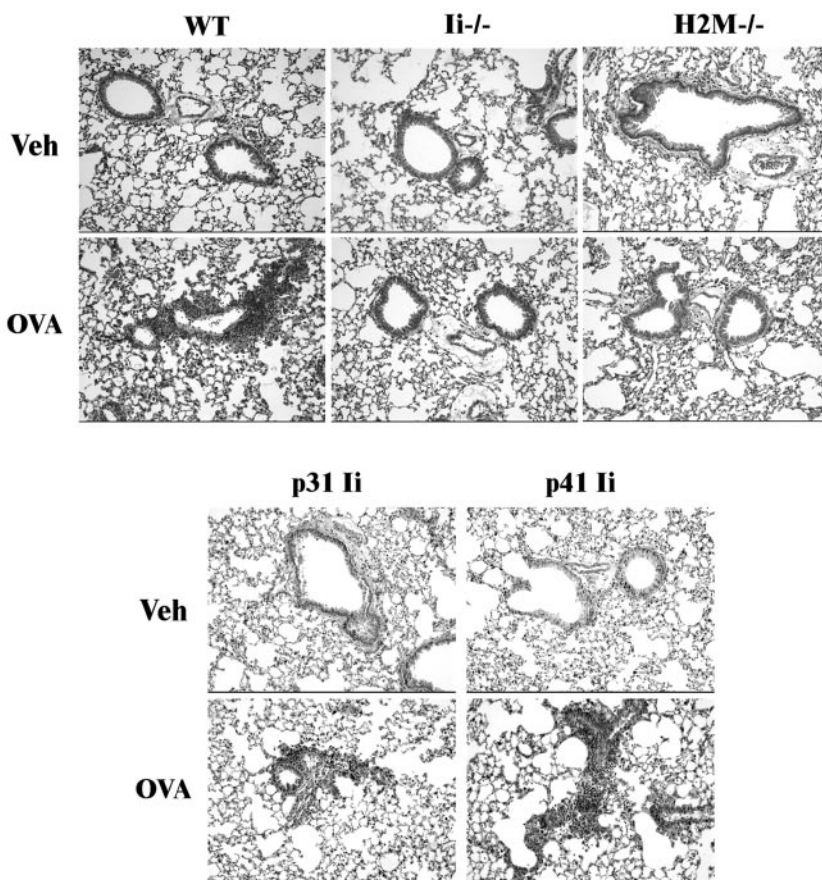


FIGURE 3. Mice expressing either p31 Ii or p41 Ii develop lung inflammation. Representative lung sections from WT, Ii^{-/-}, H2-M^{-/-}, p31 Ii, and p41 Ii mice are shown following sensitization and challenge with OVA or vehicle (veh). Sensitization and challenge with OVA generate a prominent perivascular and peribronchiolar inflammation in WT, p31 Ii, and p41 Ii animals, but not Ii^{-/-} or H2-M^{-/-} mice. Vehicle-treated animals do not develop lung inflammation.

p41 Ii selectively enhances IgE production

Previous studies demonstrate Ii and H2-M requirements during isotype switching due to B cell defects and/or Th1/Th2 bias (35, 37, 38). The role of Igs, and IgE in particular, in Th2-type immunity in the lung has been extensively documented. For example, treatment of patients suffering from allergic asthma with anti-IgE Ab ameliorates symptom scores (46, 47). To investigate how Ii and its isoforms as well as H2-M affect humoral IgE responses in OVA-induced pulmonary inflammation, serum IgE levels were measured by ELISA (Fig. 4). As expected, in WT animals, sensitization and challenge with OVA engender an increase in total IgE levels (Fig. 4A). This response is completely abrogated in mice lacking Ii or H2-M expression. Interestingly, both the total IgE (Fig. 4B) and OVA-specific IgE (Fig. 4C) levels in p41 Ii mice are significantly increased as compared with p31 Ii mice. Thus, the p41 Ii isoform more effectively promotes OVA-induced IgE production.

p41 Ii selectively augments OVA-induced AHR

Increased sensitivity to methacholine (AHR) and reversible airflow obstruction are fundamental characteristics of asthma. Allergens can also induce changes in airway physiology in animal models of allergic inflammation that may parallel features of human asthma. To measure AHR in the $Ii^{-/-}$, $H2-M^{-/-}$, p31 Ii, and p41 Ii mice, the animals were exposed to nebulized methacholine and average Penh values were measured. The ratio of mean Penh after methacholine challenge to mean Penh following vehicle exposure was used as the assessment of AHR (43, 44). WT allergen-treated mice exhibit an increased Penh ratio as compared with allergen-treated $Ii^{-/-}$ mice (Fig. 5A). The AHR of the $H2-M^{-/-}$ mice is more variable in the vehicle-treated group, but there is no significant increase in

AHR following OVA treatment, consistent with the lack of inflammation and IgE levels in these animals. Interestingly, the p41 Ii animals exhibit a significant increase in AHR compared with the p31 Ii or $Ii^{-/-}$ mice. The magnitude of this difference closely parallels that described above affecting IgE levels, further evidence of a partial immune response in p31 Ii animals in this model.

