Multiple effects of dendritic cell depletion on murine norovirus infection

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Dendritic cells (DCs) are permissive to murine norovirus (MNV) infection *in vitro* and *in vivo*. However, their roles during infection *in vivo* are not well defined. To determine the role of DCs during infection, conventional DCs were depleted from CD11c-DTR mice and infected with a persistent MNV strain. Viral titres in the intestine and secondary lymphoid organs were determined at early time points during infection, and anti-MNV antibody responses were analysed later during infection. Depletion of conventional DCs resulted in increased viral loads in intestinal tissues, impaired generation of antibody responses, and a failure of MNV to efficiently infect lymphoid tissues. These data suggest that DCs play multiple roles in MNV pathogenesis, in both innate immunity and the efficient generation of adaptive immune responses against MNV, as well as by promoting the dissemination of MNV to secondary lymphoid tissues. This is the first study to probe the roles of DCs in controlling and/or facilitating a norovirus infection *in vivo* and provides the basis for further studies aimed at defining mechanisms by which DCs control MNV replication and promote viral dissemination.

INTRODUCTION

Murine norovirus (MNV) is a small non-enveloped, plus-sense RNA virus in the genus *Norovirus* within the *Caliciviridae* family (Karst *et al.*, 2003). This gastro-intestinal virus is the single most widespread pathogen found in research mouse colonies worldwide (Pritchett-Corning *et al.*, 2009). *In vitro*, MNV productively infects mononuclear antigen-presenting cells including macrophages, dendritic cells (DCs) (Wobus *et al.*, 2004) and microglia (Cox *et al.*, 2009). *In vivo*, MNV alters DC populations (Chen *et al.*, 2011). Although wild-type mice show little to no outward signs of MNV infection (Mumphrey *et al.*, 2007), confounding effects of several MNV strains on biomedical research have been described (Cadwell *et al.*, 2010; Lencioni *et al.*, 2008; Paik *et al.*, 2011). Several of these confounding effects have been linked to disrupted DC and macrophage function in MNV-infected animals (Lencioni *et al.*, 2008; Paik *et al.*, 2011).

The earliest description of MNV established a critical role for interferon (IFN) signalling in providing protection from a potentially lethal MNV-1 infection (Karst *et al.*, 2003). More recent studies have found that IFN-α/β responses limit the replication of the tissue-culture adapted strain MNV-1 in lysozyme M- (e.g. macrophages) and CD11c- (i.e. DCs) expressing cells *in vivo* (Thackray *et al.*, 2012). Antibodies are required for clearance of MNV-1 (Chachu *et al.*, 2008b), whereas CD4^{+} and CD8^{+} T-lymphocytes limit peak levels of MNV-1 replication and facilitate the efficient clearance of MNV, respectively, but are not absolutely required for clearance (Chachu *et al.*, 2008a). However, the role(s) of DCs during MNV infection remain unknown.

DCs, the most potent class of antigen-presenting cells, are widely distributed throughout the body and are found in lymphoid tissues, such as the spleen and lymph nodes, as well as in peripheral tissues including the intestinal tract. Within intestinal tissues, DCs exist within Peyer's patches and isolated lymphoid follicles and are scattered throughout the lamina propria (Rescigno, 2010). Intestinal DCs have critical roles in promoting immunity to intestinal pathogens but must avoid generating pathological immune

responses against food antigens and commensal microbes (Denning *et al.*, 2007). In addition, certain subsets of DCs extrude dendrites between intestinal epithelial cells to the intestinal lumen and may provide an initial point-of-contact for incoming pathogens (Niess *et al.*, 2005). Despite the importance of DCs within the intestine and their ability to support MNV replication, the roles of DCs in MNV infection *in vivo* are unknown. As cells with critical functions in innate antiviral immunity in general (Kassim *et al.*, 2006) and MNV in particular (Thackray *et al.*, 2012), and the ability to prime adaptive immune responses *in vivo* (Jung *et al.*, 2002), DCs likely play important roles in controlling MNV replication and inducing anti-MNV immune responses. Conversely, while both macrophages and DCs are found in subepithelial tissues, DCs are the only cell type permissive for MNV known to make direct contact with the intestinal lumen (Niess *et al.*, 2005) and may have critical roles in the establishment and dissemination of MNV infection.

The goal of this study was to determine the role(s) of conventional DCs (cDCs) during MNV infection by studying MNV pathogenesis in transgenic CD11c-DTR mice. These mice express a high-affinity simian diphtheria toxin (DT) receptor under the control of the murine CD11c promoter (Jung *et al.*, 2002). CD11c is an integrin that is predominantly expressed by DCs, and when exogenous DT is administered to CD11c-DTR mice, DCs are systemically ablated (Jung *et al.*, 2002). Importantly, cDCs, which are most classically known for their antigen-presenting functions, are efficiently depleted in these mice, whereas plasmacytoid DCs, which express low levels of CD11c and are known for their ability to rapidly secrete large quantities of type I IFN, remain intact (Sapoznikov *et al.*, 2007). In this study, cDCs were depleted from mice, and MNV infection was analysed in the absence of cDCs. The results demonstrated that following depletion, MNV viral titres in intestinal tissues were increased early during infection, and mice had defects in the subsequent generation of anti-MNV antibody responses. In addition, cDCs were absolutely required for the efficient dissemination of MNV to secondary lymphoid tissues within the first 48 h following infection. Together, these data suggest that while cDCs play important roles in immunity to norovirus infection, MNV exploits the natural biology of cDCs to rapidly disseminate within the host.

