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**Myeloid-Specific Expression of Human Lysosomal Acid Lipase Corrects Malformation and Malfunction of Myeloid-Derived Suppressor Cells in lal<sup>−/−</sup> Mice**

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Lysosomal acid lipase (LAL) cleaves cholesteryl esters and triglycerides to generate free fatty acids and cholesterol in lysosomes. LAL deficiency causes expansion of CD11b<sup>+</sup>Gr-1<sup>+</sup> immature myeloid cells, loss of T cells, and impairment of T cell function. To test how myeloid cell LAL controls myelopoiesis and lymphopoiesis, a myeloid-specific doxycycline-inducible transgenic system was used to reintroduce human lysosomal acid lipase (hLAL) expression into LAL gene knockout (lal<sup>−/−</sup>) mice. Expression of hLAL in myeloid cells of lal<sup>−/−</sup> mice reversed abnormal myelopoiesis in the bone marrow starting at the granulocyte–monocyte progenitor stage and reduced systemic expansion of myeloid-derived suppressor cells (MDSCs). Myeloid hLAL expression inhibited reactive oxygen species production and arginine expression in CD11b<sup>+</sup>Gr-1<sup>+</sup> cells of lal<sup>−/−</sup> mice. Structural organization of the thymus and spleen was partially restored in association with reduced infiltration of CD11b<sup>+</sup>Gr-1<sup>+</sup> cells in these mice. In the thymus, reconstitution of myeloid cell LAL restored development of thymocytes at the double-negative DN3 stage. Myeloid cell LAL expression improved the proliferation and function of peripheral T cells. In vitro coculture experiments showed that myeloid hLAL expression in lal<sup>−/−</sup> mice reversed CD11b<sup>+</sup>Gr-1<sup>+</sup> myeloid cell suppression of CD4<sup>+</sup> T cell proliferation, T cell signaling activation, and lymphokine secretion. Blocking stat3 and NF-κB p65 signaling by small-molecule inhibitors in MDSCs achieved a similar effect. Injection of anti–Gr-1 Ab into lal<sup>−/−</sup> mice to deplete MDSCs restored T cell proliferation. These studies demonstrate that LAL in myeloid cells plays a critical role in maintaining normal hematopoietic cell development and balancing immunosuppression and inflammation. *The Journal of Immunology*, 2011, 187: 3854–3866.
triplex mice reversed abnormal myeloid progenitor development and differentiation in the bone marrow, reduced MDSC expansion and infiltration into the thymus and spleen, corrected abnormal T cell development in the thymus, and restored T cell proliferation and function in the spleen. These new results demonstrate that LAL in myeloid cells is critical for the development, homeostasis, and function of myeloid cells and T cells. Disruption of this pathway leads to systemic inflammation and tissue pathogenesis.

Materials and Methods

Animal care

All scientific protocols involving the use of animals have been approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine and followed guidelines established by the Panel on Euthanasia of the American Veterinary Medical Association. Protocols involving the use of recombinant DNA or biohazardous materials have been reviewed by the Biosafety Committee of Indiana University School of Medicine and followed guidelines established by the National Institutes of Health. Animals were housed under Institutional Animal Care and Use Committee-approved conditions in a secured animal facility at Indiana University School of Medicine.

FACS analysis

Single-cell suspensions from the bone marrow, spleen, and blood were prepared as previously described (11). Approximately 1 × 10^5–2 × 10^5 cells from various organs were blocked with FcR blocking Abs in FACS buffer (BD Pharmingen, San Diego, CA) followed by incubation with isotype control or primary Abs. For six-color hematopoietic progenitor cell analysis and sorting (12), a previously described procedure was followed (2). For measurement of intracellular signaling molecules, the assays were performed according to the protocols previously described (11). Anti–p-ERK, p38, NF-κB, stat1, stat3, and stat6 were purchased from Cell Signaling Technology.

Double immunofluorescence staining

The thymus and spleen were washed with PBS and dehydrated by a series of increasing ethanol concentrations, followed by paraffin embedding. Five-micrometer tissue sections were doubly stained with rabbit anti-Flag Ab (1:100, Sigma, St. Louis, MO), rabbit anti-Gr-1 Ab, and CD11b mouse Ab (eBioscience). A Cy2-conjugated donkey anti-rabbit IgG and a Cy3-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA) were used as the secondary Abs. Primary Abs or secondary Abs alone served as control and showed no positive staining.

Real-time PCR

Real-time PCR analysis was performed as previously described (13) using the TaqMan Reverse Transcription Kit and SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). GAPDH primers were used as an endogenous control to normalize all cDNA samples. The reactions were analyzed using the StepOnePlus Real-Time PCR System (Applied Biosystems).

Primers for real-time PCR, mBid: upstream 5′-GGCCCTGCTGCCTTCACTGTAG-3′, downstream 5′-TGCTCCTCTCTCTCTCTCTCTC-3′; mCasp3; upstream 5′-GCGACGGACCACTTCCATTCTTCT-3′, downstream 5′-CTGCATGTTGTTGTTGCGAGTT-3′; mCasp8; upstream 5′-GCAGGATGTTGTTGTTGCGAGTT-3′, downstream 5′-CCAGGAGGTGTTGTTGCGAGTT-3′; mCasp9; upstream 5′-TGAGGGTCTGGGCCATAGAA-3′, downstream 5′-CCCCAAAGGGATGAGAAGTTC-3′; mIFN-γ; upstream 5′-GGCGAAAAAGGATGCAT-3′, downstream 5′-CTGGACCTTGGGTTGTTGAC-3′; mTNFα; upstream 5′-CCCCAAAAGGATGAGAAGTTC-3′, downstream 5′-TGAGGGTCTGGGCCATAGAA-3′; GAPDH: upstream 5′-GGCCATCAAGCCAGACGTCT-3′, downstream 5′-CCAAACCATCAC-TGACACTCAGA-3′.

Isolation of MDSCs

Spleen single cells were placed in anti-CD11b Ab-coated culture dishes and incubated for 3 h at 37°C in a humidified 5% CO2 atmosphere. Cells were gently washed with PBS. Adherent cells were incubated with biotin-labeled primary anti-Gr-1 Ab for 20 min, followed by 20-min incubation of anti-biotin secondary Ab beads in PBS. Labeled cells were selected on an MS column using the MACS technology (Miltenyi Biotech, Auburn, CA).

