Deficiency of Autophagy in Dendritic Cells Protects against Experimental Autoimmune Encephalomyelitis*

Received for publication, April 25, 2014, and in revised form, July 28, 2014. Published, JBC Papers in Press, July 30, 2014, DOI 10.1074/jbc.M114.575860

Abhisek Bhattacharya1,4, Syanthine Parillon5, Shenyen Zeng4, Shuhua Han5, and N. Tony Eissa4,5,1
From the Departments of 1Medicine and 4Pathology & Immunology, Baylor College of Medicine, Houston, Texas 77030

Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) in the immune system. DCs present antigens to CD8 and CD4 T cells in the context of class I or II MHC. Recent evidence suggests that autophagy, a conserved intracellular degradation pathway, regulates class II antigen presentation.

Background: Recent evidence suggests that autophagy, a conserved intracellular degradation pathway, regulates class II antigen presentation.

Results: Autophagy deficiency in DCs ameliorated experimental autoimmune encephalomyelitis (EAE) by reducing in vivo priming of T cells.

Conclusion: Autophagy is required in DCs for induction of EAE.

Significance: Autophagy might be a potential target for treating CD4 T cell-mediated autoimmune conditions.

Autophagy, a conserved intracellular bulk degradation mechanism, plays several important roles in both innate and adaptive immune cells. Perturbations in the autophagy pathway have been linked to human diseases, including infection and autoimmunity (1, 2). Mature dendritic cells (DCs), the professional APCs in the immune system, are the major activators of T cells. CD4 T cells are activated predominantly by phagocytosed extracellular antigens that the APCs present in the context of MHC class II (MHC II). Recent evidence suggests that autophagy regulates this pathway of antigen presentation. Autophagy machinery is involved in delivering endogenous viral antigens to MHC II (3), and the antigen loading compartments for MHC II receive continuous input from autophagosomes (4). Pharmacological inhibition of autophagy, by class III PI3K inhibitors 3-methyladenine or wortmannin, was shown to inhibit MHC II-mediated antigen presentation (5). DC-specific deletion of an essential autophagy gene Atg5 made mice more susceptible to herpes simplex virus 2 infection due to an inability of DCs to present antigen and prime an appropriate CD4 T cell response (6). Autophagy in DCs was also found to be critical for an appropriate CD4 T cell response against infection by respiratory syncytial virus (7). Furthermore, mice vaccinated with DCs that were pretreated with rapamycin to induce autophagy showed stronger T cell response upon subsequent challenge with Mycobacterium tuberculosis (8). Rapamycin treatment has also been found to increase the severity of autoimmune experimental uveitis, and it was hypothesized that induction of autophagy mediated such an effect (9).

Autophagy in thymic epithelial cells has been shown to be essential for proper surface display of MHC-antigen complex. Mice lacking Atg5 in thymic epithelial cells showed severe autoimmune organ dysfunction, implicating a role of autophagy in the generation of proper T cell repertoire and in central tolerance (10, 11). Autophagy also modulates the presentation of citrullinated peptide, characteristically present in rheumatoid arthritis, to CD4 T cells (12). Moreover, several genome-wide association studies have implicated different autophagy genes in autoimmune diseases, such as Crohn disease, rheumatoid arthritis, and systemic lupus erythematosus (13). Several hypotheses have been put forward to explain how the autophagy pathway might mediate autoimmunity (2, 14, 15). However, the relevance of this pathway during autoimmunity remained inconclusive.

The major goal of this study was to investigate the roles of autophagy in DCs in the context of autoimmunity. To this end, we generated DC-specific Atg7 knock-out (Atg7<sup>ΔDC</sup>) mice by breeding mice bearing Atg7<sup>ΔDC</sup> alleles with CD11c-Cre trans-

* This work was supported, in whole or in part, by National Institutes of Health Grant R01 HL69033 from NHLBI. This work was also supported by the Cytometry and Cell Sorting Core at Baylor College of Medicine (supported by National Institutes of Health Grants AI036211, CA125123, and RR024574).