RESULTS

DC depletion increases MNV titres within intestinal tissues

To determine the role of cDCs during MNV infection, CD11c-DTR mice were depleted of cDCs and orally infected with MNV strain CR3. CR3 was isolated from the faeces of mice and is shed from mice for at least 35 days (Thackray *et al.*, 2007). Efficient cDC depletion in these mice initially occurs 18 h after DT administration (Probst

et al., 2005). To confirm the efficient depletion of DCs from lymphoid and intestinal tissues throughout the experimental time course, CD11c-DTR mice were injected with DT or PBS and the presence of DCs in spleens, mesenteric lymph nodes (MLNs), and intestinal tissues was determined by flow cytometry after 66 h [i.e. the time point equivalent to 48 h post-infection (p.i.)]. The 66 h time point was selected because this is the time by which DCs are reported to begin repopulating tissues by differentiating from the bone marrow (Probst *et al.*, 2005), and because this was the latest time point employed in studies of acute MNV infection (see below). Therefore, the depletion observed at the 66 h time point represented the minimal depletion that occurs during the acute MNV experiments. By administering a single intraperitoneal injection of 100 ng DT per mouse, CD11c$^+$ DCs were efficiently depleted from spleens (~70 % depletion), MLNs (~55 % depletion) and small and large intestinal tissues (~75 % depletion) (Fig. 1).

To determine the role of cDCs in controlling MNV within intestinal tissues, DT- or PBS-treated CD11c-DTR mice were orally infected with CR3 18 h after DT treatment, and tissues of the intestinal tract were collected at 24 and 48 h p.i. (or 42 and 66 h after DT administration). By 24 h p.i., the amount of infectious virus within the caeca of mice that had been depleted of cDCs was significantly increased (~20-fold) (Fig. 2a). By 48 h p.i., titres in the caeca were similar in DC- and mock-depleted mice, but DT-treated mice showed >15- to 30-fold increases in the amount of infectious virus throughout the small intestine (Fig. 2b). No significant changes were observed in viral titres in the colon at either time point in both treatment groups (Fig. 2a, b). Furthermore, there was no effect of DT administration in wild-type mice at 24 h (Fig. 2c) or at 48 h with the exception of the proximal ileum (Fig. 2d). MNV titres in the proximal ilea of DT-treated wild-type mice were slightly increased (<9-fold) relative to PBS-treated animals (Fig. 2d), but titres in the proximal ilea of CD11c-DTR mice were increased to a greater degree (>15-fold) in DT-treated mice (Fig. 2b). This suggested that the majority of the toxin's effects observed in CD11c-DTR mice were mediated by DC depletion. Taken together, these data showed that DC depletion increases MNV replication within intestinal tissues and suggests that DCs are critical for limiting MNV replication in the intestine.

DCs are required for dissemination of MNV to secondary lymphoid tissues

Since cDC depletion led to increased MNV replication in the intestine, we next sought to determine the extent to which cDCs control MNV replication in secondary lymphoid tissues, specifically the spleen and the MLNs, which drain the intestinal tract. Infectious virus was measured in the spleen and MLNs from the same animals described above. Interestingly, no infectious virus was ever recovered from either the spleen or MLNs of CD11c-DTR
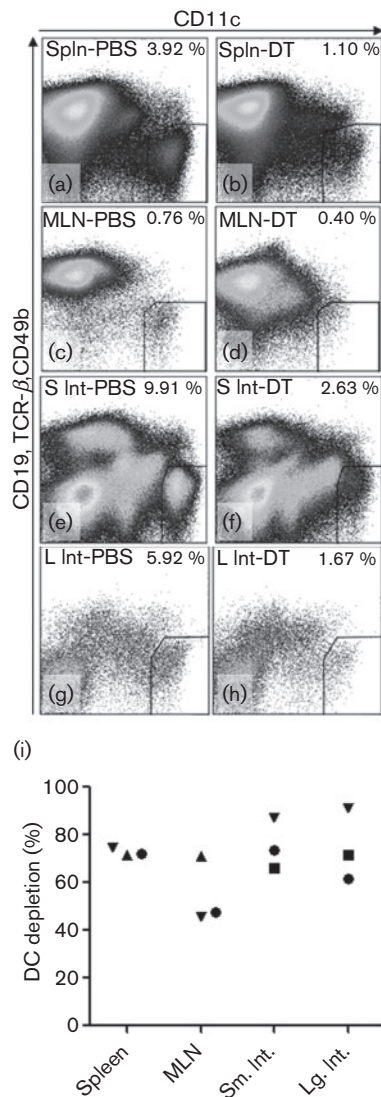
Fig. 1. DT efficiently depleted DCs from lymphoid and gastro-intestinal tissues of CD11c-DTR mice. (a–h) Mice were treated with 100 ng DT or 50 μl PBS by i.p. injection. Spleen (Spln), mesenteric lymph nodes (MLN), small (S) and large (L) intestinal (Int) tissues were removed 66 h later and single-cell suspensions stained for CD19, CD49b, and TCR-β (to exclude B-, NK and T-cells) and CD11c to identify DCs. Representative flow plots from PBS-treated (a, c, e, g) and DT-treated (b, d, f, h) mice are shown. (i) The efficiency of depletion in DT-treated mice relative to PBS-treated controls is shown, with each symbol representing the average depletion of the indicated tissue from an independent experiment.

mice depleted of DCs (Fig. 3a, b). This was in contrast to up to 3.5 logs infectious virus in the MLNs of PBS-depleted CD11c-DTR mice at 48 h p.i. (Fig. 3b). While similar trends were observed for the spleen, MNV titres in the spleens of depleted and non-depleted CD11c-DTR mice did not reach statistical significance at 24 or 48 h p.i. (Fig. 3b). DT administration did not impair dissemination of

MNV to lymphoid tissues in wild-type littermates (Fig. 3c, d). These data suggested that dissemination of MNV to secondary lymphoid organs required, and was mediated by, cDCs.

