Supplemental Data

Apoptotic Cells Promote Their Own Clearance and Immune Tolerance through Activation of the Nuclear Receptor LXR

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Supplemental Fig. 1

**A) Phagocytosis of E.Coli**

![Image of E.Coli phagocytosis](image)

**B) Total % of phagocytosis**

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<thead>
<tr>
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<th>WT</th>
<th>LXR DKO</th>
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<td>% macs with engulfed bacteria</td>
<td></td>
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<tr>
<td>% with 1 bacteria</td>
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<td>% with &gt;1 bacteria</td>
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**C) Phagocytosis of E.coli-GFP**

![Image of E.coli-GFP phagocytosis](image) 100x

**D) Phagocytosis of Microspheres**

![Image of Microsphere phagocytosis](image)

**E) Total % of phagocytosis**

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<tr>
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<th>WT</th>
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<td>% macs with engulfed spheres</td>
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**Figure S1. Loss of LXR expression does not alter macrophage phagocytosis of bacteria or fluorescent spheres.** A) Fluorescence microscopic evaluation 1 h after incubation of GFP-E.Coli with thioglycolate-elicited peritoneal macrophages from WT or LXR DKO (10:1 ratio). B) Quantification of total phagocytosis evaluated by fluorescent microscopy. C) High magnification view of WT or LXR DKO macrophages that engulfed fluorescent bacteria. D) Fluorescence microscopic evaluation 1 h after incubation of fluorescent FITC latex beads with thioglycolate-elicited peritoneal macrophages from WT or LXR DKO (10:1 ratio). E) Quantification of phagocytosis evaluated by fluorescent microscopy. F) High magnification view of WT or LXR DKO macrophages that have engulfed fluorescent latex beads.
Supplemental Fig. 2

Figure S2. LXR agonist promotes the phagocytosis of apoptotic cells *in vivo*. WT C57/BL6 mice (3 mice/group) were administered 10 mg/kg GW3965 for 3 days. Apoptotic cells ($10^8$) were fluorescently labeled and inoculated IP. After 30 or 60 min, uptake of apoptotic cells was evaluated by flow cytometry using a CD68 antibody. Experiment was repeated 3 times with comparable results.
Supplemental Fig. 3

A) Non-specific binding of AT to B cells in vivo

B) In vivo AT association

C) In vivo AT association

D) Immature DCs

In vivo AT association with peritoneal cells in vivo and AT engulfment by dendritic cells. A) AT do not associate non-specifically with peritoneal B cells. In vivo association of cfse-labeled apoptotic cells with WT or LXR DKO B cells was determined by flow cytometry. Results from 3 separate mice are shown for each genotype. B) In vivo association of cfse-labeled apoptotic cells with WT, Apoe-/- and Abcg1-/- macrophages was determined by flow cytometry. Results from 3 separate mice are averaged for each genotype. C) In vivo association of cfse-labeled apoptotic cells with background-matched (B6/129) WT and Abca1-/- macrophages was determined by flow cytometry. Note, Abca1-/- mice on B6 background are not viable and therefore Abca1-/- macrophages cannot be directly compared to C57Bl/6 mice shown in (B). D) Altered phagocytosis of AT in dendritic cells lacking LXRαβ. Quantification of phagocytosis by confocal microscopy after incubation of apoptotic thymocytes (AT) for 1 h with WT or LXR DKO dendritic cells. Cells were pretreated with vehicle or 1 μM GW3965 for 48 h as indicated.
Supplemental Fig. 4

Figure S4. Apoptotic cells do not induce apoptosis in LXR-deficient macrophages.  A) Representative thymus, lung, and testis sections (4 µm) from 40 week-old WT and LXRαβ−/− mice were analyzed by TUNEL staining (green) and staining for CD68+ macrophages (red). Free apoptotic cells are indicated by arrows (right panel).  B) Spleen sections from WT and LXR DKO mice were stained with B220 and TUNEL as indicated. TUNEL+ cells colocalize with B cells. Bar represents 25 µm. C,D) Peritoneal macrophages from WT and LXR DKO mice show no evidence of cell death after culture for 18 h with AT. C) AT were labeled with CMFDA and macrophages stained with F4/80 antibody.  D) Cultures were stained with TUNEL, DAPI and F4/80.
Figure S5. LXR WT and LXR DKO thymocytes are equally sensitive to uv irradiation- and dexamethasone-induced apoptosis. Cells obtained from thymi of 4 week-old WT and LXR DKO mice were UV irradiated (A) or stimulated with dexamethasone (B) at the indicated doses for the indicated times. Apoptosis was evaluated by flow cytometry with AnnexinV staining.
Figure S6. Identification of *Mer* as an LXR-responsive gene by transcriptional profiling. 
A) Results from microarray analysis of the expression of known LXR targets, TAM family members, and receptors involved in macrophage phagocytosis. RAW-LXRα cells were stimulated with GW3965 (1µM) and LG268 (100 nM) for 24 h. Total RNA was purified and hybridized with Affymetrix 430 v2.0 arrays at the UCLA microarray core and data analyzed with GeneSpring GX 7.3 software (Agilent Technologies). Data are represented as a heat map. B) WT and LXR DKO macrophages were cultured in vitro with GW3965 and LG268 for 24 h in the presence of 0.5% FBS. Total RNA was purified and subjected to Northern analysis using P32-labeled probes.
Supplemental Fig. 7