1 To whom correspondence should be addressed: Baylor College of Medicine, One Baylor Plaza, BCM 285 Ste, 535E, Houston, TX 77030. Tel.: 713-798-3657; Fax: 713-798-2050; E-mail: teissa@bcm.edu.

2 The abbreviations used are: DC, dendritic cell; EAE, experimental autoimmune encephalomyelitis; APC, antigen-presenting cell; BMDC, bone marrow-derived DC; DNF8, 2,4-dinitrofluorobenzene; OXA, oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one); CQ, chloroquine.
Autophagy in Experimental Autoimmune Encephalomyelitis

genic mice. We then induced experimental autoimmune encephalomyelitis (EAE), an animal model that partially mimics multiple sclerosis. EAE is considered a predominantly CD4 T cell-mediated disease in which myelin-reactive CD4 T cells are activated in the periphery and then enter the CNS (16). Here, we show that loss of autophagy in DCs significantly reduced the incidence and onset of EAE by reducing in vivo priming of T cells. Administration of chloroquine, an autophagy-lysosomal inhibitor, before EAE onset delayed disease progression and, when administered after the onset, reduced disease severity. In contrast, the severity of hapten-induced contact hypersensitivity, mediated by CD8 T cells and NK cells, remained unaltered in Atg7f/f mice. Collectively, these studies showed that autophagy in DCs is critical for CD4 T cell-mediated autoimmune component, and inhibition of autophagy might be a therapeutic approach in some autoimmune diseases.

EXPERIMENTAL PROCEDURES

Mice—C57BL/6 and CD11c-Cre transgenic mice in a C57BL/6 background were purchased from The Jackson Laboratory. Atg7f/f mice have been described previously (17, 18). All mice used were housed in a specific pathogen-free facility (BCM vivarium, biosafety level 2) and monitored daily following EAE induction. All animal protocols were approved by the institutional boards of Baylor College of Medicine.

EAE Induction and in Vivo Treatments—EAE was induced as described (16) with minor modifications. Eight to twelve-week-old mice were actively immunized with 100 μl of 1 mg/ml MOG(35–55) peptide (EZ Biolab) emulsified in Freund’s complete adjuvant (5 mg/ml Mycobacterium tuberculosis extract H37Ra in incomplete Freund’s adjuvant). At immunization and 48 h later, all mice received 500 ng of pertussis toxin (List Biological Laboratories) intraperitoneally. Mice were monitored daily for weight changes and clinical symptoms, which were scored as follows: 0, no overt abnormalities; 1, limp tail or hind limb weakness; 2, limp tail and hind limb weakness; 3, partial paralysis of hind limbs; 4, complete hind limb paralysis; 5, moribund or death from EAE. At grade 5, mice were sacrificed for humane reasons. Disease incidence was marked when an animal showed clinical signs for at least 2 consecutive days. For chloroquine treatment, mice were randomly divided into two groups and treated before or after the onset of the disease, starting from day 2 or day 8, respectively. Mice received daily intraperitoneal injection of 60 mg/kg chloroquine diphosphate (Sigma) for 13–14 consecutive days. Control groups received PBS. Investigators scoring the mice for all EAE experiments were blinded as to the type of treatment and to mouse genotypes.

Induction of Contact Hypersensitivity—Contact hypersensitivity was induced as described (19). Briefly, mice were sensitized (day 0) by applying 50 μl of 5% (w/v) oxazolone (OXA) (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one) in absolute ethanol or by applying, on day 0 and day 1, 50 μl of 0.5% DNFB in acetone/olive oil (4:1) to the shaved abdomen. Mice were challenged on day 5 by applying 20 μl of 0.2% DNFB on one ear (10 μl on both sides) or on day 6 by applying 20 μl of 1% oxazolone on one ear and solvent alone to the other ear. For mice with ear tags, the tagged ear was always used for vehicle treatment. Ear thickness was measured before and 24 h after the challenge with a micrometer (Mitutoyo), and ear swelling was calculated by using Equation 1.

\[ \text{ear thickness} = \frac{(\text{challenged ear after} - \text{challenged ear before})}{(\text{vehicle-treated ear after} - \text{vehicle-treated ear before})} \quad (\text{Eq. 1}) \]

