This is a pre- or post-print of an article published in

Ziętara, N., Łyszkiewicz, M., Puchałka, J., Pei, G., Gutierrez, M.G., Lienenklaus, S., Hobeika, E., Reth, M., Dos Santos, V.A.P.M., Krueger, A., Weiss, S.

Immunoglobulins drive terminal maturation of splenic dendritic cells

(2013) Proceedings of the National Academy of Sciences of the United States of America, 110 (6), pp. 2282-2287.

Immunoglobulins drive terminal maturation of splenic dendritic cells

Natalia Ziętara[a,b,3,*], Marcin Łyszkiewicz[a,b,*], Jacek Puchałka[c,1], Gang Pei[d], Maximiliano G. Gutierrez[d,2], Stefan Lienenklaus[a], Elias Hobeika[e], Michael Reth[e], Vitor A.P. Martins Dos Santos[c,3], Andreas Krueger[b†] and Siegfried Weiss[a†]

*, † these authors contributed equally to this work and should be considered *aequo loco*.

[a]Department of Molecular Immunology, Helmholtz Centre for Infection Research (HZI), 38124 Braunschweig, Germany; [b]Institute for Immunology, Hannover Medical School, 30625 Hannover, Germany; [c]Department of Molecular Biotechnology, Synthetic and Systems Biology Group, Helmholtz Centre for Infection Research (HZI), 38124 Braunschweig, Germany; [d]Department of Vaccinology and Applied Microbiology, Junior Research Group Phagosome Biology, Helmholtz Centre for Infection Research (HZI), 38124 Braunschweig, Germany; [e]Molecular Immunology Research Group, Max-Planck Institute of Immunobiology and Epigenetics, 79108 Freiburg, Germany;

[1]Present address: Department of Pediatrics, Dr. von Hauner Kinderspital, Ludwig Maximilian University, 81377 Munich, Germany;

[2]Present address: Mycobacterial Research, Medical Research Council, National Institute for Medical Research, The Ridgeway, London, NW7 1AA UK.

[3]Present address: Laboratory of Systems and Synthetic Biology, Wageningen University, 6703HB Wageningen, The Netherlands;

[4]To whom correspondence should be addressed. E-mail: Zietara.Natalia@mh-hannover.de

Key words: cross-presentation, soluble OVA, immunoglobulin, C-type lectin receptors;

Classification: Biological Sciences, Immunology;

Author contributions: N.Z. and M.L. designed research, performed research, analyzed data, wrote the paper; J.P. analyzed data; G.P. and M.G.G. performed research, analyzed data; S.L. performed research, analyzed data; E.H. and M.R. contributed reagents and analytic tools; V.A.P.MDS. analyzed data; A.K. and S.W. designed research, analyzed data, wrote the paper;

Abstract

Nature and physiological status of antigen-presenting cells (APC), such as dendritic cells (DC) are decisive for the immune reactions elicited. Multiple factors and cell interactions have been described that affect maturation of DC. Here we show that DC arising in the absence of immunoglobulins (Ig) *in vivo* are impaired in cross-presentation of soluble antigen. This was due to aberrant cellular targeting of antigen to lysosomes and its rapid degradation. Function of DC could be restored by transfer of Ig irrespective of antigen-specificity and isotype. Modulation of cross-presentation by Ig was inhibited by co-application of mannan and, thus, likely to be mediated by C-type lectin receptors (CLR). This unexpected dependency of splenic DC on Ig to cross-present antigen provides new insights into the interplay between cellular and humoral immunity and the immunomodulatory capacity of Ig.

\body

Introduction

Dendritic cells (DC) constitute the subset of professional antigen-presenting cells (APC) that is most potent in initiating adaptive immune responses. In order to prime naïve $CD4^+$ helper or $CD8^+$ cytotoxic T cells, DC process and present antigen in the context of MHC II or MHC I, respectively. MHC II presentation is largely restricted to exogenous antigen taken up *via* different endocytotic mechanisms. In contrast, MHC I presentation is restricted to endogenous antigen in most cells types. However DC are specifically equipped with an alternative pathway for presentation of exogenous antigen *via* MHC I, referred to as cross-presentation [1-4]. Given that many viruses do not directly infect DC, initiation of most $CD8^+$ T cell responses requires cross-priming of such cells *via* cross-presentation.

The molecular mechanisms of cross-presentation remain still largely elusive and multiple pathways of antigen transport, processing, and loading might exist, which are not mutually exclusive.

Ovalbumin (OVA) is one of the best studied model antigens in cross-presentation. Soluble OVA has been proposed to be engulfed *via* mannose receptor (MR) mediated endocytosis into specialized stable early endosomal compartments. Subsequently, antigen is exported to the cytosol, processed by proteasomal degradation and re-imported *via* TAP to early endosomes for final trimming by the insulin-regulated aminopeptidase IRAP and loaded onto MHC I molecules [3, 5-7]. However, different forms of antigen may be cross-presented *via* different routes [4].