Figure S7. Direct regulation of Mertk expression by LXR/RXR heterodimers. A) Analysis of the mouse Mertk gene promoter revealed a potential LXR response element (LXRE) 1.7kb upstream of transcription start site. Electrophoretic mobility shift assay (EMSA) analysis of in vitro translated LXR and RXR protein binding to the Mer LXRE or a mutated form of this element. Disruption of this interaction was achieved with an RXR antibody. B) Interaction of FLAG tagged LXR/RXR heterodimers with the Mer LXRE evaluated by chromatin immunoprecipitation (ChIP) assays. RAW-FLAG-LXRα cells were stimulated for 1h with GW3965 and subjected to crosslinking and immunoprecipitation with anti-IgG control, anti-FLAG and anti-RXR antibodies. C) ChIP analysis of the interaction of PolII and N-CoR with the LXREs in the Mertk and SREBP-1c promoters. RAW-LXRα cells were stimulated for 1h with GW3965 and subjected to crosslinking and immunoprecipitation with anti-IgG control, anti-PolII and anti-N-CoR antibodies.
**Figure S8. Effect of LXR activation on cytokine production.** Macrophages were incubated with AT and treated with TLR ligands as indicated. Gene expression was analyzed by realtime PCR.
Figure S9. Flow cytometric analysis of CD19+B220+ B cells from spleen and lymph node of WT and LXR DKO mice.
Supplemental Fig. 10

Figure S10. Absolute cell counts of hematopoietic populations in spleen and lymph node of WT and LXR DKO mice determined by flow cytometry. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure S11. Flow cytometric analysis of B220+CD3+ (A) and FoxP3+CD4+ (B,C) cell populations from spleen and lymph node of WT and LXR DKO mice. *P < 0.05, **P < 0.01.
Supplemental Fig. 12

Figure S12. *Lxrαβ/-* mice accumulate IgG-containing immune complexes in multiple tissues. A) Kidneys sections from LXR DKO mice of 12, 24, 36 and 48 weeks of age were stained with anti-mouse IgG antibody or DAPI. Bar represents 50 µm. B) Skin sections from WT and LXR DKO mice at 48 weeks of age were stained with H&E, anti-mouse IgG and DAPI. Bar represents 100 µm. C) Lung sections from WT and LXR DKO mice at 48 weeks of age were stained with H&E, anti-mouse IgG, anti-CD68 or anti-CD4. Bars represent 100 µm in top panels and 25 µm in the rest of the panels.
Supplemental Fig. 13

A

WT  LXRα-/-  LXRβ-/-  LXRαβ-/-

IgG

DAPI

B

WT  LXRαβ-/-  ApoE-/-  ABCG1-/-

H&E

IgG

ABCA1+/+  ABCA1-/-

H&E

IgG
Figure S13. A) Combined loss of both LXRα and LXRβ is required for full manifestation of the autoimmune phenotype. Kidney sections from WT, Lxrα-/- Lxrβ-/- and LXR DKO mice at 48 weeks of age were stained with anti-mouse IgG antibody or DAPI. B) Mice lacking key LXR target genes involved in cholesterol metabolism do not exhibit the autoimmune phenotype of Lxrαβ-/- mice. Kidney sections from WT, LXR DKO, ApoE-/-, or Abcg1-/- mice (C57Bl/6 background) at 40 weeks of age were stained with anti-mouse IgG antibody or H&E. Kidney sections from Abca1+/+ and Abca1-/- mice (C57Bl/6/sv129 mixed strain) at 52 weeks of age stained with anti-mouse IgG antibody or H&E. Bars represent 50 μm.
Table S1. Glomerulonephritis and altered renal function in LXR deficient mice.

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<th>Glomerular Pathology Parameters</th>
<th>LXR WT</th>
<th>LXR DKO</th>
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<tr>
<td>Survival (%)</td>
<td>100</td>
<td>60</td>
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<tr>
<td>Glomerulonephritis (scale 0-4)</td>
<td>0 ± 0</td>
<td>2.5 ± 0.7</td>
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<tr>
<td>Diuresis (ml per mouse/day)</td>
<td>0.3 ± 0.11</td>
<td>1.1 ± 0.4</td>
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<td>Urine Urea (mg/dL)</td>
<td>6150 ± 540</td>
<td>3200 ± 423</td>
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<tr>
<td>Urine Albumin/Creatinine (ug/mg)</td>
<td>7.4 ± 2.5</td>
<td>24.7 ± 8.2</td>
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WT and LXRαβ-/- mice (12/group) were evaluated for survival after 48 weeks. Glomerular damage was evaluated on a scale 0-4. Daily diuresis, urea, creatinine and proteinuria values were evaluated in metabolic cages with mice housed individually.