Single Cell Suspension and Flow Cytometry—Single cell suspensions from spleen, thymus, and lymph node were prepared by mechanical disruption over a cell strainer (BD Falcon), followed by RBC lysis using ACK lysis buffer (Quality Biological, Inc.). Intracellular staining for Foxp3 was performed using Cytofix/Cytoperm Plus kit (BD Biosciences). The following antibodies were used: CD8-APC (17-0081-81), MHC-II (I-A/I-E)-FITC (11-5321), CD40-PE (12-040181), all from eBioscience; CD11c-Alexa Fluor700 (560583), CD11c-APC-Cy7 (561241), CD11b-PerCP-Cy5.5 (550993), CD11b-APC-Cy7 (561039), CD4-APC (535051), CD4-PE (553048), CD8-FITC (553030), CD3e-PE-Cy7 (561100), and Foxp3-PE (560414), all from BD Biosciences. Flow cytometry was performed with LSR Fortessa (BD Biosciences), and FACSDiva software was used for data collection, and FlowJo software was used for analysis.

T Cell Proliferation—Single cell suspensions from spleen were incubated with the indicated concentration of MOG(35–55) peptide for 5–6 days, and supernatant was collected. IL-2 was measured in the supernatant by ELISA (eBioscience). For nonspecific stimulation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Invitrogen) was used to measure proliferation. Anti-CD3 and anti-CD28 (eBioscience) and concanavalin A (Sigma) were used for stimulation.

Antigen Presentation Assay—BMDCs were generated by culturing single cell suspension from bone marrow for 7 days in the presence of 20 ng/ml GM-CSF and 10 ng/ml IL-4. BMDCs were plated at 1 × 10^5 cells/well in a 96-well plate and left in culture overnight. 1 × 10^5 B3Z cells/well were added along with the indicated concentration of ovalbumin (Sigma). Colorimetric assessment was performed after 24 h as described (20). B3Z cells were a kind gift from Dr. Nilabh Shastri, University of California at Berkeley.

BMDC Stimulation and Ovalbumin Endocytosis—BMDCs were stimulated with LPS for 24 h. TNF-α and IL-6 in the supernatant were measured by ELISA (eBioscience). Cells were collected, and Fcs were blocked and stained for flow cytometry to check surface expression of various molecules. For ovalbumin endocytosis, BMDCs were incubated with 25 μg/ml OVA-Alexa Fluor488 conjugate (Molecular Probes), washed with ice-cold PBS, treated with trypsin/EDTA, and analyzed by flow cytometry.

Immunoblotting—Cell lysis was performed on ice using RIPA buffer with protease inhibitor mixture (BD Biosciences). Dounce homogenizer (Wheaton) was used for preparing lysates from isolated organs. DCs from spleen were isolated by MACS LS column, using CD11c microbeads (Miltenyi Biotec), and lysed. Lysates were boiled in Laemmli sample buffer for 5 min at 95 °C and resolved by SDS-PAGE. Following transfer onto nitrocellulose membrane by Mini Trans-Blot Cell (Bio-Rad),
membranes were incubated with antibodies, and imaging was performed using an Odyssey Infrared Imaging System (LI-COR Bioscience). The following antibodies were used: anti-Atg7 (600-401-487, Rockland Immunochemicals); anti-actin (AM43020, Ambion); anti-p62 (03-GP62-C-1, American Research Products); and anti-LC3 was described previously (17, 18).

Statistical Analysis—Normally distributed data were analyzed by Student’s t test. Welch correction was applied in case of significant difference in variance between the groups, and non-parametric comparisons were performed by Mann-Whitney test. For EAE data, curves were compared by analysis of variance, incidence by $\chi^2$ test, and Kaplan-Meier curves by log-rank test. Analyses were performed by GraphPad Prism Version 6.00 for Windows, GraphPad Software, La Jolla, CA.

