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Leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1) deficient mice have an altered immune cell phenotype

Xiaobin Tang[*,§], **Linjie Tian**[*], **Gloria Esteso**[*], **Seung-Chul Choi**[*], **Alexander D. Barrow**[†,¶], **Marco Colonna**[†], **Francisco Borrego**[‡], and **John E. Coligan**[*,‖]

[*]Receptor Cell Biology Section, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD 20852

[†]Department of Pathology and Immunology, Washington University School of Medicine, Campus Box 8118, 660 South Euclid Avenue. St. Louis, MO 63110, USA

[‡]Laboratory of Molecular and Developmental Immunology, Division of Monoclonal Antibodies, CDER, FDA

Abstract

Cross-linking of the collagen binding receptor LAIR-1 *in vitro* delivers an inhibitory signal that is able to down-regulate activation-mediated signals. To study the *in vivo* function of LAIR-1, we generated LAIR-$1^{-/-}$ mice. They are healthy and fertile, and have normal longevity; however, they show certain phenotypic characteristics distinct from wild-type mice, including increased numbers of splenic B, T regulatory and dendritic cells. As LAIR-$1^{-/-}$ mice age, the splenic T cell population shows a higher frequency of activated and memory T cells. Since LAIR-$1^{+/+}$ and LAIR-$1^{-/-}$ T cells traffic with equal proficiency to peripheral lymphoid organs, this is not likely due to abnormal T lymphocyte trafficking. LAIR-$1^{-/-}$ mice have lower serum levels of IgG1 and, in response to T-dependent immunization with TNP-OVA, switch less efficiently to antigen specific IgG2a and IgG2b, while switching to IgG1 is not affected. Several mouse disease models, including EAE and colitis, were utilized to examine the effect of LAIR-1 deficiency and no differences in the responses of LAIR-$1^{-/-}$ and LAIR-$1^{+/+}$ mice were observed. Taken together, these observations indicate that LAIR-1 plays a role in regulating immune cells and suggest that any adverse effects of its absence may be balanced *in vivo* by other inhibitory receptors.

Introduction

Immune responses are tightly controlled by the opposing actions of activating and inhibitory signals. Activation receptors on immune cells recognize “stressed” cells, including transformed and pathogen-infected, and induce a hyper-inflammatory state to combat the danger to the host; inhibitory receptors are expressed to dampen the immune response to prevent unwarranted or excessive inflammation (1). The impairment of inhibitory signals, e.g. the absence of inhibitory immune receptors or the down-regulation of ligands for these receptors, can lead to a state of hyper-responsiveness that facilitates the development of

[‖]Address correspondence and reprint requests to John E. Coligan, 12441 Parklawn Drive, Twinbrook II, Room 205, Rockville, MD 20852, Phone: 1-301-496-8247, Fax: 1-301-480-2818, jcoligan@niaid.nih.gov.
[§]Current address: Division of Immunology and Rheumatology, Department of Medicine, Stanford University School of Medicine.
[¶]Current address: Department of Biochemistry, University of Cambridge, Downing Site, Cambridge CB2 1QW, United Kingdom.

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autoimmune diseases. For example, CD200$^{-/-}$ mice develop myeloid cell dysregulation and enhanced susceptibility to autoimmune inflammation, such as experimental autoimmune encephalitis (EAE) and collagen-induced arthritis (CIA) (2), PD-1$^{-/-}$ (Programmed Death 1) mice develop glomerulonephritis and arthritis (3), BTLA$^{-/-}$ mice develop autoimmune hepatitis-like disease and produce auto-antibodies to nuclear antigen (4), and FcγRIIb$^{-/-}$ mice develop a lupus-like syndrome with fatal glomerulonephritis (5).

The mouse leukocyte-associated immunoglobulin-like receptor (LAIR)-1 or CD305 localizes to the Leukocyte Receptor Complex (LRC) at the proximal end of mouse chromosome 7. The human LRC region is syntenic with the mouse, but encodes more genes, including LAIR-2, a secreted protein highly homologous to LAIR-1 (6). LAIR-1 possesses one immunoglobulin domain in its extracellular region and two ITIM motifs in the cytoplasmic tail that mediate its inhibitory capacity through interaction with Src homology 2 domain-containing protein tyrosine phosphatase (SHP)-1, SHP-2 and C-terminal Src kinase (7, 8). LAIR-1 is reported to be expressed on the majority of cells of the immune system, including T cells, NK cells, monocytes, dendritic cells and on human B cells, but not on B cells from mice (9–12). *In vitro* experiments show that ligation of LAIR-1 with mAb or collagens inhibits the cytotoxic activity of NK and CD8 T cells, BCR induced B cell activation and proliferation, and CD3 signaling and cytokine production by T cells (9–11, 13–16).

Collagens, the most abundant proteins in the body, have been identified as high affinity functional ligands for LAIR-1 and LAIR-2 (14, 17). LAIR-1 interacts with the glycine-proline-hydroxyproline (GPO) repeats that are present in all collagens and a synthetic trimeric peptide of 10 GPO repeats alone can inhibit immune cell activation *in vitro* (14, 18). Twenty-eight different types of collagens have been identified in vertebrates, plus there are more than 20 other proteins that contain collagenous domains (19). The collagen-rich extracellular matrix is important for maintenance of tissue structures, cell adhesion and migration during growth, differentiation, morphogenesis and wound healing (20). Several collagen receptors have been shown to have important biological functions. Examples are the discoidin domain receptor 1 (DDR1) that promotes leukocyte migration and facilitates the differentiation/maturation and cytokine/chemokine production by macrophages and dendritic cells (DC) (21, 22), GP-VI that plays a central role in the hemostatic plug formation at sites of vascular injury (23, 24) and VLA-1 that plays a role in regulating inflammation during rheumatoid arthritis and DTH responses (25). VLA-1 also potentiates CD8 T cell mediated immune protection against influenza infection (26). Considering the abundance and biological importance of collagens, and the broad expression of LAIR-1 on immune cells, it is reasonable to suspect that LAIR-1 plays a role in regulating the responses of immune cells in both normal and pathological situations.

Although the inhibitory potential of LAIR-1 is well known, the actual *in vivo* function is unknown. To explore the *in vivo* role of this receptor, we generated LAIR-1 deficient mice. These animals show some phenotypic characteristics distinct from wild type (wt) mice, but are healthy and show normal longevity, and they develop similar pathology states as wt mice in induced autoimmune diseases.

Materials and Methods

Mice

LAIR-1$^{-/-}$ and LAIR-1$^{fl/fl}$ mice were generated by OZgene (Australia) as shown in Supplemental Figure 1. Briefly, exon 4 to 8 of LAIR-1 encoding the DNA-binding domain was flanked by loxP sites. A PGK-neo cassette flanked by Flp recombinase target sites was used for selection. Following homologous recombination of the vector in embryonic stem

cells, clones bearing the LAIR-$1^{fl/fl}$ locus were established after deletion of PGK-neo selection cassette by Cre recombination, and clones with the LAIR-$1^{-/-}$ locus were generated after deletion of the LoxP sites flanking regions (exon 4–8) together with the PGK-neo cassette using Cre recombinase. Identified targeted ES clones were microinjected into the blastocysts of C57BL/6 mice. Chimeric mice were mated with C57BL/6 female mice to produce heterozygous mice and their wt littermates, and LAIR-$1^{fl/fl}$ mice were bred to CD4 cre mice to get T-cell specific LAIR-1 deficient mice. C57BL/6 and Rag$1^{-/-}$ mice were obtained from Jackson Laboratories and OT-II, CD4 cre transgenic mice were from Taconic. FcγRIIB$^{-/-}$ mice were a kind gift of Dr. Silvia Bolland (NIH, NIAID). All experiments were performed with littermate mice: LAIR-$1^{-/-}$ with LAIR-$1^{+/-}$ or LAIR-$1^{+/+}$, and CD4 cre LAIR-$1^{fl/fl}$ with LAIR-$1^{fl/fl}$. LAIR-$1^{+/-}$ and LAIR-$1^{+/+}$ were shown to express equivalent levels of LAIR-1. All mice were maintained on a C57BL/6 genetic background and housed in a pathogen-free environment in the NIAID animal facility. All experimental protocols were approved by NIAID Animal Care and Use Committee (ACUC).