T cell proliferation assay

CD4+ T cells were isolated from the spleen of various mouse groups and purified with anti-CD4 mAb-coated magnetic beads and MACS-LS columns according to the manufacturer’s instructions. Isolated CD4+ T cells were labeled with CFSE (Molecular Probes). Labeled cells were cultured and stimulated with 1 μg/ml plate-bound anti-CD3 Ab and 1 μg/ml soluble anti-CD28 Ab (BD Pharmingen) for 4 d at 37°C in a humidified 5% CO2 incubator. CFSE profiles of CD4+ T cell proliferation was evaluated by FACS. Activation of cultured CD4+ T cells was assessed by expression of surface activation marker CD69 with anti-CD69 Ab by FACS.

Annexin V binding

Dual staining with FITC–annexin V and propidium iodide (PI) was performed to detect cells undergoing apoptosis using an annexin V–FITC kit (BD Biosciences, Bedford, MA) following a procedure previously described (1). Cultured and stimulated CD4+ T cells were stained with surface markers and washed twice with PBS. After resuspension of labeled cells in annexin V-binding buffer containing FITC-conjugated annexin V, PI was added into samples and incubated on ice for 10 min. Cells were analyzed on an LSRII within 1 h. Viable cells were defined by FITC+ and PI− population. Early apoptotic cells were defined by FITC+ and PI− population. Nonspecific binding was blocked by preincubating with rat IgG (10 μg/ml) and anti-FcII/III.

Lin− bone marrow cell purification

A previously described procedure was used (2). Briefly, bone marrow cells were isolated from various mouse groups. Erythrocytes were lysed. Bone marrow cells were labeled with a mixture of biotin-coupled Abs raised against lineage-specific Ags: CD11b, Gr-1, B220, TER-119, and CD3ε (mouse lineage panel kit; BD Pharmingen) for 20 min at 4°C. Unlabeled cells were separated on a depletion column using the MACS technology according to the manufacturer’s instructions (Miltenyi Biotech). This method resulted in >95% Lin− (lineage negative) cells.

In vitro MDSC suppression assay and lymphokine measurement

CD4+ T cells were isolated from the spleen of wild-type (H2b+) mice with anti-CD4 mAb-coated magnetic beads and MACS-LS columns according to the manufacturer’s instructions. Isolated wild-type CD4+ T cells were labeled with CFSE and cultured with 1 μg/ml plate-bound anti-CD3 Ab and 1 μg/ml soluble anti-CD28 Ab for 4 d in the presence or absence of CD11b+Gr-1− cells from various mouse groups at 37°C in a humidified 5% CO2 incubator. The ratio of MDSCs/CD4+ T cells was 1:5. Proliferation of CD4+ T cells was measured by FACS. T cell activation was also assessed with anti-CD90 Ab. The lymphokine concentrations in the cultured medium were measured by OptEIA ELISA kits according to the protocols by Pharmingen (San Diego, CA) or R&D Systems (Minneapolis, MN).

Measurement of p-CD3ε chain

CD4+ T cells isolated from the wild-type mouse spleen were cocultured (10^6/ml T cells per well) with CD11b+Gr-1+ cells from various mouse groups for 4 d in RPMI 1640 medium containing 1 μg/ml plate-bound anti-CD3 Ab and 1 μg/ml soluble anti-CD28 Ab at 37°C in a humidified 5% CO2 incubator. The ratio of MDSCs/CD4+ T cells was 1:5. Proliferation of CD4+ T cells was measured by FACS. T cell activation was assessed by measuring activation of CD4+ cells with anti-CD3ε Ab (BD Pharmingen). Rat IgG2 Ab (BD Pharmingen) was used as isotype control.

Arginase activity

Arginase activity was measured as previously described (14). Briefly, cells were lysed for 30 min at room temperature with 50 μl 0.1% Triton X-100.
PBS containing 5 μg pepstatin, 5 μg aprotinin, and 5 μg antipain protease inhibitors. Subsequently, 50 μl 10 mM MnCl₂ and 50 μl 50 mM Tris-HCl (pH 7.5) were added, and the enzyme was activated by heating for 10 min at 56°C. Arginine hydrolysis (100 μl) was used by incubating the lysate with 100 μl 0.5 M L-arginine (pH 9.7) at 37°C for 60–120 min. The reaction was stopped with 400 μl H₂SO₄ (96%)/H₃PO₄ (85%)/H₂O (1:3:7, v/v/v). The urea concentration was measured at 540 nm after addition of 25 μl 9% a-isonitrosopropiophenone (dissolved in 100% ethanol), followed by heating at 95°C for 45 min and 10 min in the dark. One unit of enzyme activity is defined as the amount of enzyme that catalyzes the formation of 1 μmol urea per minute.

**Reactive oxygen species production**

MDSCs were isolated from the spleen of lal⁺/⁺ mice, lal⁻/⁻ mice, 3-mo doxycycline-treated or doxycycline-untreated c-fms-rtTA/(TetO) 7-CMV-hLAL;lal⁻/⁻ (TgKO) triple mice using cell sorting. The purity of cell populations was >95%. Oxidation-sensitive dye DCFDA (Molecular Probes/Invitrogen), was used to measure reactive oxygen species (ROS) production. Cells were incubated at 37°C in prewarmed RPMI 1640 in the presence of 2.5 μM DCFDA for 20 min. Cells were then labeled with allophycocyanin-conjugated anti–Gr-1 and PE-conjugated anti-CD11b Abs on ice and analyzed by flow cytometry.

**stat3 and NF-κB inhibition**

stat3 inhibitor cucurbitacin B (C8499) and NF-κB inhibitor ammonium pyrrolidinedithiocarbamate (PDTC; P8765) were purchased from Sigma.