Ii expression is required for the OVA-induced cytokine generation within the lung

OVA-induced pulmonary inflammation in mice leads to a predominant increase in Th2-type cytokines, including IL-4 and IL-13 (48). Other mediators, including IL-10 and IFN- γ , may also be important in modulating immune responses within the lung. For example, mice lacking IL-10 exhibit AHR, but fail to develop pulmonary inflammation (49). To compare cytokine profiles in p31 Ii and p41 Ii animals, RNA was extracted from lung tissue following induction of pulmonary inflammation, and RT-PCR analysis was performed (Fig. 6A). Both p31 and p41 Ii, but not $Ii^{-/-}$, animals express increased levels of IL-4 and IL-13 mRNA following exposure to OVA. IL-10 and IFN- γ mRNA levels were less dramatically altered in either the p31 Ii or p41 Ii mice, although both animals exhibited higher levels of these mRNAs than $Ii^{-/-}$ mice. These data support the findings above showing similar levels of lung inflammation in p31 Ii and p41 Ii animals.

To investigate whether protein levels of Th1- and Th2-type cytokines were altered in the lungs of our allergen-treated animals, IL-13 (Th2-type) and IFN- γ (Th1-type) concentrations were measured in BAL fluid. Consistent with the results above, both the p31 Ii and p41 Ii mice exhibit a large increase in IL-13 following induction of lung inflammation (Fig. 6B), and $Ii^{-/-}$ mice fail to

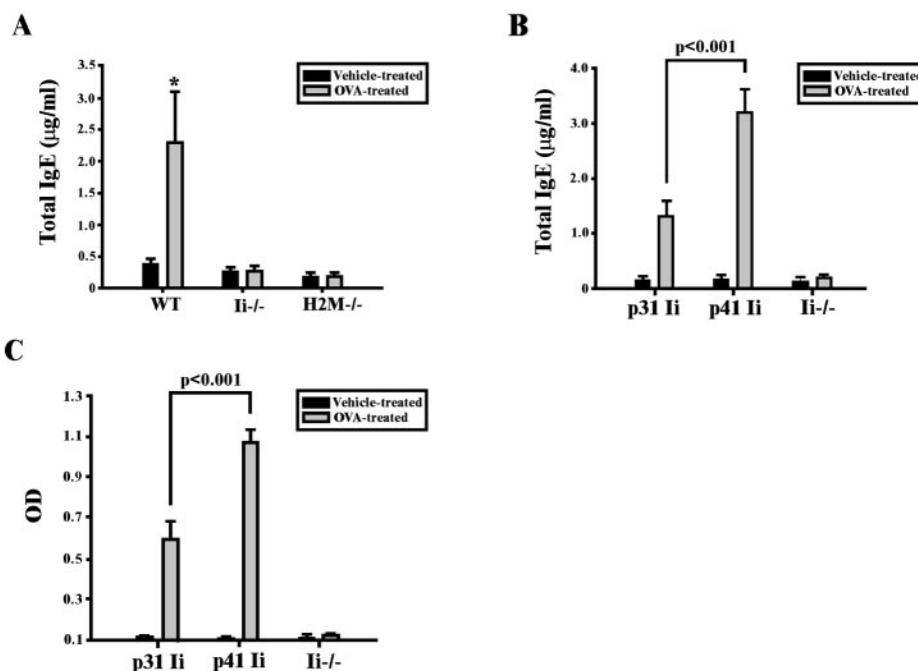


FIGURE 4. p41 Ii selectively promotes IgE generation. *A*, Serum total IgE levels were measured by sandwich ELISA from WT, $Ii^{-/-}$, and $H2-M^{-/-}$ mice following sensitization and challenge with OVA or vehicle. Data represent the mean $\pm$ SEM IgE ($n = 5$ for OVA-treated groups, $n = 3$ for $Ii^{-/-}$ and $H2-M^{-/-}$ vehicle-treated animals, and $n = 2$ for WT vehicle-treated mice). *B*, Serum total IgE levels from p31 Ii, p41 Ii, and $Ii^{-/-}$ mice treated as in *A* above. p41 Ii mice exhibited increased IgE production as compared with p31 Ii or $Ii^{-/-}$ mice. Bars represent the mean $\pm$ SEM IgE ($n = 10$ for p31 Ii and p41 Ii OVA-treated groups, $n = 9$ for $Ii^{-/-}$ OVA-treated animals, $n = 5$ for p31 Ii vehicle-treated mice, and $n = 4$ for p41 Ii and $Ii^{-/-}$ vehicle-treated animals). Results were pooled from two separate experiments. *C*, Serum OVA-specific IgE titers from p31 Ii, p41 Ii, and $Ii^{-/-}$ mice treated as in *A* and *B* above. p41 Ii mice exhibited increased OVA-specific IgE production as compared with p31 Ii or $Ii^{-/-}$ animals. Bars represent the mean $\pm$ SEM OD ($n = 10$ for p31 Ii OVA-treated animals, $n = 9$ for $Ii^{-/-}$ and p41 Ii OVA-treated groups, $n = 5$ for p31 Ii vehicle-treated mice, $n = 4$ for p41 Ii vehicle-treated mice, and $n = 3$ for $Ii^{-/-}$ vehicle-treated animals). Results were pooled from two separate experiments. *, $p < 0.02$ as compared with $Ii^{-/-}$ and $H2-M^{-/-}$ OVA-treated animals.