DC-depleted mice have increased MNV shedding in faeces

The cell type and location of MNV shedding are unknown. To determine whether cDCs were an important source of transmissible virus, cDC-depleted and non-depleted CD11c-DTR mice were infected with MNV as described above, and viral shedding in faeces was determined by measuring viral genomes in faeces by quantitative reverse transcription PCR (qRT-PCR) at 1, 2, 4, 7, 21 and 28 days p.i. (Fig. 4). Viral shedding in cDC-depleted mice was significantly increased at 2 days p.i., but became similar to shedding in non-depleted animals thereafter. These data demonstrate that transient DC depletion does not prevent shedding of MNV in faeces. Instead, increased numbers of viral genome were shed in the faeces of depleted mice, which is likely a result of increased viral replication within the intestinal tract.

DCs are required for the efficient generation of antibodies against MNV

In addition to their functions in innate immunity, DCs are important stimulators of adaptive immune responses (Jung et al., 2002). To determine whether cDCs contribute to adaptive immune responses against MNV, DC-depleted and non-depleted CD11c-DTR mice were infected with MNV, and anti-MNV IgG in the serum was measured prior to infection and 4, 7, 14, 21 and 28 days p.i. by ELISA. In the absence of cDCs, mice had significantly less anti-MNV IgG for the first 2 weeks following infection (Fig. 5a). In addition, when sera from CD11c-DTR mice depleted of cDCs were tested for their ability to neutralize MNV infection in tissue culture, sera had significantly decreased ability to neutralize MNV at 4 and 7 days p.i. (Fig. 5b). These data suggested that the generation of neutralizing antibodies was delayed and indicated that cDCs are critical for the efficient generation of antibody responses against MNV.

DISCUSSION

MNV represents a versatile model for the analysis of mechanisms of pathogenesis and immunity for a norovirus in its natural host (Wobus et al., 2006). While the roles of IFN, B-cells and T-cells have been described previously (Chachu et al., 2008a; Chachu et al., 2008b; Mumphrey et al., 2007; Thackray et al., 2012), this is the first study to describe the dual role of cDCs in both controlling and promoting MNV infection. Furthermore, this study elucidated distinct roles for cDCs in innate and adaptive immunity and the dissemination of MNV.
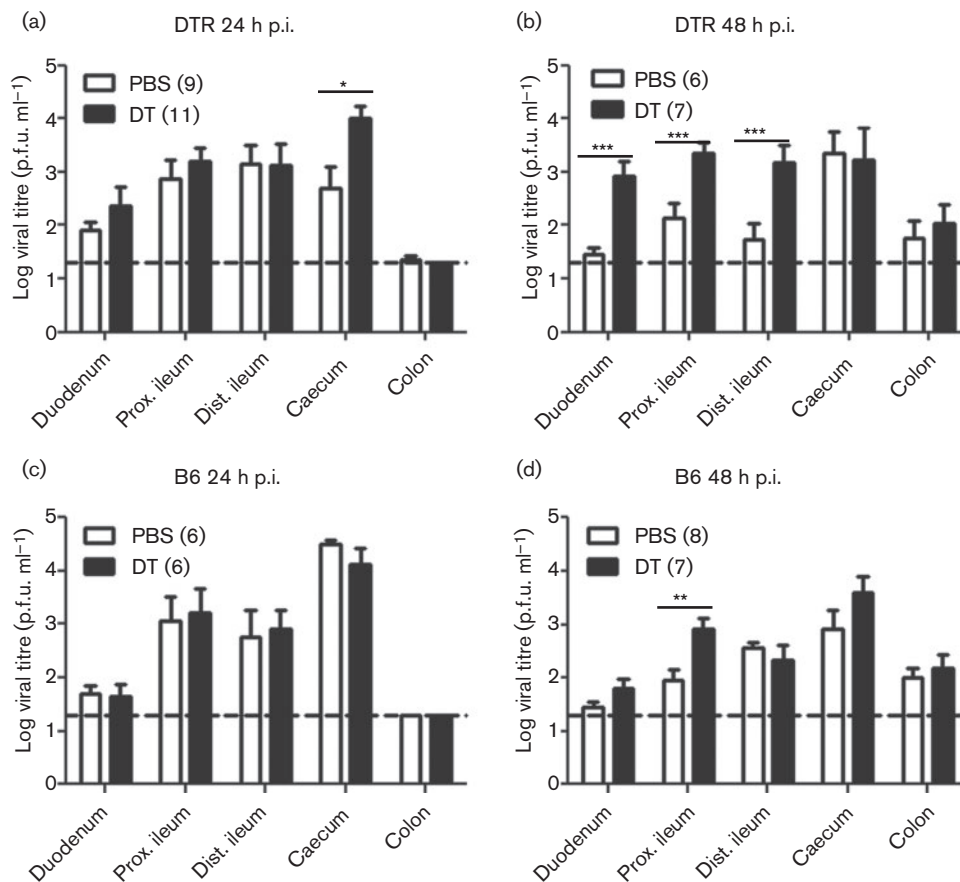
Fig. 2. DCs control MNV replication within the intestinal tract. CD11c-DTR mice (a, b) or littermates (c, d) were treated with PBS (white) or DT (black) 18 h prior to infection with 7.5×10^6 p.f.u. CR3. Intestinal tissues were harvested 24 (a, c) or 48 (b, d) h p.i. Viral titres were determined by plaque assay. The number of mice in each group is indicated in parentheses. Data are shown as mean $\pm$ SEM for three independent experiments. The dashed line indicates the limit of detection. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