**Results**

**Myeloid expression of hLAL–Flag fusion protein in Tg/KO triple mice**

The previously established Tg/KO triple mouse model (7) was treated with or without doxycycline for 3 mo and analyzed by FACS to assess the profile of hLAL–Flag fusion protein expression. Single-cell suspensions from the bone marrow, blood, and spleen were doubly stained with fluorochrome-conjugated Flag Ab and Abs specific for macrophages, dendritic cells (DCs), neutrophils, or T cells. CD11b⁺ myeloid cells, Gr-1⁻ neutrophils, and CD11c⁺ DCs showed significant hLAL–Flag expression in all tested organs of doxycycline-treated triple mice compared with organs of untreated triple mice and doxycycline-treated wild-type mice (Fig. 1).
FIGURE 2. Reverse of abnormal hematopoietic progenitor development and MDSC expansion by hLAL in Tg/KO triple mice. A, The percentage and total numbers of LSK, LK, CMP, GMP, and MEP populations in the bone marrow of *lat*+/+ mice, *lat*−/− mice, 3-mo doxycycline-treated or doxycycline-ununtreated c-fms-rtTA/(TetO)7-CMV-hLAL bi-transgenic mice, and 3-mo doxycycline-treated or doxycycline-untreated Tg/KO triple mice were analyzed by FACS. Results are mean ± SD from four mice in each group (*n* = 4). *p < 0.05, **p < 0.01, *lat*+/+ and *lat*−/− served as control. B, A representative and statistical analysis of CD11b+Gr-1+ cells in the bone marrow, blood, and spleen from *lat*+/+ mice, *lat*−/− mice, 3-mo doxycycline-treated or doxycycline-ununtreated c-fms-rtTA/(TetO)7-CMV-hLAL bi-transgenic mice, and 3-mo doxycycline-treated or doxycycline-untreated Tg/KO triple mice. Results are mean ± SD from five mice in each group (*n* = 5). *p < 0.05. C, Statistical analysis of Ly6C+ and Ly6G+ cells in gated CD11b+ cells of the bone marrow, blood, and spleen from *lat*+/+ mice, *lat*−/− mice, and 3-mo doxycycline-treated or doxycycline-untreated Tg/KO triple mice. Results are mean ± SD from five mice in each group (*n* = 5). *p < 0.05. KO, *lat*−/− mice; Tg/KO OFF, 3-mo doxycycline-untreated Tg/KO triple mice; Tg/KO ON, 3-mo doxycycline-treated Tg/KO triple mice; Tg OFF, 3-mo doxycycline-untreated c-fms-rtTA/(TetO)7-CMV-hLAL bi-transgenic mice; Tg ON, 3-mo doxycycline-treated c-fms-rtTA/(TetO)7-CMV-hLAL bi-transgenic mice; WT, *lat*+/+ mice.
was no hLAL–Flag protein expression in CD3+ T lymphocytes regardless of doxycycline treatment. This result confirmed that hLAL–Flag fusion protein expression in Tg/KO triple mice was myeloid cell specific. No hLAL–Flag fusion protein was detected in lal+/+ mice regardless of doxycycline treatment, suggesting that induction of hLAL–Flag fusion protein was not caused by doxycycline alone. The morphological shape of CD11b+ myeloid cells in doxycycline-treated lal−/− triple mice was different from that in untreated lal−/− triple mice.

hLAL expression reversed abnormal increase of granulocyte–monocyte progenitors and MDSCs in Tg/KO triple mice

Abnormal development of hematopoietic progenitor cells causes significant expansion of CD11b+Gr-1+ immature myeloid cells

FIGURE 3. Reverse of abnormal MDSC formation and function by hLAL in Tg/KO triple mice. A, Myeloid-specific expression of hLAL reduced differentiation of Lin− BM cells in vitro with GM-CSF and IL-4. Whole, Lin−, and Lin+ bone marrow cells were isolated from lal+/+ mice, lal−/− mice, 3-mo doxycycline-treated or doxycycline-untreated Tg/KO triple mice. Cells were cultured with GM-CSF and IL-4 for 5 d. Representative (%) and statistical analysis (total cell numbers) of CD11b+Gr-1+ cells analyzed by FACS. Results are mean ± SD from five mice in each group (n = 5). *p < 0.05. B, CD11b+Gr-1+ cells were isolated from lal+/+ mice, lal−/− mice, 3-mo doxycycline-treated or doxycycline-untreated Tg/KO triple mice. Cells were cultured with GM-CSF and IL-4 for 7 d. CD11c+ and MHCII+ cells were analyzed by FACS. A representative (%) and statistical analysis of CD11c+ and MHCII+ cells is demonstrated. Results are mean ± SD from five mice in each group (n = 5). *p < 0.05, **p < 0.01. C, MDSCs were isolated from lal+/+ mice, lal−/− mice, 3-mo doxycycline-treated (On) or doxycycline-untreated (Off) Tg/KO triple mice. Cells were cultured with or without 1 μg/ml LPS for 24 h. The cultured supernatants were harvested, and TGF-β, IL-6, IL-10, and MIP-2 were analyzed by ELISA. Results are mean ± SD from five mice in each group (n = 5). *p < 0.05. KO, lal−/− mice; Tg/KO OFF, 3-mo doxycycline-untreated Tg/KO triple mice; Tg/KO ON, 3-mo doxycycline-treated Tg/KO triple mice; WT, lal+/+ mice.
in \(lal^{-/-}\) mice (2). To test whether LAL expression in myeloid cells reverses this problem, \(lal^{+/+}\), \(lal^{-/-}\), and \(lal^{-/+}\) mice, 3-mo doxycycline-treated or doxycycline-untreated c-fms-rtTA/(TetO)_2-CMV-hLAL bi-transgenic mice, and 3-mo doxycycline-treated or doxycycline-untreated Tg/KO triple mice were used. The percentage and total numbers of hematopoietic progenitor LSK (IL-7R\(^{-/-}\)Lin\(^{-/-}\)Sca-1\(^{-/-}\)c-Kit\(^{-/-}\)), common myeloid progenitors (CMP; IL-7R\(^{-/-}\)Lin\(^{-/-}\)Sca-1\(^{-/-}\)c-Kit\(^{-/-}\)CD34\(^{-/-}\)FcRII/III\(^{low}\)), granulocyte–monocyte progenitors (GMP; IL-7R\(^{-/-}\)Lin\(^{-/-}\)Sca-1\(^{-/-}\)c-Kit\(^{-/-}\)CD34\(^{-/-}\)FcRII/III\(^{+}\)\)), and megakaryocyte–erythroid progenitors (MEP; IL-7R\(^{-/-}\)Lin\(^{-/-}\)Sca-1\(^{-/-}\)c-Kit\(^{-/-}\)CD34\(^{-/-}\)FcRII/III\(^{+}\)\)) populations were analyzed by FACS. Similar to that reported previously (2), frequencies of LSK, LK, and GMP were increased in \(lal^{-/-}\) mice compared with those in \(lal^{+/+}\) wild-type mice in the bone marrow. No change was observed in c-fms-rtTA/(TetO)_2-CMV-hLAL bi-transgenic mice regarding doxycycline treatment. Tg/KO triple mice without doxycycline treatment showed similar progenitor frequencies compared with those of \(lal^{-/-}\) mice. However, Tg/KO mice with doxycycline treatment reduced GMP progenitor frequencies close to the level of those of \(lal^{+/+}\) mice (Fig. 2A, 2B). LK and LSK frequencies were slightly affected with no significance after doxycycline treatment in triple mice. Doxycycline treatment also reversed the MEP decrease in triple mice (Fig. 2A). CMP showed relatively no change in all groups. These results indicate that myeloid-specific expression of hLAL is able to rescue hematopoietic developmental defects at the GMP developmental stage but not at the earlier LK, LSK, and CMP stages in the bone marrow.

