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Preferential Th1 Immune Response in Invariant Chain-Deficient Mice

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MHC class II molecules associate with the invariant chain (Ii) molecule during biosynthesis. Ii facilitates the folding of class II molecules, interferes with their peptide association, and is involved in MHC class II transport. In this study, we have investigated the in vitro and in vivo immune response of Ii-deficient mice (Ii−/−). Our results have demonstrated that CD4⁺ T cells from Ii−/− mice proliferate normally in vitro after in vivo immunization with protein Ags. However, cytokine secretion profiles of Ag-primed CD4⁺ T cells from Ii−/− mice differ from CD4⁺ T cells from wild-type mice. Whereas cells from wild-type mice secrete IFN-γ and IL-4, cells from Ii−/− mice secrete mostly IFN-γ. Moreover, Ii−/− mice exhibit a normal Th1 response in the delayed-type hypersensitivity and trinitrobenzene sulfonic acid colitis models; however, these mice lack an in vivo Th2 response, as demonstrated in the asthma model. Therefore, we suggest that defective Ag presentation in Ii−/− mice leads selectively to a Th1 effector response. The Journal of Immunology, 2002, 168: 1610–1617.

The MHC class II molecules are heterodimeric complexes that display the foreign antigenic peptides on the cell surface of APCs to CD4⁺ T cells (1–3). MHC class II synthesis and assembly begins in the endoplasmic reticulum (ER), with the noncovalent association of the α- and β-chains with trimers of the invariant chain (Ii) (4). Briefly, three αβ dimers bind sequentially to a trimer of the Ii to form a nonameric complex (αβIi)₃ (5–7), which then exits the ER. After being transported to the trans-Golgi, the αβIi complex is diverted from the secretory pathway to the endocytic system (8–11) and ultimately to acidic endosome/lysosome-like structures called MHC class II compartments (12–16). Once in this pathway, the luminal domain of the Ii is proteolytically degraded, leaving a small fragment, the class II-associated Ii peptide (CLIP), which is bound to the released αβ dimers. Interaction of the αβCLIP complexes in the specialized lysosome-like compartment with another class II-related molecule, HLA-DM in humans and H2-M in mice, drives out the residual αβ CLIP, ultimately rendering the Ii complex competent to bind exogenous Ags (4). Our results have demonstrated that CD4⁺ T cells from Ii−/− mice lack the ability to present exogenous Ags, whereas cells from wild-type mice secrete IFN-γ and IL-4, cells from Ii−/− mice secrete mostly IFN-γ. Moreover, Ii−/− mice exhibit a normal Th1 response in the delayed-type hypersensitivity and trinitrobenzene sulfonic acid colitis models; however, these mice lack an in vivo Th2 response, as demonstrated in the asthma model. Therefore, we suggest that defective Ag presentation in Ii−/− mice leads selectively to a Th1 effector response.

It has become possible to analyze the role of Ii in authentic class II-positive cell types using li-deficient mice (li−/−) generated by gene targeting. Cells from mutant mice show aberrant transport of MHC class II molecules, resulting in reduced levels of class II complexes at the surface, which do not have the typical compact conformation indicative of tight peptide binding. Although these complexes do not bind endogenously processed Ags, class II molecules that reach the surface are able to bind exogenous peptides added to the medium. In addition, deletion of the Ii gene was found to greatly diminish the ability of splenic APCs to present exogenous protein Ag in a class II-restricted fashion in vitro and to reduce the CD4⁺ T cell maturation in vivo (19–21). Ii−/− mice clearly have a deficiency in the CD4⁺ T cell compartment as a result of defective positive selection in the thymus. More specifically, their CD4⁺ T cells express high levels of CD44 and CD69 and low levels of TCR and CD45RB (22, 23). This activated phenotype may result from an abnormal selection of CD4⁺ T cells. Alternatively, the high percentage of phenotypically mature CD4⁺ T cells could reflect the low number of CD4⁺ T cells in the periphery that responded to a normal antigenic load and therefore developed a high percentage of cells with a memory or activated phenotype.

