Supporting Information

SI Materials and Methods

Mice

Female wild type (WT) C57BL/6 mice were purchased from Harlan-Winkelmann (Borchem, Germany and AN Venvay, The Netherlands). OT I mice, $RAG1^{-/-}$ [1], $RAG2^{-/-}$ C57BL/6 [2] and $RAG2^{-/-}\gamma c^{-/-}$ [3] mice were bred at the animal facility of the Helmholtz Centre for Infection Research (HZI). Mb1-cre mice have been described previously [4]. $FcR\gamma^{-/-}$ [5], $Fc\gamma RII^{-/-}$ [6] and $Fc\gamma RI/II/III^{-/-}$ mice were provided by J. Sjef Verbeek, Leiden University Medical Center, Netherlands. $C3^{-/-}$ mice [7] were provided by Andreas Klos, Hannover Medical School, Germany. $MR^{-/-}$ mice [8] were provided by Christian Kurts, Bonn University, Germany. Mice were used between 8 to 12 weeks of age. All mice were bred and maintained in specific pathogen free conditions.

Isolation and culture of splenocytes, T cells and BM cells

Splenocytes: Spleen cells were prepared by gentle flushing out the spleens with complete IMDM. Erythrocytes were lysed for 2 min in ACK lysis buffer (0.15M NH4Cl, 10mM KHCO3, 0.1mM EDTA) and washed two times in PBS. Cell clumps were removed by passage through a 50µm nylon filter. Splenocytes preparation was carried out strictly on ice. Cells were further used for staining with different Abs, flow cytometry analysis or cell sorting.

T cells: OT I ($OVA_{257-264}$ specific $CD8^+$ T cells) cells were isolated from lymph nodes (subcutaneous and mesenteric) and spleen. Single cell suspension was

purified using the CD8 negative isolation kits (Dynal) containing Abs against B220, CD11b, Ter-119, CD16/32 and CD4 following the protocol provided by the manufacturer. Cell preparations contained more than 90% of the desired cell population and were essentially free of $CD11c^{hi}$ cells as determined by flow cytometry using Abs specific for CD8 and CD11c. For antigen presentation assays OT I cells were stained with 1μM CFSE (Molecular Probes) for 10 minutes at 37°C according to the manufacturer's protocol.

BMDC: Bone marrow cells were cultured in complete RPMI medium 1640 containing 10% FCS and mouse granulocyte macrophage-colony stimulating factor (20ng/ml, Peprotech) for 10 days. On day 9 BMDCs were stimulated with LPS (0.1μg/ml) for 18h.

Flow cytometry and cell sorting

Single cell suspensions were treated with anti-mouse CD16/CD32 BD Fc Block (2.4G2, Becton Dickinson) for 10min followed by staining with appropriate mAbs for 20min on ice. Abs used in this work included anti-mouse CD11c (clone N418) conjugated with allophycocyanin (APC) or phycoerythrin-Cy7 (PE-Cy7), CD11b (M1/70) PE-Cy7, CD8α (53-6.7) PacificBlue, fluorescein isothiocyanate (FITC) or phycoerythrin (PE), B220 (RA3-6B2) APC-Alexa Fluor 750, CD4 (GK1.5) PE, CD86 (GL1) FITC, CD40 (HM40-3) FITC, CD80 (16-10A1) PE, CD1d (1B1) PE, ICAM-1 (YN1/1.7.4) PE; all purchased from eBioscience. H-$2K^b$ (Y-3) FITC, I-A^b (M5/114.15.2) FITC were purified and conjugated with FITC in our laboratory. Anti-mouse CD14 (Sa14-2) APC and CD64 (FcγRI) (X54-5/7.1) PE were

purchased from BioLegend. For analysis of surface expression of C-type lectin receptors the following Abs were used: anti-mouse dectin-1 (218820) APC (R&D Systems), CD209b (SIGN-R1) (22D1) APC (eBioscience), CD209a (DC-SIGN) (MMD3) eFluor-660 (eBioscience), CD206 (MR) (C068C2) PE (BioLegend). Flow cytometric analysis and sorting were performed using FACSCanto, LSRII and FACSAria (Becton Dickinson). All samples during the sorting procedure were kept at 4^{o}C. The data were analyzed using FACSDiva, version 6.1.1 software (Becton Dickinson) and FlowJo, version 9.4.8 (Tree Star).

Analysis of antigen presentation

IgG-OVA immunocomplexes were prepared by incubation of OVA (Profos) with polyclonal rabbit anti-OVA IgG (ICN Biomedicals) for 30 min at 37^{o}C in a mass ratio 1:50. DCs were loaded with 10 times concentrated IgG-OVA for 1h at 37^{o}C, further washed intensively. In order to prepare cell associated antigen EG7-OVA and EL4 cells were harvested from culture bottles and counted. Further 1×10^{6} cell were UV irradiated, using an UV Crosslinker, with 9mJ/cm^{2}. Next, cells were washed intensively with PBS and plated (1×10^{4}/well) on 96-well plates together with splenic cDC (1×10^{4}/well). Subsequently, the cells were washed three times and resuspended in complete IMDM containing $2x10^{5}$ CFSE labeled OT I cells. For inhibition of endosomal acidification chloroquine (InvivoGen) was titrated into DC-T cell co-cultures.

Determination of antigen uptake and processing

Sorted cDC were incubated with 62.5µg/ml of DQ-OVA (conjugated with BODIPY FL, Molecular Probes) for 45min at 37^{o}C or on ice. Further, cDC were washed carefully and analyzed by FACS. For analysis of uptake of OVA-Cy5 soluble OVA was conjugated to Cy5. The labeling procedure involved gel filtration as a final step for removal of low molecular mass molecules such as unbound fluorochrome. The concentration of OVA-Cy5 was determined by measurement of OD_{280}. Sorted DC were incubated with indicated concentrations of OVA-Cy5 for 1h, than washed carefully. Uptake of fluorescent OVA was determined by FACS.