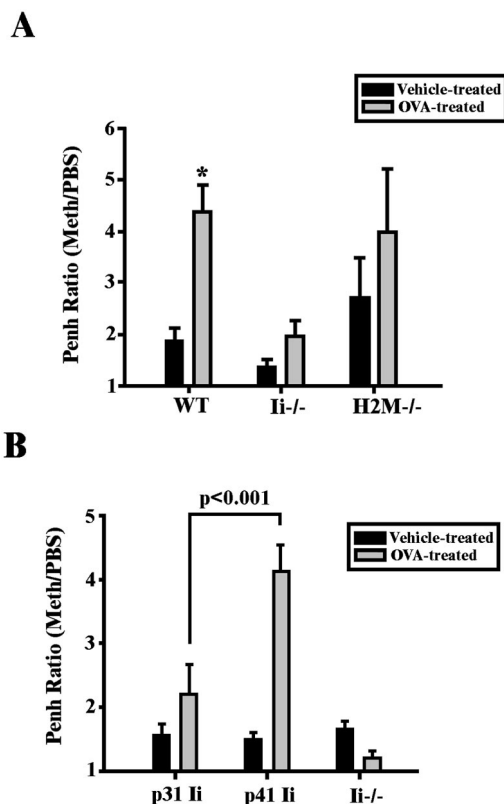


FIGURE 5. p41 Ii expression augments development of AHR. **A**, Penh values were measured following exposure to nebulized 0.5× PBS and 100 mg/ml methacholine in WT, Ii^{-/-}, and H2-M^{-/-} mice as a determination of AHR. OVA-treated WT animals showed a significant increase in Penh ratio as compared with OVA-treated Ii^{-/-} mice. Data represent the mean ± SEM Penh ratio ($n = 5$ for OVA-treated mice, $n = 3$ for Ii^{-/-} and H2-M^{-/-} vehicle-treated animals, and $n = 2$ for WT vehicle-treated mice). **B**, Penh values were measured as in **A** above in p31 Ii, p41 Ii, and Ii^{-/-} mice following exposure to 0.5× PBS and 100 mg/ml methacholine. p41 Ii animals showed a significantly increased AHR to methacholine as compared with p31 Ii and Ii^{-/-} mice. Data represent mean ± SEM Penh ratio (in the OVA-treated groups, $n = 15$ for p31 Ii, $n = 14$ for p41 Ii, and $n = 9$ for Ii^{-/-} mice; in the vehicle-treated cohorts, $n = 7$ for p31 Ii and p41 Ii, and $n = 4$ for Ii^{-/-} mice). Results were pooled from three separate experiments. *, $p < 0.05$ as compared with Ii^{-/-} OVA-treated mice.

demonstrate any increase. The changes in IFN- γ concentrations are less dramatic. The Ii^{-/-} mice do not show any alteration in IFN- γ , whereas both the p31 Ii and p41 Ii animals exhibit a trend toward decreased IFN- γ following OVA sensitization and challenge, again consistent with the alterations observed in mRNA IFN- γ expression. In conclusion, both p31 Ii and p41 Ii animals show similar patterns of cytokine responses following allergen challenge, accurately reflecting the alterations in lung inflammation observed in these mice (Figs. 2 and 3).

Discussion

Ii and H2-M are critical for generation of class II-peptide complexes on professional APC within the MHC class II-restricted Ag presentation pathway. Thus, absence of either of these components may be expected to have a significant impact on the development of the integrative immune response. The present experiments demonstrate Ii and H2-M are both essential to mediate OVA-induced pulmonary inflammation, IgE generation, and AHR in a Th2-dependent immune response. These data are consistent with and extend prior studies, and provide further evidence that class II-de-

pendent Ag presentation and CD4⁺ T cell activation are required for development of inflammation and AHR in this model (20).