A main finding of this study was that MNV titres in the intestinal tract were increased significantly for the first 48 h following MNV infection in mice depleted of cDCs (Fig. 2). While cDCs are best known for their ability to present antigen and prime T-cells (Jung *et al.*, 2002), they also possess important roles in innate immunity (Kassim *et al.*, 2006; Loof *et al.*, 2007). For example, cDCs are important producers of type I IFN at early times following adenovirus infection (Fejer *et al.*, 2008). Since IFN are important for protection from MNV lethality (Karst *et al.*, 2003), and type I IFN responses within macrophages and DCs limit MNV infection *in vivo* (Thackray *et al.*, 2012), it is likely that a lack of cDC-derived IFN in the intestinal tissues resulted in the observed increase in MNV replication. Due to the short time frame, it is unlikely that the observed increases in viral titres were the result of impaired T-cell-mediated immunity from a lack of antigen presentation.

An alternative explanation for the increased viral titres within the intestinal tract is that MNV could have increased replication within cells that are recruited to

tissues in which DCs have been depleted. However, we think that this alternative is less likely. First, it has recently been shown that CD11c-DTR mice undergo a strong neutrophilia within the first 72 h following DC depletion (Tittel *et al.*, 2012); however, there is no evidence that MNV is able to replicate within neutrophils. Secondly, CD11b$^+$ CD11c$^-$ myeloid cell populations (which could include macrophages, monocytes and neutrophils) were quantified in PBS- and DT-treated mice in the current study, and although the proportion of these cells was increased by roughly threefold in the spleen and MLNs, intestinal tissues had only a slight increase (1.2–1.4-fold) in the proportion of CD11b$^+$ CD11c$^-$ cells (data not shown). In contrast, viral titres were increased 15–30-fold in intestinal tissues. If the increased viral titres were due to a greater frequency of permissive cells, then one would expect a significantly more robust recruitment of cells into intestinal tissues.

In contrast to the increased viral titres seen in the intestinal tract, no infectious MNV was detected in MLNs in the absence of cDCs for the first 48 h following MNV infection
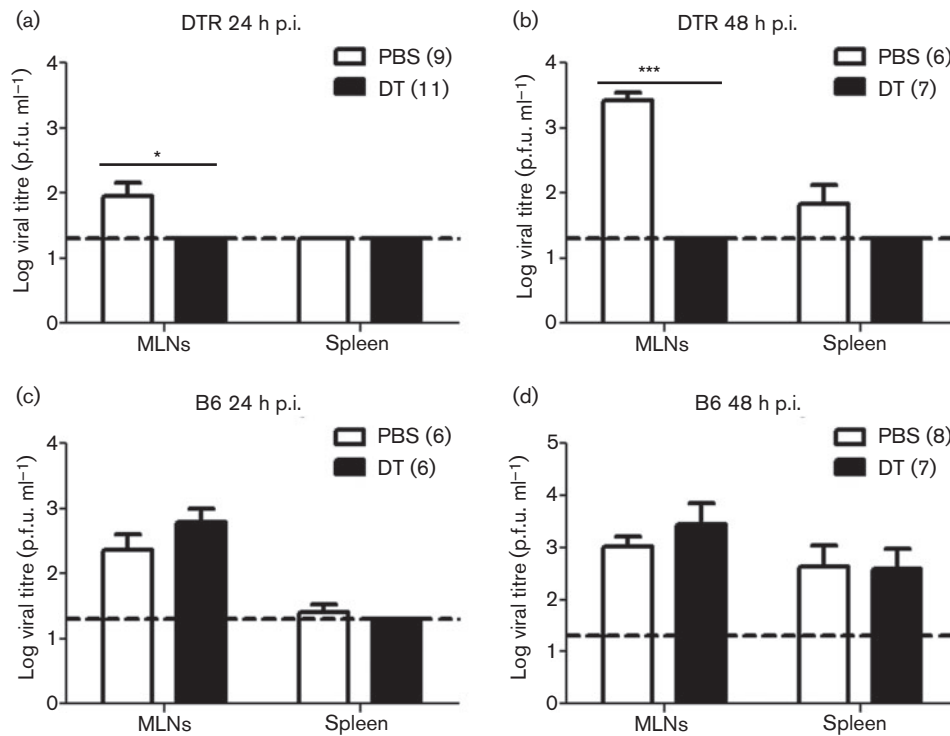
Fig. 3. DCs are required for efficient dissemination of MNV to secondary lymphoid tissues. CD11c-DTR mice (a, b) or littermates (c, d) were treated with PBS (open bars) or DT (filled bars) 18 h prior to infection with 7.5×10^{6} p.f.u. CR3. Spleens and MLN were harvested at 24 (a, c) or 48 (b, d) h p.i. and viral titres determined by plaque assay. The number of mice in each group is indicated in parentheses. Data are shown as means ± SEM for three independent experiments. The dashed line indicates the limit of detection. *P<0.05, ***P<0.001.
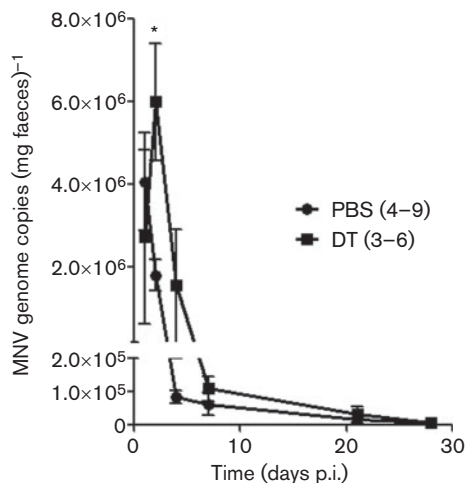

Fig. 4. DC depletion transiently increases MNV shedding. CD11c-DTR mice were treated with PBS (●) or DT (■) 18 h prior to infection with 7.5×10^{6} p.f.u. CR3. Faeces were collected from mice at 1, 2, 4, 7, 21 and 28 days p.i. and MNV RNA was quantified by qRT-PCR. The number of mice in each group is indicated in parentheses. Data are shown as means ± SEM for three independent experiments. *P<0.05.