Antibodies

Labeled antibodies to CD3 (145-2C11), CD4 (RM4-5), CD8 (53-6.7), CD11b (M1/70), CD11c (N418), CD23 (B3B4), CD25 (PC61.5), CD40 (1C10), CD44 (IM7), CD62L (MEL-14), CD69 (H1.2F3), CD80 (16-10A1), CD86 (GL1), B220 (RA3-6B2), C-kit (ACK2), F4/80 (BM8), Gr-1 (RB6-8C5), I-A^b (AF6-120.1), IFNγ (XMG1.2), NK1.1 (PK136), DX5 (DX5), Sca-1 (D7) and LAIR-1 (113), along with specific isotype-matched control antibodies were from eBioscience. Labeled anti-CD21 (7G6) and anti-IgM (R6-60.2) were from BD PharMingen. Labeled anti-120G8.04 (120G8.04) from Imgenex Corp was used for detection of pDC.

Flow cytometry

Specific cell-types were identified by flow cytometry according to the expression of surface molecules as follows: CD4 T (CD4$^+$ CD8$^-$ CD3$^+$), CD4 naive T (CD4$^+$ CD8$^-$ CD44lo CD62Lhi), CD4 memory T (CD4$^+$ CD8$^-$ CD44hi), CD4 effector memory T (CD4 T_{EM}, CD4$^+$ CD8$^-$ CD44hi CD62Llo), CD4 central memory T (CD4 T_{CM}, CD4$^+$ CD8$^-$ CD44hi CD62Lhi), CD8 T (CD8$^+$ CD4$^-$ CD3$^+$), CD8 naïve T (CD8$^+$ CD4$^-$ CD44lo CD62Lhi), CD8 memory T (CD8$^+$ CD4$^-$ CD44hi), CD8 effector memory T (CD8 T_{EM}, CD8$^+$ CD4$^-$ CD44hi CD62Llo) and CD8 central memory T (CD8 T_{CM}, CD8$^+$ CD4$^-$ CD44hi CD62Lhi), NK (NK1.1$^+$ CD3$^-$ or DX5$^+$ CD3$^-$), NKT (NK1.1$^+$ CD3$^+$), marginal zone B (MZ B, B220$^+$ CD21hi CD23lo), transitional 1 (T1)/B-1 B (T1/B1, B220$^+$ CD21$^-$ CD23$^-$), follicular B (FO, B220$^+$ CD21lo CD23hi), granulocytes (Gr-1$^+$), macrophages (F4/80$^+$), conventional dendritic cells (cDC, CD11c$^+$ B220$^-$), plasmacytoid dendritic cells (pDC, CD11clo 120G8$^+$), bone marrow pro-B/pre-B (B220$^+$ IgM$^-$), immature B (B220lo IgM$^+$), mature B (B220hi IgM$^+$), hematopoietic stem (HSC, Lin$^-$ c-Kit$^+$ Sca-1$^+$) and committed progenitor (CPC, Lin$^-$ c-Kit$^+$ Sca-1$^-$) cells. To determine cytokine production, stimulated cells were incubated in the presence of GolgiStop (BD Biosciences) for the last 4–6 h. Then, cell surface receptor expression and intracellular cytokine staining were determined with BD Cytofix/Cytoperm Plus kits (BD Biosciences). Data were acquired on a FACSCalibur (BD Biosciences) and were analyzed with FlowJo software (Tree Star, Inc.).

Competitive homing analyses

Competitive homing assays were performed with isolated LAIR-$1^{+/+}$ and LAIR-$1^{-/-}$ T cells (Miltenyi Biotec) and differentially labeled with 5-(and 6-) carboxyfluorescein, succinimidyl ester (FAM-SE; Molecular Probes) or tetramethylrhodamine isothiocyanate (TRITC; Molecular Probes), as previously described (27). Mice were sacrificed 2.5 and 19 h after intravenous injection of cells, and single-cell suspensions from inguinal and mesenteric

lymph nodes, spleen, and blood were isolated and analyzed by flow cytometry. To avoid possible bias related to the dyes toxicity, LAIR-$1^{+/+}$ and LAIR-$1^{-/-}$ cells were labeled with the opposite dyes in different experiments. The homing ratio of LAIR-$1^{-/-}$ versus LAIR-$1^{+/+}$ T cells was calculated as follows: {(LAIR-$1^{-/-}$ T cells-FAM-SE)/(LAIR-$1^{+/+}$ T cells-TRITC) + (LAIR-$1^{-/-}$ T cells-TRITC)/(LAIR-$1^{+/+}$ T cells-FAM-SE)}/2.

Experimental autoimmune diseases

Experimental colitis was induced by naïve CD4 T cells transfer (28). Briefly, FACS sorted 4×10^5 naive CD4 T cells ($CD4^+$ $CD8^-$ CD44lo CD62Lhi) were injected intraperitoneally into Rag$1^{-/-}$ mice. Mice were weighed weekly and monitored for signs of disease. For EAE induction (29), mice were immunized subcutaneously with 200μg MOG 35–55 peptide (Anaspec, Inc) emulsified in 200μl of CFA (DIFCO Laboratories) containing killed Mycobacterium (DIFCO Laboratories) plus 200ng pertussis toxin (List Biological Laboratories) by intraperitoneal injection on days 0 and 2, and EAE disease scoring was determined daily (29). Mononuclear cells from spinal cords were isolated as described previously (30). IFNγ and IL-17 production by mononuclear cells isolated from spinal cords was determined on day 28, after stimulation with PMA and ionomycin for 4h.

T-dependent and -independent immunizations

To elicit a T-dependent immune response, CD4cre LAIR-$1^{fl/fl}$ and LAIR-$1^{fl/fl}$ mice were immunized with 100μg of TNP-OVA in alum, and afterwards were re-challenged with 100ug TNP-OVA on day 28. To elicit a T-independent immune response, LAIR-$1^{-/-}$ and LAIR-$1^{+/-}$ mice were immunized with 50ug TNP-LPS (TI-I, T-independent type I) or 25ug TNP-ficoll (TI-II, T-independent type II).

ELISA analysis for immunizations

Mouse Ig isotype-specific ELISA was carried out by using isotype-specific goat anti-mouse Ig (Southern Biotech) to coat 96 well plates. Serially diluted sera from mice were added to the coated plates and bound immunoglobulins were detected by alkaline phosphatase-conjugated detection antibodies to specific mouse isotypes (Southern Biotech). For T-dependent immunization, sera were collected 2 weeks after the primary immunization or 1 week after re-challenge on day 28. The serum levels of TNP-specific antibodies for different Ig subclasses and isotypes were determined using ELISA plates coated with TNP-BSA (Biosearch Technologies Inc). T-independent immunization Ig isotype antibodies in serum were determined 7 days after immunization by TNP specific ELISA. Anti-nuclear antibodies (ANA) in the serum of mice at 13 months of age were measured with an ANA ELISA screen kit (Diamedix).

Generation of bone marrow derived dendritic cells (BMDC)

Dendritic cells were generated from bone marrow cells according to Inaba *et al.* with slight modification (31). In brief, HSC were purified from bone marrow cells obtained from femurs and tibias of LAIR-$1^{+/+}$and LAIR-$1^{-/-}$ mice by c-kit^+ isolation kits (Miltenyi Biotech). Cells (5×10^5 cell/well) were incubated in RPMI 1640 complete medium supplemented with 10% FBS, 10 ng/ml recombinant murine GM-CSF and IL-4 (R&D systems) in 24-well plates. Media containing rmGM-CSF and rmIL-4 was renewed every 2 days. On day 6, cells were harvested by gentle swirling, and stimulated with LPS (1 μg/ml), lipoteichoic acid (LTA) (25 μg/ml) or poly I:C (25 μg/ml) for 24 h (Sigma).

pDC *in vitro* functional assay

pDCs were isolated from splenocytes by MACS kit (Miltenyi Biotec); purity was at least 90%. 4×10^4 purified pDCs were cultured in 100 μl RPMI 1640 supplemented with 10% FBS

(Atlanta Biologicals), penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), HEPES (10 mM) and sodium pyruvate (1 mM) (Mediatech, Inc). 10 μg/ml CpG ODN 1585 (InvivoGen) was added to the cultures for stimulation. Supernatants were collected after 24 h for IFNα analyses by ELISA according to the manufacturer's instructions (eBioscience).

Analysis of bone

For the μCT analysis of trabecular bone, the trabecular volume in the distal femoral metaphysis from 8 week old wild-type and LAIR-$1^{-/-}$ male mice was measured using a Scanco μCT40 scanner (Scanco Medical AG). A threshold of 200 was used for the evaluation of scans.