Microarrays

RNA isolation, cDNA preparation and DNA microarray analysis of gene expression was performed at the gene array facility of the Helmholtz Centre for Infection Research (HZI) in Braunschweig. Fluorescent images of hybridized microarrays (Affymetrix, MOE-430 version 2.0) were obtained using an Affymetrix Genechip Scanner. Microarray data were analyzed using BioConductor Suite 2.1 software. All samples were repeated two times with individually sorted cells and averaged.

Reconstitution of RAG-deficient mice with T cells

WT and RAG-deficient mice were injected i.v. with $CD3^+CD4^+$ and $CD3^+CD8^+$ splenic T cells (3×10^6/mouse). 21 days after reconstitution splenic cDC were sorted from recipient mice and used as APCs in OT I antigen presentation assay.

***In vivo* i.v. administration of serum, immunoglobulins and mannan**

Serum: WT and $RAG^{-/-}$ mice were i.v. injected with 100µl of serum isolated from blood of WT or RAG-deficient mice. Injection was repeated once per week for 3 weeks to allow complete turnover of the DC pool in the spleen. Further, recipient mice were sacrificed and splenic cDC were sorted and tested for their ability to cross-present OVA.

Immunoglobulins: WT and $RAG^{-/-}$ mice were i.v. injected with 7.5µg of Functional Grade Purified IgG1, κ (clone P3.6.2.8.1, eBioscience), 7.5µg Functional Grade Purified IgM (clone 11E10, reactive with LPS, eBioscience), IgM+IgG (7.5µg + 7.5µg) or PBS on day 0 and day 7. After 21 days mice were sacrificed and splenic cDC were sorted. The cells were loaded with soluble OVA and used as APCs in OT I assays.

Mannan: WT and $RAG^{-/-}$ mice were i.v. injected with 100µl (2mg/ml) of mannan from *Saccharomyces cerevisiae* (Sigma Aldrich), 4h later some groups were additionally injected i.v. with Ig as described above. Mannan was injected twice per week within 3 weeks. Next, mice were sacrificed and sorted splenic cDC were tested in cross-presentation assay.

Preparation of Fab fragments from rat IgG

Purified rat anti-mouse CD16/32 IgG2b (clone FCR-4G8, in-house preparation) was digested for 16h at 37^{o}C with immobilized papain (Pierce) in papain digestion buffer (20mM NaPi pH 7.2, 10mM EDTA pH 8.0, 20mM Cysteine). The digest was purified by FPLC on HiTrap DEAE Sepharose fast flow column (GE Healthcare). The purity of preparation was validated by SDS-PAGE.

Binding of immunoglobulins to CLR

WT and MR-deficient BMDC were counted, washed twice with PBS and incubated for 15min at 37^{o}C after each wash. Further cells were incubated with Fab fragments generated from anti-CD16/32 rat IgG. Next, cells (5x10^{5}/sample) were incubated on ice with mouse IgG labeled with PE (P3.6.2.8.1, eBioscience) for 1h. Cells were analyzed by flow cytometry.

Western Blot

Splenic cDC from WT and RAG-deficient mice were sorted as described in “Flow cytometry and cell sorting section”. 1*10^{6} cells were lysed in 100µl of sample buffer and boiled for 10 min at 96^{o}C. Next, 15 µl of protein extracts was subjected to electrophoresis in 10% SDS-PAGE gels, transferred to a nitrocellulose membrane, and blocked with 0.1% Tween 20/5% of milk TBS. The nitrocellulose membrane was then incubated overnight with primary Abs (goat anti-mouse Cathepsin B, Cathepsin D and Cathepsin E all from R&D Systems), washed, and incubated with secondary antibody donkey anti-goat conjugated to HRP. The

membrane was subsequently developed using the ECL Western Blotting Detection Kit (Amersham Biosciences). Quantification of bands intensity was performed with ImageJ and GraphPad Prism softwares.

Fluorescence microscopy

Splenic cDC were sorted from WT and RAG-deficient mice as described above. Further, cells were plated at 5×10^5/well on a glass slides in a 24 well plate and incubated overnight at 37^{o}C. Next day, cDC were loaded with 62.5µg/ml of DQ-OVA (Invitrogen) for 40 min at 37^{o}C in complete IMDM. After three washes cells were further incubated for 2h at 37^{o}C, washed in PBS and fixed in 3% paraformaldehyde at room temperature for 15 minutes. The following antibodies were used: rat anti-LAMP-2 (Hybridoma Bank), mouse anti-EEA-1 (BD Biosciences, Germany), anti-mouse and anti-rat Alexa546 conjugated (Molecular Probes). Cathepsins B, D and E were detected using biotinylated Abs (all from R&D Systems).

Fixed cells on cover slips were quenched for 15 min in 50 mM Glycin in PBS followed by 30 min incubation with 1 % Bovine Serum Albumin (BSA, Sigma, USA) and 0.01 % Saponin (Sigma) in PBS. The primary and secondary antibodies were diluted in PBS and incubated for 1 h. Nuclear staining was performed using 4'.6-diamidino-2-phenylindole (DAPI). After staining, coverslips were mounted on slides using aqueous mounting medium (Dako Cytomation). Samples were analyzed by confocal fluorescence microscopy using a Leica SP5 microscope (Leica Microsystems).

SI References

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