

# Functionally relevant neutrophilia in CD11c diphtheria toxin receptor transgenic mice

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**Transgenic mice expressing the diphtheria toxin receptor (DTR) in specific cell types are key tools for functional studies in several biological systems. *B6.FVB-Tg(Itgax-DTR/EGFP)57Lan/J (CD11c.DTR)* and *B6.Cg-Tg(Itgax-DTR/OVA/EGFP)1Gjh/Crl (CD11c.DOG)* mice express the DTR in CD11c<sup>+</sup> cells, allowing conditional depletion of dendritic cells. We report that dendritic-cell depletion in these models caused polymorphonuclear neutrophil (PMN) release from the bone marrow, which caused chemokine-dependent neutrophilia after 6–24 h and increased bacterial clearance in a mouse pyelonephritis model. We present a transgenic mouse line, *B6.Cg-Tg(Itgax-EGFP-CRE-DTR-LUC)2Gjh/Crl (CD11c.LuciDTR)*, which is unaffected by early neutrophilia. However, *CD11c.LuciDTR* and *CD11c.DTR* mice showed late neutrophilia 72 h after dendritic cell depletion, which was independent of PMN release and possibly resulted from increased granulopoiesis. Thus, the time point of dendritic cell depletion and the choice of DTR transgenic mouse line must be considered in experimental settings where neutrophils may be involved.**

Dendritic cells are the main inducers of adaptive immunity. A milestone in dendritic cell research was the development of *CD11c.DTR* transgenic mice<sup>1</sup>, which express the DTR gene under control of a cloned *Itgax* promoter (here referred to as the *CD11c* promoter) and thus allow conditional dendritic cell depletion upon injection of diphtheria toxin. *CD11c.DTR* mice, the first of these lines, were key for clarifying the roles of dendritic cells in numerous fundamental immune processes<sup>1–3</sup>, the defense against infections<sup>4–7</sup> or in immune-mediated diseases<sup>8–14</sup>. However, like all experimental tools, *CD11c.DTR* mice are not perfect. For example, the *CD11c* promoter is active not only in dendritic cells, but also in macrophages in the splenic marginal zone and in alveoli<sup>15</sup>, and in activated CTL cells<sup>1</sup>; consequently, these cells are also targeted in *CD11c.DTR* mice<sup>2</sup>. In contrast, plasmacytoid dendritic cells or NK cells expressing only low *CD11c* levels are not targeted<sup>2</sup>. Moreover, *CD11c.DTR* mice allow depletion only for 2–3 d, and do not survive a second diphtheria toxin injection within less than 7 d (refs. 10,15), unless bone marrow chimeras are created<sup>4,5</sup>.

*CD11c.DOG* mice are a recently described bacterial artificial chromosome (BAC) transgenic line that allows prolonged dendritic cell depletion<sup>16</sup>, which enabled extended studies on dendritic cell homeostasis<sup>17,18</sup>. We have recently used *CD11c.DTR* mice to study the role of kidney dendritic cells in bacterial pyelonephritis<sup>7</sup>, a prevalent kidney infection caused mostly by uropathogenic *Escherichia coli* (UPEC)<sup>19</sup>. Immune defense relies on PMNs, which are recruited to kidneys by chemokines, especially by CXCL2 (IL-8, also known as MIP-2 in the mouse)<sup>20</sup>. Using a mouse model of pyelonephritis, we had previously observed that depletion of kidney dendritic cells resulted in reduced CXCL2 production, and delayed PMN recruitment and bacterial clearance from the kidney<sup>7</sup>. Following up on these studies, we noted a hitherto undescribed side effect in *CD11c.DTR* and *CD11c.DOG* mice: at 24 h after dendritic cell depletion, these mice showed neutrophilia, that is, increased PMN levels in the blood, which was due to PMN release from the bone marrow and is functionally relevant for the defense against pyelonephritis. We describe a new transgenic line that is unaffected by neutrophilia until 48 h after dendritic-cell depletion. Our results provide a basis for the rational design of experiments under conditions where PMNs may be relevant.