Results

Expression of LAIR-1 on mouse immune cells

Mouse LAIR-1 was reported to be expressed on most immune cells, except B cells(9). We found that LAIR-1 is constitutively expressed on all immune cell types examined, including a population of B cells (Figure 1A and 1B). The LAIR-1 expression profile of cells isolated from lymph nodes is similar to that of splenocytes (data not shown). We then checked the expression of LAIR-1 on splenic T cell subsets (Figure 1C) and found that LAIR-1 is constitutively expressed on $CD4^+$ $CD25^+$ T cells, and both naive and memory CD4 and CD8 T cells. LAIR-1 is expressed at similar levels on naive and memory CD4 T cells, but is expressed at higher levels on CD8 memory cells than on naïve CD8 T cells. Examination of splenic B cell subsets revealed that LAIR-1 is mainly expressed on marginal zone B, but not on T1/B1 ($CD21^-$ $CD23^-$) and FO B (CD21lo CD23hi) cells (Figure 1D).

Phenotypic studies of LAIR-$1^{-/-}$ mice

LAIR-$1^{-/-}$ mice were born at the expected Mendelian ratios, developed normally and appeared healthy in a pathogen free housing environment. To determine if the absence of LAIR-1 affected lymphoid cell development and homeostasis, we performed flow cytometry analysis of various cell types. Thymi of young (2–4 months old) LAIR-$1^{-/-}$ mice did not show any gross defect in T cell development, having normal levels of $CD4^+/CD8^+$ (DP), $CD4^-/CD8^-$ (DN), and $CD4^+$ or $CD8^+$ cells (Figure 2A). CD69 expression on LAIR-$1^{-/-}$ DP cells is normal and development of LAIR-$1^{-/-}$ DN thymocytes does not show any sign of abnormality (data not shown). These results indicate that LAIR-$1^{-/-}$ mice have normal T lymphopoiesis. Likewise, LAIR-$1^{-/-}$ mice showed normal B cell development in the bone marrow. The pro-B/pre-B, immature and mature B cell populations in the bone marrow of LAIR-$1^{-/-}$ mice are comparable to those found in LAIR-$1^{+/+}$ mice (Figure 2B). There are also no defects in the HSC and CPC compartments in LAIR-$1^{-/-}$ mice (Figure 2B).

We also checked splenic (Figure 2C, Table 1) and lymph node cell subsets (Figure 2D) in young mice. In the spleen, the frequency of LAIR-$1^{-/-}$ CD3 T cells is slightly, but significantly, lower than those of LAIR-$1^{+/+}$ mice (~12% less), which is reflected in both the CD4 (~10% less) and CD8 T (~13% less) cells; on the other hand, the frequency of $CD4^+CD25^+$ T cells, the majority of which are regulatory T (Treg) cells, is increased about 23% in LAIR-$1^{-/-}$ mice. Other more defined T cell populations showed no differences in younger mice.

The percentage of splenic LAIR-$1^{-/-}$ B cells is increased by around 10% compared to B cells from LAIR-$1^{+/+}$ mice. FO B cells are re-circulating cells that home mainly to B cell follicles in secondary lymphoid organs including spleen and lymph nodes, and play an important role in T cell-dependent responses in follicles and T cell-independent responses in the bone marrow (32); and MZ B cells are non-circulating mature B cells enriched primarily

in the marginal zone of the spleen, which function in both T cell-dependent and -independent responses to blood-borne pathogens (33). Most of the B cell population increase in LAIR-1$^{-/-}$ mice is due to MZ B cells that are elevated about 23% (from 8.1% in LAIR-1$^{+/+}$ to 10.0% in LAIR-1$^{-/-}$), as the frequency of FO and T1/B-1 B cells are similar in LAIR-1 $^{-/-}$ and LAIR-1$^{+/+}$ mice (Figure 2C, Table 1).

LAIR-1$^{-/-}$ mice have an increased frequency of splenic cDC (about 22% more) (Figure 2C) and BM cDC (about 13% more) (Supplemental Figure 2A), whereas the frequency of lymph node cDCs are not statistically different compared to LAIR-1$^{+/+}$ (Figure 2D). Human pDCs are reported to express high levels of LAIR-1 (34). We confirm that mouse pDCs (CD11clo 120G8^{+}) also express very high levels of LAIR-1 (data not shown). However, when we analyzed the pDC populations in spleen, lymph node and bone marrow, we found that LAIR-1$^{-/-}$ mice have similar amounts of pDCs as LAIR-1$^{+/+}$ mice (Supplemental Figure 2A).

The frequencies of granulocytes and macrophages are not perturbed by the absence of LAIR-1, and the expression of the activation markers CD80 and CD86 on macrophages is comparable between LAIR-1$^{-/-}$ and LAIR-1$^{+/+}$ mice (Figure 2C). As shown in Figure 2D, the phenotypes of lymph node cells in LAIR-1$^{-/-}$ mice also tend to have less T cells, more Treg cells, even though not statistically significant. The altered peripheral populations in young LAIR-1$^{-/-}$ mice do not lead to pathological conditions (unpublished observations).

We also analyzed splenocytes from more than 10 pairs of littermates that were older than 12 months. We found that in comparison to LAIR-1$^{+/+}$, as in younger mice, LAIR-1$^{-/-}$ mice tend to have less CD3^{+} T cells with significantly less CD8 T cells. More strikingly, while the analyses of CD69 expression by peripheral T cells revealed no differences between LAIR-1$^{-/-}$ and LAIR-1$^{+/-}$ mice in their activation state when the mice are young (2 to 4 months old) (Figure 3A, Table 1), older LAIR-1$^{-/-}$ mice have markedly increased CD69 expression on both splenic CD4 and CD8 T cells populations (Figure 3A, Table 2). The frequency of CD69^{+} CD4 and CD69^{+} CD8 cells was elevated about 20% and 40%, respectively, in LAIR-1$^{-/-}$ compared to LAIR-1$^{+/-,\ +/+}$ mice (Figure 3A, Table 2). Compared to wt mice, young LAIR-1$^{-/-}$ mice did not show any difference in the frequencies of peripheral CD4 T_{EM}, CD4 T_{CM}, CD8 T_{EM} and CD8 T_{CM} cell subsets (Figure 3B and Table 1). On the other hand, older LAIR-1$^{-/-}$ mice have increased population within the memory CD4 T cell pool, due to a ~20% increase in CD4 T_{EM} population, as the CD4 T_{CM} population is less in older mice (Figure 3B and Table 2). A similar accumulation of CD8 memory T cells was evident in older LAIR-1$^{-/-}$ with increased frequencies of both CD8 T_{EM} and CD8 T_{CM} (Figure 3B and Table 2). Reflecting this, the LAIR-1$^{-/-}$ naive CD4 and CD8 T cell populations were smaller in older LAIR-1$^{-/-}$ than those in the LAIR-1$^{+/-,\ +/+}$ mice (Figure 3B and Table 2). As with younger mice, splenic B cells tend to be elevated in older LAIR-1$^{-/-}$ mice, which is accounted for by the presence of more marginal zone B cells but not follicular and T1/B-1 B cells (Table 2). The frequencies of LAIR-1$^{-/-}$ granulocytes and macrophages and their activated state do not show differences in aged mice compared with LAIR-1$^{+/+}$ populations. Despite the presence of higher percentages of activated and effector T cell subsets, older LAIR-1$^{-/-}$ animals appear to be healthy and normal.

LAIR-1$^{-/-}$ mice do not spontaneously develop autoimmune diseases

Two year old LAIR-1 $^{-/-}$ mice housed in a pathogen free environment are visibly normal, and 18 month old LAIR-1$^{-/-}$ mice necropsy reports did not show significant differences from wt mice (data not shown). Complete blood count (CBC) analyses of young (2–5 month old) versus older (9 month old) mice revealed that eosinophils were significantly elevated (2-fold) in younger LAIR-1$^{-/-}$ mice, but not different in older mice. Older LAIR-1$^{-/-}$ mice

had slightly elevated platelet levels. There were no other differences in the blood cell numbers between LAIR-$1^{-/-}$ and wt (Tables S1 and S2). We also compared LAIR-$1^{-/-}$ to wt mice for serum immunoglobulin levels and found that the levels of IgA, IgG2a, IgG2b and IgM are not different, whereas the level of IgG1 is significantly less (23%) in LAIR-$1^{-/-}$ mice. The lower amounts of IgG3 in LAIR-$1^{-/-}$ mice are not significant (Figure 4A).