To see whether correction of the GMP abnormality reverses abnormal myelopoiesis in the bone marrow and other organs, the bone marrow, blood (PBMCs), and spleen were harvested from \(lal^{+/+}\), \(lal^{-/-}\), and \(lal^{-/+}\) mice, c-fms-rtTA/(TetO)_2-CMV-hLAL bi-transgenic mice, and Tg/KO mice. MDSCs were stained with fluorochrom-conjugated anti-CD11b and anti–Gr-1 Abs for FACS analysis. In the blood, the percentage numbers of CD11b\(^{+}\)Gr-1\(^{+}\) MDSCs were drastically increased in \(lal^{-/-}\) mice compared with those in \(lal^{+/+}\) mice, similar to previous reports (2). The numbers of CD11b\(^{+}\)Gr-1\(^{+}\) MDSCs in c-fms-rtTA/(TetO)_2-CMV-hLAL bi-transgenic mice were similar to those in \(lal^{-/-}\) mice regardless of doxycycline treatment. Tg/KO mice without doxycycline treatment showed modestly decreased MDSCs (19.3%) compared with those in \(lal^{-/-}\) mice (31.84%). However, doxycycline treatment for 3 mo further decreased MDSCs (7.40%), closer to that in \(lal^{+/+}\) mice (4.97%) (Fig. 2B). The same trend was also observed in the bone marrow and spleen (Fig. 2B), except that there was no statistical difference in the bone marrow. Because bi-transgenic mice with or without doxycycline treatment showed very little differences from those in \(lal^{+/+}\) mice, bi-transgenic mice were not further analyzed. Only \(lal^{+/+}\) and \(lal^{-/-}\) mice were used as controls in the following studies. MDSCs can be divided into monocytic (CD11b\(^{+}\)Ly6C\(^{-}\)) and granulocytic (CD11b\(^{+}\)Ly6G\(^{+}\)) MDSCs. Both populations were further analyzed for LAL effect. In gated CD11b\(^{+}\) cells, Gr-1\(^{+}\) cells appeared as Ly6C\(^{-}\)Ly6G\(^{+}\) (double positive) in most organs, except in the lung where a significant portion of Gr-1\(^{+}\) cells only appeared as Ly6G\(^{-}\) (Fig. 2C).

**hLAL expression reversed malformation and malfunction of MDSCs in Tg/KO mice**

To test the differentiation ability of bone marrow progenitor cells into MDSCs, Lin\(^{-}\) bone marrow cells were isolated from \(lal^{+/+}\), \(lal^{-/-}\), and \(lal^{-/+}\) mice, doxycycline-untreated and doxycycline-treated Tg/KO mice and cultured in vitro. Five days after GM-CSF and IL-4 stimulation, the CD11b\(^{+}\)Gr-1\(^{+}\) population in cultured cells was increased in \(lal^{-/-}\) mice (79.75%) compared with the hLAL in Tg/KO triple mice. CD11b\(^{+}\)Gr-1\(^{+}\) population from doxycycline-treated mice (60.76%) was partially reduced compared with that from doxycycline-untreated mice (79.00%) (Fig. 3A). A similar study was performed in Lin\(^{-}\) and whole bone marrow subpopulations. The same observation was made in whole bone marrow cells but not in Lin\(^{-}\) bone marrow cells (Fig. 3A). Therefore, LAL deficiency in Lin\(^{-}\) bone marrow cells was partially responsible for MDSC homeostasis.

To examine whether the expanded CD11b\(^{+}\)Gr-1\(^{+}\) population from \(lal^{-/-}\) mice was an immature proliferating precursor population and sensitive to myeloid growth factors (15), CD11b\(^{+}\)Gr-1\(^{+}\) cells isolated from the spleen of \(lal^{+/+}\), \(lal^{-/-}\), and doxycycline-treated Tg/KO mice were...
cultured in vitro and stimulated with GM-CSF. In the absence of GM-CSF, these cells rapidly died (data not shown). In contrast, 7 d after GM-CSF stimulation, 53.83% of MDSCs differentiated into mature CD11c+MHC class II+ DCs in lal−/− mice but only 6.30% in lal+/+ mice, suggesting that there were more immature CD11b+Gr-1+ myeloid cells in the spleen of lal−/− mice than in the spleen of lal+/+ mice and that these precursors possess the ability to differentiate into mature myeloid cells. Most of CD11b+Gr-1+ myeloid cells in lal−/− mice were mature cells (e.g., neutrophils), which lack the ability to convert into DCs. Expression of hLAL in doxycycline-treated Tg/KO mice was able to reduce the immature population. The conversion rate into DCs in doxycycline-treated Tg/KO mice was 15.04% after GM-CSF stimulation compared with 34.99% in untreated mice (Fig. 3B). Because DCs are major APCs, MHC class II expression was used to confirm the identity of these cells.

Functionally, when in vitro-cultured MDSCs from these mice were stimulated with LPS, secretion of immunomodulators TGF-β, IL-6, IL-10, and MIP-2 in the culture medium was significantly increased in lal−/− mice compared with that in lal+/+ mice. Expression of hLAL in doxycycline-treated Tg/KO mice reversed abnormal secretion of these cytokines compared with that in untreated mice (Fig. 3C). TGF-β, IL-6, and IL-10 are requisites for T cell suppression and Th2 cell polarization (16).

