Generation and culture of bone marrow-derived dendritic cells (bmDCs)

BmDCs were generated as described by a modified protocol of Inaba et al \(^{51}\). Briefly, bone marrow cells from murine tibias and femurs were passed through a nylon mesh to remove debris, and \(3 \times 10^6\) cells were placed in 6-well plates (BD Biosciences, Germany) containing 5 ml DC-media (RPMI supplemented with 5% FCS, 1x nonessential amino acids, 2mM L-glutamine, 500nM 2-ME, 100U/ml penicillin/streptomycin, 20µg/ml gentamycin), 150 U/ml GM-CSF (R&D Systems, Germany) and 75 U/ml IL-4 (BD Biosciences, Germany). After 3 days, 70% of the media was exchanged. On day 6, \(2 \times 10^6\) non-adherent cells were transferred into a new six-well plate with 3ml DC-media/well. On day 7, cells were stimulated for 48h with \(\alpha\)CD40 (1C10 \(^{52}\), a kind gift from S. Amigorena, Paris, France). After a total of 9 days of culture, mature bmDCs were harvested and used in all subsequent experiments of the manuscript.

Quantitative RT-PCR

Antigen-pulsed bmDCs and OT-II T cells were cocultivated for 24 hours in a 3-D collagen matrix. Following isolation of cells by gel digestion with collagenase, total RNA was extracted according to the manufacturer's instructions using TriReagent (Sigma-Aldrich, Deisenhofen, Germany) and reverse transcription was performed using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Burlington, Canada). IL-2, Tbet, GATA3 and FoxP3 mRNA were quantified by real time PCR using TaqMan Gene Expression Assays and TaqMan PCR Master Mix (both Applied Biosystems, Foster City, USA). A two-step PCR procedure (15 sec at 95°C, 60 sec at 60°C; 40 cycles) was performed using the ABI PRISM 7300 sequence detection system (Applied Biosystems). RNA expression levels were normalised to expression of endogenous \(\beta\)-actin mRNA.
**Silencing of CYTIP with small interfering RNA (siRNA)**

Downregulation of CYTIP expression by siRNA transfection was done as described by Hofer et al. with some slight modifications. Briefly, 5x10^5 d7 bmDCs were transfected with 80pM siRNA (ready to use 21-bp duplexes, selected from the Dharmacon database [Dharmacon, Lafayette, CO]) using GeneSilencerTM siRNA Transfection Reagent (PeqLab, Erlangen, Germany) according to manufacturer’s manual. After 4 hours cells were stimulated with αCD40 antibody and used after 24 hours for further experiments. CYTIP knockdown was controlled by immune fluorescence and quantified by RT-PCR. Therefore, total RNA was isolated from control siRNA transfected or CYTIP siRNA transfected bmDCs by Trizol Reagent (Invitrogen, Karlsruhe, Germany). Subsequently, cDNA synthesis was performed using oligo-dT primers and cDNA reversed transcriptase (Promega, Mannhein, Germany) and CYTIP knockdown was detected by quantitative RT-PCR (5´GAT GGA AGA CAA CCG AAG G´3 and 3´CC TTT TCG TCC TGT TAC CTT`5) using the ABI PRISM 7000 cycler (Applied Biosystem, Vienna, Austria). RNA expression levels were normalised to expression of endogenous β-actin mRNA.

**Immunofluorescence microscopy**

CYTIP knockdown was controlled by fluorescence microscopy using αCYTIP antibody (rat IgG1, clone 2F9; gift of Prof. W. Kolanus), an αcytohesin-1 antibody (C-19, goat polyclonal; Santa Cruz Biotechnology, Santa Cruz, USA) followed by an anti-rat-Alexa Fluor 488nm and an anti-goat-Alexa 647nm (both Invitrogen, Karlsruhe, Germany), respectively. Samples were analysed on a Leica LSM confocal microscope using a 63x lens. Background staining with secondary mAbs was negligible.
References


Figure S1

Cell surface activation markers are expressed to a similar extend in mature wildtype and LFA-1<sup>dd</sup> bmDCs. Mean fluorescence intensity (MFI) of indicated cell surface markers on CD11c-positive mature bmDCs is depicted (n≥6).