There are reports that deficiencies in inhibitory receptors can cause development of autoimmune diseases that are characterized by the presence of ANA in the serum (35, 36). We found that aged (13 months) LAIR-$1^{-/-}$ mice have similar amounts of ANA compared to wt mice (Figure 4B). Inhibitory receptors can cooperate to regulate autoimmune diseases. For instance, lymphocyte activation gene 3 (LAG-3) can synergistically act with PD-1 to prevent autoimmune diseases (37). FcγRIIB is another ITIM containing receptor and FcγRIIB$^{-/-}$ mice on C57BL/6 background develop auto-antibodies and autoimmune glomerulonephritis (5). Moreover, duplication of the TLR7 gene accelerates the development of this autoimmune disease (38). We wondered if the absence of LAIR-1 would also exacerbate the disease process in FcγRIIB$^{-/-}$ mice; however, LAIR-$1^{-/-}$ FcγRIIB$^{-/-}$ mice did not show any differences in the levels of ANA, urine protein or the activated lymphocyte phenotype (data not shown), and the pathology of double knockout mice was similar to the FcγRIIB$^{-/-}$ mice, as were the survival rates (Figure 4C, D). Taken together, we found no evidence that LAIR-$1^{-/-}$ mice develop spontaneous autoimmune diseases and LAIR-1 deficiency does not accelerate the development of autoimmunity in FcγRIIB$^{-/-}$ mice.

LAIR-1 deficiency does not exacerbate the development of colitis or EAE

Because LAIR-$1^{-/-}$ T cells from older mice have elevated populations of activated T cells (Table 2, Figure 3), we pursued the possibility that LAIR-1 deficiency might make mice more susceptible to the induction of autoimmune diseases. To study colitis susceptibility, we employed a model in which naive $CD4^+$ cells are transferred into lymphopenic hosts (Rag$1^{-/-}$ mice) and colitis development is monitored by measuring body-weight changes (39). We found that transfer of LAIR-$1^{-/-}$ or LAIR-$1^{+/-}$ naive T cells resulted in equally severe bodyweight loss through the ninth week after transfer (Figure 5A), which indicates that LAIR-$1^{-/-}$ naive CD4 T cells induce similar colitis as LAIR-$1^{+/-}$ naive CD4 T cells.

We also investigated the role of LAIR-1 in an EAE model because a critical step in the pathogenesis of EAE is the extravasation of leukocytes from the blood stream into the central nervous system (CNS) parenchyma, which involves autoagressive T cell adhesion to and migration through the endothelial monolayer of the post capillary venules where they encounter the endothelial cell basement membrane that includes collagen-type IV (29). Thus, it is reasonable to suspect that T cells and other leukocytes will interact with collagens in their trafficking towards the CNS, and that this interaction might somehow affect their activation status as a consequence of the engagement of LAIR-1. We found that similar EAE pathogenesis progression was induced in LAIR-$1^{-/-}$ and LAIR-$1^{+/-}$ mice (Figure 5B), and IFNγ and IL-17 production by mononuclear cells, isolated from the spinal cords on day 21 after disease induction, was also similar (data not shown). We also did not observe any difference in the induction of serum (K/BxN)-induced arthritis between LAIR-$1^{-/-}$ and LAIR-$1^{+/+}$ mice (data not shown).

LAIR-1 deficiency does not affect T cell trafficking

Several surface collagen receptors, like integrins and DDR tyrosine kinases, are reported to be involved in cell adhesion and migration (19). Therefore, since we observed some small alterations in the peripheral LAIR-$1^{-/-}$ T cell subsets in the spleen (see Tables 1 and 2), we

did competitive homing experiments to compare the migration of LAIR-$1^{-/-}$ and LAIR-$1^{+/+}$ T cells to peripheral lymphoid organs. The results show that LAIR-$1^{-/-}$ T cells are not altered in their entrance to peripheral lymphoid tissues (measurement at 2.5h) or their re-circulation (measurement at 19h) through the peripheral lymphoid tissues, lymph nodes, spleen and peripheral blood (Figure 5C).

LAIR-$1^{-/-}$ NK cells have normal functional potential

We compared the *in vitro* functional status of LAIR-$1^{-/-}$ and LAIR-$1^{+/-}$ NK cells by degranulation assays. Briefly, splenocytes from such mice were stimulated for 5h with the anti-NK1.1 or mouse IgG2a in the presence of soluble anti-CD107a antibodies. Cells were then stained with anti-DX5 and anti-CD3 mAb and for intracellular IFN-γ. The frequency of $DX5^{+}$CD3 cells that were $CD107a^{+}$ IFN-γ^{+} from LAIR-$1^{-/-}$ mice was comparable to that from LAIR-$1^{+/-}$ controls (Supplemental Figure 2B).

LAIR-$1^{-/-}$ T cells are less efficient in helping B cells switch to antigen specific IgG2a and IgG2b

The ability of LAIR-$1^{-/-}$ T cells to provide B cell help was determined by comparing the responses of CD4 cre LAIR-$1^{fl/fl}$ and LAIR-$1^{fl/fl}$ mice to immunization with TNP-OVA in alum, which induces a T dependent response. CD4 cre LAIR-$1^{fl/fl}$ mice showed a significantly diminished TNP-specific IgG2a and IgG2b response (Figure 6A), indicating that LAIR-$1^{-/-}$ T cells are less efficient in helping B cells to switch to produce these antigen specific isotypes.

MZ B cells are LAIR-1^{+} and they have been functionally linked to TI-II immune responses to multivalent antigens due to an established requirement for the spleen in these responses (40, 41). We tested if LAIR-1 plays a role in TI-I or TI-II immune responses. The ELISA results showed that upon both TI-I and TI-II immunizations, TNP-specific Ig antibodies in serum were similar between LAIR-$1^{-/-}$ and control mice. The level of TNP-IgG3 tended to be higher in LAIR-$1^{-/-}$ mice with the TI-I immunization; however, it was not statistically significant (Figure 6B). Taken together, the lack of LAIR-1 did not affect the T-independent B cell responses.

In vitro DC differentiation capability and IFN-α production by pDCs are not affected in LAIR-$1^{-/-}$ mice

Human LAIR-1 was reported to inhibit the differentiation of $CD14^{+}$ peripheral blood precursors to DC (42). We addressed if LAIR-1 deficiency affects the differentiation of bone marrow precursors into DC. We found that the *in vitro* generation of BMDC was not affected by LAIR-1 deficiency (Figure 7A), and that upon stimulation with LPS, LTA or poly I:C, the upregulation of CD11b, CD40, CD80, CD86 and MHC class II is comparable between LAIR-$1^{+/+}$ and LAIR-$1^{-/-}$ BMDC (Figure 7B). LAIR-1 alone or in combination with NKp44 was also reported to inhibit IFNα production by human pDCs (34). Therefore, we investigated if LAIR-1 absence has an effect on the production of IFNα by pDCs. Our results show that LAIR-$1^{-/-}$ and LAIR-$1^{+/+}$ pDCs produce similar amounts of IFNα when stimulated with CpG (Figure 7C).

LAIR-1 deficiency does not affect bone mass

Osteoclasts are giant multinucleated cells that resorb the bone matrix. Osteoclasts are dependent on ITAM signals for differentiation (43). Interestingly, the Osteoclast-associated receptor (OSCAR), which associates with the Fc receptor common γ chain (FcRγ), has recently been shown to be a collagen receptor and to contribute to osteoclastogenesis in vivo (44). Like OSCAR, LAIR-1 is also expressed on osteoclast precursors and is also encoded in

the Leukocyte Receptor Complex. LAIR-1 could therefore negatively regulate OSCAR signaling during osteoclastogenesis with a possible effect on bone mass (45). We therefore analyzed the bones of wt and LAIR-$1^{-/-}$ mice by μCT to see if there were any differences in bone mass. No significant differences in bone mass could be observed between wt and LAIR-$1^{-/-}$ mice (Figure 8).

Discussion

Immune cell responses are tightly regulated by the balance of signals from activating and inhibitory receptors that they express. All else being equal inhibitory signals tend to predominate over activating signals serving to prevent hyper-responsiveness/autoimmunity against self-antigens that could damage the host. A large number of ITIM-bearing inhibitory molecules with diverse tissue distribution and ligand recognition have been shown to negatively regulate cell activation. ITIM-containing molecules are involved in the control of a large spectrum of immune functions (46). LAIR-1 is an ITIM inhibitory bearing receptor expressed by the majority of immune cells, whose cross-linking *in vitro* by Ab or collagens delivers an inhibitory signal that can down-regulate activating signals (9, 10, 47); however, the function of LAIR-1 *in vivo* remains largely a mystery. In order to begin to address this, we generated and characterized LAIR-$1^{-/-}$ and LAIR-$1^{fl/fl}$ mice.