In this study, we have investigated the in vitro and in vivo immune responses of T cells in Ii−/− mice. Our results have demonstrated that CD4⁺ T cells from Ii−/− mice can proliferate normally in vitro after in vivo immunization with protein Ags. However, cytokine secretion profiles of Ag-primed CD4⁺ T cells from Ii−/− mice are quite different from CD4⁺ T cells from wild-type mice. Whereas cells from wild-type mice secrete IFN-γ and IL-4, cells from Ii−/− mice secrete mostly IFN-γ. Moreover, Ii−/− mice exhibit a normal Th1 response in vivo in delayed-type hypersensitivity (DTH) and trinitrobenzene sulfonic acid (TNBS) colitis models; however, they failed to show a Th2 response in vivo using the OVA asthma model.

Importantly, these results suggest that the efficiency of Ag presentation dictates the nature of the immune response and that inefficient Ag presentation in li-deficient mice leads to a preferential Th1 response.

Materials and Methods

**Animals**

C57BL/6 (control), li-deficient on a C57BL/6 background (li−/−) (21) and p41 transgenic on the C57BL/6 li−/− background (p41), mice (22) were
used in this study. The Animal Research Committee at the Weizmann Institute of Science (Rehovot, Israel) approved all animal procedures.

**Immunization and CD4+ T cell priming**

Three to five mice were immunized with 100 μg of keyhole limpet hemocyanin (KLH) (Calbiochem, La Jolla, CA) in a 1:1 emulsion with CFA containing 1 mg/ml Mycobacterium tuberculosis strain H37Ra (Difco Laboratories, Detroit, MI) injected in the hind footpads. After 9 days, the draining lymph nodes were removed and cell suspensions were prepared.

**In vitro proliferative recall response**

After total lymph node cells were removed 9 days after injection, the cells were cultured in 96-well plates at 5 × 10^5 cells/well in Bruf medium supplemented with 5% FCS, 2 mM of glutamate, 100 U/ml penicillin, 100 μg/ml streptomycin, and different concentrations of KLH. Approximately 1 × 10^5 purified CD4+ T cells were incubated with 5 × 10^5 irradiated wild-type APCs in triplicates in 96-well plates with increased concentrations of KLH. Proliferation was measured by adding of 1 μCi of [3H]thy midine (International Chemical and Nuclear, Irvine, CA) in the last 18 h of a 4-day culture.

**Purification of CD4+ T cells**

Total CD4+ T cells were isolated from spleen and lymph nodes by negative selection as previously described (24).

**Cytokine ELISA analysis**

ELISAs were performed using anti-IL-4 or anti-IFN-γ as the primary Ab and the corresponding biotinylated anti-IL-4 and IFN-γ mAbs (BD Pharmingen, San Diego, CA), following the recommended protocol.

**Polyclonal stimulation**

CD4+ T cells were cultured in the presence of irradiated wild-type APCs in the presence of different concentrations of Con A or anti-CD3. Proliferation was measured after 4 days by incorporation of [3H]thy midine.

**DTH response**

Mice were immunized in the dorsal flanks with 100 μg of KLH in CFA. Five days later, soluble KLH (10 μg) was injected in the right ear and PBS in the left ear. Ear swelling was measured 24 h later.

**OVA sensitization and challenge**

C57BL/6 (control) and Ii–/– mice were immunized i.p. on days 0, 7, and 14 with 10 or 100 μg of chicken egg albumin (OVA; Sigma-Aldrich, St. Louis, MO) mixed with 2 mg of aluminum hydroxide (Pierce, Rockford, IL) in 300 μl of PBS. Beginning on day 15 after the initial sensitization, the animals were challenged every day for 5 days (days 15–19) by inhaling 4% OVA in PBS, administered by an ultrasonic nebulizer (DeVilbiss, Somerset, PA) for 20 min. For each inhalation, the animals were placed in a 0.5-L plastic chamber connected to the nebulizer.

**Bronchoalveolar lavage (BAL)**

BAL was performed on day 19, 4 h after the last OVA challenge. After deep anesthesia with 2,2,2 tribromoethanol solution, a midline celiotomy was performed into the proximal trachea and secured with a 3-0 silk suture. The animals were killed by cervical dislocation 3–5 days after TNBS administration.