Ii^{-/-} mice exhibit defective CD4⁺ T cell thymic selection, leading to a decreased CD4⁺ T cell population in the periphery. Thus, the diminished lung immune response observed in the Ii^{-/-} mice may result from a relative dearth of the effector CD4⁺ T cells. However, several pieces of data argue against this simplistic view. First, C57BL/6 Ii^{-/-} animals are able to mount a normal Th1-type immune response to *Leishmania major* infection and are able to control the infection as effectively as control mice (50). Second, BALB/c Ii^{-/-} mice exhibit a similar course of disease progression and Th2-type immune development following infection with *L. major* as control BALB/c animals. Finally, C57BL/6 Ii^{-/-} animals develop comparable inflammation as control animals in a Th1-dependent murine model of colitis (20). Thus, the attenuated pulmonary inflammatory response observed in the Ii^{-/-} animals may be more related to their inability to process and present the critical class II-dependent Ags required to drive the immune response within the lung, rather than defective CD4⁺ T cell selection. The findings that both Th1-type and Th2-type cytokines within the lungs and BAL fluid of Ii^{-/-} animals are markedly decreased support this naive mechanism. Also, the similar phenotype obtained with the H2-M^{-/-} animals provides further evidence that inhibition of Ag presentation is playing a vital role in this animal model.

There is increasing evidence that the role of the p31 and p41 Ii isoforms in Ag presentation and immunity is not completely redundant. As shown in Fig. 1, the relative expression of p41/p31 Ii ratios is relatively increased in the lung among the tissues examined. Thus, the lung is a logical site to investigate potential differences in immunity between the p31 and p41 Ii mice. Importantly, the p31 Ii and p41 Ii mice used in this study were generated by a hit and run gene-targeting strategy so that expression of these Ii isoforms is under the control of the endogenous regulatory elements responsible for constitutive and inducible Ii expression. The expression of Ii, MHC class II molecules and selection of CD4⁺ T cells are equivalent in these two mouse strains (29, 41). Both the p31 and p41 Ii animals are able to generate a normal inflammatory response within the lung, as judged by BAL cell infiltration, histology, and lung cytokine mRNA levels. However, the p41 Ii is required for normal levels of IgE production and AHR. This augmentation of the immune response by p41 Ii within the lung is similar to that observed in a Th2-type mouse model of CNS autoimmunity, in which the expression of the p41 Ii molecule enhances disease severity (35). Thus, these data support and extend the current literature that expression of p41 Ii appears to extend or enhance the immune response as compared with animals that solely express p31 Ii.

Although the exact mechanism by which p31 Ii and p41 Ii differentially regulate the immune response is not known, at least two possibilities exist. One potential mechanism is predicated on the finding that the additional exon present in the p41 Ii isoform, exon 6b, is a potent and selective inhibitor of cathepsin L (30, 31). More recent data suggest that the p41 exon 6b can act as a chaperone for cathepsin L, potentiating its stability and activity, especially at neutral pH (32). In either case, expression of the p41 Ii may alter the endosomal proteolytic milieu by modifying cathepsin L activity and, as a consequence, peptide processing in professional APC, leading to enhanced presentation of some antigenic determinants (33, 34, 51). A second potential mechanism explaining alterations in immunity between the p31 Ii and p41 Ii animals is that the p41 Ii may have a distinct mode of class II occupancy that augments Ag presentation of poorly presented peptides, leading to amplification of T cell stimulation and enhanced immunity. Indeed, several studies show that the Ii interacts with MHC class II outside of