Homeostasis and function of the immune system requires complex interactions between its components. Accordingly, T and B cells influence development, function and maturation status of DC. In addition to well-established role of T cells in shaping

DC function [8-10] B cells appear to be able to modulate the functional maturation of DC [11]. Thus, lack of B cells skews the T cell response towards Th1 by promoting expression of IL-12 by DC. Such regulatory function is likely to be mediated *via* secretion of cytokines [11]. Immunoglobulins (Ig) constitute the largest fraction of secretory molecules from B cells. They, mostly in the form of immune complexes (IC) or acting *via* Fc receptors, have been suggested to influence DC function and, in particular, cross-presentation [12, 13]. However, the mechanism and extent how Ig and/or IC affect DC maturation and antigen presentation remain poorly understood.

Therefore, we tested the hypothesis that development of fully functional DC depends on the presence of a functional adaptive immune system. We observed that cross-presentation of soluble antigen by splenic conventional DC (cDC) generated in lymphopenic mice was severely impaired. This inefficient cross-presentation in the absence of T and B cells was due to aberrant antigen trafficking and rapid degradation of antigen, thus preventing efficient loading and antigen presentation by MHC I. We showed that efficient cross-presentation depended on serum Ig, which presumably acts *via* C-type lectin receptors (CLR). Taken together, our results reveal a novel mechanism for regulation of DC development *via* soluble Ig.

Results

Impaired cross-presentation by splenic cDC generated in a lymphopenic environment. Function of DC critically depends on their maturation status. Therefore, first we re-assessed how lack of components of the adaptive immune system affects maturation of splenic DC to full function. To this end, we examined splenic conventional DC (cDC) from RAG-deficient mice that lack T and B cells and WT mice for their maturation status and capacity to present antigen. No major differences in surface expression of MHC I, MHC II, CD1d, ICAM-1 and other co-stimulatory molecules were observed (Fig. 1A), indicating an overall similar maturation status of splenic cDC from $RAG^{-/-}$ and WT mice. Next, we sensitized cDC from either mouse strain with soluble ovalbumin (OVA), which requires cross-presentation, or with cognate peptide (SIINFEKL, $OVA_{257-264}$), which is independent of cross-presentation. OVA protein-sensitized splenic cDC isolated from RAG-deficient mice were impaired in priming OT I T cells compared to OVA-loaded WT cDC (Fig. 1B). In contrast, peptide-pulsed cDC from RAG-deficient and WT mice primed OT I T cells equally well. Thus, while displaying a comparable maturation status at the cell surface, splenic cDC from RAG-deficient mice cannot cross-present soluble antigen effectively.

Absence of T and B cells during cDC development *in vivo* leads to different antigen trafficking and enhanced degradation. We set out to identify the mechanism underlying the deficiency in cross-presentation by cDC from $RAG^{-/-}$ mice. First, we performed gene expression analysis using sorted splenic $CD8\alpha^-$ and $CD8\alpha^+$ cDC from $RAG^{-/-}$ and WT mice. This revealed that amongst others several C-type lectin receptors (CLR) were expressed at elevated levels in cDCs from lymphopenic mice as were genes encoding endolysosomal enzymes including Cathepsins (Fig.

2A). Since Cathepsins require proteolytic processing for activation, the analysis of mRNA expression and immunofluorescent microscopy (Fig. S1) are only of limited value. Western blot analysis revealed that in splenic cDC from RAG-deficient mice Cathepsins D, E and B are indeed present at highly elevated levels in catalytically active form when compared to cDC from WT mice (Fig. 2B). Thus, more efficient antigen uptake and more aggressive degradation of soluble antigen could be a possible consequence. Indeed, analysis of antigen uptake using fluorophore-coupled OVA (OVA-Cy5) demonstrated increased endocytosis of OVA by cDCs from RAG$^{-/-}$ mice (Fig. 2C). Similarly, as predicted, antigen processing was increased in RAG$^{-/-}$ cDC (Fig. 2D). This was assessed by measuring fluorescence of DQ-OVA, the emission of which is dependent on degradation. Together this suggests that in the absence of T and B cells, cDC acquire antigen more efficiently but their increased proteolytic activity ultimately results in reduced presence of MHC I-peptide complexes. To substantiate this hypothesis, we visualized OVA trafficking in cDC using fluorescence microscopy. Co-localization of DQ-OVA degradation products with EEA1-positive early endosomes was observed only in WT cDC (Fig. 2E, left panel). In contrast, cDC from RAG-deficient mice exhibited DQ-OVA degradation products mainly in LAMP2 positive late endosomal/lysosomal compartments (Fig. 2E, right panel). These results prompted us to test the hypothesis that inhibition of lysosomal acidification should restore cross-presentation ability in cDC from RAG-deficient mice. To this end, we treated sorted DC with graded concentrations of chloroquine prior to loading with soluble OVA and tested their ability to activate OT I T cells. Consistent with our hypothesis, inhibition of lysosomal acidification in cDC from RAG-deficient mice improved their capacity to cross-present soluble OVA (Fig. 2F).