We found that the deficiency of LAIR-1 did not affect the Mendelian ratio of the offspring, nor their longevity or visible normality in a pathogen free housing environment. Old (18 months) LAIR-$1^{-/-}$ mice necropsy reports did not show significant pathologic differences from LAIR-$1^{+/+}$ mice. We performed CBC tests on young (2–5 months old) and older (9 months old) mice (Supplementary Tables 1 and 2) as a means of evaluating the physiological peripheral immune profile (48). Younger LAIR-$1^{-/-}$ mice have 2-fold elevated eosinophils compared to LAIR-$1^{+/+}$ mice. Increased eosinophil counts (eosinophilia) can signify infections, allergic or autoimmune diseases (49). In LAIR-$1^{-/-}$ mice the elevated eosinophil counts subside with age with no evidence of other pathologic conditions. LAIR-1 was reported to negatively regulate the *in vitro* maturation of primary megakaryocytic progenitors (50) that upon maturation produce platelets. However, we observed only slightly increased the platelet count in 9 month old LAIR-$1^{-/-}$ mice (Supplemental Tables 1 and 2).

The absence of LAIR-1 expression has no evident pathological consequences. However, a sign that LAIR-1 may have an inhibitory role *in vivo* is reflected by the fact that with age LAIR-$1^{-/-}$ mice exhibit a higher frequency of activated and effector/memory T cells (Figure 3). The importance of the expression of LAIR-1 on T cells is also shown by the low efficiency that LAIR-$1^{-/-}$ T cells have in helping B cells to effectively switch to antigen specific IgG2a and -2b (Figure 6). Altogether, these data indicate that LAIR-1 has a small role, albeit noticeable, in controlling T cell functions, although its absence does not result in an observable pathologic effect. Although our data showed that LAIR-1 expression on T cells may have a role in helping B cells in switching to certain antigen specific IgG isotypes, we do not know if the expression of LAIR-1 on B cells may also have a role. Our data showed that LAIR-1 expression is restricted to MZ B cells (Figure 1D). MZ B cells are sessile and reside in the outer white pulp of the spleen between the marginal sinus and the red pulp. They have been functionally linked to T-independent type II immune responses to multivalent antigens due to an established requirement for the spleen in these responses (40, 41). We showed the deficiency of LAIR-1 had no effect in the generation of antigen specific Igs upon either TI-I or TI-II immunizations, except that TNP-IgG3 tended to be higher in LAIR-$1^{-/-}$ mice with the TI-I immunization (Figure 6B). This indicates that the effect of LAIR-1 deficiency on marginal zone B cells has a marginal role in T-independent B-cell responses.

It was very surprising to us not to find a role for LAIR-1 in the autoimmune disease models that we tested, specifically in EAE (Figure 5B). It is well known that during extravasation to inflamed tissues, autoagressive T cells migrate through the endothelial monolayer and encounter the endothelial cell basement membrane (29). Collagen type IV is an important component of the basement membranes, and it has been shown before that LAIR-1 is able to interact with collagen type IV that is in matrigel, a preparation of basement membranes (18). Encephalitogenic T cells entering the central nervous system apparently interact with components of the basement membranes, as laminins have been shown to regulate the extravasation of T cells into the brain (51, 52). Why the interaction of LAIR-1 with collagens present in the basement membranes does not occur or has no impact remains to be explained. In this same vein, it was surprising to find that LAIR-1 has no obvious impact on bone mass stimulated by OSCAR generated ITAM signaling in osteoclasts or their precursors, as both receptors are expressed by these cells and both recognize collagens (44). One possible explanation is that there is redundancy in inhibitory receptor pathways that can negatively regulate OSCAR e.g. PECAM-1, PIR-B or SIRPα (53–55).

The deficiency of the ITIM containing receptor LAIR-1 results in higher frequencies of activated and effector/memory T cells in aged mice, yet this apparently does not lead to hyper-activated immune response. In apparent contradiction, LAIR-1 deficiency results in lower levels of serum IgG1 and IgG3 in LAIR-$1^{-/-}$ mice and a lower efficiency of LAIR-$1^{-/-}$ T cells in providing help to B cells for switching to antigen specific IgG2a and IgG2b following immunization. There are several potential explanations for these observations: (i) LAIR-1 is not a pivotal regulator of the immune system, and/or other inhibitory receptors compensate for its inhibitory function; (ii) LAIR-1 needs to cooperate with other receptors to effect its inhibitory function; (iii) LAIR-1 functions only if mice are faced with overwhelming challenges like infections and septic shock [our preliminary data do not show any differences between LAIR-$1^{+/+}$ and LAIR-$1^{-/-}$ mice after LCMV infection and LPS injection (data not shown)]; (iv) higher numbers of Tregs observed in LAIR-$1^{-/-}$ mice may suppress activation of the immune system; (v) mouse LAIR-1 may not function *in vivo* as an “inhibitory” receptor. Unlike human LAIR-1, mouse LAIR-1 recruits SHP-2 but not SHP-1 when tyrosine phosphorylated (6), and it has been shown that SHP-2 may play a positive regulatory role in T cells (56). Additional studies are required to investigate these or other possibilities. In summary, while murine LAIR-1 plays a role in the natural development of the immune system, its absence does not affect survival in a controlled pathogen free environment or even the severity of induced ailments where exposure to collagen might be expected to be involved. Similar findings were made for CD94 deficient mice in that unexpectedly these mice developed normally and responded normally in a variety of disease models (57); however, recently, these mice have been shown to lack resistance to mousepox, caused by the *Orthopoxvirus* ectromelia virus infection (58). Whether a critical circumstantial role for LAIR-1 will ultimately unveil itself remains to be determined.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ANA	anti-nuclear antibodies
BDMC	bone marrow derived dendritic cells
CBC	complete blood count
cDC	conventional dendritic cells
CPC	committed progenitor cell
DC	dendritic cells
DDR1	discoidin domain receptor 1
EAE	experimental autoimmune encephalitis
FO	follicular
GPO	glycine-proline-hydroxyproline
HSC	hematopoietic stem cells
LAIR	leukocyte-associated immunoglobulin-like receptor
LAG-3	lymphocyte activation gene 3
LRC	Leukocyte Receptor Complex
LTA	lipoteichoic acid
MZ	marginal zone
PD-1	Programmed Death 1
pDC	plasmacytoid dendritic cells
SHP	Src homology 2 domain-containing protein tyrosine phosphatase
T1/B1	transitional 1/B-1
$\mathbf{T_{CM}}$	central memory T
$\mathbf{T_{EM}}$	effector memory T
TI-I	T-independent type I
TI-II	T-independent type II
wt	wild type

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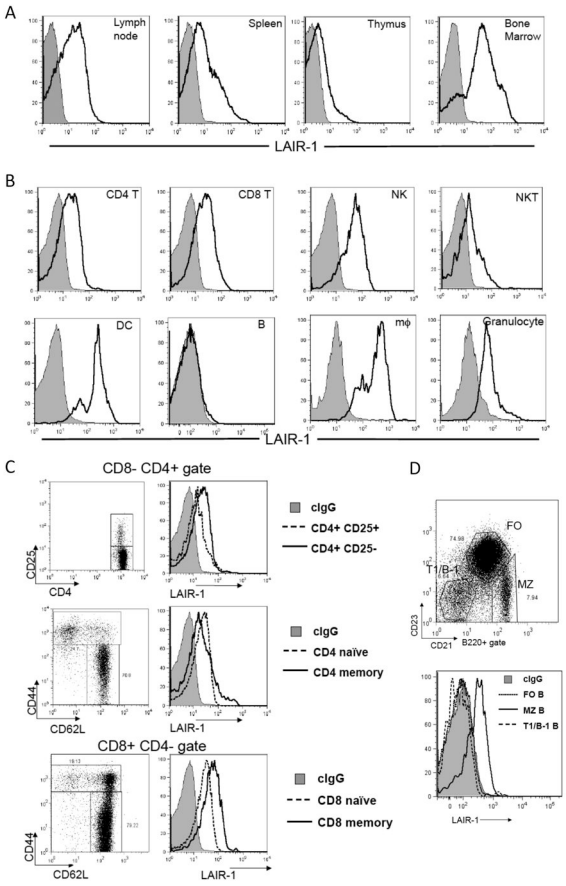

Figure 1.