**Macroscopic and histologic assessment of colitis**

The colon was examined under ×5 magnification to evaluate the macroscopic lesions according to the Wallace criteria. The Wallace score ranks macroscopic colon lesions on a scale from 0 to 13 based on criteria reflecting inflammation, such as hyperemia, thickening of the bowel, and the extent of ulceration (25, 26). The colon was cut lengthwise and fixed overnight in 10% paraformaldehyde acid and embedded in paraffin, stained with H&E, and examined randomly by a pathologist and ranked according to the Ameho criteria (27). This score, on a scale from 0 to 6, takes into account the degree of the inflammatory infiltrate, the presence of erosion, ulceration or necrosis, and the depth and surface extension of the lesions.

**Results**

**In vitro proliferative responses**

It has been shown previously that, in the absence of Ii, total lymph node cells (LNCs) responded poorly to immunization with exogenous proteins (19, 22, 28–30). Briefly, control (C57BL/6) and Ii–/– mice were immunized with KLH, and their total LNCs were analyzed for their recall response. As expected, LNCs from mice lacking Ii proliferated poorly compared with control cells after immunization (Fig. 1A). The low proliferative response could be explained by inefficient selection of CD4+ T cells in the absence of Ii, which could result in a lower precursor naive T cell frequency for a given Ag (28) or in T cells with TCRs of lower avidity. Alternatively, the weak response could reflect the low level of the total CD4+ T cell population, which is otherwise normal in the periphery of Ii–/– mice, or the poor Ag presentation by the Ii–/– APCs.

To determine whether CD4+ T cells from Ii–/– mice are intrinsically defective, we analyzed the in vitro responsiveness of these cells. Ii–/– and control-enriched splenic CD4+ T cells were stimulated by the mitogen Con A (Fig. 1B) or anti-CD3 mAbs (Fig. 1C), and their proliferation was measured. As was previously shown (28), both populations proliferated almost the same under these conditions. These results showed that the Ii–/– CD4+ T cells were competent to transduce a mitogenic signal and could respond to this signal at a level comparable to the wild-type cells.

To analyze the responsiveness of Ii–/– CD4+ T cells to antigenic protein, we injected control (C57BL/6) and Ii–/– mice with KLH/CFA. Nine days later, CD4+ T cells were purified from draining lymph nodes and analyzed for their recall response to KLH. Equal numbers of control and Ii–/– CD4+ T cells were incubated in the presence of wild-type splenic APCs in the presence of different concentrations of KLH. As shown in Fig. 2A, control and Ii–/– CD4+ T cells had a similar proliferative response. Furthermore, no difference in proliferation was detected when these CD4+ T cells were titrated in the presence of control APCs and 10 μg/ml of KLH (Fig. 2B). These results implied that the low numbers of CD4+ T cells from the Ii–/– mice were selected efficiently. These Ii–/– CD4+ T cells were qualitatively similar and showed a response to Ag similar to the control T cells. Therefore, the low proliferative responsiveness of total LNCs is probably due to the low numbers of the CD4+ T cells in the periphery of the Ii–/– mice.