We conclude that in splenic cDC from lymphopenic mice antigen is miss-targeted to late endosomal/lysosomal compartments resulting in enhanced degradation of antigen and, consequently, in inefficient cross-presentation.

Soluble immunoglobulin is sufficient to restore efficient cross-presentation by cDC from lymphopenic mice. We next wanted to delineate the critical factors required for proper maturation of splenic cDC that are lacking in RAG-deficient mice. First, we determined the individual contribution of B and T cells. OVA -sensitized cDC from B-cell deficient $mb1^{cre/cre}$ mice were hampered to support proliferation of OT I T cells to a similar extent as cDC from $RAG^{-/-}$ mice, in contrast to cDC from WT and B-cell sufficient $mb1^{cre/+}$ mice (Fig. 3A). Similar to cDC from $RAG^{-/-}$ mice cDC from $mb1^{cre/cre}$ mice did not display any defect in presenting exogenously administered cognate peptide (Fig. S2A). T cells however, when transferred into RAG-deficient mice were not able to fully reconstitute the cross-presentation capacity of splenic cDC (Fig. 3B). These data indicate that B cells alone are able to modulate the efficiency of cross-presentation of soluble antigen by splenic cDC.

$RAG^{-/-}$ mice still contain NK cells, which could negatively influence cDC. However, cDC derived from $RAG2^{-/-}\gamma c^{-/-}$ mice, which lack NK cells in addition to T and B cells, had not acquired cross-presentation capacity (Fig. 3C). Thus, NK cells do not negatively influence cross-presentation by cDC in RAG-deficient mice.

To evaluate candidate factors of B cells able to modulate cross-presentation, we first reconstituted $RAG^{-/-}$ mice or WT mice with serum from RAG-deficient or normal mice. Cross-presentation ability of $RAG^{-/-}$ cDC was completely restored by transfer of WT serum in contrast to transfer of $RAG^{-/-}$ serum (Fig. 3D). This indicates that soluble factors rather than cell-cell interactions between B cells and cDC modulate cross-presentation. Importantly, transfer of serum from RAG-deficient mice into WT

mice did not reduce cross-presentation by WT cDC, excluding the presence of inhibitory factors in serum of RAG-deficient mice.

Ig, a major component of serum, could act on such cDC. Thus, WT and $RAG^{-/-}$ mice were reconstituted with purified monoclonal IgG, IgM or both. Either type of Ig restored the cross-presentation capacity of cDC from RAG-deficient mice to WT levels (Fig. 3E). Of note, purified IgM even enhanced cross-presentation by splenic cDC isolated from WT mice. Addition of serum or Ig did not alter the presentation of exogenously added peptide (Fig. S2B, C). Taken together, secreted Ig from B cells directly promotes the capacity of splenic cDC to cross-present soluble antigen both by restoring cross-presentation in cDC from Ig-deficient hosts as well as by enhancing cross-presentation in cDC from Ig-competent hosts.

Secreted immunoglobulin promotes cross-presentation by cDC *via* interaction with lectin receptors *in vivo*. Gene expression analysis revealed several differentially regulated candidate genes whose products might serve as receptors for Ig. The genes encoding FcγRI, FcγRIIb, FcγRIII, as well as several CLR, including mannose receptor (MR, CD206), were upregulated in cDC from $RAG^{-/-}$ mice (Fig. 4A). Surface staining for Fcγ receptors (CD64), CLR (CD206, CD209a,b), and CD14 essentially confirmed our mRNA expression data (Fig. 4B). We also included the CLR Clec9a in our analysis, since it had been shown to bind dead-cell associated antigens and regulate cross-presentation [14, 15]. Whereas in WT mice a large fraction of $CD8^{+}$ DC was virtually Clec9a-negative, this population of Clec9a-negative $CD8^{+}$ DC was almost absent from RAG-deficient mice (Fig. 4B).

Next, we addressed a possible contribution of activating or inhibitory FcγR to Ig-dependent regulation of cross-presentation. cDC isolated from $FcR\gamma^{-/-}$, $Fc\gamma RII^{-/-}$, and

FcγRI/II/III$^{-/-}$ mice were able to cross-present OVA as efficiently as WT cDC suggesting that signals provided *via* FcγR are not required for functional maturation of splenic cDC (Fig. 4C). These results are in line with our finding that not only IgG, but also IgM, can restore cross-presentation. Consistent with previous reports [12, 13], cDC isolated from FcRγ$^{-/-}$ and FcγRI/II/III$^{-/-}$ mice were impaired in cross-presenting OVA-IC (Fig. 4D). Alternatively, IC associated with components of the complement system could engage complement receptors present on splenic cDC [16, 17]. The component C3 is essential for the activation of the complement system and is required for all three pathways - the classical, the alternative, and the lectin pathway. Therefore, we analyzed cross-presentation of cDC from complement component 3 (C3) deficient mice. However, splenic cDC from such mice were as efficient as WT cDC in cross-presentation of OVA, thus, excluding IC and engagement of complement receptors as modulators of cross-presentation (Fig. 4E). Of note, presentation of other than soluble Ag, like cell associated OVA, was not affected in cDC from RAG$^{-/-}$ mice (Fig. 4F). Taken together, these data indicate that Ig affects DC function at at least two distinct levels: IC-FcR interaction and, more fundamentally, FcR-independent in a non-complexed form. The dichotomy of Ig-dependent and Ig-independent cross-presentation of soluble antigen and apoptotic cells, respectively, is consistent with the assumption that humoral immunity has a greater role in clearance of soluble antigen than in removal of infected cells.