LAIR-1 expression on immune cells. Isolated single cell suspensions were analyzed by flow cytometry with PE conjugated anti-LAIR-1 mAb (open histograms) or Armenian hamster isotype control IgG (closed histograms). (A) LAIR-1 expression on cells isolated from C57BL/6 lymph node, spleen, thymus and bone marrow; (B) LAIR-1 expression on C57BL/6 splenocyte populations: CD4 T ($CD4^+$ $CD8^-$ $CD3^+$) and CD8 T ($CD8^+$ $CD4^-$ $CD3^+$) cells, B cells ($B220^+$), NK cells ($NK1.1^+$ $CD3^-$), NKT cells ($NK1.1^+$ $CD3^+$), conventional DCs ($CD11c^+$ $B220^-$), granulocytes ($Gr\text{-}1^+$) and macrophages ($F4/80^+$); (C) LAIR-1 expression on splenic T cell subsets. $CD4^+$ $CD25^+$ T cells, naive T ($CD4^+$ $CD8^-$ CD44lo CD62Lhi; $CD8^+$ $CD4^-$ CD44lo CD62Lhi), memory T cells ($CD4^+$ $CD8^-$ CD44hi; $CD8^+$ $CD4^-$ CD44hi) were gated as shown by the indicated antibodies. (D) LAIR-1 is mainly expressed on MZ B cells. MZ B ($B220^+$ CD21hi CD23lo), FO B ($B220^+$ CD21lo CD23hi) and transitional 1 zone T1/B-1 (T1/B-1) B cells ($B220^+$ $CD21^-$ $CD23^-$) were delineated by CD21, CD23 and B220 staining.

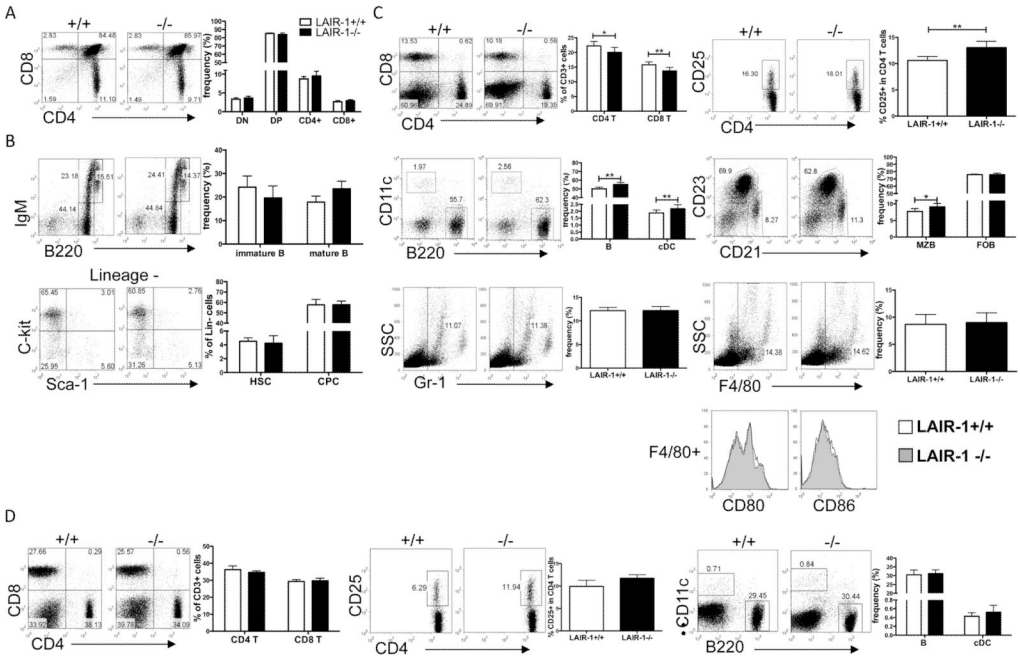

Figure 2.

Flow cytometric analyses of immune cells from LAIR-1$^{-/-}$ and wt mice. FACS analyses of cells obtained from (A) thymus, (B) bone marrow, (C) spleen, (D) inguinal lymph nodes of LAIR-1$^{+/+}$ and LAIR-1$^{-/-}$ mice using flurochrome-conjugated antibodies. Bone marrow cell types were identified by flow cytomety according to the expression of surface molecules: pro-B/pre-B (B220^{+} IgM^{-}), immature B cell (B220lo IgM^{+}), mature B cell (B220hi IgM^{+}), hematopoietic stem (Lin^{-} c-Kit^{+} Sca-1^{+}, HSC) and committed progenitor cells (Lin^{-} c-Kit^{+} Sca-1^{-}, CPC). Numbers indicate percentage of lymphocytes. Data shown are representative of more than 10 independent experiments.

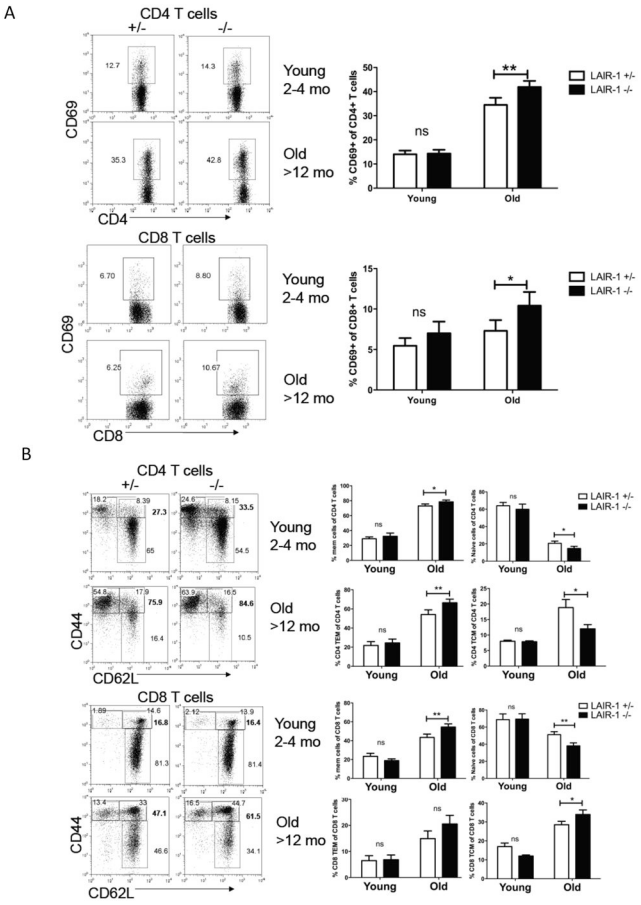

Figure 3.

Increased levels of activated and effector/memory T cells in older LAIR-$1^{-/-}$ mice. (A) Levels of activated $CD69^{+}$ $CD4^{+}$ (upper panel) and $CD69^{+}$ $CD8^{+}$ (lower panel) T cells in young versus old LAIR-$1^{-/-}$ and LAIR-$1^{+/-}$ mice. T cells were analyzed from young (2–4 months) or old (>12 months) LAIR-$1^{+/-}$ or LAIR-$1^{-/-}$ mice (n=6, young; n=17, old). Frequencies for each population are shown on the right. (B) Levels of CD4 (upper panel) and CD8 (lower panel) naïve and memory T cells in young versus old LAIR-$1^{-/-}$ and LAIR-$1^{+/-}$ mice. Memory (CD44hi, bold number in dot plots), naive (CD44lo CD62Lhi) T_{EM} (CD44hi CD62Llo) and T_{CM} CD44hi CD62Lhi splenic T cells were quantified from young (2–4 months) or old (>12 months) LAIR-$1^{+/-}$ or LAIR-$1^{-/-}$ mice (n=6, young; n=17, old). Frequencies for each population are shown on the right. Data are presented as the mean ± SEM. Statistical comparison of the data was performed using the paired two-tailed Student's t test. Group differences with $P<0.05$ was considered statistically significant. One star indicates $P<0.05$ and two stars indicate $P<0.01$.

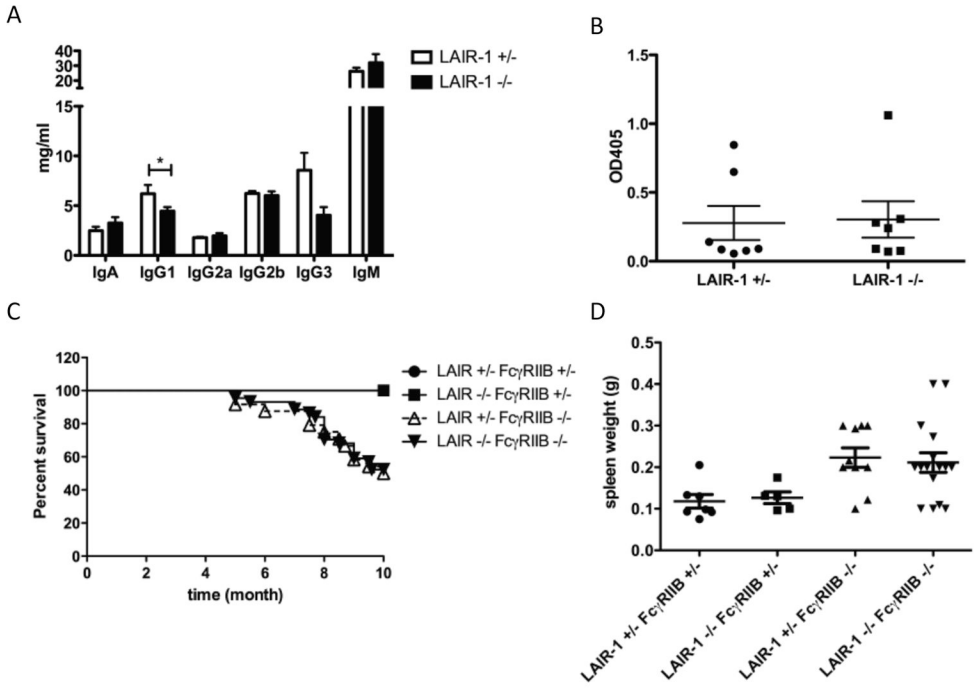

Figure 4.