**Cytokine secretion by CD4+ T cells from Ii–/– mice**

Surprisingly, although T cell proliferation was indistinguishable between the control and Ii–/– mice, analysis of cytokines secreted by the KLH/CFA-primed CD4+ T cell populations in the recall response to KLH revealed significant differences. Whereas wild-type CD4+ T cells secreted a mixture of IFN-γ and IL-4, Ii–/– CD4+ T cells did not produce any detectable IL-4 (Fig. 3A) and instead secreted higher levels of IFN-γ than those of the wild-type T cells (Fig. 3B). These results suggested that, although the CD4+ T cells from both mice had the same ability to respond to Ag by...
proliferating, they were skewed to differentiate into different effector populations. Specifically, the results suggested that when a mixed population of Th1 and Th2 effector cells had been elicited in the wild-type mice, in the absence of Ii, most of the cells were Th1. To determine whether CD4$^+$ T cells from the Ii$^{-/-}$ mice had the intrinsic potential to become either Th1 or Th2 cells, we isolated and stimulated total CD4$^+$ T cells from wild-type or Ii$^{-/-}$ mice to differentiate with Con A with or without IL-4, a Th2-inducing cytokine, or IL-12, a Th1-inducing cytokine. After 4 days, the cells were washed and restimulated with Con A for 24 h. The supernatant was then analyzed for cytokine production. CD4$^+$ T cells from both groups secreted IL-4 and IFN-γ (Fig. 3D), therefore implying that CD4$^+$ T cells from Ii$^{-/-}$ had the same potential to become effector CD4$^+$ T cells from the wild-type mice and that the skewing in the T cell effector population is probably due to defective peripheral Ag presentation or a different cytokine profile in the peripheral lymph nodes.

**Normal in vivo Th1 responses in Ii$^{-/-}$ mice**

To determine whether the skewing to the Th1 population occurs in vivo, we monitored the Th1 and Th2 responses in Ii$^{-/-}$ mice. For Th1, we used the DTH and TNBS colitis models. DTH is a form of

![Figure 1](http://www.jimmunol.org/DownloadedFrom/)

**FIGURE 1.** *A*, Ii$^{-/-}$ CD4$^+$ T cells respond to mitogenic signal. In vitro proliferative recall response of C57BL/6 (B6) and Ii$^{-/-}$ to KLH. Mice were immunized with 100 mg of KLH in 1:1 emulsion with CFA in the footpads. Nine days later, lymph nodes were removed and total cell suspension was prepared. Proliferation response to KLH was measured by addition of 1 μCi of $[^3H]$thymidine for the last 18 h of a 4-day culture. One experiment representative of five was depicted ($B$ and $C$). CD4$^+$ T cells purified from C57BL/6 (B6) and Ii$^{-/-}$ mice were cultured in the presence of elevated concentrations of Con A ($B$) or anti-CD3 ($C$) in the presence of wild-type APCs. Proliferation was determined by addition of 1 μCi of $[^3H]$thymidine for the last 18 h of a 4-day culture. One experiment representative of three was depicted.

![Figure 2](http://www.jimmunol.org/DownloadedFrom/)

**FIGURE 2.** Ii$^{-/-}$ CD4$^+$ T cells respond to antigenic stimulation in a recall response. C57BL/6 (B6) and Ii$^{-/-}$ mice ($A$) or C57BL/6, Ii$^{-/-}$, and p41 mice ($B$) were immunized with 100 μg of KLH in 1:1 emulsion with CFA in the footpads. Nine days later, lymph nodes were removed and cell suspension was prepared. CD4$^+$ T cells were purified, and the cells were incubated in the presence of wild-type APCs with elevated concentrations of KLH ($A$) or titrated in the presence of 10 μg/ml of soluble KLH. $B$, Proliferation was determined by addition of 1 μCi of $[^3H]$thymidine for the last 18 h of a 4-day culture. One experiment representative of four was depicted.
cell-mediated immunity elicited by Ag in the skin, which is mediated by the inflammatory CD4+ (Th1) cell. The in vivo DTH responsiveness of Ii−/− and wild-type mice was analyzed by immunizing both groups with 100 μg of KLH in CFA in the dorsal flanks. As shown in Fig. 4, 5 days later soluble KLH (10 μg) was injected in the right ear and PBS in the left ear. Ear swelling was measured 24 h later. The magnitude of the DTH response in the Ii−/− mice by the fifth day after injection almost reached wild-type levels (p value of KLH vs PBS of control or Ii−/− < 0.01). The fact that the DTH response reached almost normal levels suggested that the in vivo CD4+ Th1 cell response is only marginally affected in the Ii−/− mice. To further evaluate the in vivo Th1 response in Ii−/− mice, we used the TNBS colitis model. Inflammatory bowel diseases are a group of diseases characterized by chronic destruction of the colon (in ulcerative colitis) or the small and large bowel (in Crohn’s disease), due to the transmural infiltration of the bowel wall by a mixed inflammatory infiltrate. This model is particularly useful in elucidating the primary role of the CD4+ Th1, because Th1 response induces fatal, acute, transmural, and focal types of lesions in the early stage of the disease, whereas Th2 response plays a role only in the later stages of the disease (31). We have studied the early stage of the disease by intrarectal installation of TNBS to control and Ii−/− mice. The mice were killed 3–5 days after TNBS administration, and macroscopic and microscopic evaluations were performed. The specimens were given a macroscopic score from 0 to 13 based on the Wallace criteria and a microscopic score based on the Ameho criteria. All groups (wild-type and Ii−/− mice) exhibited a transmural inflammatory infiltrate of neutrophils, macrophages, and lymphocytes (Fig. 5A). There were no significant differences in the Ameho or Wallace scores (Fig. 5B). These results further proved the normal in vivo Th1 response of Ii−/− mice.