CLR, like DC-SIGN or SIGN-R1, constitute a large group of surface receptors, more than 20 of which can be expressed on myeloid cells [18]. Such receptors have been shown to engage Ig *via* their carbohydrates and potentially interfere with immune reactions [19]. Hence, they could be involved in the functional maturation of splenic DC. Mannan derived from *S. cerevisiae* is widely considered to be a specific inhibitor

of CLR [20]. Thus, we administered mannan to RAG$^{-/-}$ or WT mice prior to administration of IgG and tested the influence on the recovery of cross-presentation capacity by cDC. Importantly, administration of mannan alone did not induce maturation of DC as assessed by surface staining for MHC I, CD40 as well as the co-stimulatory ligands CD80 and CD86 (Fig. S3). Interestingly, in the presence of mannan, IgG could not restore cross-presentation in cDC from RAG$^{-/-}$ mice (Fig. 4G). Mannan treatment reduced cross-presentation to some extent in cDC from WT mice, since uptake of soluble OVA depends on MR [6]. In order to closer investigate the receptor candidates, we tested bone marrow derived DC (BMDC) from MR-deficient mice. However, in the absence of MR binding of Ig was not impaired when compared to WT BMDC (Fig. S4). This indicates that MR alone is not responsible for functional Ig binding to DC. It also suggests that the contribution of a particular CLR might be masked by redundancy.

These experiments show that non-specific Ig, independent of IC formation engage mannan-inhibitable receptors, possibly of the CLR family, on splenic cDCs to induce functional maturation by dampening excessive degradation of soluble antigen and promoting efficient cross-presentation.

Discussion

Investigation of signals that control the function of DC is critical to understand their role in regulation of the immune response and homeostasis. Mutual interactions between DC with T and B cells during induction of adaptive immune response have been described [2, 10, 21]. Nevertheless, still little is known how the separate lymphocyte populations modulate the development and function of tissue resident DC in steady state. Cross-presentation of exogenous self antigens is necessary to delete autoreactive $CD8^+$ T cells but also in triggering $CD8^+$ T cell responses against pathogens that do not directly infect DC [22, 23]. Therefore DC, the main APC that possess the ability to cross-present, play a crucial role in maintaining the subtle balance between tolerance and autoimmunity as well as protective T cell responses. Factors that influence this balance might be very important targets for therapeutic interventions.

In our studies, we used the lymphopenic mouse model where a deficiency in the RAG gene leads to a developmental blockade and results in the complete absence of mature T and B lymphocytes. We could show that tissue resident DC, which developed in such lymphopenic hosts exhibit severely impaired capacity to cross-present soluble OVA and prime $CD8^+$ T cells. Interestingly, splenic cDC isolated from B cell deficient mice, $mb1^{cre/cre}$ [24] showed similar dysfunction like cDC isolated from RAG-deficient mice. Consistent with the importance of B cells injection of WT serum or soluble Ig into RAG knockout mice could recover deficiency in cross-presentation of cDC isolated from such lymphopenic hosts. Therefore we concluded that mature B cells and B cell derived Ig are necessary to maintain proper function of splenic cDC in steady state.

Our experiments showed that transfer of naïve T cells into RAG-deficient mice only mildly enhanced the cross-presenting abilities of splenic cDC. This finding does not correlate with a previous report by Shreedhar et al., [25] in which the authors showed that adoptive transfer of immune T cells can restore the disturbed antigen presentation capacity of DC from lymphopenic mice. However, the two systems are difficult to compare. Immune T cells were essential in the *in vivo* assays and hapten was used as antigen for skin sensitization. Thus, most likely skin derived Langerhans cells from lymphopenic mice were targeted. Such DC are known to follow a different developmental pathway compared to normal cDC [26]. In our case, the DC from lymphopenic hosts showed properties of their monocytic precursor. For instance, they still expressed CD14 and exhibited a high capacity for lysosomal degradation. The signal elicited by circulating Ig was presumably required for a full differentiation after acquisition of residency in the spleen.