RESULTS

Atg7 Deficiency in DCs Reduced the Incidence and Severity of EAE—We hypothesized that autophagy deficiency in DCs would result in reduced antigen presentation to CD4 T cells in the context of an autoimmune disease. To test this hypothesis, we bred $\text{Atg7}^{\text{ flox}}$ mice (henceforth, $\text{Atg7}^{\text{ flox}}$ mice) with transgenic CD11c-cre mice and generated mice with DC-specific deletion of $\text{Atg7}$ (called $\text{Atg7}^{\text{DC\Delta}}$ mice hereafter). $\text{Atg7}^{\text{DC\Delta}}$ mice did not present with any gross abnormalities and had substantial reduction of ATG7 levels in their BMDCs (Fig. 1A). EAE was induced in $\text{Atg7}^{\text{DC\Delta}}$ mice and their littermate $\text{Atg7}^{\text{ flox}}$ controls, and the mice were monitored daily for signs of EAE. Disease severity was measured in a scale of 0 to 5, and incidence was confirmed when signs of EAE were observed for at least 2 consecutive days (described in detail under “Experimental Procedures”).

$\text{Atg7}^{\text{DC\Delta}}$ mice showed a significant reduction in severity of EAE compared with their littermate control (Fig. 1B). Furthermore, the incidence of EAE was also reduced in $\text{Atg7}^{\text{DC\Delta}}$ mice and their littermate $\text{Atg7}^{\text{ flox}}$ controls, and the mice were monitored daily for signs of EAE. Disease severity was measured in a scale of 0 to 5, and incidence was confirmed when signs of EAE were observed for at least 2 consecutive days (described in detail under “Experimental Procedures”).

$\text{Atg7}^{\text{DC\Delta}}$ mice were observed to be weight loss, cumulative disease score (area under the curve), and peak disease score significantly attenuated (Fig. 1, E–G).

We then wanted to rule out the possibility that amelioration of EAE in $\text{Atg7}^{\text{DC\Delta}}$ mice was due to global immunosuppression and not specific to the pathophysiology of EAE. We utilized models of hapten-induced contact hypersensitivity, which is predominantly mediated by NK and CD8 T cells but not by
Autophagy in Experimental Autoimmune Encephalomyelitis

CD4 T cells (19, 21). Control and Atg7DC−/− mice were sensitized in the abdomen area with OXA or DNFB and then challenged after 5 or 6 days in one ear with the respective hapten. Disease severity was estimated by measuring swelling of the hapten-challenged ear compared with the vehicle-treated ear. Severity of hapten-induced contact hypersensitivity in response to OXA or DNFB was similar between Atg7DC−/− mice and their littermate controls (Fig. 2A). Thus, the reduction in severity of EAE in Atg7DC−/− mice was not due to a global reduction in immune functions in vivo, but it was likely specific to EAE.

To further rule out the possibility that autophagy deficiency reduced global DC functions, we evaluated LPS-induced cytokine secretion and up-regulation of costimulatory molecules as well as the endocytic functions of BMDCs. There were no significant differences between BMDCs from control and Atg7DC−/− mice, in endocytosis of ovalbumin, up-regulation of MHC-II and CD40 (Fig. 2C), or production of IL-6 or tumor necrosis factor-α (TNF-α) in response to LPS (Fig. 2, B–D). These results are in agreement with a previous study showing that Atg5 deficiency in DCs did not produce gross alterations in global DCs functions (6). Taken together, these data suggest that there were no global functional defects in autophagy-deficient DCs.

Atg7DC−/− Mice Exhibited Reduced Priming of T Cells during EAE—The findings that Atg7DC−/− mice showed specific defects in CD4 T cell-mediated EAE, while maintaining an intact NK cell and CD8 T cell-mediated response in contact hypersensitivity, suggested two possibilities. The Atg7DC−/− mice might have intrinsic T cell defects, particularly in the CD4 T cell compartment. The other possibility is that Atg7 deficiency in DCs resulted in reduced antigen presentation to CD4 T cells, thereby reducing their activation in Atg7DC−/− mice. To rule out the first possibility, we evaluated the weight and cellularity of spleen and thymus. We also checked frequencies and development of total T cells and CD4 T cells in Atg7DC−/− mice. Analyses of these compartments did not reveal any gross defects in Atg7DC−/− mice. Furthermore, there was no difference, between Atg7DC−/− and control mice, in the regulatory T cell compartment (Fig. 3).