Autoimmune disease is not detected in LAIR-$1^{-/-}$ mice and LAIR-1 deficiency does not accelerate the development of autoimmune disease in FcγRIIB$^{-/-}$ mice. (A) Serum samples from LAIR-$1^{+/-}$ (open bars) or LAIR-$1^{-/-}$ (solid bars) mice at 13 months of age were analyzed for serum immunoglobulin levels. Results are for seven animals per group. Bar graphs represent the average ± SEM. (B) Levels of ANA in the serum of LAIR-$1^{+/-}$ (circle) or LAIR-$1^{-/-}$ (square) mice at 13 months of age (n=7). (C) Mortality curves for LAIR-$1^{+/-}$ FcγRIIB$^{+/-}$ (n=11), LAIR-$1^{-/-}$ FcγRIIB$^{+/-}$(n=7), LAIR-$1^{+/-}$ FcγRIIB$^{-/-}$ (n>20) and LAIR-$1^{-/-}$ FcγRIIB$^{-/-}$ mice (n>20). (D) Spleen weight of LAIR-$1^{+/-}$ FcγRIIB$^{+/-}$, LAIR-1−/− FcγRIIB$^{+/-}$, LAIR-$1^{+/-}$ FcγRIIB$^{-/-}$ and LAIR-$1^{-/-}$ FcγRIIB$^{-/-}$ mice at 10 months of age. Data are presented as the mean ± SEM. Statistical comparison of the data was performed using the paired two-tailed Student's t test. Group differences with $P<0.05$ were considered statistically significant.

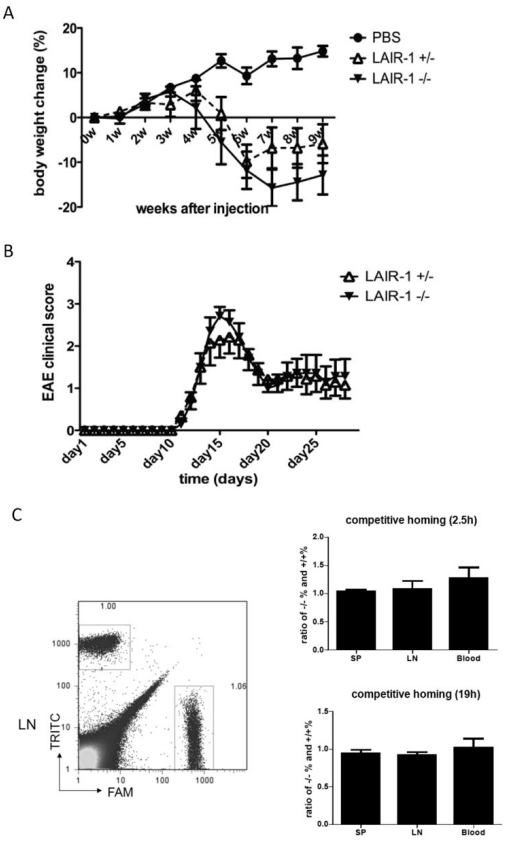

Figure 5.

LAIR-1 deficiency does not affect the progression of experimental EAE or colitis, or T cell migration. (A) Naive LAIR-$1^{-/-}$ and LAIR-$1^{+/-}$ CD4 T cells induce similar degrees of colitis. Purified LAIR-$1^{+/-}$ (open triangles) and LAIR-$1^{-/-}$ (filled triangles) naive $CD4^{+}$ T cells were transferred into Rag$1^{-/-}$ mice. The body weight change of these mice is plotted as mean ± SEM. PBS was injected as a control group (filled dots) (n=5). This is a representative result of three independent experiments. (B) LAIR-1 deficiency does not affect the development of EAE. EAE disease course in LAIR-$1^{+/-}$ and$^{-/-}$ mice (n=8). Disease scores are plotted as mean ± SEM. These results are representative of two independent experiments. (C) LAIR-$1^{-/-}$ and LAIR-$1^{+/+}$ T cells migrate similarly. Equal numbers of purified LAIR-$1^{+/+}$ and LAIR-$1^{-/-}$ T cells were labeled with FAM or TRITC and injected i.v. into C57BL/6 mice. After 2.5 and 19 h, mice were sacrificed and lymphocytes were isolated from tissues of the recipient mice (n =3 or 4). Cells were analyzed by flow cytometry for FAM or TRITC. The homing ratio of LAIR-$1^{-/-}$ versus LAIR-$1^{+/+}$ T cells was calculated as described in the Methods. Data are presented as the mean ± SEM. Statistical comparison of the data was performed using the paired two-tailed Student's t test. Group differences with $P<0.05$ were considered statistically significant.

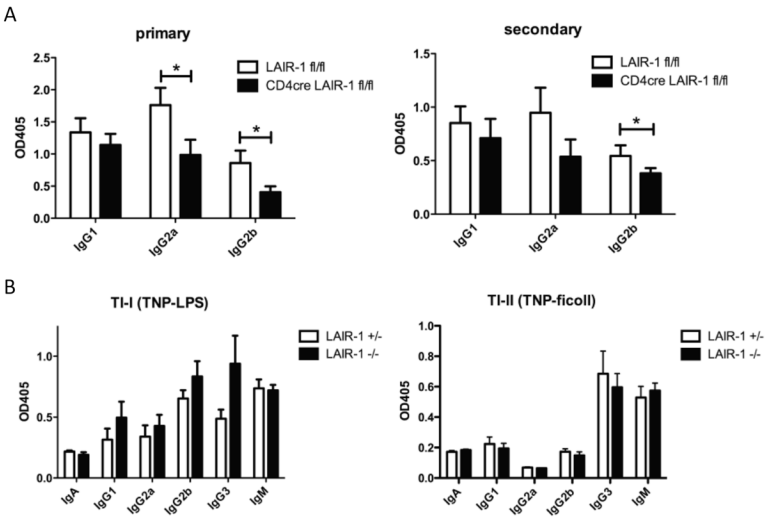

Figure 6.

Humoral responses in LAIR-$1^{-/-}$ (filled bars) and LAIR-$1^{+/+}$ (open bars) mice. (A) CD4 cre LAIR-$1^{fl/fl}$ T cells are less efficient in helping B cells to switch to antigen specific IgG2a and IgG2b with T-dependent immunization. Mice were immunized with 100 μg of TNP-OVA with alum, and the levels of TNP-specific antibody subclasses in the sera were quantified 2 wk after the primary immunization (*Left*) or 1 week after re-challenge (day 28) (*Right*). Bar graphs represent the average ± SEM (n=10). (B) T-cell-independent B-cell responses in LAIR-$1^{-/-}$ mice are comparable to LAIR-$1^{+/+}$ mice. LAIR-$1^{+/-}$ and LAIR-$1^{-/-}$ mice were immunized with 50μg TNP-LPS (TI-I, left) or 25μg TNP-ficoll (TI-II, right), and TNP specific antibodies in serum were quantified 7 days after immunization by ELISA. Bar graphs represent the average ± SEM (n=7). Statistical comparison of the data was performed using the paired two-tailed Student's t test. Group differences with $P<0.05$ were considered statistically significant.