## RESULTS

### A paradox during pyelonephritis studies in *CD11c.DTR* mice

We studied kidney infiltration by dendritic cells, macrophages and PMNs by flow cytometry (Fig. 1a) in a mouse pyelonephritis model that we have previously described<sup>7</sup>. Briefly, the model requires two transurethral instillations of UPEC at a 3-h interval and causes kidney infection in about 85% of mice. Injection of diphtheria toxin into *CD11c.DTR* mice at the time point of transurethral infection reduced kidney dendritic cell numbers by about 50%, decreased PMN recruitment and impaired bacterial clearance (Fig. 1b–e), confirming our previous study<sup>7</sup>. Although dendritic cell numbers were reduced only by ~50% 6 h after administration of diphtheria toxin, the remaining dendritic cells were defunct, as they no longer produced PMN-attracting chemokines<sup>7</sup>. To obtain clearer results at the time point of maximal dendritic cell depletion, we infected *CD11c.DTR* mice with

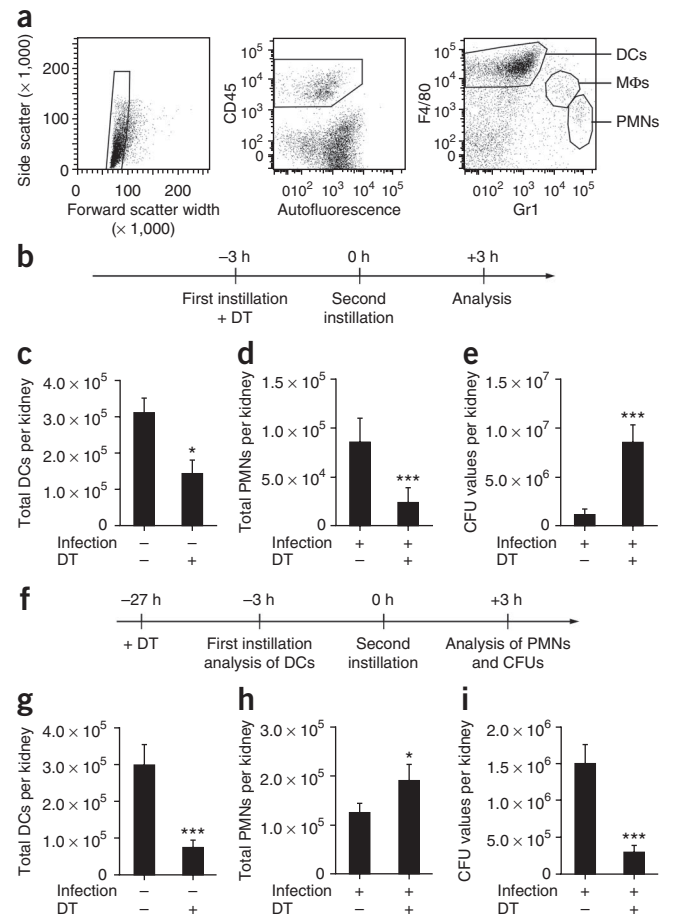
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**Figure 1** | Paradoxical effect of dendritic cell depletion on bacterial clearance. (a) Flow cytometry gating strategy to identify dendritic cells (DCs), PMNs and macrophages (MΦs) by determining forward scatter, side scatter and expression of CD45, F4/80 and Gr1 by antibody staining. (b–e) Outline of the experiment (instillation indicates infection by transurethral UPEC instillation; b), and total numbers of DCs (c), PMNs (d) and of bacterial CFU values (e) per kidney in *CD11c.DTR* mice at 6 h after injection of 8 ng per gram body weight diphtheria toxin (DT). (f–i) Outline of the experiment (f) and numbers of intrarenal DCs 24 h after injection (g), and numbers of PMNs (h) and bacterial CFU values (i) 30 h after injection of 8 ng DT per gram body weight into *CD11c.DTR* mice. Results are shown for one representative of three individual experiments using 6 mice per group. Error bars, s.d. ( $n = 6$  mice); \* $P < 0.05$ ; \*\*\* $P < 0.001$ .