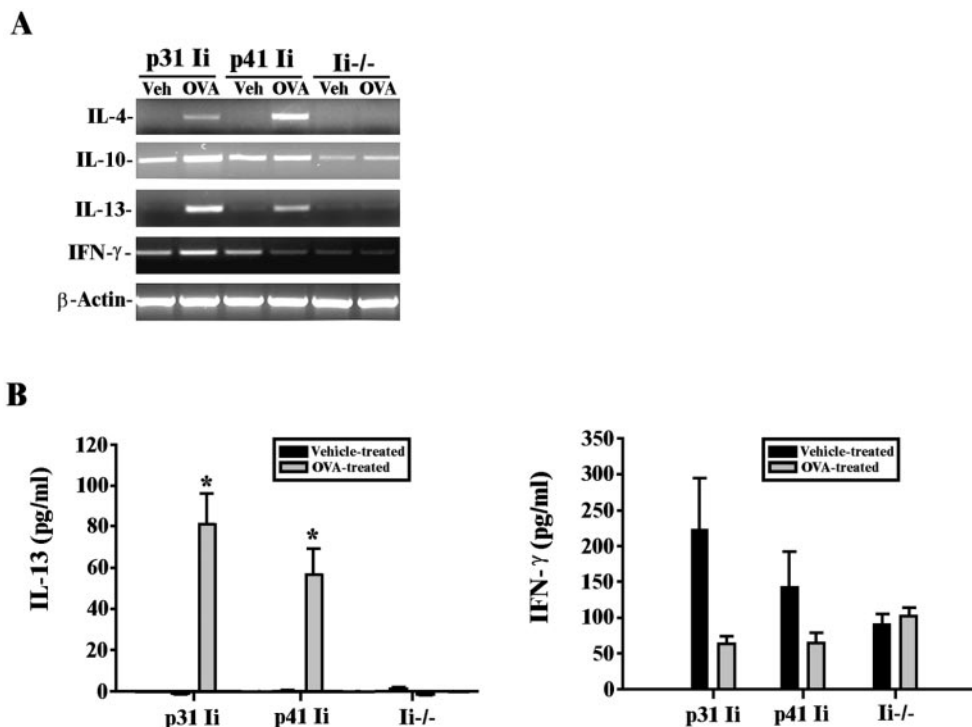


FIGURE 6. Ii expression is required for the allergen-induced cytokine generation within the lung. **A**, RT-PCR of cytokine transcripts from lung RNA (2 μ g) isolated from p31 Ii, p41 Ii, and Ii^{-/-} mice following sensitization and challenge with OVA or vehicle (veh). Both p31 Ii and p41 Ii, but not Ii^{-/-}, mice demonstrate an increase in Th2-type cytokine mRNA levels (IL-4 and IL-13) following allergen challenge. **B**, IL-13 and IFN- γ protein levels were measured by sandwich ELISA in the BAL fluid of p31 Ii, p41 Ii, and Ii^{-/-} mice following exposure to OVA or vehicle. p31 Ii and p41 Ii mice show an increase in IL-13 concentrations following allergen exposure. Data represent the mean $\pm$ SEM of IL-13 levels (in the OVA-treated group, $n = 10$ for p31 Ii, $n = 9$ for p41 Ii, and $n = 8$ for Ii^{-/-} mice; in the vehicle-treated sets, $n = 5$ for p31 Ii, and $n = 3$ for p41 Ii and Ii^{-/-} mice). Results were pooled from two separate experiments. *, $p < 0.005$ compared with Ii^{-/-} OVA-treated mice.

the CLIP binding region, including regions C terminus to CLIP (52–55). Also, the observation that p41/H2-M^{-/-}, but not p31/H2-M^{-/-}, splenocytes are capable of presenting some class II-restricted Ags lends support to a unique p41 Ii-class II interaction and suggests that these effects extend beyond alterations in the endosomal proteolytic milieu (36).

Eosinophilic airway inflammation, increased IgE, and AHR are the defining characteristics of asthma. In many studies, attenuation of lung inflammation and cytokine levels are linked to decreased AHR, suggesting a causative role for inflammation of the AHR response (56–59). Other studies demonstrate a clear dissociation of lung inflammation and AHR in both human and animal studies (60–62). For example, administration of pentoxifylline during allergen sensitization markedly attenuates AHR without altering inflammation (44). Also, mice deficient in IL-10 fail to develop AHR, but do exhibit normal lung inflammation when sensitized and challenged with OVA (49). Our data showing normal lung eosinophilia and cytokine levels (mRNA and protein) in the p31 mice, but decreased AHR and IgE, are another example of this dissociation of AHR from pulmonary inflammation. Interestingly, intratracheal injection of splenic dendritic cells pulsed with the immunodominant OVA_{323–339} peptide is capable of inducing AHR and Th2-type cytokines within the lung, but not pulmonary eosinophilia or inflammation (63). These data together suggest that presentation of different antigenic epitopes may differentially regulate AHR and pulmonary inflammation.

In summary, disruption of MHC class II-restricted Ag presentation by absence of Ii or H2-M expression markedly disrupts inflammatory cell infiltration and AHR in Th2-dependent, allergen-induced lung immunity. Inflammation, but not AHR, is rescued by

sole expression of the p31 Ii isoform, whereas expression of p41 Ii restores both inflammation and AHR. Thus, manipulation of class II-restricted Ag presentation by expression of Ii and its isoforms can lead to distinct alterations in the immune response.

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References

- Cresswell, P. 1994. Assembly, transport, and function of MHC class II molecules. *Annu. Rev. Immunol.* 12:259.
- Germain, R. N. 1994. MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. *Cell* 76:287.
- Watts, C. 1997. Capture and processing of exogenous antigens for presentation on MHC molecules. *Annu. Rev. Immunol.* 15:821.
- Wolf, P. R., and H. L. Ploegh. 1995. How MHC class II molecules acquire peptide cargo: biosynthesis and trafficking through the endocytic pathway. *Annu. Rev. Cell. Dev. Biol.* 11:267.
- Riese, R. J., and H. A. Chapman. 2000. Cathepsins and compartmentalization in antigen presentation. *Curr. Opin. Immunol.* 12:107.
- Riese, R. J., P. R. Wolf, D. Bromme, L. R. Natkin, J. A. Villadangos, H. L. Ploegh, and H. A. Chapman. 1996. Essential role for cathepsin S in MHC class II-associated invariant chain processing and peptide loading. *Immunity* 4:357.
- Villadangos, J. A., R. J. Riese, C. Peters, H. A. Chapman, and H. L. Ploegh. 1997. Degradation of mouse invariant chain: roles of cathepsins S and D and the influence of major histocompatibility complex polymorphism. *J. Exp. Med.* 186:549.
- Villadangos, J. A., R. A. Bryant, J. Deussing, C. Driessen, A. M. Lennon-Dumenil, R. J. Riese, W. Roth, P. Saftig, G. P. Shi, H. A. Chapman, et al. 1999. Proteases involved in MHC class II antigen presentation. *Immunol. Rev.* 172:109.
- Sloan, V. S., P. Cameron, G. Porter, M. Gammon, M. Amaya, E. Mellins, and D. M. Zaller. 1995. Mediation by HLA-DM of dissociation of peptides from HLA-DR. *Nature* 375:802.
- Sherman, M. A., D. A. Weber, and P. E. Jensen. 1995. DM enhances peptide binding to class II MHC by release of invariant chain-derived peptide. *Immunity* 3:197.