Differential expression of CLR and endolysosomal enzymes in splenic cDC that had developed in a lymphopenic environment suggested that such cDC might exhibit altered antigen uptake and target soluble antigens into degradation pathways different from WT DC. Indeed, we could show that cDC isolated from RAG-deficient mice have a slightly higher ability to acquire soluble antigen in comparison to WT cDC. However, cDC from lymphopenic mice degraded antigen much more vigorously. In addition, microscopic analysis showed that degradation of OVA takes place in different cellular compartments in the cDC from RAG-deficient mice compared to WT cDC. Splenic cDC isolated from $RAG^{-/-}$ mice targeted OVA into lysosomes while in WT cDC antigen remained in early endosomes. It had previously been shown that early endosomes provide a milder environment for antigen degradation that enables DC to form MHC-peptide complexes [27]. In contrast, Mφ

and other phagocytes target Ag into lysosomes where degradation is more efficient. More rapid degradation of the antigen prevents formation of MHC-peptide complexes and blocks efficient T cell stimulation [28].

FcγR had been shown to regulate the maturation of DC. Especially FcγRII was proposed to prevent spontaneous maturation of DC, thereby promoting steady state tolerance [13, 29]. Nevertheless, experiments employing splenic cDC isolated from different FcγR-deficient mice did not support the hypothesis that intravenous administration of Ig provides a FcγR-dependent signal to restore the impaired function of cDC from RAG-deficient mice. An FcR-independent mechanism of Ig function in this context was also suggested by our observation that IgM acted essentially identical to IgG. Recently, it was shown that expression of the Fc receptor for IgM, FcμR, is restricted to B cells and not expressed on T cells or DC [30]. Taken together, this report and our data strongly suggest that Ig acts independently of Fc receptors, FcγR or FcμR.

As our experiments essentially excluded Fc receptors and complement as effectors of Ig and also pointed towards mechanisms different from the uptake of IC and apoptotic cells, we explored whether CLR might mediate Ig-induced amelioration of cross-presentation. This was further prompted by our observation that CLR were upregulated on DC from RAG-deficient mice. Moreover, in a recent study Anthony et al demonstrated that anti-inflammatory activity of intravenous immunoglobulin (IVIG) is mediated by CLR, like DC-SIGN and SIGN-R1 [31, 32]. To test the hypothesis of a CLR-dependent effect we performed a bulk blockade of CLR by injection of mannan prior to administration of Ig. We observed that Ig were able to rescue efficient cross-presentation only when CLR were not blocked. Mannan is widely considered to be a specific inhibitor for CLR [20, 33, 34]. However, it cannot ultimately be excluded that

an intervention using mannan might result in non-specific effects beyond inhibition of CLR. Thus, it has been proposed by others that mannan induced functional maturation of bone-marrow derived DC *in vitro* and of lymph node DC *in vivo* [35]. However, these effects were evoked at much higher doses of mannan than those employed in our experiments. Of note, the reported effects of mannan on maturation of DC were dramatically lower in comparison to effects mediated by LPS and accordingly, we did not observe any phenotypic maturation of DC after administration of mannan at the concentration used throughout our study.

More than 20 CLR have been reported to be expressed on myeloid cells, which exhibit partially overlapping binding capacities for N-glycans and also engage similar downstream signaling pathways [18]. Thus, the CLR system is likely to be highly redundant also with respect to Ig binding. We detected elevated expression of the MR, SIGN-R1, SIGN-R2, and Clec9a on DC from RAG-deficient mice, raising the possibility that one or more of these or a different receptor at all might mediate Ig-induced amelioration of cross-presentation. Analysis of Ig-binding to DC from MR-deficient mice did not reveal any difference in binding when compared to WT DC. However, uptake of soluble OVA depends on MR, thereby precluding functional analysis of MR-deficiency in this experimental system [6].

In summary, our studies excluding FcR-dependent and complement-dependent mechanisms as well as mechanisms mediating the uptake of IC or apoptotic cells in addition to our findings that the Ig-induced capacity to cross-present can be inhibited by the *bona fide* CLR-specific inhibitor mannan, leads us to suggest that interaction of soluble Ig with CLR is required to endow splenic DC with an antigen-processing machinery optimized for cross-presentation.

Why is such a simple signal required for functional maturation of splenic DC? We attribute this to the plasticity of the monocyte/macrophage/DC lineage. The highly diversified functions of cells from this lineage requiring a multitude of differentiation and activation stages have to be matched by a multitude of differentiation and maturation signals. Thus, migratory and homing molecules might be giving directives and finally the Ig molecules *via* CLR might give the final functional cue for such DC.

Materials and Methods

Mice

Mouse lines used in this study were C57BL/6 (WT), OT I, RAG1$^{-/-}$, RAG2$^{-/-}$, RAG2$^{-/-}$ γc$^{-/-}$, mb1-cre, FcRγ$^{-/-}$, FcγRII$^{-/-}$, FcγRI/II/III$^{-/-}$, and C3$^{-/-}$ and are described in detail in SI Materials and methods. All animal experiments were conducted under approval of the local authority LAVES (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit) #33.9-42502-04-10/0148.