(Fig. 3). The most plausible explanation for this finding is that cDCs were infected by MNV in either the lamina propria or in the Peyer's patches and subsequently migrated to the draining MLNs of non-depleted mice while carrying infectious virus. CD103^{+} DCs constitutively migrate from the intestine to MLNs and are thought to play a role in maintaining tolerance to commensal bacteria and/or food-derived antigens (Coombes *et al.*, 2007). These cells have previously been implicated in facilitating the dissemination of a non-invasive strain of *Salmonella* from the intestinal tract to the MLNs (Bogunovic *et al.*, 2009). In addition, migratory DCs have been demonstrated or implicated to promote dissemination of viral pathogens as well, including influenza virus (Moltedo et al., 2011), human immunodeficiency virus (Geijtenbeek *et al.*, 2000) and the enteric pathogen mouse mammary tumour virus (Courreges *et al.*, 2007). Taken together, these studies support the hypothesis whereby migratory DCs provide a mechanism by which MNV disseminates from the initial sites of replication to the draining MLNs. However, future studies are needed to test whether CD103^{+} DCs or other subsets of migratory DCs provide the mechanism for dissemination of MNV *in vivo*.

Our data also provide evidence that MNV replication in DCs is not required for MNV shedding. Similar or higher amounts of MNV genomes were detected in the faeces of
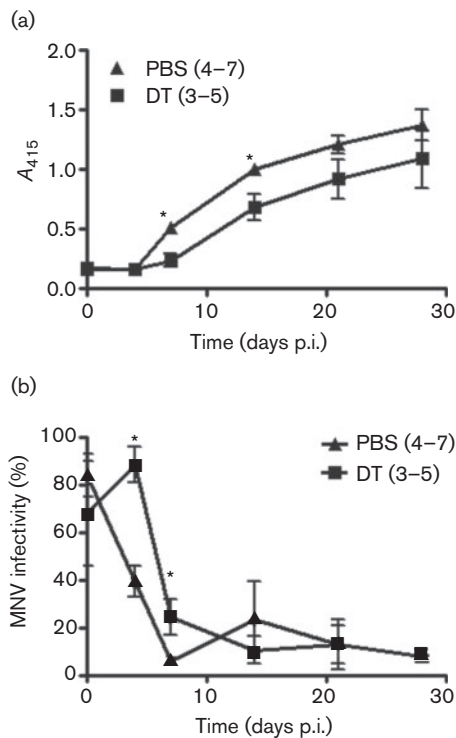
(a)

(b)

Fig. 5. DCs facilitate the efficient generation of anti-MNV antibodies. Mice were treated with PBS or DT 18 h prior to infection with 7.5×10^6 p.f.u. CR3. Blood was collected from mice prior to infection and 4, 7, 14, 21, and 28 days p.i. (a) Anti-MNV IgG was measured by ELISA using 1 : 100 dilutions of sera. (b) To measure the ability of serum to neutralize MNV, 10^4 p.f.u. CR3 was incubated with a 1 : 100 dilution of serum in a volume of 100 μl. Infectious virus was detected by plaque assay and is presented as a percentage of input virus. The number of mice in each group is indicated in parentheses. Data are shown as mean ± SEM for three independent experiments. *$P < 0.05$.

DC-depleted mice compared with non-depleted mice (Fig. 4), suggesting transient DC depletion was insufficient to limit MNV transmission. In addition, replication of MNV in the MLNs was not required for MNV shedding early during the infection since MNV was unable to replicate in the MLNs during the first 48 h following infection (Fig. 3) at times when MNV shedding occurred (Fig. 4). Interestingly, the significant increase in MNV shedding observed on day 2 was temporally associated with an increased amount of infectious MNV within the intestines (compare Figs 2 and 4). This transient increase in shedding was likely due to the transience of cDC depletion in the CD11c-DTR model (Probst et al., 2005). Thus, as cDCs repopulated the tissues over time, 'normal' control of MNV replication was probably restored and MNV shedding reduced to amounts found in the faeces of non-depleted mice. Future studies are needed to determine the mechanism of MNV shedding.