We then tested whether the reduction in the incidence and severity of EAE resulted from defective antigen presentation to CD4 T cells by Atg7−/− deficient DCs. To evaluate antigen-specific recall response, we isolated splenocytes from control and Atg7DC−/− mice following induction of EAE and incubated them with MOG peptide. IL-2 production by splenocytes was measured to detect antigen-specific T cell recall response. We found that IL-2 production was significantly reduced in splenocytes from the Atg7DC−/− mice, suggesting reduced recall response in those mice (Fig. 4A). This reduction was not due to any intrinsic defects in T cells themselves as splenocytes from Atg7DC−/− mice showed similar proliferation to that observed in splenocytes from control mice in response to anti-CD3, anti-CD3 and anti-CD28 combination, or concanavalin A (Fig. 4B). Taken together, these data suggest that autophagy is critical for the ability of DCs to prime CD4 T cells to initiate EAE.

We then tested the ability of Atg7-deficient DCs to present antigen to CD8 T cells in the context of MHC class I. We used B3Z cells, a lacZ-inducible CD8 T cell hybridoma that recognizes OVA(257–264) (SIINFEKL) in context of MHC-I (H-2Kb) (20). Antigen recognition by B3Z cells results in their proliferation that can be measured colorimetrically. There was no significant difference between BMDCs isolated from control or Atg7DC−/− mice with respect to their abilities to present antigen in the context of MHC-I (Fig. 4C). These data suggested that autophagy deficiency reduced antigen presentation to CD4 T cells but not to CD8 T cells.

Autophagy-Lysosomal Pathway Inhibitor Chloroquine Reduced Severity of EAE—Our data suggested that autophagy plays an important role in the pathogenesis of EAE. We then sought to investigate the effect of pharmacological inhibition of the autophagy-lysosomal pathway on EAE. We induced EAE in mice and started chloroquine (CQ) treatment before disease onset, 2 days after immunization. CQ administration delayed disease incidence and reduced severity, cumulative disease score, and weight loss (Fig. 5, A–D). To test the effect of CQ treatment on a pre-existing disease, we induced EAE and started CQ injection after the onset of EAE, 8 days after immunization (Fig. 5, upper panel). CQ treatment significantly reduced disease severity and cumulative diseases score (Fig. 5, E and F).

FIGURE 2. Atg7DC−/− mice had no global defects in DC functions. A, mice were sensitized and challenged after 5–6 days with OXA or DNFB. Ear swelling was measured 24 h after challenge. Each symbol represents one mouse. B–D, functional characterization of BMDCs from Atg7DC−/− mice. BMDCs were incubated with 25 μg/ml of OVA-Alexa Fluor 488 for 90 or 180 min. Endocytosis was measured by flow cytometry (B). In additional experiments, BMDCs were stimulated with LPS for 24 h; cells were stained for flow cytometry (C), and supernatant was collected for ELISA (D). n = 3–9/group; data are mean ± S.E. No significant differences were detected by Student’s t test.
Previous studies have shown that expression of \textit{Atg5} was increased during EAE in mice and in subjects with multiple sclerosis (22). To test whether autophagy is induced during EAE, we analyzed levels of the autophagy marker microtubule-associated protein 1 light chain 3α (LC3)-type II and of the autophagy substrate sequestosome (SQSTM1), also known as p62, in the spinal cord of naive or diseased mice at different stages of EAE. We observed that SQSTM1 was reduced at all stages of EAE, whereas levels of LC3-II were increased during early EAE and reduced during severe EAE (Fig. 6A). Together, these data suggest induction of autophagy in spinal cord with progressive autophagy flux during EAE. The causal association of autophagy induction, however, is uncertain. It is possible that autophagy induction might simply be caused by neuronal damage in EAE.

Although CQ treatment, prior to EAE onset, delayed disease progression and attenuated its severity, the clinical scores and weight loss became similar to that of the PBS-treated mice following cessation of CQ treatment (Figs. 5B and 6B). Moreover, no significant difference in weight loss was observed between PBS-treated and CQ-treated groups when CQ treatment was initiated after onset of EAE (Fig. 6C). In this CQ regime, both sets of mice eventually reached similar peak scores (Fig. 6D). These data suggest that CQ treatment following disease onset could not completely protect against the progression of EAE.