Flow cytometry and cell sorting

Conventional DC (cDC) were sorted as cells that were CD11c^{hi}CD8α$^{- /+}$CD11b$^{-/+}$. All B cells were CD19^{+}. Abs used in this work and detailed procedures are described in SI Materials and methods. Purity of antigen presenting cells (APC) was always >97% as judged by reanalysis.

Analysis of antigen presentation

For the experiments using soluble OVA or peptides, cDC were plated in 96-well plates (Nunc) at 1x10^{4} cells/well with the indicated amount of soluble EndoGrade OVA (Profos) or OVA$_{257-264}$ (Ana Spec Inc.) for 1h. Proliferation of T cells was analyzed by flow cytometry after 1.5 or 2.5 days of culture. The number of divided cells (CFSE^{lo} CD8^{+}) was determined as described [36] (SI Materials and methods).

Microarrays

Data sets have been deposited in NCBI's GEO: ID GSE17989. See also SI Materials and methods.

Additional methods

A detailed description of assays for antigen uptake and processing, cell isolation and culture, Western blot, fluorescence microscopy, and in vivo administration of cells and reagents is described in SI Materials and methods.

Acknowledgements

We thank Regina Lesch and Susanne zur Lage for excellent technical assistance. We thank J. Sjef Verbeek, Andreas Klos and Christian Kurts for providing mouse strains. The authors thank Gordon D. Brown, University of Aberdeen, UK, Luisa Martinez-Pomares, University of Nottingham, UK, and Philip R. Taylor, Cardiff University, UK, for providing cell lines.

We are grateful for critical reading of the manuscript to Harald von Boehmer from Harvard Medical School and Jonathan Yewdell from NIAID.

This work was supported in part by the Kultusministerium of Niedersachsen - Georg Lichtenberg PhD program to (N.Z.), the Marie Curie Action Miditrain MEST-CT-2004-504990 (to M.L.), the Helmholtz Gemeinschaft *via* HIRSIB, the Hannover Biomedical Reasearch School (HBRS), the Deutsche Krebshilfe and the German Research Council (DFG) (to S.W.), German Research Foundation (DFG), Emmy-Noether Program (KR2320/2-1), SFB738-A7, and EXC62, "Rebirth" (to A.K.) and Helmholtz Young Investigator grant to M.G.G and G.P.

Conflict-of-interest disclosure: The authors declare no competing financial interest.

References

1. Joffre OP, Segura E, Savina A and Amigorena S (2012) Cross-presentation by dendritic cells. *Nat Rev Immunol* 12: 557-569.

2. Heath WR, et al (2004) Cross-presentation, dendritic cell subsets, and the generation of immunity to cellular antigens. *Immunol Rev* 199: 9-26.

3. Burgdorf S, Scholz C, Kautz A, Tampe R and Kurts C (2008) Spatial and mechanistic separation of cross-presentation and endogenous antigen presentation. *Nat Immunol* 9: 558-566.

4. Segura E and Villadangos JA (2011) A modular and combinatorial view of the antigen cross-presentation pathway in dendritic cells. *Traffic.* 12: 1677-1685.

5. Burgdorf S, Kautz A, Bohnert V, Knolle PA and Kurts C (2007) Distinct pathways of antigen uptake and intracellular routing in CD4 and CD8 T cell activation. *Science* 316: 612-616.

6. Burgdorf S, Lukacs-Kornek V and Kurts C (2006) The mannose receptor mediates uptake of soluble but not of cell-associated antigen for cross-presentation. *J Immunol* 176: 6770-6776.

7. Saveanu L, et al (2009) IRAP identifies an endosomal compartment required for MHC class I cross-presentation. *Science* 325: 213-217.

8. Banchereau J, et al (2000) Immunobiology of dendritic cells. *Annu Rev Immunol* 18: 767-811.

9. Mellman I and Steinman RM (2001) Dendritic cells: specialized and regulated antigen processing machines. *Cell* 106: 255-258.

10. Wykes M and MacPherson G (2000) Dendritic cell-B-cell interaction: dendritic cells provide B cells with CD40-independent proliferation signals and CD40-dependent survival signals. *Immunology* 100: 1-3.

11. Bayry J, et al (2005) Modulation of dendritic cell maturation and function by B lymphocytes. *J Immunol* 175: 15-20.

12. den Haan JM and Bevan MJ (2002) Constitutive versus activation-dependent cross-presentation of immune complexes by CD8(+) and CD8(-) dendritic cells in vivo. *J Exp Med* 196: 817-827.

13. Regnault A, et al (1999) Fcgamma receptor-mediated induction of dendritic cell maturation and major histocompatibility complex class I-restricted antigen presentation after immune complex internalization. *J Exp Med* 189: 371-380.

14. Sancho D, et al (2009) Identification of a dendritic cell receptor that couples sensing of necrosis to immunity. *Nature* 458: 899-903.

15. Zhang JG, (2012) The dendritic cell receptor Clec9A binds damaged cells via exposed actin filaments. *Immunity* 36: 646-657.