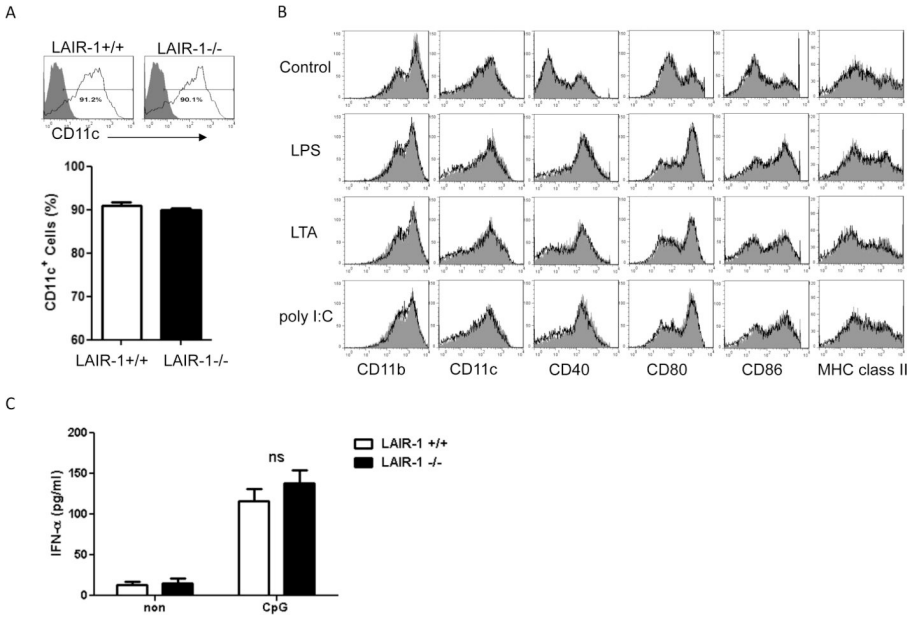

Figure 7.

In vitro DC differentiation from bone marrow precursors and IFNα production by pDCs are not affected by the absence of LAIR-1. (A) DCs were generated from c-kit^{+} HSC purified from the bone marrow of LAIR-1$^{+/+}$ and LAIR-1$^{-/-}$ mice with rmGM-CSF (10 ng/ml) and rmIL-4 (10 ng/ml) for 6 days. BMDC differentiation was evaluated by the frequency of CD11c^{+} cells. Filled histogram: control IgG-PE and open histogram: anti-mouse CD11c-PE. Data are presented as mean ± SD and represent three independent experiments. (B) BMDC were stimulated with LPS (1 μg/ml), LTA (25 μg/ml) or poly I:C (25 μg/ml) for 24 h. The expression of cell surface molecules on immature and mature DCs was evaluated by flow cytometry. Filled histograms correspond to LAIR-1$^{+/+}$ mice and open histograms to LAIR-1$^{-/-}$ mice. Data are representative of three independent experiments. (C) Purified LAIR-1$^{+/+}$ (open bars) and LAIR-1$^{-/-}$ (solid bars) pDCs were cultured for 24 h with or without CpG (10 μg/ml). Supernatants were collected and IFNα production was measured. Bar graphs represent the average ± SEM from 5 independent experiments.

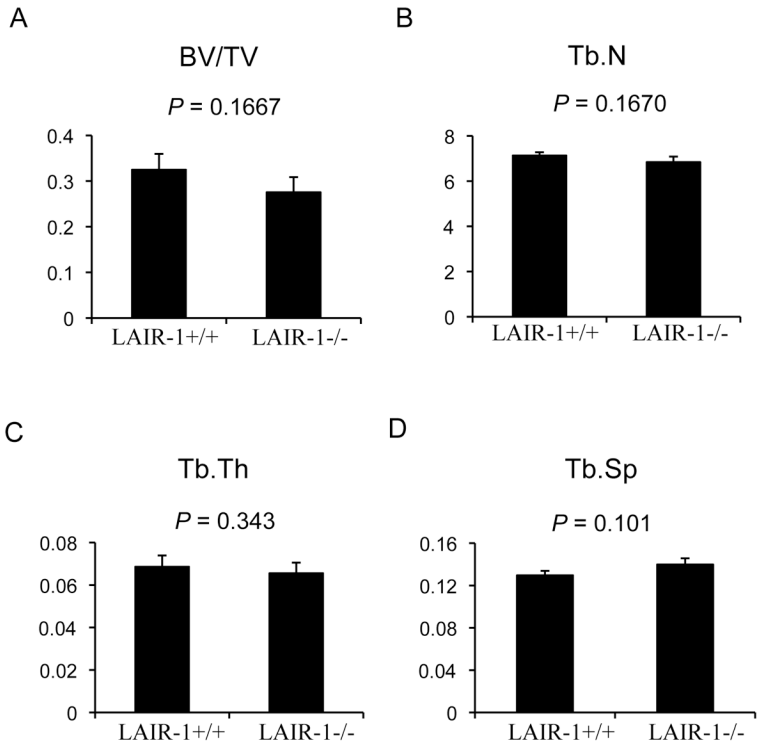

Figure 8.

The bone masses of LAIR-1$^{-/-}$ mice do not differ from wild-type mice. No significant differences were observed between the percentage of (A) trabecular bone volume/tissue volume (BV/TV), (B) trabecular number (Tb.N), (C) trabecular thickness (Tb.Th) or (D) trabecular spacing (Tb.Sp), between the femurs of wild-type and LAIR-1$^{-/-}$ mice (8 weeks old), as determined by μCT. Data are presented as average (n=5) ± SEM; P values are indicated above each graph.

Table 1

Percentage of splenic cell types in 2–4 month old LAIR-1$^{-/-}$ compared to wt mice.

	LAIR-1+/+, +/−	LAIR-1−/−
CD3 T cell	39.29 ± 2.35	34.68 ± 2.88 **
CD4 T cell	22.21 ± 1.53	20.05 ± 1.68 *
CD4+ CD25+ T cell	10.6 ± 0.75	13.06 ± 1.19 *
CD4+ CD69+ T cell	14.02 ± 1.55	14.33 ± 1.58
CD4 naïve T cell	59.92 ± 6.00	64.04 ± 3.81
CD4 mem T cell	32.28 ± 4.34	28.94 ± 2.53
CD4 T_{CM} cell	7.84 ± 0.30	7.98 ± 0.36
CD4 T_{EM} cell	21.80 ± 3.94	24.44 ± 4.08
CD8 T cell	15.8 ± 0.99	13.69 ± 1.24 **
CD8+ CD25+ T cell	1.33 ± 0.36	2.04 ± 1.06
CD8+ CD69+ T cell	5.45 ± 0.95	7.00 ± 1.44
CD8 naïve T cell	68.76 ± 6.66	69.36 ± 6.24
CD8 mem T cell	23.41 ± 3.20	18.81 ± 1.86
CD8 T_{CM} cell	16.95 ± 1.84	11.97 ± 0.56
CD8 T_{EM} cell	6.46 ± 1.89	6.84 ± 1.80
B cell	50.04 ± 1.98	55.01 ± 2.46 **
MZ B cell	7.71 ± 0.85	9.10 ± 0.99 *
Conventional DC cell (CD11c+ B220−)	1.85 ± 0.41	2.27 ± 0.47 **

Numbers indicate mean percentage of cells ± SEM. Data collected from 6~15 pairs of mice in more than 6 independent analyses. Statistical comparison of the data was performed using the paired two-tailed Student's t test.

* indicates P<0.05 and

** indicates P<0.01.

Table 2

Percentage of splenic cell types in greater than one year old LAIR-$1^{-/-}$ compared to wt mice.

	LAIR-1+/+, +/−	LAIR-1−/−
CD3 T cell	32.05 ± 2.09	27.41 ± 2.78
CD4 T cell	16.94 ± 0.86	17.00 ± 0.99
CD4+ CD25+ T cell	22.93 ± 1.51	26.37 ± 1.86
CD4+ CD69+ T cell	34.49 ± 2.93	41.89 ± 2.56 **
CD4 naïve T cell	20.63 ± 2.59	14.72 ± 2.44 *
CD4 mem T cell	72.96 ± 2.68	78.45 ± 2.51 *
CD4 T_{CM} cell	18.84 ± 2.60	12.00 ± 1.36 *
CD4 T_{EM} cell	54.12 ± 4.82	66.45 ± 3.70 **
CD8 T cell	14.71 ± 1.13	12.26 ± 1.38 *
CD8+ CD25+ T cell	0.93 ± 0.11	1.05 ± 0.13
CD8+ CD69+ T cell	7.31 ± 1.33	10.42 ± 1.70 *
CD8 naïve T cell	51.21 ± 3.51	38.11 ± 3.45 **
CD8 mem T cell	43.46 ± 3.48	54.42 ± 3.36 **
CD8 T_{CM} cell	28.51 ± 1.80	33.87 ± 2.40 *
CD8 T_{EM} cell	14.95 ± 2.92	20.55 ± 3.30
B cell	57.84 ± 2.04	61.84 ± 2.86
MZ B cell	6.01 ± 0.55	7.08 ± 0.43 *

Numbers indicate mean percentage of cells ± SEM. Data collected from more than 17 pairs of mice in 8 independent analyses. Statistical comparison of the data was performed using the paired two-tailed Student's t test.

* indicates P<0.05 and

** indicates P<0.01.