**FIGURE 5.** Reverse of T cell developmental and maturation defects by hLAL in Tg/KO triple mice. A. Total cells were isolated from the thymus of lal+/+ mice, lal−/− mice, 3-mo doxycycline-treated or doxycycline-untreated Tg/KO triple mice. Anti-CD44 and anti-CD25 Abs were used to define DN T cell subsets by FACS. Representative and statistical analysis of DN1 (CD44+CD25−), DN2 (CD44+CD25+), DN3 (CD44−CD25+), and DN4 (CD44−CD25−) subsets demonstrated by FACS. Results are mean ± SD from four mice in each group (n = 4). *p < 0.05. B. Peripheral CD4+ and CD8+ profiles in the spleen and blood (PBMC) of lal+/+ mice, lal−/− mice, 3-mo doxycycline-treated or doxycycline-untreated Tg/KO triple mice were stained with CD4+ and CD8+ Abs. A representative (%) and statistical analysis (total cell number) of CD4+ and CD8+ populations was performed by FACS. Results are mean ± SD from six mice in each group (n = 6). *p < 0.05. C. The percentage numbers of Foxp3+ Tregs among total CD4+ T cells from the spleen of lal+/+ mice, lal−/− mice, 3-mo doxycycline-treated or doxycycline-untreated Tg/KO triple mice were analyzed by FACS. Results are mean ± SD from six mice in each group (n = 6). *p < 0.05. KO, lal−/− mice; Tg/KO OFF, 3-mo doxycycline-untreated Tg/KO triple mice; Tg/KO ON, 3-mo doxycycline-treated Tg/KO triple mice; WT, lal+/+ mice.
hLAL expression reversed pathogenic disorganization of the thymus and spleen in Tg/KO mice

LAL deficiency results in disorganization of the thymus and spleen structures in lat−/− mice (1). To determine if this disorganization correlates with infiltration of immature CD11b+Gr-1+ cells, the thymus and spleen were harvested from lat+/+ mice, lat−/− mice, and doxycycline-untreated and doxycycline-treated Tg/KO mice. In lat+/+ mice, the cortical and medullary regions were readily distinguished from one another in the thymus with few Gr-1+ and CD11b+ cells present. The medullary area was difficult to identify in the thymus of lat−/− mice (2) and doxycycline-untreated Tg/KO mice with massive Gr-1+ and CD11b+ cell infiltrations. In age-matched doxycycline-treated Tg/KO mice, a readily identifiable medulla area reappeared with significantly reduced numbers of CD11b+Gr-1+ double-positive cells (Fig. 4). In the spleen of lat−/− mice and doxycycline-untreated Tg/KO mice, massive accumulation of CD11b+Gr-1+ double-positive cells were present in the abnormal red pulp area. In age-matched doxycycline-treated Tg/KO mice, the number of CD11b+Gr-1+ double-positive cells was significantly reduced with restoration of the spleen organization (Fig. 4).

hLAL expression reversed abnormal T cell development and peripheral T cell maturation in Tg/KO mice

Our previous studies revealed deficiency in T cell development (in the thymus) and peripheral T cell maturation (mainly in the spleen) in lat−/− mice. In addition to the intrinsic defect in T cells, massive MDSC infiltration into the thymus and spleen may also contribute to the abnormal T cell development and maturation in lat−/− mice. To test this point, T cell development and maturation in lat+/+ mice, lat−/− mice, doxycycline-untreated and doxycycline-treated Tg/KO mice were analyzed by FACS.

T cell development in the thymus can be divided into CD4+CD8− double-negative (DN), CD4+CD8+ double-positive (DP), and CD4−CD8−/CD4−CD8+ single-positive (SP) stages. Our previous data showed that T cells in almost all these stages were significantly reduced in lat−/− mice compared with those in lat+/+ mice (except that the decrease in CD4+CD8− SP cells was only observed at 9 mo of age). We also reported that this blockage in T cell development occurred initially at the DN3 (CD44+CD25−) to DN4 (CD44+CD25+) transition in the thymus (1). When analyzed by CD4 and CD8 markers, myeloid hLAL expression partially reversed this defect. The percentage number of DN3 subset cells was lower (56.13 ± 2.01 versus 69.36 ± 3.91%), whereas the percentage number of DN4 subset cells was higher (27.94 ± 3.06 versus 16.45 ± 2.64%) in the thymus of doxycycline-treated Tg/KO mice compared with that in the thymus of doxycycline-untreated Tg/KO mice (Fig. 5A). hLAL expression also partially reversed the deficiency of T cells at other development stages, including DN, DP, and SP stages when analyzed by CD4, CD8 markers (Supplemental Fig. 1).

The numbers of T cells localized within other organs were also analyzed using these mouse strains. Compared with 3-mo doxycycline-untreated Tg/KO mice, the numbers of CD4+ T cells...
were increased in the blood (from 2.93 to 5.64%) and the spleen (from 7.71 to 12.90%) of age-matched doxycycline-treated Tg/KO mice. CD8+ T cells were also increased in the blood (from 0.63 to 4.02%) and the spleen (from 2.82 to 4.24%) of doxycycline-treated Tg/KO mice compared with that in doxycycline-untreated mice (Fig. 5B). In contrast, the numbers of CD4+CD25+ regulatory T cells (Tregs) were decreased in the spleen of doxycycline-treated Tg/KO mice compared with those in doxycycline-untreated Tg/KO mice (Fig. 5C). Previously, we reported that Tregs were increased in lal−/− mice (1).

hLAL expression reversed abnormal T cell function in Tg/KO mice

In addition to inhibiting T cell proliferation, LAL deficiency-induced MDSC expansion may also suppress T cell function, such as response to TCR stimulation. CD4+ splenic T cells from lal+/+ mice, lal−/− mice, doxycycline-untreated and doxycycline-treated Tg/KO mice were cultured in vitro with anti-CD3 Ab plus anti-CD28 Ab. T cell proliferation was measured by CFSE labeling dilution (cell division) and FACS. As demonstrated in Fig. 6A, LAL deficiency abolished TCR-stimulated CD4+ T cell