16. Ben Nasr A, et al (2006) Critical role for serum opsonins and complement receptors CR3 (CD11b/CD18) and CR4 (CD11c/CD18) in phagocytosis of Francisella tularensis by human dendritic cells (DC): uptake of Francisella leads to activation of immature DC and intracellular survival of the bacteria. *J Leukoc Biol* 80: 774-786.

17. Morelli AE, et al (2003) Internalization of circulating apoptotic cells by splenic marginal zone dendritic cells: dependence on complement receptors and effect on cytokine production. *Blood* 101: 611-620.

18. Sancho D and Reis e Sousa C (2012) Signaling by myeloid C-type lectin receptors in immunity and homeostasis. *Annu Rev Immunol* 30: 491-529.

19. Geijtenbeek TB and Gringhuis SI (2009) Signalling through C-type lectin receptors: shaping immune responses. *Nat Rev Immunol* 9: 465-479.

20. Geijtenbeek TB, et al (2003) Mycobacteria target DC-SIGN to suppress dendritic cell function. *J Exp Med* 2003. 197: 7-17.

21. Moulin V, et al (2000) Lymphocytes regulate dendritic cell (DC) function in vivo: increased interleukin 12 production by DCs from B cell-deficient mice results in T helper cell type 1 deviation. *J Exp Med* 192: 475-482.

22. Kurts C, et al (1997) CD4+ T cell help impairs CD8+ T cell deletion induced by cross-presentation of self-antigens and favors autoimmunity. *J Exp Med* 186: 2057-2062.

23. Kurts C, Kosaka H, Carbone FR, Miller JF and Heath WR (1997) Irestricted cross-presentation of exogenous self-antigens leads to deletion of autoreactive CD8(+) T cells. *J Exp Med* 186: 239-245.

24. Hobeika E, et al (2006) Testing gene function early in the B cell lineage in mb1-cre mice. *Proc Natl Acad Sci USA* 103: 13789-13794.

25. Shreedhar V, et al (1999) Dendritic cells require T cells for functional maturation in vivo. *Immunity* 11: 625-636.

26. Kaplan DH, et al (2007) Autocrine/paracrine TGFbeta1 is required for the development of epidermal Langerhans cells. *J Exp Med* 204: 2545-2552.

27. Bird PI, Trapani JA and Villadangos JA (2009) Endolysosomal proteases and their inhibitors in immunity. *Nat Rev Immunol* 9: 871-882.

28. Savina A and Amigorena S (2007) Phagocytosis and antigen presentation in dendritic cells. *Immunol Rev* 219: 143-156.

29. Nimmerjahn F and Ravetch JV (2006) Fcgamma receptors: old friends and new family members. *Immunity* 24: 19-28.

30. Ouchida R, et al (2012) Critical role of the IgM Fc receptor in IgM homeostasis, B-cell survival, and humoral immune responses. *Proc Natl Acad Sci U S A* 109: E2699-2706.

31. Anthony RM, Wermeling F, Karlsson MC and Ravetch JV (2008) Identification of a receptor required for the anti-inflammatory activity of IVIG. *Proc Natl Acad Sci USA* 105: 19571-19578.

32. Anthony RM, Kobayashi T, Wermeling F and Ravetch JV (2011) Intravenous gammaglobulin suppresses inflammation through a novel T(H)2 pathway. *Nature* 475: 110-113.

33. McGreal EP, et al (2006) The carbohydrate-recognition domain of Dectin-2 is a C-type lectin with specificity for high mannose. *Glycobiology* 16: 422-430.

34. Turville SG, Vermeire K, Balzarini J and Schols D (2005) Sugar-binding proteins potently inhibit dendritic cell human immunodeficiency virus type 1 (HIV-1) infection and dendritic-cell-directed HIV-1 transfer. *J Virol* 79: 13519-13527.

35. Sheng KC, et al (2006) Mannan derivatives induce phenotypic and functional maturation of mouse dendritic cells. *Immunology* 118: 372-383.

36. Wilson NS, et al (2003) Most lymphoid organ dendritic cell types are phenotypically and functionally immature. *Blood* 102: 2187-2194.

Figure Legends

Fig. 1. Selectively impaired cross-presentation by splenic cDC generated in a lymphopenic environment. (A) Splenocytes of WT and $RAG^{-/-}$ mice were electronically gated as $CD11c^{hi}CD11b^{+/-}CD8\alpha^{+/-}B220^{-}$ population analyzed for expression of the indicated markers. Data are representative of at least 4-5 mice per group in six independent experiments. **(B)** Splenic cDC isolated from WT or $RAG^{-/-}$ mice were loaded with $OVA_{257-264}$ peptide or OVA protein for 1h. CFSE-labeled OT I T cells were incubated for 1.5 days (peptide) or 2 days (OVA) with cDC at a 10:1 ratio. The proliferative response of T cells was enumerated by flow cytometry. Numbers of proliferating cells are shown (mean +SEM). Data are representative of multiple independent experiments with minimum three mice per each group.