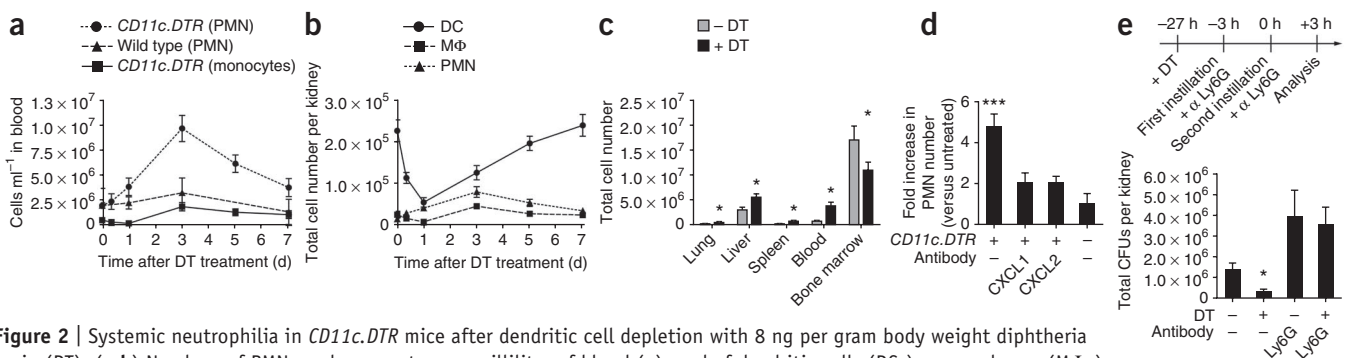
UPEC 1 d after dendritic cell depletion, when kidney dendritic cell numbers were reduced by >90% (Fig. 1f,g). We anticipated that the delay of PMN recruitment and bacterial clearance would be more pronounced under these conditions. However, the kidneys contained more PMNs and fivefold less colony forming units (CFU) of UPEC than nondepleted controls (Fig. 1h,i), indicating that mice depleted of dendritic cells 1 d before infection could paradoxically control pyelonephritis better. Thus, depending on the time point of dendritic-cell depletion, we observed diametrically opposed results for bacterial clearance (Fig. 1e,i).

### Dendritic cell depletion released PMNs from bone marrow

To clarify these opposing effects, we analyzed the numbers of PMNs, dendritic cells, monocytes and macrophages in dendritic cell-depleted, non-infected mice. We observed slightly increased PMN numbers in the blood at 6 h after diphtheria toxin injection and a twofold increase after 24 h (Fig. 2a). At 72 h after diphtheria toxin injection, PMN levels peaked at fivefold increased numbers, and after 7 d they were still about twofold increased (Fig. 2a). We did not observe neutrophilia after injecting diphtheria toxin into wild-type control mice (Fig. 2a). The number of blood monocytes also increased in dendritic cell-depleted mice, peaking at two- to threefold greater numbers over the value in the control after 72 h (Fig. 2a). These higher monocyte and neutrophil blood counts (monocytosis and neutrophilia, respectively) were mirrored by increased intrarenal macrophage and PMN numbers (Fig. 2b). The intrarenal PMN increase was significant after 24 h and peaked after 72 h at fivefold increased values ( $P \leq 0.001$  and  $P \leq 0.05$ , respectively; Fig. 2b).



We next investigated the cause of neutrophilia in diphtheria toxin-treated *CD11c.DTR* mice. PMN numbers were increased not only in kidney and blood, but also in the spleen, liver or lung at 24 h after diphtheria toxin injection, excluding mobilization from these tissues as the underlying reason. By contrast, PMN numbers were decreased in the bone marrow (Fig. 2c), suggesting the bone marrow as the origin of the neutrophilia. *CD11c.DTR* mice backcrossed to the BALB/c background also showed neutrophilia in the blood and reduced PMN numbers in