**FIGURE 7.** Reverse of the MDSC immunosuppressive function by hLAL in Tg/KO triple mice. CD4+ T cells were isolated from the wild-type spleen and labeled with CFSE. MDSCs were isolated from the spleen of lal−/− mice, lal−/− mice, 3-mo doxycycline-treated or doxycycline-untreated Tg/KO triple mice. CFSE-labeled wild type CD4+ T cells were cocultured with MDSCs in vitro on anti-CD3 plus anti-CD28 Abs-coated plates for 4 d. The ratio of MDSC/CD4+ T cells was 1:5. A, CFSE cell division of CD4+ T cells was measured by FACS. B, CD69 expression on CD4+ T cells was measured by FACS. A statistical analysis is presented. Results are mean ± SD from three mice in each group (n = 3). *p < 0.05. C, Phosphorylation of p-CD3ε chain on CD4+ T cells was measured by FACS. A statistical analysis is presented. Results are mean ± SD from three mice in each group (n = 3). *p < 0.05. D, The concentrations of IL-2, IL-4, and IFN-γ in cocultured medium were measured by ELISA. Results are mean ± SD from three mice in each group (n = 4). *p < 0.05. E, Phosphorylation of p-ZAP70 in CD4+ T cells was measured by FACS with anti–p-ZAP70 and anti-CD4 Abs. Results are mean ± SD from three mice in each group (n = 4). *p < 0.05. KO, lal−/− mice; Tg/KO OFF, 3-mo doxycycline-untreated Tg/KO triple mice; Tg/KO ON, 3-mo doxycycline-treated Tg/KO triple mice; WT, lal−/− mice.
proliferation (represented by peaks) in *lal<sup>−/−</sup>* mice compared with that in *lal<sup>+/+</sup>* mice. However, myeloid hLAL expression partially restored T cell response to TCR stimulation in doxycycline-treated Tg/KO mice, whereas a minor restoration was observed in doxycycline-untreated mice. Furthermore, myeloid hLAL expression partially restored CD69 expression on T cells from doxycycline-treated Tg/KO mice compared with that on T cells from doxycycline-untreated mice (Fig. 6B). CD69 is the earliest inducible cell surface glycoprotein during lymphoid activation and a signal-transmitting receptor in lymphocyte proliferation. Myeloid hLAL expression also reduced apoptosis by annexin V staining and decreased expression of proapoptotic signaling molecules in T cells from doxycycline-treated Tg/KO mice compared with those in T cells from doxycycline-untreated mice (Fig. 6C). Functionally, expression of multiple CD<sup>4+</sup> and CD<sup>8+</sup> T cell secreting lymphokines was measured by real-time PCR using sequence-specific oligonucleotide primers after stimulation with anti-CD3 Ab plus anti-CD28 Ab. Myeloid hLAL expression partially restored mRNA levels of Th1 and Th2 lymphokines in doxycycline-treated Tg/KO mice compared with those in doxycycline-untreated mice. The expression of Th17 lymphokine IL-17 partially restored in doxycycline-treated Tg/KO mice compared with that on T cells from doxycycline-untreated mice (Fig. 6D). The expression of Th17 lymphokine IL-17 remained relatively unchanged (Fig. 6D).

**hLAL expression reversed MDSC immunosuppressive function in Tg/KO mice**

To determine directly whether hLAL expression in myeloid cells is responsible for controlling MDSC immunosuppression of T cell proliferation and function, an in vitro MDSC and T cell coculturing experiment was performed. CFSE-labeled *lal<sup>−/−</sup>* splenic CD<sup>4+</sup> T cells were cultured in vitro and stimulated with anti-CD3 Ab plus anti-CD28 Ab in the presence or absence of CD11b<sup>Gr-1<sup>−</sup></sup> MDSCs (MDSC/T ratio = 1:5) that were isolated from the spleen of *lal<sup>−/−</sup>* mice, *lal<sup>+/+</sup>* mice, or doxycycline-treated or doxycycline-untreated Tg/KO mice. Proliferation of T cells was evaluated as CFSE dilution (cell division) by FACS. MDSCs from *lal<sup>−/−</sup>* mice. MDSCs from doxycycline-treated Tg/KO triple mice reversed this inhibitory effect, whereas MDSCs from doxycycline-untreated Tg/KO mice still displayed strong inhibitory activity (Fig. 7A). MDSCs from doxycycline-treated Tg/KO mice also partially restored CD69 expression and p-CD3<sup>+</sup> activation (phosphorylation) compared with MDSCs from doxycycline-untreated mice after TCR stimulation (Fig. 7B, 7C). The TCR–CD3 complex plays a critical role in T cell signaling. Dissociation between TCR and CD3<sup>+</sup> prevents TCR-mediated signaling (17). In addition, expression levels of TCR downstream IL-2, IL-4, and IFN-γ mRNA levels in T cells were partially restored in doxycycline-treated Tg/KO mice compared with those in untreated triple mice after TCR stimulation (Fig. 7D), indicating restoration of CD4<sup>+</sup> T cell function. When the TCR signaling linked to lymphokine production in CD4<sup>+</sup> T cells was investigated, coculture with MDSCs from *lal<sup>−/−</sup>* mice strongly inhibited p-ZAP70/Syk activation (phosphorylation) in *lal<sup>−/−</sup>* CD4<sup>+</sup> T cells compared with MDSCs from *lal<sup>+/+</sup>* mice. T cell culture with MDSCs from doxycycline-treated Tg/KO mice partially restored phosphorylation of p-ZAP70, whereas MDSCs from doxycycline-untreated mice again inhibited cytokine synthesis after TCR stimulation (Fig. 7E). These results suggest that LAL deficiency-induced MDSC malfunction alters integrity of the

**FIGURE 8.** Reverse of abnormal ROS production and arginase expression by hLAL in Tg/KO triple mice. A, Myeloid-specific expression of hLAL reduced ROS production in spleen MDSCs. MDSCs were isolated from the spleen of *lal<sup>−/−</sup>* mice, *lal<sup>+/+</sup>* mice, or doxycycline-treated or doxycycline-untreated Tg/KO triple mice. n = 4. Cells were incubated with 2 μm DCFDA. A representative of ROS concentration in CD11b<sup>Gr-1<sup>−</sup></sup> cells by FACS analysis is presented. Results from statistical analysis are mean ± SD from three mice in each group (n = 3). *p < 0.05. B, Myeloid-specific expression of hLAL reduced arginase expression in spleen MDSCs. Statistical analysis of arginase activity in CD11b<sup>Gr-1<sup>−</sup></sup> cells from the spleen of *lal<sup>−/−</sup>* mice, *lal<sup>+/+</sup>* mice, or doxycycline-treated or doxycycline-untreated Tg/KO triple mice. Results are mean ± SD from four mice in each group (n = 5). *p < 0.05. KO, *lal<sup>−/−</sup>* mice; Tg/KO OFF, 3-mo doxycycline-untreated Tg/KO triple mice; Tg/KO ON, 3-mo doxycycline-treated Tg/KO triple mice; WT, *lal<sup>+/+</sup>* mice.
TCR complex and its downstream intracellular signaling in CD4+ T cells to downregulate lymphokine synthesis.

hLAL expression reversed abnormal ROS production and arginase expression in Tg/KO triple mice