Abnormal in vivo Th2 response in Ii−/− mice
The in vivo Th2 responsiveness of Ii−/− mice was evaluated by analyzing their response to an OVA challenge in an asthma model.
Asthma is a chronic inflammatory disorder of the airways characterized by intermittent episodes of dyspnea due to airway obstruction. The inflammatory response in the asthmatic lung is characterized by infiltration of the airway wall with lymphocytes and eosinophils. Recent advances suggest that the T lymphocytes, and in particular CD4+ T cells, producing the Th2 pattern of cytokines have a major effect in the pathogenesis of this disease (32–35). Control and Ii−/− were immunized with 10 μg of OVA (days 0, 7, and 14) and then were given OVA inhalations for five consecutive days (days 15–19). Inflammatory cell recruitment in the BAL and histologic lung sections of the control and Ii−/− mice was compared. As shown in Fig. 6A, the inhalation of aerosolized OVA caused a massive infiltration of eosinophils into the BAL of control mice, whereas the same challenge caused no inflammatory response in the Ii−/− mice (82.3% control vs 1.76% Ii−/−; *p = 1.7 × 10−10).

Histopathologic examination of the lung tissue from OVA-challenged control mice revealed a pleomorphic peribronchial and perivascular infiltrate consisting of eosinophils, macrophages, lymphocytes, and neutrophils. However, examination of the lung tissue from OVA-challenged Ii−/− mice revealed normal lung tissue with no such infiltrate (Fig. 6B). Microscopic slides were examined randomly by a pathologist, and an inflammatory score from 0 to 4 was given (0, no inflammatory infiltrate; 1, slight accumulation of scattered inflammatory cells; 2, mild homogeneous infiltrate; 3, moderate homogeneous infiltrate; 4, severe inflammation). The inflammatory infiltrate in the C57BL/6 group was significantly high; however, almost no inflammatory response was detected in the Ii−/− group (control 3.33 vs Ii−/− 0.25; *p = 9.6 × 10−5) (Fig. 6C). To determine whether the reduced inflammatory response was due to ineffective priming of the T cells, the OVA doses injected into the mice were increased (Fig. 6D). A 10-fold increase in the OVA dosage injected caused no peribronchial or perivascular inflammation in Ii-deficient mice (55.8% control vs 1.85% Ii−/−), suggesting a severely defective in vivo CD4+ Th2 response in Ii−/− mice.

Besides the II role in Ag presentation, we have previously demonstrated that B cells derived from II-deficient mice are arrested at the immature stage (36, 37). To determine whether the B cell population affects the skewing to the Th1 population in the II−/− mice, we have analyzed the inflammatory response to OVA in the II−/−, which have been reconstituted with transgenically expressed low levels of II p41 isoform (p41 mice) (22). The low levels of II expressed in these mice are sufficient to fully reconstitute the T cell repertoire, which exhibits a normal recall proliferative response (22). CD4+ T cells derived from these mice proliferate similarly to control and II−/− cells (Fig. 2B) and secrete IL-4 in the recall response to KLH (Fig. 4C); however, these low levels cannot mediate the full B cell differentiation in the spleen, and the B cells are arrested at the immature stage (36). As shown in Fig. 6, C and D, the inhalation of aerosolized OVA caused a massive infiltration of eosinophils into the BAL of the p41 transgenic mice. Histopathologic examination of the lung tissue from OVA-challenged p41 mice revealed a pleomorphic peribronchial and perivascular infiltrate consisting of eosinophils, macrophages, lymphocytes, and neutrophils (Fig. 6B). These results suggest that the Ag presentation, and not the B cell developmental stage, controls the skewing to the T cell effector population.