**Figure 2** | Systemic neutrophilia in *CD11c.DTR* mice after dendritic cell depletion with 8 ng per gram body weight diphtheria toxin (DT). (a,b) Numbers of PMNs and monocytes per milliliter of blood (a), and of dendritic cells (DCs), macrophages (MΦs) and PMNs per kidney (b) in noninfected male *CD11c.DTR* mice at the indicated time points after DT injection. (c) PMN numbers in the indicated organs of *CD11c.DTR* mice injected 24 h earlier with diphtheria toxin (+DT) or not (-DT). (d) Splenic neutrophilia in *CD11c.DTR* mice after antibody-mediated neutralization of CXCL1 or CXCL2 at the time of dendritic cell depletion. Shown results were obtained 24 h after DT and antibody administration. (e) Total number of CFUs per kidney in *CD11c.DTR* mice treated as indicated. Shown is a representative of three individual experiments. Error bars, s.d. ( $n = 3-5$  mice); \* $P < 0.05$ ; \*\*\* $P < 0.001$ .

the bone marrow (**Supplementary Fig. 1**), indicating that this phenomenon did not depend on mouse strain differences.

To study the underlying mechanisms, we determined serum levels of cytokines and chemokines after dendritic cell depletion. We observed an increase in the amount of CXCL2 but not of IL-1, IL-4, IL-5, IL-17, TNF, CCL2, CCL3 and CCL5 (data not shown). CXCL2 and the related chemokine CXCL1 are plausible candidates to trigger PMN release from the bone marrow because they bind the chemokine receptors CXCR1 and CXCR2, which regulate PMN migration<sup>21</sup>. Indeed, 24 h after antibody blockade of either chemokine, PMN numbers in the spleen were no longer fivefold but only twofold increased over numbers in nondepleted mice (**Fig. 2d**).

We speculated that dendritic cell depletion-induced neutrophilia might be the cause for the improved clearance of UPEC from the kidney. To test this, we depleted dendritic cells from *CD11c.DTR* mice 24 h before infection, and prevented neutrophilia with the PMN-depleting antibody to Ly6G (1A8) (**Fig. 2e**), which in our hands eliminated 81% of the circulating PMNs (data not shown). Considering the danger of urosepsis in the absence of PMNs, we analyzed the mice 3 h after the second instillation of bacteria. Intrarenal CFU in mice depleted of PMNs and in mice depleted of both PMNs and dendritic cells did not differ, and were similarly increased over intrarenal CFU in mice depleted of dendritic cells alone (**Fig. 2e**). Thus, PMNs were required for the improved UPEC clearance we observed 24 h after dendritic cell depletion (**Fig. 1i**).

### *CD11c.DOG* mice also showed neutrophilia

To investigate whether neutrophilia was unique to *CD11c.DTR* mice, we examined a different line that allowed conditional depletion of dendritic cells. *CD11c.DOG* BAC transgenic mice express DTR, ovalbumin and enhanced (e)GFP in dendritic cells<sup>16</sup>. Kidney dendritic cell depletion, neutrophilia and intrarenal PMN counts quantitatively resembled that in *CD11c.DTR* mice, except that neutrophilia declined after 72 h (**Fig. 3a,b** and **Supplementary Fig. 2a**). Indeed, bacterial clearance was improved 1 d after dendritic cell depletion also in *CD11c.DOG* mice (**Supplementary Fig. 2**).

*CD11c.DOG* mice offer the advantage of tolerating higher diphtheria toxin doses than *CD11c.DTR* mice without signs of toxicity<sup>17</sup>. We found that a dose of 40 ng diphtheria toxin per gram body weight, instead of the standard 8 ng per gram body weight, depleted kidney dendritic cell numbers slightly more efficiently in *CD11c.DOG* mice (**Fig. 3a**). The higher dose caused neutrophilia

that persisted beyond 72 h, peaked earlier at 24 h and was significant 6 h after diphtheria toxin injection, reaching a value of  $3.9 \pm 0.84 \times 10^6$  PMN per ml of blood ( $P \leq 0.05$  **Fig. 3b**). This value was very similar to the  $3.8 \pm 0.86 \times 10^6$  PMN per ml blood seen in *CD11c.DTR* or *CD11c.DOG* mice injected 24 h before with 8 ng per gram body weight diphtheria toxin (**Fig. 3b**), in other words to the level that had improved bacterial clearance (**Fig. 1i** and **Supplementary Fig. 2b**). *CD11c.DOG* mice treated with the higher dose of diphtheria toxin at the time of infection showed higher intrarenal PMN numbers and less UPEC CFUs compared to nondepleted mice (**Fig. 3c–e**). This demonstrated that the same level of neutrophilia improved bacterial clearance to a similar extent, regardless of the interval between dendritic cell depletion and infection.