**DISCUSSION**

T cell activation, both at the periphery and in the CNS, is required for induction of EAE (16). DCs infiltrate the CNS during inflammation and have been shown to be the major APCs within CNS during EAE (23). However, the role of DCs as APCs in the periphery during EAE induction is much less understood.
Dermal DCs are required for EAE induction as depletion of these DCs conferred resistance to EAE (24). However, in other studies, depletion of DCs increased the severity of EAE, a result attributed to the tolerogenic effect of DCs (25). Most of the above studies relied on DC depletion mechanisms either genetically or by using depleting antibodies or diphtheria toxin in a cell type-specific diphtheria toxin receptor mouse. Although depletion of DCs can reduce antigen presentation, it might also remove other potential regulatory mechanisms exerted by DCs. In such an all-or-none model, the contribution of DC-mediated antigen presentation in a complex autoimmune disease becomes difficult to assess. It is also possible that in a DC-depletion model, other cells might increase their participation in antigen presentation.

Atg7 deficiency in DCs significantly reduced the onset and severity of EAE. The underlying mechanism is likely due to reduced T cell priming, as evidenced by reduced MOG-specific recall response. These data are consistent with prior studies suggesting that autophagy controls class II antigen presentation to CD4 T cells. They also support reports implicating DCs as the major antigen-presenting cells in the periphery during the onset of EAE.
Atg7 deficiency in DCs did not completely abolish the incidence or severity of EAE. This finding could be due to two possibilities. Cre-mediated gene deletion is often incomplete (26, 27). Moreover, there are many subsets of DCs, and CD11c-cre-mediated gene deletion has been shown to be incomplete in plasmacytoid DCs (28). Plasmacytoid DCs play a major role in priming of T cells during EAE (29). Thus, CD11c-cre-mediated deletion of Atg7 might be leaky and could not completely abolish Atg7 from all subsets of dendritic cells in all Atg7DC−/− mice. The other possibility is that antigen presentation by other types of APCs might occur in EAE (25).

CQ treatment at different phases of EAE could delay disease onset or reduce severity. CQ, an autophagy-lysosomal inhibitor, is a widely used anti-malarial drug that has been used for treating other autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosus (2). As autoimmunity results from uncontrolled activation of adaptive immune system and CD4 T cells are the central component of adaptive immunity, autophagy might modulate autoimmune diseases through antigen presentation and activation of CD4 T cells. CQ treatment could also affect other cell types, including regulatory T cells, which might also affect in vivo development of EAE (30). CQ treatment could not completely abolish the disease. These data are consistent with a previous clinical trial showing that oral CQ treatment in established cases of MS could not provide long term benefit over placebo (31). Several possibilities might explain such outcomes. CQ is not a specific autophagy inhibitor, as it inhibits the later stages of lysosomal degradation. At the late stages of EAE, CD8 T cells might become important as effector cells, and hence, the beneficial effect of CQ could not be sustained. Recent studies have suggested that, similar to multiple sclerosis in humans, EAE in mice has a strong CD8 T cell component (32). We did not find defects in two models of hapten-induced contact hypersensitivity, mediated predominantly by CD8 T cells, in Atg7DC−/− mice. Thus, future treatment of multiple sclerosis might have to include specific autophagy inhibitors in combination with additional agents. Discovery of more specific autophagy inhibitors for long term in vivo use would be essential to answer these questions.

Acknowledgments—We thank Dr. Nilabh Shastri for the B3Z cells. We acknowledge members of the Eissa laboratory for stimulating discussions and for assisting with experiments. We thank Joel M. Sederstrom for expert assistance.