Fig. 2. Absence of T and B cells during DC development leads to aberrant trafficking and enhanced degradation of antigen. (A) Expression analysis of endolysosomal enzymes in splenic cDC. **(B)** Western blot analysis of Cathepsins D, E and B in DC sorted from WT and RAG-deficient mice. Numbers below plots indicate relative quantification of band intensity of Cathepsins D and B – 25kD bands and for Cathepsin E – 40kD band. Data are representative of 2 independent experiments. **(C)**WT and $RAG^{-/-}$ splenic cDC were incubated for 1h with the indicated concentrations of OVA-Cy5. **(D)** WT and $RAG^{-/-}$ splenic cDC were loaded with 62.5µg/ml of DQ-OVA for 45 min, incubated at $37^{o}C$ for the indicated time points, and analyzed by flow cytometry. Data are representative of three independent experiments with minimum three mice per each group. **(E)** WT and $RAG^{-/-}$ cDCs were loaded with DQ-OVA, incubated at $37^{o}C$ for 2h, fixed, and stained for EEA1 (left panel) or LAMP2 (right panel). Pictures are representative of multiple cells

(sorted from 12 animals per group) analyzed in two independent experiments. Far right panels show co-localization scatter plots. DAPI was used as nuclear staining. Bars: 10 µm. **(F)** Splenic cDC from WT and $RAG^{-/-}$ mice were pre-treated with the indicated concentrations of chloroquine, loaded with OVA (1mg/ml) and incubated with CFSE-labeled OT I cells for 2.5 days. The proliferative response of T cells was enumerated by flow cytometry. Numbers of proliferating cells are shown (mean +SEM). Data are representative of two independent experiments.

Fig. 3. Soluble immunoglobulin is sufficient to restore efficient cross-presentation by cDC from lymphopenic mice. (A) Splenic cDC from WT, $RAG^{-/-}$, $mb1^{cre/cre}$ and $mb1^{cre/+}$ mice were loaded with OVA (500 µg/ml) and incubated with CFSE-labeled OT I cells for 2 days. **(B)** WT and $RAG^{-/-}$ mice were i.v. injected with splenic WT T cells ($CD3^{+}CD4^{+}$ and $CD3^{+}CD8^{+}$). Three weeks after T cell transfer splenic DC were sorted from recipient mice, loaded with OVA (500 µg/ml) and incubated with CFSE labeled OT I cells for 2 days. **(C)** Splenic cDC isolated from WT, $RAG^{-/-}$ or $RAG2^{-/-}\gamma c^{-/-}$ were loaded with OVA (500 µg/ml) and incubated with CFSE labeled OT I cells for 2 days. **(D)** WT and $RAG^{-/-}$ mice were injected three times within 21 days i.v. with serum collected from WT and $RAG^{-/-}$ mice. **(E)** WT and $RAG^{-/-}$ mice were injected twice within 21 days i.v. with murine IgG (7.5 µg/mouse) and/or murine IgM (7.5 µg/mouse). The proliferative response of T cells was enumerated by flow cytometry. Numbers of proliferating cells are shown (mean +SEM). Data are representative of two or three independent experiments with minimum three mice per group. Statistical significance was determined using paired Student *t* test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.

Fig. 4. Immunoglobulin promotes cross-presentation by cDC *via* interaction with lectin receptors. (A) Expression analysis of candidate surface receptors in splenic cDC. **(B)** Splenic cDC of WT and $RAG^{-/-}$ mice were analyzed for expression of the indicated C-type lectin receptors. Data are representative of 4 mice per group in 2 independent experiments. **(C)** Splenic cDC from WT, $FcR\gamma^{-/-}$, $Fc\gamma RII^{-/-}$, or $Fc\gamma RI/II/III^{-/-}$ mice were loaded with OVA (500 µg/ml) and incubated with CFSE-labeled OT I cells for 2 days. **(D)** Sorted splenic cDC from $RAG^{-/-}$, WT, $Fc\gamma c^{-/-}$, $RcR\gamma IIB^{-/-}$, $FcR\gamma I/II/III^{-/-}$ were loaded with αOVA IgG+OVA. Cross-presentation abilities were tested by co-incubation with OT I cells. **(E)** Splenic cDC from WT or $C3^{-/-}$ mice were analyzed as in (C). **(F)** Sorted splenic cDC from $RAG^{-/-}$ and WT mice were loaded for 1h with $5x10^4$ UV-irradiated EG7-OVA or EL4 cells. Further cells were coincubated with CFSE-labeled OT I cells. **(G)** WT and $RAG^{-/-}$ recipient mice were first injected i.v. with mannan (200 µg/mouse) and, subsequently, with IgG (7.5 µg/mouse) every 3 days for 21 days. Data are representative of two independent experiments with minimum three mice per group. The proliferative response of T cells was enumerated by flow cytometry. Numbers of proliferating cells are shown (mean +SEM). Statistical significance was determined using paired Student *t* test. * $p< 0.05$; ** $p<0.01$; *** $p<0.005$.