### Generation and characterization of *CD11c.LuciDTR* mice

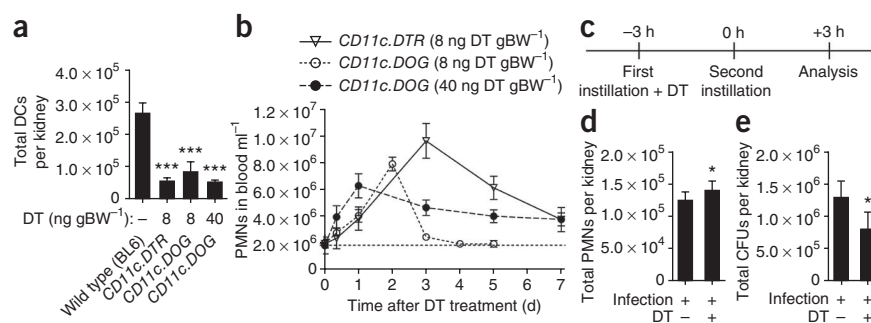
We generated a line of BAC transgenic mice termed *CD11c.LuciDTR* mice, which express eGFP, Cre recombinase, DTR and luciferase as individual proteins under the control of the *CD11c* promoter (**Fig. 4a**). Using whole-body bioluminescence imaging, we observed luciferase expression throughout *CD11c.LuciDTR* mice unless dendritic cells were depleted (**Fig. 4b**). The transgenic expression profile of these mice resembled that of *CD11c.DTR* mice, as evidenced by expression of the eGFP reporter in splenic dendritic cells, including CD11b<sup>+</sup> and CD8<sup>+</sup> conventional dendritic cells, low expression in plasmacytoid dendritic cells (**Fig. 4c**) and no expression in NK cells, NKT cells, B cells, macrophages and granulocytes (**Supplementary Fig. 3a** and data not shown). Only a minor percentage of CD4<sup>+</sup> and CD8<sup>+</sup> splenic T cells expressed both eGFP and CD11c (0.11% and 0.31%, respectively) (**Supplementary Fig. 3a**). NK cells expressed intermediate levels of CD11c but did not express eGFP (**Supplementary Fig. 3a**).

To investigate the potential of Cre recombinase-mediated recombination in splenic conventional dendritic cells from *CD11c.LuciDTR* mice, we crossed these mice with the eGFP reporter RA/EG mice in which a *flox-stop-eGFP* cassette is under the control of a knock-in thymidine kinase promoter<sup>22</sup>. In these mice, all cells expressed high levels of eGFP after recombination, as opposed to the low endogenous eGFP levels in *CD11c.LuciDTR* mice (**Fig. 4d**). Recombination in *CD11c.LuciDTR* × RA/EG mice occurred in about 71% of conventional dendritic cells as indicated by high eGFP expression (**Fig. 4d**), which was less than in previously described *Tg(Ilgax-cre)1-1Reiz* mice<sup>23</sup>.

The depletion of splenic conventional dendritic cells in these mice 24 h after 8 ng per gram body weight diphtheria toxin

**Figure 3** | *CD11c.DOG* mice showed early neutrophilia and improved bacterial clearance.

(a) Dendritic cell (DC) numbers per kidney 1 d after injection of the indicated amount of diphtheria toxin (DT) into *CD11c.DOG* or *CD11c.DTR* mice. gBW, gram body weight. (b) Numbers of PMNs in the blood of the indicated mice after injection of DT. The dashed line indicates the average PMN count in male BL6 mice. (c–e) Outline of the experiment indicating when mice were infected by transurethral UPEC instillation and given DT (c), PMN counts (d) and UPEC CFU values (e) per kidney in *CD11c.DOG* mice injected with 40 ng per gram body weight DT 6 h before analysis. Shown are data for a representative of three individual experiments. Error bars, s.d. ( $n = 5$  mice); \* $P < 0.05$ ; \*\*\* $P < 0.001$ .







also monocyte) numbers were unchanged in the blood after 24 h but there was a 66% increase in intrarenal PMN after injecting 40 ng per gram body weight diphtheria toxin, which was significant ( $P \leq 0.01$ ; **Fig. 5b,c**).