ROS play a very important role in regulating MDSC immunosuppressive function on T cells (18). To evaluate ROS production, purified CD11b+Gr-1+ cells from the spleen of lal+/+ mice, lal−/− mice, doxycycline-untreated and doxycycline-treated Tg/KO mice were measured for ROS production by DCFDA labeling and FACS. As demonstrated in Fig. 8A, LAL deficiency significantly increased ROS production in CD11b+Gr-1+ cells of lal−/− mice compared with that in lal+/+ mice (76.94 versus 22.38%). Myeloid hLAL expression partially inhibited ROS production in CD11b+ Gr-1+ cells of doxycycline-treated Tg/KO mice compared with that in untreated mice (41.96 versus 72.03%). Increased arginase expression is another important mechanism in regulating MDSC immunosuppressive function on T cells (19). In the same purified spleen CD11b+Gr-1+ cells, LAL deficiency significantly increased arginase expression in lal−/− mice compared with that in lal+/+ mice (Fig. 8B). Myeloid hLAL expression partially inhibited arginase expression in CD11b+Gr-1+ cells of doxycycline-treated Tg/KO triple mice. Therefore, both ROS production and arginase upregulation are two critical mechanisms for MDSCs in lal−/− mice.

hLAL expression reversed abnormal activation of stat3 and NF-κB in Tg/KO mice

The functional abnormality of MDSCs in lal−/− mice was also reflected at the activation levels of stat3 and NF-κB p65 signaling molecules as we previously reported (2). Abnormal activation of these molecules serves as hallmarks for MDSC malformation and malfunction in multiple animal models (2, 11, 20–22). To test if activation of stat3 and NF-κB p65 is required for MDSC differentiation, inhibitors were used to block these two pathways. First, Lin− bone marrow cells were isolated from lal−/− mice and cultured in vitro. Five days after GM-CSF and IL-4 stimulation in the presence or absence of stat3 inhibitor cucurbitacin B or NF-κB inhibitor ammonium pyrrolidinedithiocarbamate (PDTC), CD11b+Gr-1+ populations were analyzed by FACS. Both cucur-

![Figure 9](http://www.jimmunol.org/)

**FIGURE 9.** stat3 and NF-κB inhibitors blocked proliferation and function of MDSCs in lal−/− mice. A. Lin− bone marrow cells were isolated from lal−/− mice (KO). Cells were cultured with GM-CSF and IL-4 for 5 d in the presence of 2 μm cucurbitacin B or 5 μm PDTC. Statistical analysis of total cell numbers of CD11b+Gr-1+ cells by FACS. Results are mean ± SD from six mice in each group (n = 6). *p < 0.05, **p < 0.01. B. MDSCs were isolated from the spleen of lal−/− mice and cocultured with 2 μm cucurbitacin B or 5 μm PDTC for 2 d. CFSE-labeled wild-type CD4+ T cells were cocultured with MDSCs in vitro on anti-CD3 plus anti-CD28 Abs-coated plates for 4 d. The ratio of MDSC/CD4+ T cells was 1:5. CFSE cell division of CD4+ T cells was measured by FACS. A representative histogram of five experiments is presented. C. CD69 expression on CD4+ T cells was measured by FACS in the above study. Results are mean ± SD from six mice in each group. n = 6. **p < 0.01. D. Cells from the bone marrow (BM), blood (PBMC), and spleen of lal+/+ mice (WT), lal−/− mice (KO), 3-mo doxycycline-treated (Tg/KO ON) or doxycycline-untreated (Tg/KO OFF) Tg/KO triple mice were stained with CD11b and Gr-1 Abs, followed by intranuclear staining with p-stat3 or p–NF-κB Ab. Percentages of p-stat3+ and p–NF-κB+ cells from three independent experiments analyzed by FACS. n = 3. *p < 0.05.
bacitracin B and PDTC inhibited MDSC differentiation from Lin− bone marrow cells of lat−/− mice (Fig. 9A). Next, in the CFSE labeling study, cucurbitacin B and PDTC reversed suppressive function of spleen MDSCs from lat−/− mice on wild-type T cell proliferation (Fig. 9B). They also reversed CD69 expression on wild-type T cells (Fig. 9C). Importantly, expression of hLAL in doxycycline-treated Tg/KO mice significantly reduced activation of stat3 and NF-κB p65 in MDSCs in various compartments compared with that in MDSCs in untreated mice (Fig. 9D).

**Gr-1 depletion reversed T cell proliferation and decreased T cell apoptosis in lat−/− mice**

To address the specific effect of MDSCs on T cell proliferation, anti–Gr-1 Ab was injected into lat−/− mice to deplete MDSCs. After 2 wk of injection, the CD4+ T cell population was partially recovered (Fig. 10A). This observation was associated with decreased apoptosis by annexin V analysis (Fig. 10B) and increased proliferation by BrdU analysis (Fig. 10C). Even though there was no significant improvement in the CD8+ T cell population, decreased apoptosis and increased proliferation were also observed (Fig. 10B, 10C). This clearly proves that MDSCs are partially responsible for decrease of T cell population in lat−/− mice.

**Discussion**

One major manifestation in lat−/− mice is the massive expansion and infiltration of myeloid cells into multiple organs, including the thymus and spleen (2). By contrast, T cell development, proliferation, and function are dramatically decreased, leading to impairment of the immune surveillance system (1). As a consequence, LAL deficiency results in severe pathogenic phenotypes in multiple organs as we previously reported (1–6). Based on these observations, we hypothesize that LAL in myeloid cells is required for sustaining normal myeloid cell formation and function in multiple steps in multiple compartments. To prove directly that LAL deficiency in myeloid cells is a causative factor for lat−/− pathogenesis, hLAL was specifically introduced into myeloid lineage cells in lat−/− mice by the myeloid-specific c-fms 7.2-kb promoter/intron2 DNA sequence. The rationale for this study is that LAL is a critical enzyme that controls neutral lipid metabolism. Downstream metabolic derivatives of these lipids serve as hormonal ligands for anti-inflammatory nuclear receptors (e.g., peroxisome proliferator-activated receptor-γ, or PPAR-γ), which belong to a special group of transcription factors. PPAR-γ is a negative modulator for multiple proinflammatory molecules (e.g., IL-6, IL-1β, TNF-α, MMP12, Api6, etc.) that stimulate MDSC expansion through intracellular signaling molecule (e.g., stat3) (21). LAL deficiency leads to insufficient ligands for PPAR-γ activation and promotes upregulation of multiple inflammatory molecules. This has been confirmed by the Affymetrix GeneChip microarray study (6). Overexpression of several of these PPAR-γ downstream genes (i.e., MMP12, Api6) causes MDSC expansion and cancer in vivo (11, 20, 22). By reconstitution of LAL in lat−/− mice, LAL expression may restore ligand production that leads to inhibition of proinflammatory cytokines and limits MDSC expansion to prevent T cell apoptosis.