Beyond the immediate effects of transient cDC depletion on innate immunity to MNV and dissemination to secondary lymphoid tissues, DCs were also required for the efficient generation of an antibody response against MNV (Fig. 5). There are several possible mechanisms that may underlie the observed impairment of an antibody response against MNV. Since DCs are important for priming $CD4^+$ T-cell responses (Lemos et al., 2003), it is likely that T-cell responses against MNV are impaired in DC-depleted mice. However, $CD4^+$ T-cells are not required for clearance of the acute, laboratory-adapted strain MNV-1 (Chachu et al., 2008a), while B-cells are required (Chachu et al., 2008b), suggesting that B-cell responses to MNV can occur in the absence of $CD4^+$ T-cell help. If this is the case, then it is possible that the impaired antibody response observed in depleted animals was not due to impaired antigen presentation. However, it is important to note that this study used the field isolate, CR3, which is not cleared from wild-type mice (Thackray et al., 2007). Thus, the role of T-cells in B-cell activation may differ between MNV strains. In our study, we found that serum concentrations of anti-MNV IgG were reduced in DT-treated mice, while the ability of their sera to neutralize MNV occurred to a similar extent, albeit with delayed kinetics, relative to PBS-treated controls (Fig. 5b). This supports a role for DC in the generation of a T-cell-dependent antibody response. It is also possible that B-cell responses were impaired because of a lack of MNV antigen within the MLNs in DC-depleted mice. If this is the case, then the 'failure' of MNV to disseminate may not have been beneficial for the host, but instead might have resulted in weaker control of MNV by the adaptive immune system. Alternatively, DCs may produce cytokines that are necessary for the optimal activation of B-cell responses (Zhu et al., 2007). DC-dependent antibody responses to influenza (Gonzalez et al., 2010) and Friend murine leukemia virus (Browne & Littman, 2009) have been described to require diverse DC functions including pathogen detection and antigen transport. However, the exact role of DCs in promoting B-cell responses to MNV remains unknown.

Overall, we have found evidence that DCs have differing roles at distinct anatomical sites during MNV infection. DC depletion led to impaired control of MNV replication within the intestinal tract and weaker and slower antibody responses, but DCs were required for the rapid dissemination of MNV to secondary lymphoid tissues. These studies highlight a critical innate role for cDCs in controlling norovirus replication within intestinal tissues, while reinforcing the idea that enteric viruses can exploit cDCs to facilitate their dissemination within the host.

METHODS

Cell culture, mice, and virus. RAW 264.7 cells were purchased from the ATCC and maintained as described previously (Wobus et al., 2004). Wild-type C57BL/6 mice were purchased from the Jackson Laboratory (000664). CD11c-DTR mice (Jung et al., 2002) were purchased from the Jackson Laboratory (004509) and bred at the University of Michigan animal facilities. All mice in this study were

cared for and used in accordance with federal and university guidelines. All mice were tested for anti-MNV antibodies by ELISA as described previously (Wobus *et al.*, 2004) and were seronegative. MNV strain CR3 (GV/CR3/2005/USA) was used at passage 6 (Thackray *et al.*, 2007). For *in vivo* infections, CR3 lysate was concentrated by ultracentrifugation and resuspended in PBS as previously described (Chachu *et al.*, 2008b). A mock lysate was similarly concentrated and used as control.

DC depletion and MNV infection. DCs were depleted from CD11c-DTR mice by intraperitoneal injection of 100 ng DT in a volume of 50 µl PBS per mouse (John *et al.*, 2009). Control mice received an injection of PBS instead. Eighteen hours after DT or PBS treatment, mice were orally infected with 7.5×10^6 p.f.u. CR3 in a volume of 25 µl PBS or an equal volume of concentrated mock lysate.

Leukocyte isolation. To determine depletion efficiency, spleens, MLNs and small and large intestines were removed from DT- or PBS-treated CD11c-DTR mice 66 h after DT administration. Spleens and MLNs were finely minced, digested using 2 mg collagenase D ml^{-1} (Roche) for 30 min at 37 °C, and disrupted by pushing through 40 µm cell strainers to obtain single-cell suspensions. Red cells were lysed by treating splenocytes with ACK lysing buffer (BioWhittaker) for 5 min at room temperature. To obtain intestinal leukocytes, intestinal tissues were washed extensively and minced. Lamina propria mononuclear cells (LPMCs) were isolated from large intestines as described previously (Franchi *et al.*, 2012). LPMCs were isolated from small intestines in a similar manner. Small intestinal tissues were treated with 1 mM dithiothreitol for 15 min to remove mucus. Intestinal tissues were then washed three times with 5 mM EDTA at 37 °C for a total of 90 min to remove epithelial cells. Intestinal tissues were then digested using 200 U collagenase, type 3 ml^{-1} and 0.01 mg DNase I ml^{-1} (both from Worthington) for 90–180 min. Digested intestinal cell suspensions were filtered through 70 µm cell strainers, centrifuged on a 40:75 % Percoll gradient (GE Life Sciences), and LPMCs were collected from the interface, similar to cells from the large intestine.

Flow cytometry. Splenocytes, MLN cells and intestinal leukocytes were treated with 2.4G2 hybridoma supernatant to block F$_C$ receptor-mediated antibody binding (Unkeless, 1979). Cells were then stained for markers of B-cells (CD19, clone 6D5; BioLegend), NK cells (CD49b, clone DX5; BioLegend) and T-cells (TCR-β, clone H57-597; BioLegend) to exclude these cells from further analysis and CD11c (clone N418; eBioscience) to identify DCs. Additional myeloid cells (monocytes, macrophages, or neutrophils) were identified from the CD11c$^-$ population by staining for CD11b (clone M1/70, BD Pharmingen). Flow cytometry was performed using a FACSCanto (Becton Dickinson) and data were analysed using FlowJo software (Treestar). DCs were identified as CD11c$^+$, CD19$^-$, CD49b$^-$, TCR-β^- cells.

Quantification of MNV. At 24 or 48 h p.i., tissues were removed, including duodenum, proximal and distal ileum, caecum, colon, spleen and MLN. The entire MLN, one-half of the caecum and spleen, and 1 cm of various portions of the intestinal tract were homogenized in 1 ml medium. To measure infectious virus, plaque assays were performed on RAW 264.7 cells as previously described (Gonzalez-Hernandez *et al.*, 2012; Mumphrey *et al.*, 2007; Wobus *et al.*, 2004). Titres are presented as p.f.u. ml^{-1}. To measure genome titres in faeces, samples were submitted to the Research Animal Diagnostic Services (Charles River Laboratories, Wilmington, MA) for qRT-PCR and analysed as described previously (Thackray *et al.*, 2007).