11. Ghosh, P., M. Amaya, E. Mellins, and D. C. Wiley. 1995. The structure of an intermediate in class II MHC maturation: CLIP bound to HLA-DR3. *Nature* 378:457.
12. Fung-Leung, W. P., C. D. Surh, M. Liljedahl, J. Pang, D. Leturcq, P. A. Peterson, S. R. Webb, and L. Karlsson. 1996. Antigen presentation and T cell development in H2-M-deficient mice. *Science* 271:1278.
13. Miyazaki, T., P. Wolf, S. Tourne, C. Waltzinger, A. Dierich, N. Barois, H. Ploegh, C. Benoist, and D. Mathis. 1996. Mice lacking H2-M complexes, enigmatic elements of the MHC class II peptide-loading pathway. *Cell* 84:531.
14. Viville, S., J. Neefjes, V. Lotteau, A. Dierich, M. Lemeur, H. Ploegh, C. Benoist, and D. Mathis. 1993. Mice lacking the MHC class II-associated invariant chain. *Cell* 72:635.
15. Bikoff, E. K., L. Y. Huang, V. Episkopou, J. van Meerwijk, R. N. Germain, and E. J. Robertson. 1993. Defective major histocompatibility complex class II assembly, transport, peptide acquisition, and CD4⁺ T cell selection in mice lacking invariant chain expression. *J. Exp. Med.* 177:1699.
16. Elliott, E. A., J. R. Drake, S. Amigorena, J. Elsemore, P. Webster, I. Mellman, and R. A. Flavell. 1994. The invariant chain is required for intracellular transport and function of major histocompatibility complex class II molecules. *J. Exp. Med.* 179:681.
17. Martin, W. D., G. G. Hicks, S. K. Mendiratta, H. I. Leva, H. E. Ruley, and L. Van Kaer. 1996. H2-M mutant mice are defective in the peptide loading of class II molecules, antigen presentation, and T cell repertoire selection. *Cell* 84:543.
18. Bikoff, E. K., R. N. Germain, and E. J. Robertson. 1995. Allelic differences affecting invariant chain dependency of MHC class II subunit assembly. *Immunity* 2:301.
19. Shachar, I., and R. A. Flavell. 1996. Requirement for invariant chain in B cell maturation and function. *Science* 274:106.
20. Topilski, I., A. Harmelin, R. A. Flavell, Y. Levo, and I. Shachar. 2002. Preferential Th1 immune response in invariant chain-deficient mice. *J. Immunol.* 168:1610.
21. Matza, D., O. Wolstein, R. Dikstein, and I. Shachar. 2001. Invariant chain induces B cell maturation by activating a TAF(II)105-NF- κ B-dependent transcription program. *J. Biol. Chem.* 276:27203.
22. Matza, D., F. Lantner, Y. Bogoch, L. Flaishon, R. Hershkovich, and I. Shachar. 2002. Invariant chain induces B cell maturation in a process that is independent of its chaperonic activity. *Proc. Natl. Acad. Sci. USA* 99:3018.
23. Strubin, M., C. Berte, and B. Mach. 1986. Alternative splicing and alternative initiation of translation explain the four forms of the Ia antigen-associated invariant chain. *EMBO J.* 5:3483.
24. Koch, N., W. Lauer, J. Habicht, and B. Dobberstein. 1987. Primary structure of the gene for the murine Ia antigen-associated invariant chains (Ii): an alternatively spliced exon encodes a cysteine-rich domain highly homologous to a repetitive sequence of thyroglobulin. *EMBO J.* 6:1677.
25. O'Sullivan, D. M., D. Noonan, and V. Quaranta. 1987. Four Ia invariant chain forms derive from a single gene by alternate splicing and alternate initiation of transcription/translation. *J. Exp. Med.* 166:444.
26. Romagnoli, P., C. Layet, J. Yewdell, O. Bakke, and R. N. Germain. 1993. Relationship between invariant chain expression and major histocompatibility complex class II transport into early and late endocytic compartments. *J. Exp. Med.* 177:583.
27. Arunachalam, B., C. A. Lamb, and P. Cresswell. 1994. Transport properties of free and MHC class II-associated oligomers containing different isoforms of human invariant chain. *Int. Immunol.* 6:439.
28. Stockinger, B., U. Pessara, R. H. Lin, J. Habicht, M. Grez, and N. Koch. 1989. A role of Ia-associated invariant chains in antigen processing and presentation. *Cell* 56:683.