We observed neutrophilia in *CD11c.LuciDTR* mice at later time points. PMN numbers in the blood were twice as high 3 d after injecting 8 ng per gram body weight diphtheria toxin than after 24 h or in homeostasis, but we observed no such increase within 2 d of injecting diphtheria toxin. Blood monocyte counts were also doubled after 3 d in *CD11c.LuciDTR* mice (**Fig. 5c**). Both monocyte and PMN levels had decreased by day 5 (**Fig. 5c**).

As blood and kidney PMN numbers were hardly affected 24 h after 8 ng per gram body weight diphtheria toxin injection (**Fig. 5a,b**), we predicted that *CD11c.LuciDTR* mice should not show improved bacterial clearance at that time point. To test this, we injected these mice with 8 ng per gram body weight diphtheria toxin 24 h before infecting them. Kidney dendritic cell and PMN numbers were lower and UPEC CFU were higher in depleted compared to nondepleted *CD11c.LuciDTR* mice (**Fig. 5d,e**), indicating delayed bacterial clearance in the absence of dendritic cells. This supported our conclusion that improved bacterial clearance in *CD11c.DTR* and *CD11c.DOG* mice (**Figs. 1i, 3e and 5e**) was due to neutrophilia induced by dendritic cell depletion.

### Early and late neutrophilia are mechanistically distinct

The results above established that PMN release from the bone marrow caused early neutrophilia 24 h after dendritic cell depletion in *CD11c.DTR* and *CD11c.DOG* mice. We next determined the cause of the late neutrophilia at 72 h in *CD11c.DTR* and *CD11c.LuciDTR* mice (**Figs. 2a and 5c**). A previous study reported increased myelopoiesis in mice with constitutive depletion of dendritic cells via transgenic diphtheria toxin expression, indicating a dendritic cell-mediated feedback suppression of myelopoiesis<sup>24</sup>. To test whether increased proliferation of PMNs (granulopoiesis) was responsible for late neutrophilia, we stained bone marrow granulocytes for the proliferation marker Ki67. This marker was expressed primarily by  $CD11b^+ Ly6G^{low}$  PMN precursors<sup>25</sup>, rather than by mature  $Ly6G^{high}$  PMN (**Fig. 6a**). At 24 h after diphtheria toxin injection, the number of proliferating PMN precursors was not altered (**Fig. 6b**), which is an argument against a major effect of granulopoiesis on early neutrophilia. Dendritic cell numbers were reduced in all three transgenic lines, whereas PMN

numbers were decreased only in *CD11c.DTR* and *CD11c.DOG* lines (**Supplementary Fig. 6**), consistent with PMN bone marrow release in these two lines. At 72 h, dendritic cells had started to reappear in all three lines, and no loss of PMN from the bone marrow was evident in any line at that time point (**Supplementary Fig. 6c,d**). We observed more proliferating PMN precursors (**Fig. 6c**) in *CD11c.LuciDTR* mice but not in *CD11c.DTR* and *CD11c.DOG* mice, pointing to a correlation between granulopoiesis and late neutrophilia in *CD11c.LuciDTR* mice.