Serology. Serum was collected from DT- or PBS-treated mice prior to and at various times following CR3 infection by retro-orbital bleeding. CR3 and MNV-1 are part of a single serotype (Thackray

et al., 2007). Thus, ELISA was performed on serially diluted sera using plates coated with CsCl-purified MNV-1 as described (Wobus *et al.*, 2004). For microneutralization experiments, a 1:100 dilution of serum was incubated with 10^4 p.f.u. CR3 in a volume of 100 µl for 30 min at room temperature. Infectious virus was then measured by plaque assay as previously described (Gonzalez-Hernandez *et al.*, 2012). The percentage infectivity was calculated by dividing the observed titre of serum-treated virus by the titre of virus incubated with PBS.

REFERENCES

Bogunovic, M., Ginhoux, F., Helft, J., Shang, L., Hashimoto, D., Greter, M., Liu, K., Jakubzick, C., Ingersoll, M. A. & other authors (2009). Origin of the lamina propria dendritic cell network. *Immunity* **31**, 513–525.

Browne, E. P. & Littman, D. R. (2009). Myd88 is required for an antibody response to retroviral infection. *PLoS Pathog* **5**, e1000298.

Cadwell, K., Patel, K. K., Maloney, N. S., Liu, T. C., Ng, A. C., Storer, C. E., Head, R. D., Xavier, R., Stappenbeck, T. S. & Virgin, H. W. (2010). Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16L1 phenotypes in intestine. *Cell* **141**, 1135–1145.

Chachu, K. A., LoBue, A. D., Strong, D. W., Baric, R. S. & Virgin, H. W. (2008a). Immune mechanisms responsible for vaccination against and clearance of mucosal and lymphatic norovirus infection. *PLoS Pathog* **4**, e1000236.

Chachu, K. A., Strong, D. W., LoBue, A. D., Wobus, C. E., Baric, R. S. & Virgin, H. W., IV (2008b). Antibody is critical for the clearance of murine norovirus infection. *J Virol* **82**, 6610–6617.

Chen, X., Leach, D., Hunter, D. A., Sanfelippo, D., Buell, E. J., Zemple, S. J. & Grayson, M. H. (2011). Characterization of intestinal dendritic cells in murine norovirus infection. *Open Immunol J* **4**, 22–30.

Coombes, J. L., Siddiqui, K. R., Arancibia-Cárcamo, C. V., Hall, J., Sun, C. M., Belkaid, Y. & Powrie, F. (2007). A functionally specialized population of mucosal CD103$^+$ DCs induces Foxp3$^+$ regulatory T cells via a TGF-β and retinoic acid-dependent mechanism. *J Exp Med* **204**, 1757–1764.

Courreges, M. C., Burzyn, D., Nepomnaschy, I., Piazzon, I. & Ross, S. R. (2007). Critical role of dendritic cells in mouse mammary tumor virus in vivo infection. *J Virol* **81**, 3769–3777.

Cox, C., Cao, S. & Lu, Y. (2009). Enhanced detection and study of murine norovirus-1 using a more efficient microglial cell line. *Virol J* **6**, 196.

Denning, T. L., Wang, Y. C., Patel, S. R., Williams, I. R. & Pulendran, B. (2007). Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. *Nat Immunol* **8**, 1086–1094.

Fejer, G., Drechsel, L., Liese, J., Schleicher, U., Ruzsics, Z., Imelli, N., Greber, U. F., Keck, S., Hildenbrand, B. & other authors (2008). Key

role of splenic myeloid DCs in the IFN-$\alpha\beta$ response to adenoviruses *in vivo*. *PLoS Pathog* **4**, e1000208.

Franchi, L., Kamada, N., Nakamura, Y., Burberry, A., Kuffa, P., Suzuki, S., Shaw, M. H., Kim, Y. G. & Núñez, G. (2012). NLRC4-driven production of IL-1β discriminates between pathogenic and commensal bacteria and promotes host intestinal defense. *Nat Immunol* **13**, 449–456.

Geijtenbeek, T. B., Kwon, D. S., Torensma, R., van Vliet, S. J., van Duijnhoven, G. C., Middel, J., Cornelissen, I. L., Nottet, H. S., KewalRamani, V. N. & other authors (2000). DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances *trans*-infection of T cells. *Cell* **100**, 587–597.

Gonzalez, S. F., Lukacs-Kornek, V., Kuligowski, M. P., Pitcher, L. A., Degn, S. E., Kim, Y. A., Cloninger, M. J., Martinez-Pomares, L., Gordon, S. & other authors (2010). Capture of influenza by medullary dendritic cells via SIGN-R1 is essential for humoral immunity in draining lymph nodes. *Nat Immunol* **11**, 427–434.

Gonzalez-Hernandez, M. B., Bragazzi Cunha, J. & Wobus, C. E. (2012). Plaque assay for murine norovirus. *J Vis Exp* **66**, e4297.

John, B., Harris, T. H., Tait, E. D., Wilson, E. H., Gregg, B., Ng, L. G., Mrass, P., Roos, D. S., Dzierszinski, F. & other authors (2009). Dynamic Imaging of CD8$^+$ T cells and dendritic cells during infection with *Toxoplasma gondii*. *PLoS Pathog* **5**, e1000505.