29. Takaes, N. T., J. A. Lower, E. J. Robertson, and E. K. Bikoff. 1995. Major histocompatibility class II peptide occupancy, antigen presentation, and CD4⁺ T cell function in mice lacking the p41 isoform of invariant chain. *Immunity* 3:385.
30. Ogrinc, T., I. Dolenc, A. Ritonja, and V. Turk. 1993. Purification of the complex of cathepsin L and the MHC class II-associated invariant chain fragment from human kidney. *FEBS Lett.* 336:555.
31. Bevec, T., V. Stoka, G. Pungercic, I. Dolenc, and V. Turk. 1996. Major histocompatibility complex class II-associated p41 invariant chain fragment is a strong inhibitor of lysosomal cathepsin L. *J. Exp. Med.* 183:1331.
32. Lennon-Dumenil, A. M., R. A. Roberts, K. Valentijn, C. Driessen, H. S. Overkleef, A. Erickson, P. J. Peters, E. Bikoff, H. L. Ploegh, and P. Wolf Bryant. 2001. The p41 isoform of invariant chain is a chaperone for cathepsin L. *EMBO J.* 20:4055.
33. Peterson, M., and J. Miller. 1992. Antigen presentation enhanced by the alternatively spliced invariant chain gene product p41. *Nature* 357:596.
34. Fineschi, B., K. Sakaguchi, E. Appella, and J. Miller. 1996. The proteolytic environment involved in MHC class II-restricted antigen presentation can be modulated by the p41 form of invariant chain. *J. Immunol.* 157:3211.
35. Slavina, A. J., J. M. Soos, O. Stuve, J. C. Patarroyo, H. L. Weiner, A. Fontana, E. K. Bikoff, and S. S. Zamvil. 2001. Requirement for endocytic antigen processing and influence of invariant chain and H-2M deficiencies in CNS autoimmunity. *J. Clin. Invest.* 108:1133.
36. Bikoff, E. K., G. Kenty, and L. Van Kaer. 1998. Distinct peptide loading pathways for MHC class II molecules associated with alternative Ii chain isoforms. *J. Immunol.* 160:3101.
37. Alfonso, C., J. O. Han, G. S. Williams, and L. Karlsson. 2001. The impact of H2-DM on humoral immune responses. *J. Immunol.* 167:6348.
38. Swier, K., D. R. Brown, J. J. Bird, W. D. Martin, L. Van Kaer, and S. L. Reiner. 1998. A critical, invariant chain-independent role for H2-M in antigen presentation. *J. Immunol.* 160:540.
39. Krinzman, S. J., G. T. De Sanctis, M. Cernadas, L. Kobzik, J. A. Listman, D. C. Christiani, D. L. Perkins, and P. W. Finn. 1996. T cell activation in a murine model of asthma. *Am. J. Physiol.* 271:L476.
40. Lambrecht, B. N., B. Salomon, D. Klatzmann, and R. A. Pauwels. 1998. Dendritic cells are required for the development of chronic eosinophilic airway inflammation in response to inhaled antigen in sensitized mice. *J. Immunol.* 160:4090.
41. Takaes, N. T., J. A. Lower, D. Yelon, E. J. Robertson, and E. K. Bikoff. 1997. In vivo functions mediated by the p41 isoform of the MHC class II-associated invariant chain. *J. Immunol.* 158:187.
42. Bikoff, E. K. 1992. Formation of complexes between self-peptides and MHC class II molecules in cells defective for presentation of exogenous protein antigens. *J. Immunol.* 149:1.
43. Hamelmann, E., J. Schwarze, K. Takeda, A. Oshiba, G. L. Larsen, C. G. Irvin, and E. W. Gelfand. 1997. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am. J. Respir. Crit. Care Med.* 156:766.
44. Fleming, C. M., H. He, A. Ciota, D. Perkins, and P. W. Finn. 2001. Administration of pentoxifylline during allergen sensitization dissociates pulmonary allergic inflammation from airway hyperresponsiveness. *J. Immunol.* 167:1703.
45. Momburg, F., N. Koch, P. Moller, G. Moldenhauer, G. W. Butcher, and G. J. Hammerling. 1986. Differential expression of Ia and Ia-associated invariant chain in mouse tissues after in vivo treatment with IFN- γ . *J. Immunol.* 136:940.
46. Milgrom, H., R. B. Fick, Jr., J. Q. Su, J. D. Reimann, R. K. Bush, M. L. Watrous, and W. J. Metzger. 1999. Treatment of allergic asthma with monoclonal anti-IgE antibody: rhuMAB-E25 Study Group. *N. Engl. J. Med.* 341:1966.