Figure S2

Expression of active LFA-1 on dendritic cells leads to accumulation of antigen-specific T cells on the dendritic cell surface. Mature wildtype or LFA-1<sup>dd</sup> bmDCs were cocultivated with naïve CD4<sup>+</sup> OT-II T cells in a 3-D collagen gel and their interaction was visualised using time-lapse videomicroscopy. Two examples of DC/T cell interactions are shown demonstrating that in contrast to the profound T cell accumulation on LFA-1<sup>dd</sup> DCs only few T cells associated with wildtype DCs.

Figure S3

CYTIP silencing in CD18 deficient dendritic cells does not impair antigen-specific T cell proliferation. Mature bmDCs from wildtype and CD18-/ mice were transfected with control siRNA or CYTIP specific siRNA. Subsequently, naïve CD4<sup>+</sup> OT-II T cells were cocultivated with DCs (ratio: 1 DC: 1000 T cells) and T cell proliferation was assessed by incorporation of [<sup>3</sup>H]-thymidine. Whereas, antigen-specific T cell proliferation induced by CYTIP silenced wildtype DCs was reduced, T cell proliferation upon coculture with CD18-/ DCs transfected with control siRNA or CYTIP specific siRNA was comparable. Assays were performed in triplicates. One representative assay out of two is shown.

Figure S4

CYTIP and cytohesin-1 are co-expressed in mature dendritic cells. Expression of CYTIP and cytohesin-1 in mature wildtype bmDCs was analysed by immune fluorescence and is
demonstrated either as individual staining or as a merged picture. Leica LSM confocal, original magnification x63.

**Figure S5**

**CYTIP expression is induced upon maturation of dendritic cells.** BmDCs were cultivated for 0, 3, 6, 8 and 9 days in conditions known to induce maturation (GM-CSF + IL-4). Subsequently, expression of CYTIP was analysed by immune fluorescence (A), western blot (α CYTIP mAb clone 2F9) (B) and quantitative RT-PCR (C). CYTIP expression was found to be induced upon maturation of DCs. Additional stimulation of mature DCs (d9) with LPS did not further increase CYTIP expression.
Figure S1

The figure shows the measurement of various cell surface markers in WT and LFA-1<sup>ΔΔ</sup> mice, with and without CD40 stimulation.

- **IA/IE**: The MFI (x1000) for WT is higher than for LFA-1<sup>ΔΔ</sup>, with CD40 stimulation increasing the MFI for both genotypes.
- **CD54**: The MFI for WT is higher than for LFA-1<sup>ΔΔ</sup>, with no significant differences between stimulated and unstimulated conditions.
- **CD80**: The MFI for WT is higher than for LFA-1<sup>ΔΔ</sup>, with no significant differences between stimulated and unstimulated conditions.
- **CD86**: The MFI for WT is higher than for LFA-1<sup>ΔΔ</sup>, with CD40 stimulation increasing the MFI for both genotypes.
- **CD40**: The MFI for WT is higher than for LFA-1<sup>ΔΔ</sup>, with no significant differences between stimulated and unstimulated conditions.
- **CD18**: The MFI for WT is higher than for LFA-1<sup>ΔΔ</sup>, with no significant differences between stimulated and unstimulated conditions.
Figure S2

WT

LFA-1<sup>d/d</sup>
Figure S3

cytohesin-1  cytip  Overlay

20 µm
Figure S4

Bar graph showing cpm for control siRNA, cytip siRNA, control siRNA, and cytip siRNA with p < 0.05 indicated.

Legend:
- WT
- CD18−/−
Figure S5

A)

d3  d6  d8  d9  d9 + LPS

B)

C)

x-fold increase (normalized against β-actin)