In the bone marrow of lat−/− mice, LAL deficiency caused abnormal development of hematopoietic progenitor cells at multiple stages, including Lin− LSK, LK, and GMP populations (Fig. 2) (2). Myeloid-specific expression of hLAL corrected the abnormal increase of the GMP population in Tg/KO mice but not LSK and LK populations (Fig. 2). It is more likely that abnormal development of LSK and LK progenitor cells is due to the intrinsic defect. In lat−/− mice, differentiation from Lin− progenitor cells to CD11b+Gr-1+ immature cells was abnormally increased. Myeloid hLAL expression successfully reversed this abnormality (Fig. 3A). More systematic analysis showed myeloid hLAL expression reversed abnormal expansion of CD11b+Gr-1+ MDSCs in the bone marrow, blood, and spleen (Fig. 2B). MDSCs can be divided into monocytic (CD11b+Ly6C+) and granulocytic (CD11b+Ly6G+) MDSCs (8). Notably, most gated CD11b+ cells showed Ly6C+ and Ly6G+ double positive in lat−/− mice (Fig. 2C). Taken together, these results clearly support that LAL expression in myeloid lineage cells is critical for normal hematopoiesis and myelopoiesis.

MDSCs are a heterogeneous population of cells consisting of myeloid progenitor cells and immature myeloid cells. In healthy individuals, immature myeloid cells quickly differentiate into mature granulocytes, macrophages, or DCs. In pathological conditions, blockade of the differentiation of immature myeloid cells into mature myeloid cells results in the expansion of this population (8–10). The mechanisms responsible for this disruption are poorly understood. In lat−/− mice, abnormalities in myeloid cells were evident, as stat3 and NF-κB intracellular signaling molecules were aberrantly activated (Fig. 9D). Inhibition of both molecules by inhibitors resulted in reduced MDSC proliferation and suppressive function (Fig. 9A–C). LAL deficiency blocked differentiation of immature myeloid cells to mature DCs (Fig. 3C). Myeloid hLAL expression in Tg/KO mice rescued differentiation of immature myeloid cells to mature DCs and reduced abnormal activation of intracellular signaling molecules in MDSCs. Therefore, the LAL pathway in myeloid cells is essential for maintaining differen-
tiation of immature cells to mature cells. Thus, MDSC expansion in \( \text{lal}^{-/-} \) mice results from both an abnormal increase in myelopoiesis in the bone marrow and inhibition of differentiation to mature myeloid cells.

Infiltration of MDSCs in the thymus and spleen of \( \text{lal}^{-/-} \) mice implicates the interrelationship between MDSCs and T cells both in terms of differentiation and function. When MDSCs were depleted by anti–Gr-1 Ab injection, T cell numbers were recovered accordingly (Fig. 10). This clearly shows the role of MDSCs in the maturation of T cells in \( \text{lal}^{-/-} \) mice. LAL inhibits MDSC suppressive functions to influence T cells at four critical steps. First, LAL expression in monocyteic cells blocks MDSC infiltration into the immune organs such as the thymus and spleen. Loss of the LAL function leads to massive infiltration of MDSCs into these organs, causing structural disorganization in \( \text{lal}^{-/-} \) mice (1). Myeloid hLAL expression significantly reduced MDSC infiltration and partially restored organization of the thymus and spleen (Fig. 4).

Second, LAL deficiency leads to impaired T cell development in the thymus of \( \text{lal}^{-/-} \) mice. Myeloid hLAL expression reversed abnormal T cell development in \( \text{lal}^{-/-} \) thymus (Fig. 5, Supplemental Fig. 1). Third, LAL deficiency leads to reduced T cell proliferation in the spleens of \( \text{lal}^{-/-} \) mice. MDSCs in \( \text{lal}^{-/-} \) mice generate abnormally high levels of immunomodulators (e.g., cytokines, ROS, arginase, etc.) that are involved in suppressing T cell activity (Figs. 3C, 8). Myeloid hLAL expression diminished these immunomodulators and increased T cell maturation and proliferation in \( \text{lal}^{-/-} \) mice (Figs. 6A–C, 7A, 7B). Fourth, LAL deficiency leads to substantial increase of CD4^+Foxp3^+ Tregs in \( \text{lal}^{-/-} \) mice. It is well known that Tregs inhibit CD4^+ T cell functions such as cytokine production and proliferation (23). Myeloid hLAL expression suppressed Tregs in \( \text{lal}^{-/-} \) mice (Fig. 5C).

LAL deficiency-induced MDSC expansion not only suppresses T cell proliferation but also inhibits T cell cytokine production. T cell dysfunction in \( \text{lal}^{-/-} \) mice is characterized by a failure to respond to TCR stimulation, loss of expression of TCR \( \zeta \) chain and CD69, inactivation of p-ZAP70/Syk intracellular signaling, and lymphocyte production. Myeloid hLAL expression enhanced T cell responses to TCR stimulation by restoring expression of TCR \( \zeta \) chain and CD69, activation of T cell signaling, and lymphokine production (Figs. 6, 7). Although MDSCs play an important role in T cell proliferation and function, intrinsic defect due to LAL deficiency in T cells may also contribute to T cell malformation and malfunction. This may explain why myeloid hLAL expression only partially corrects T cell pathogenic phenotypes.

MDSCs were originally identified through their role in suppressing immunity to tumors. Studies in \( \text{lal}^{-/-} \) mice suggest that MDSCs also contribute to other diseases in multiple organs. Myeloid hLAL expression significantly ameliorated pathogenic phenotypes in the liver, small intestine, and lung of \( \text{lal}^{-/-} \) organs as reported previously (7, 21). Yet, in general, LAL serves an anti-inflammatory function by negatively regulating proinflammatory cytokines/chemokines, MMPs, apoptosis inhibitors, and oncogenes through the anti-inflammatory PPAR-\( \gamma \) pathway (5, 6).

When several of these genes (e.g., Apip and MMP12) were overexpressed in myeloid cells or epithelial cells, they triggered regional or systemic MDSC expansion, T cell suppression, and cancer formation in transgenic mice (11, 20, 22). In summary, neutral lipid metabolism by LAL and its downstream pathways in myeloid cells are critical in controlling hematopoiesis, myelopoiesis, and lymphopoiesis.

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Disclosures

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