**Discussion**

II-deficient mice have a decreased number of thymic and peripheral CD4+CD8− T cells (19–21). The low levels of peripheral CD4+ T cells express TCR at a reduced level, displaying high levels of CD44 and CD69 and lower levels of CD45RB (22, 23, 28). It was previously suggested that the abnormal expression of surface molecules could be explained as a consequence of differences in the selection of the CD4+ T cells by the epithelial cells in the thymus (28). Alternatively, the high percentage of phenotypically activated or memory CD4+ T cells could reflect the low levels of CD4+ T cells in the periphery, which have had a higher probability to respond to the normal antigenic load and therefore have a higher frequency of activated cells. As presented in this study, our results demonstrate that purified CD4+ T cells from II−/− mice are able to respond to mitogenic stimuli in vitro as well as CD4+ T cells from control mice. Moreover, equal numbers of CD4+ T cells from C57BL/6 or II−/− mice proliferate similarly in a recall response to antigenic protein after immunization.

Notably, we have found that the main difference between the wild-type and II−/− CD4+ T cells is in their cytokine secretion. Whereas wild-type cells secrete IFN-γ and IL-4, CD4+ T cells from II−/− mice secrete mostly IFN-γ and only low doses of IL-4. This result could be explained as follows. First, these T cells were selected differently in the thymus; Ag presentation in the thymus is probably affected by the lack of II, and therefore these cells might be selected with TCRs that intrinsically caused a propensity to yield Th1 effector cells. For example, if only high-affinity T cells were selected as a result of limiting MHC-peptide in the thymus, such cells may yield Th1 cells upon activation in the periphery. Alternatively, the naive T cell repertoire might be normal, but the priming environment of the immune response might be different in the two strains and the T cells could have been affected by the dose of the Ag, the type of APC, the costimulatory pathways (38–42), and the cytokine environment (43–45), all of which have been...
postulated to be some of the polarizing factors. Our findings support the theory that Ii−/− T cells are selected qualitatively, similarly to wild-type cells. We have shown that CD4+ T cells from both Ii−/− and wild-type mice can produce IL-4 or IFN-γ in the presence of polarizing cytokines. Therefore, we suggest that the T cells have no intrinsic difference in their ability to become either Th1 or Th2 and that they were probably not selected differently. We favor the hypothesis that the lymphoid microenvironment or the way the Ag is presented during the immune response might influence the differentiation of the cells into a given effector T cell population. Bretscher et al. (38) and Hosken et al. (40) previously proposed that low to moderate doses of Ag presented to CD4 naive T cells would switch their development into a Th1 effector response, whereas very high or very low doses of Ag presented would switch their response into a Th2 effector response. The absence of Ii alters the nature of peptide binding to class II molecules. Specifically, the few class II complexes that escape the ER have an aberrant conformation, forming no compact dimers and instead floppy dimers are observed (19–21). The compact conformer seen in the wild-type mice probably bears a tightly bound peptide, but the floppy form found in the Ii−/− mice is likely to be empty or to carry a low-affinity peptide in a loosely bound state. Most proteins are probably presented less efficiently or are not presented at all in the Ii−/− mice. Defective Ag presentation in Ii−/− mice might lead selectively to Th1 effector CD4+ T cells, possibly as a result of the delivery of a low antigenic signal.