47. Hamelmann, E., K. Takeda, A. Oshiba, and E. W. Gelfand. 1999. Role of IgE in the development of allergic airway inflammation and airway hyperresponsiveness: a murine model. *Allergy* 54:297.
48. Hamelmann, E., U. Wahn, and E. W. Gelfand. 1999. Role of the Th2 cytokines in the development of allergen-induced airway inflammation and hyperresponsiveness. *Int. Arch. Allergy Immunol.* 118:90.
49. Makela, M. J., A. Kanehiro, L. Borish, A. Dakhama, J. Loader, A. Joetham, Z. Xing, M. Jordana, G. L. Larsen, and E. W. Gelfand. 2000. IL-10 is necessary for the expression of airway hyperresponsiveness but not pulmonary inflammation after allergic sensitization. *Proc. Natl. Acad. Sci. USA* 97:6007.
50. Brown, D. R., K. Swier, N. H. Moskowitz, M. F. Naujokas, R. M. Locksley, and S. L. Reiner. 1997. T helper subset differentiation in the absence of invariant chain. *J. Exp. Med.* 185:31.
51. Hsieh, C. S., P. deRoos, K. Honey, C. Beers, and A. Y. Rudensky. 2002. A role for cathepsin L and cathepsin S in peptide generation for MHC class II presentation. *J. Immunol.* 168:2618.
52. Siebenkotten, I. M., C. Carstens, and N. Koch. 1998. Identification of a sequence that mediates promiscuous binding of invariant chain to MHC class II allotypes. *J. Immunol.* 160:3355.
53. Wilson, N. A., P. Wolf, H. Ploegh, L. Ignatowicz, J. Kappler, and P. Marrack. 1998. Invariant chain can bind MHC class II at a site other than the peptide binding groove. *J. Immunol.* 161:4777.
54. Thayer, W. P., L. Ignatowicz, D. A. Weber, and P. E. Jensen. 1999. Class II-associated invariant chain peptide-independent binding of invariant chain to class II MHC molecules. *J. Immunol.* 162:1502.
55. 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.
56. Krinzman, S. J., G. T. De Sanctis, M. Cernadas, D. Mark, Y. Wang, J. Listman, L. Kobzik, C. Donovan, K. Nassr, I. Katona, et al. 1996. Inhibition of T cell costimulation abrogates airway hyperresponsiveness in a murine model. *J. Clin. Invest.* 98:2693.
57. Cernadas, M., G. T. De Sanctis, S. J. Krinzman, D. A. Mark, C. E. Donovan, J. A. Listman, L. Kobzik, H. Kikutani, D. C. Christiani, D. L. Perkins, and P. W. Finn. 1999. CD23 and allergic pulmonary inflammation: potential role as an inhibitor. *Am. J. Respir. Cell Mol. Biol.* 20:1.
58. Haczku, A., K. Takeda, E. Hamelmann, J. Loader, A. Joetham, I. Redai, C. G. Irvin, J. J. Lee, H. Kikutani, D. Conrad, and E. W. Gelfand. 2000. CD23 exhibits negative regulatory effects on allergic sensitization and airway hyperresponsiveness. *Am. J. Respir. Crit. Care Med.* 161:952.
59. Haczku, A., K. Takeda, I. Redai, E. Hamelmann, G. Cieslewicz, A. Joetham, J. Loader, J. J. Lee, C. Irvin, and E. W. Gelfand. 1999. Anti-CD86 (B7.2) treatment abolishes allergic airway hyperresponsiveness in mice. *Am. J. Respir. Crit. Care Med.* 159:1638.
60. Crimi, E., A. Spanevello, M. Neri, P. W. Ind, G. A. Rossi, and V. Brusasco. 1998. Dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma. *Am. J. Respir. Crit. Care Med.* 157:4.
61. Wilder, J. A., D. D. Collie, B. S. Wilson, D. E. Bice, C. R. Lyons, and M. F. Lipscomb. 1999. Dissociation of airway hyperresponsiveness from immunoglobulin E and airway eosinophilia in a murine model of allergic asthma. *Am. J. Respir. Cell Mol. Biol.* 20:1326.
62. Lilly, C. M., R. W. Chapman, S. J. Sehrging, P. J. Mauser, R. W. Egan, and J. M. Drazen. 1996. Effects of interleukin 5-induced pulmonary eosinophilia on airway reactivity in the guinea pig. *Am. J. Physiol.* 270:L368.
63. Sung, S., C. E. Rose, and S. M. Fu. 2001. Intratracheal priming with ovalbumin and ovalbumin 323-339 peptide-pulsed dendritic cells induces airway hyperresponsiveness, lung eosinophilia, goblet cell hyperplasia, and inflammation. *J. Immunol.* 166:1261.