

(A) Flow cytometric-based analysis of the expression of of the indicated co-stimulatory molecules or activation markers on wild-type (Alox15^{+/+}) and Alox15^{-/-} CD11c⁺ bone marrow-derived dendritic cells (BMDCs) after LPS-induced maturation (100ng LPS/ml). (B) Effect of the 15-lipoxygenase inhibitor Baicalein on the expression of indicated surface molecules on human monocyte-derived DCs in the absence and presence of maturation cocktail. Data shown are representative for at least 3 independent experiments (n=3). Error bars represent SEM. *p<0.05, **p<0.005, Students *t* test.



(A) Flow-cytometric-based analysis of the expression of the indicated co-stimulatory molecules or activation markers on wild-type (Alox15^{+/+}) and Alox15^{-/-} CD11c⁺ bone marrow-derived dendritic cells after LPS-induced maturation (100ng LPS/ml) in the absence and presence of oxydized phosphatidylcholine (oxPC) (50µg/ml). Data shown are representative for at least 3 independent experiments (n=3). Error bars represent SEM. *p<0.05, **p<0.005, Students *t* test.



(A) Measurement of T-cell proliferation by [methyl-3H] thymidine incorporation after allogeneic stimulation of BALB/c lymphocytes with immature or maturated (LPS-treated) bone marrow-derived dendritic cells (BMDCs) isolated from wild-type (WT) and Alox15^{-/-} mice (both C57BL/6 background). Data shown are representative for at least 3 indenpendent experiments (n=3).

Mixed lymphocyte reaction (MLR)

For the MLR, allogenic lymphoid LN-T cells (4 x 10⁵/well) were incubated in 96-well cell culture plates with graded numbers of mature WT and Alox15^{-/-} DCs for 5 days. The assay was performed in triplicates. Proliferation of T cells was monitored by measuring [methyl-3H]thymidine incorporation, added on day 4 of culture. Cells were pulsed with 1 µCi/well [methyl-3H]thymidine (Hartmann Analytik) for 16 h, harvested onto glass fiber filters (Printed Filtermat A; Wallac, Turcu, Finnland) using an ICH-110 harvester (Inotech, Dottikon, Switzerland), and [methyl-3H]thymidine incorporation was determined using a microplate counter (Wallac).



(A) Flow-cytometric-based measurement of the 12/15-LO expression in splenocytes of naive wild-type (Alox15^{+/+}) and Alox^{-/-} mice. (B) Quantification of the Alox15 mRNA expression levels in enriched CD19⁺ or CD4⁺ splenocytes isolated from naive wild-type (+/+) and Alox^{-/-} (-/-) mice, compared to mRNA expression levels of wild-type bone marrow-derived DCs. (C) Agarose gel electrophoresis of the corresponding products of the performed real-time PCR of the Alox15 and GAPDH genes in the indicated cell types. Data shown are representative for at least 3 independent experiments (n=3).



(A) Real-time PCR-based quantification of mRNA expression levels of COX-2 and in (B) ELISA-based analysis of the PGE2 production by Alox15+/+ and Alox15-/-CD11c⁺ bone marrow-derived dendritic cells after LPS-induced maturation (100ng LPS/ml for 16 hours). (C) Effect of the Cyclooxygenase-2 inhibitor Indomethacin on the expression levels of the IL-23 specific subunit p19 and (D) the IL-12 specific subunit p35 in Alox15^{+/+} and Alox15^{-/-} CD11c⁺ bone marrow-derived dendritic cells after LPS-induced maturation (100ng/ml) with or without pretreament (4h) of the cells with 10µM Indomethacin. Data shown are representative for at least 3 indenpendent experiments (n=3). Error bars represent SEM. *p<0.05, **p<0.005, Students t test.