Jung, S., Unutmaz, D., Wong, P., Sano, G., De los Santos, K., Sparwasser, T., Wu, S., Vuthoori, S., Ko, K. & other authors (2002). In vivo depletion of CD11c$^+$ dendritic cells abrogates priming of CD8$^+$ T cells by exogenous cell-associated antigens. *Immunity* **17**, 211–220.

Karst, S. M., Wobus, C. E., Lay, M., Davidson, J. & Virgin, H. W., IV (2003). STAT1-dependent innate immunity to a Norwalk-like virus. *Science* **299**, 1575–1578.

Kassim, S. H., Rajasagi, N. K., Zhao, X., Chervenak, R. & Jennings, S. R. (2006). In vivo ablation of CD11c-positive dendritic cells increases susceptibility to herpes simplex virus type 1 infection and diminishes NK and T-cell responses. *J Virol* **80**, 3985–3993.

Lemos, M. P., Fan, L., Lo, D. & Laufer, T. M. (2003). CD8alpha$^+$ and CD11b$^+$ dendritic cell-restricted MHC class II controls Th1 CD4$^+$ T cell immunity. *J Immunol* **171**, 5077–5084.

Lencioni, K. C., Seamons, A., Treuting, P. M., Maggio-Price, L. & Brabb, T. (2008). Murine norovirus: an intercurrent variable in a mouse model of bacteria-induced inflammatory bowel disease. *Comp Med* **58**, 522–533.

Loof, T. G., Rohde, M., Chhatwal, G. S., Jung, S. & Medina, E. (2007). The contribution of dendritic cells to host defenses against *Streptococcus pyogenes*. *J Infect Dis* **196**, 1794–1803.

Moltedo, B., Li, W., Yount, J. S. & Moran, T. M. (2011). Unique type I interferon responses determine the functional fate of migratory lung dendritic cells during influenza virus infection. *PLoS Pathog* **7**, e1002345.

Mumphrey, S. M., Changotra, H., Moore, T. N., Heimann-Nichols, E. R., Wobus, C. E., Reilly, M. J., Moghadamfalahi, M., Shukla, D. &

Karst, S. M. (2007). Murine norovirus 1 infection is associated with histopathological changes in immunocompetent hosts, but clinical disease is prevented by STAT1-dependent interferon responses. *J Virol* **81**, 3251–3263.

Niess, J. H., Brand, S., Gu, X., Landsman, L., Jung, S., McCormick, B. A., Vyas, J. M., Boes, M., Ploegh, H. L. & other authors (2005). CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* **307**, 254–258.

Paik, J., Fierce, Y., Mai, P. O., Phelps, S. R., McDonald, T., Treuting, P., Drivdahl, R., Brabb, T., LeBoeuf, R. & other authors (2011). Murine norovirus increases atherosclerotic lesion size and macrophages in Ldlr$^{-/-}$ mice. *Comp Med* **61**, 330–338.

Pritchett-Corning, K. R., Cosentino, J. & Clifford, C. B. (2009). Contemporary prevalence of infectious agents in laboratory mice and rats. *Lab Anim* **43**, 165–173.

Probst, H. C., Tschannen, K., Odermatt, B., Schwendener, R., Zinkernagel, R. M. & Van Den Broek, M. (2005). Histological analysis of CD11c-DTR/GFP mice after in vivo depletion of dendritic cells. *Clin Exp Immunol* **141**, 398–404.

Rescigno, M. (2010). Functional specialization of antigen presenting cells in the gastrointestinal tract. *Curr Opin Immunol* **22**, 131–136.

Sapoznikov, A., Fischer, J. A., Zaft, T., Krauthgamer, R., Dzionek, A. & Jung, S. (2007). Organ-dependent in vivo priming of naive CD4$^+$, but not CD8$^+$, T cells by plasmacytoid dendritic cells. *J Exp Med* **204**, 1923–1933.

Thackray, L. B., Wobus, C. E., Chachu, K. A., Liu, B., Alegre, E. R., Henderson, K. S., Kelley, S. T. & Virgin, H. W., IV (2007). Murine noroviruses comprising a single genogroup exhibit biological diversity despite limited sequence divergence. *J Virol* **81**, 10460–10473.

Thackray, L. B., Duan, E., Lazear, H. M., Kambal, A., Schreiber, R. D., Diamond, M. S. & Virgin, H. W. (2012). Critical role for interferon regulatory factor 3 (IRF-3) and IRF-7 in type I interferon-mediated control of murine norovirus replication. *J Virol* **86**, 13515–13523.

Tittel, A. P., Heuser, C., Ohliger, C., Llanto, C., Yona, S., Hämmerling, G. J., Engel, D. R., Garbi, N. & Kurts, C. (2012). Functionally relevant neutrophilia in CD11c diphtheria toxin receptor transgenic mice. *Nat Methods* **9**, 385–390.

Unkeless, J. C. (1979). Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. *J Exp Med* **150**, 580–596.

Wobus, C. E., Karst, S. M., Thackray, L. B., Chang, K. O., Sosnovtsev, S. V., Belliot, G., Krug, A., Mackenzie, J. M., Green, K. Y. & Virgin, H. W. (2004). Replication of Norovirus in cell culture reveals a tropism for dendritic cells and macrophages. *PLoS Biol* **2**, e432.

Wobus, C. E., Thackray, L. B. & Virgin, H. W., IV (2006). Murine norovirus: a model system to study norovirus biology and pathogenesis. *J Virol* **80**, 5104–5112.

Zhu, J., Huang, X. & Yang, Y. (2007). Type I IFN signaling on both B and CD4 T cells is required for protective antibody response to adenovirus. *J Immunol* **178**, 3505–3510.