REFERENCES
1. Choi, A. M., Ryter, S. W., and Levine, B. (2013) Autophagy in human health and disease. *N. Engl. J. Med.* 368, 1845–1846
2. Bhattacharya, A., and Eissa, N. T. (2013) Autophagy and autoimmunity crosstalks. *Front. Immunol.* 4, 88
3. Paludan, C., Schmid, D., Landthaler, M., Vockerodt, M., Kube, D., Tuschl, T., and MüNZ, C. (2005) Endogenous MHC class II processing of a viral nuclear antigen after autophagy. *Science* 307, 593–596
4. Schmid, D., Pypaert, M., and MüNZ, C. (2007) Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. *Immunity* 26, 79–92
5. MüNZ, C. (2009) Enhancing immunity through autophagy. *Annu. Rev. Immunol.* 27, 423–449
6. Lee, H. K., Mattei, L. M., Steinberg, B. E., Alberts, P., Lee, Y. H., Chervonsky, A., Mizushima, N., Grinstein, S., and Iwasaki, A. (2010) *In vivo* requirement for Atg5 in antigen presentation by dendritic cells. *Immunity* 33, 227–239
7. Reed, M., Morris, S. H., Jang, S., Mukherjee, S., Yue, Z., and Lukacs, N. W. (2013) Autophagy-inducing protein beclin-1 in dendritic cells regulates CD4 T cell responses and disease severity during respiratory syncytial virus infection. *J. Immunol.* 191, 2526–2537
8. Jagannath, C., Lindsey, D. R., Dhandayuthapani, S., Xu, Y., Hunter, R. L., Jr., and Eissa, N. T. (2009) Autophagy enhances the efficacy of BCG vaccine by increasing peptide presentation in mouse dendritic cells. *Nature Med.* 15, 267–276
9. Zhang, Z., Wu, X., Duan, J., Hinrichs, D., Wegmann, K., Zhang, G. L., Hall, M., and Rosenbaum, J. T. (2012) Low dose rapamycin exacerbates autoimmune experimental uveitis. *PLoS One* 7, e36589
10. Aichinger, M., Wu, C., Nedjic, J., and Klein, L. (2013) Macroutophagy substrates are loaded onto MHC class II of medullary thymic epithelial cells for central tolerance. *J. Exp. Med.* 210, 287–300
11. Nedjic, J., Aichinger, M., Emmerich, J., Mizushima, N., and Klein, L. (2008) Autophagy in thymic epithelial shape the T-cell repertoire and is essential for tolerance. *Nature* 455, 396–400
12. Ireland, J. M., and Unanue, E. R. (2011) Autophagy in antigen-presenting cells results in presentation of citrullinated peptides to CD4 T cells. *J. Exp. Med.* 208, 2625–2632
13. Pierdominici, M., Vomero, M., Barbati, C., Colasanti, T., Maselli, A., Vacirca, D., Giovannetti, A., Malorni, W., and Ortona, E. (2012) Role of autophagy in immunity and autoimmunity, with a special focus on systemic lupus erythematosus. *FASEB J.* 26, 1400–1412
14. Levine, B., Mizushima, N., and Virgin, H. W. (2011) Autophagy in immunity and inflammation. *Nature* 469, 323–335
15. Zhou, X. J., Lu, X. L., Lv, J. C., Yang, H. Z., Qin, L. X., Zhao, M. H., Su, Y., Li, Z. G., and Zhang, H. (2011) Genetic association of PRDM1-ATG5 intergenic region and autophagy with systemic lupus erythematosus in a Chinese population. *Ann. Rheum. Dis.* 70, 1330–1337
16. Stromnes, I. M., and Goverman, J. M. (2006) Active induction of experimental allergic encephalomyelitis. *Nat. Protoc.* 1, 1810–1819
17. Bonilla, D. L., Bhattacharya, A., Sha, Y., Xu, Y., Xiang, Q., Kan, A., Jagannath, C., Komatsu, M., and Eissa, N. T. (2013) Autophagy regulates
phagocytosis by modulating the expression of scavenger receptors. Immunity 39, 537–547
18. Shin, J. N., Fattah, E. A., Bhattacharya, A., Ko, S., and Eissa, N. T. (2013) Inflammasome activation by altered proteostasis. J. Biol. Chem. 288, 35886–35895