The fact that Ii−/− mice have an almost normal DTH response 5 days after injection supports the finding that these mice have a higher percentage of Th1 cells. These Th1 cells, which are the main effector CD4+ T cell population that can be found in the Ii−/− mice, respond effectively to antigenic stimulation. Our results in the TNBS colitis model, exhibiting a known Th1-dependent inflammatory response (31), show a similar inflammatory colitis reaction in both the wild-type and Ii−/−. Thus, these results strengthen our conclusion that, although Ii−/− mice have a low CD4+ T cell population, their Th1 response is normal. Ii−/− mice exhibit a complete loss of OVA-challenged response in the asthma model. These results could be explained by the lack of IL-4 secretion in the in vitro studies, indicating that Ag presentation might be a crucial factor in the skewing to one effector cell population. Alternatively, the lack of a Th2 response might be attributed to the lack of a mature B cell population in the Ii-deficient mice. Previously we have shown that B cells do not mature normally in mice lacking Ii. These mice accumulate IgMhigh, CD23low, heat-stable Aghigh, and IgDlow immature B cells in their periphery, which mediates a low T-independent Ab response (36, 37). Thus, B cells might play an important role in this model. The role of B cells in T cell priming is still controversial. Several studies have revealed a critical role for B cells in the T cell response in vivo (46–48). These results were challenged by reports showing that the absence of B cells had little impact on CD4+ T cell proliferation and cytokine production in response to protein Ags (49–
51). More recently, a few studies have suggested that B cells are not required for T cell sensitization but play a role in the induction of IL-4 gene expression (52–54). In this study, we show that transgenic mice expressing the li p41 isoform exclusively on the li−/− background (22), which have a normal T cell population but a defect in their B cell differentiation, could develop a normal T cell activation and eosinophils infiltration in response to OVA stimulation. In addition, it was previously reported that B cell-deficient mice (μM−/−) exhibited a normal T cell activation and eosinophils infiltration in the peribronchial regions of the airways, with signs of eosinophil activation and degranulation (55). Therefore, we conclude that defective Ag presentation in mice lacking li might lead selectively to Th1 effector CD4+ T cells, possibly as a result of the delivery of low antigenic signal. Previously, Brown et al. (56) studied the T helper differentiation in mice lacking li. They analyzed the response of C57BL/6 and BALB/c li−/− mice to Leishmania major and followed their CD4+ T cell differentiation. Control of the disease is dependent on class II-restricted Th1 cells and their production of IFN-γ, which is required to activate macrophages to restrain intracellular replication of the organism. In contrast to most strains of mice, BALB/c animals are unable to contain L. major due to the development of an aberrant Th2 response during infection (57). In their studies, it was shown that C57BL/6 li-deficient mice controlled L. major infection similarly to wild-type mice and had a normal Th1 subset. In addition, BALB/c li−/− mice exhibited a progressive course of infection and Th2 effector cell development that were comparable to that seen in the wild-type mice. Therefore, their conclusion was that li-deficient mice develop both Th1 and Th2 responses. The ability of li−/− mice to mount a Th2 response contradicts the results presented in this study, which could be addressed by several explanations. First, the ability of li-deficient mice to develop the various effector populations was analyzed in two strains of mice: C57BL/6 and BALB/c. It was previously concluded that MHC genotypes predetermine Th phenotype based upon the selection of TCR affinity (58). Therefore, these two strains respond differently to Ag stimulation and their outcome populations vary. Our studies focused only on the C57BL/6 background, and the differences observed are correlated to the inability of this strain to respond equally to various treatments. It will be interesting in the future to follow the skewing of the effector populations in the BALB/c strain with these different treatments. Furthermore, L. major is an obligate intracellular parasite that infests the phagocyte system. The parasite replicates productively only in the host’s macrophages within an endolysosomal compartment that contains MHC class II molecules. Whereas we used an exogenous Ag in a measured dose, Brown et al. (56) used a replicating parasite in a susceptible genus (BALB/c). Therefore, continuous parasite replication exposes the mice to very high doses of Ag, which might enable sufficient Ag to be presented and to overcome the block on Th2 differentiation.

In conclusion, our results suggest that the low levels of CD4+ T cells in the periphery of the li−/− mice are selected appropriately in the thymus and respond equally to the Ag in the periphery when compared with wild-type mice. However, the immune response of these mice is skewed to a Th1 response in vitro and in vivo, probably due to aberrant Ag presentation in the li−/− mice.

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References