19. Paust, S., Gill, H. S., Wang, B. Z., Flynn, M. P., Moseman, E. A., Senman, B., Szczepanik, M., Telenti, A., Askename, P. W., Companis, R. W., and von Andrian, U. H. (2010) Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigen-specific memory of haptenes and viruses. Nat. Immunol. 11, 1127–1135
20. Shastri, N., and Gonzalez, F. (1993) Endogenous generation and presentation of the ovalbumin peptide/Kb complex to T cells. J. Immunol. 150, 2724–2736
21. Vocanson, M., Hennino, A., Rozieres, A., Poyet, G., and Nicolas, J. F. (2009) Effector and regulatory mechanisms in allergic contact dermatitis. Allergy 64, 1699–1714
22. Alirezaei, M., Fox, H. S., Flynn, C. T., Moore, C. S., Hebb, A. L., Frausto, R. F., Bhan, V., Kiosses, W. B., Whitton, J. L., Robertson, G. S., and Crocker, S. J. (2009) Elevated ATG5 expression in autoimmune demyelination and multiple sclerosis. Autophagy 5, 152–158
23. Greter, M., Heppner, F. L., Lemos, M. P., Odermatt, B. M., Goebels, N., Laufer, T., Noelle, R. J., and Becher, B. (2005) Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. Nat. Med. 11, 328–334
24. King, I. L., Kroenke, M. A., and Segal, B. M. (2010) GM-CSF-dependent, CD103+ dermal dendritic cells play a critical role in Th effector cell differentiation after subcutaneous immunization. J. Exp. Med. 207, 953–961
25. Yogeit, N., Frommer, F., Lukas, D., Kauth-Neu, K., Karram, K., Ielo, D., von Stebut, E., Probst, H. C., van den Broek, M., Riethmacher, D., Birnberg, T., Blank, T., Reizis, B., Korn, T., Wiendl, H., Jung, S., Prinz, M., Kurschus, F. C., and Waisman, A. (2012) Dendritic cells ameliorate autoimmunity in the CNS by controlling the homeostasis of PD-1 receptor(+) regulatory T cells. Immunity 37, 264–275
26. Harno, E., Cottrell, E. C., and White, A. (2013) Metabolic pitfalls of CNS Cre-based technology. Cell Metab. 18, 21–28
27. Schulz, T. J., Glaubitz, M., Kuhlow, D., Thierbach, R., Biringer, M., Steinberg, P., Pfeiffer, A. F., and Ristow, M. (2007) Variable expression of Cre recombinase transgenes precludes reliable prediction of tissue-specific gene disruption by tail-biopsy genotyping. PLoS One 2, e1013
28. Wang, Y., Huang, G., Vogel, P., Neale, G., Reizis, B., and Chi, H. (2012) Transforming growth factor-β-activated kinase 1 (TAK1)-dependent checkpoint in the survival of dendritic cells promotes immune homeostasis and function. Proc. Natl. Acad. Sci. U.S.A. 109, E343–E352
29. Isaksson, M., Ardesjo, B., Ronnblom, L., Kämpe, O., Lassmann, H., Erolanta, M. L., and Lobell, A. (2009) Plasmacytoid DC promote priming of autoimmune Th17 cells and EAE. Eur. J. Immunol. 39, 2925–2935
30. Thome, R., Moraes, A. S., Bombeiro, A. L., Farias Ados, S., Francelin, C., da Costa, T. A., Di Gangi, R., dos Santos, L. M., de Oliveira, A. L., and Verinaud, L. (2013) Chloroquine treatment enhances regulatory T cells and reduces the severity of experimental autoimmune encephalomyelitis. PLoS One 8, e65913
31. Miller, H. G., Foster, J. B., Newell, D. J., Barwick, D. D., and Brewis, R. A. (1963) Multiple sclerosis: therapeutic trials of chloroquine, soluble aspirin, and gammaglobulin. Br. Med. J. 2, 1436–1439
32. Ji, Q., Castelli, L., and Goverman, J. M. (2013) MHC class I-restricted myelin epitopes are cross-presented by Tip-DCs that promote determinant spreading to CD8+ T cells. Nat. Immunol. 14, 254–261