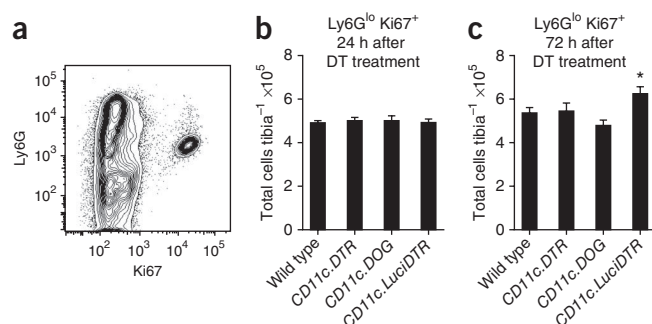
### DISCUSSION

*CD11c.DTR* and *CD11c.DOG* mice developed neutrophilia 24 h after dendritic cell depletion owing to chemokine-dependent PMN release from the bone marrow. This side effect mechanistically differs from previously reported neutrophilia resulting from myeloproliferation as a consequence of loss of dendritic cells<sup>24</sup>, which becomes evident later, 72 h after dendritic cell depletion. We provide several independent lines of evidence that the early neutrophilia 24 h after diphtheria toxin injection caused improved bacterial clearance in a pyelonephritis model.

The molecular mechanism underlying early neutrophilia involves the chemokines CXCL1 and CXCL2, which are known to regulate PMN release from the bone marrow and their migration in the periphery<sup>21</sup>. The mechanistic cause for the chemokine increase in *CD11c.DTR* and *CD11c.DOG* mice but not *CD11c.LuciDTR* mice remains to be clarified. *CD11c.DTR* mice use the short *CD11c* promoter<sup>26</sup>, in contrast to the other two lines which are BAC transgenic mice. However, the occurrence of neutrophilia in one of these two lines, *CD11c.DOG* mice, argues against the promoter as the underlying cause. Future studies are needed to clarify whether ectopic transgene expression or the site of transgene integration into the genome mechanistically underlie neutrophilia.

The majority of previous studies using *CD11c.DTR* mice reported immunogenic or pro-inflammatory roles of dendritic cells, such as the production of cytokines or the stimulation of T cells<sup>1,4,5,7,8,11,12</sup>. One implication of our present findings is that these studies might have underestimated pro-inflammatory dendritic cell functions, because the reduction of these functions after dendritic cell depletion may have been partially compensated by neutrophilia. In contrast, some studies using *CD11c.DTR* mice reported anti-inflammatory functions. For example, dendritic cell depletion aggravated early experimental glomerulonephritis<sup>10</sup>, cisplatin-induced tubulotoxicity<sup>14</sup>, experimental cryptococcal pneumonia<sup>6</sup>, liver ischemia/reperfusion injury<sup>13</sup> and dextran sodium sulfate-induced colitis in one study<sup>9</sup>. Notably, in some of these studies, neutrophil influx into affected tissues has been reported<sup>6,9,14</sup>. Our observations suggest that some inflammatory events observed after dendritic cell depletion may in fact have been due to neutrophilia, although whether and to what extent this is true remains speculative. It is possible that the monocytosis in the mice studied here might also affect disease models in which monocytes or macrophages are important.

Transgenic expression of the DTR has been used to construct mice allowing depletion of other cell types, such as Langerhans cells<sup>27</sup>, regulatory T cells<sup>28</sup>, kidney podocytes<sup>29</sup> or distinct neuronal subsets<sup>30</sup>. Furthermore, inducible DTR mice allow depletion of any cell type when crossed to transgenic mice expressing Cre recombinase under a cell type-specific promoter<sup>31</sup>. It remains to



**Figure 6** | Granulopoiesis in dendritic cell-depleted mice.

(a) Representative dot plot of bone marrow  $CD11b^+$  cells stained for Ly6G and Ki67. (b,c) Numbers of proliferating PMN precursors per tibia in the indicated strains injected 24 h (b) or 72 h (c) earlier with 8 ng per gram body weight diphtheria toxin. Shown are data for a representative of two individual experiments. Error bars, s.d. ( $n = 5$  mice);  $*P < 0.05$ .

be studied whether these DTR transgenic mouse lines develop neutrophilia. If so, then experiments in such transgenic models, perhaps especially in mice allowing conditional ablation of immunosuppressive cells, might be prone to misinterpretation.

In conclusion, the time point of dendritic cell depletion and the choice of the DTR transgenic line need to be considered when planning experiments under conditions that may be affected by PMNs. We recommend *CD11c.LuciDTR* mice for analysis up to 48 h after dendritic cell depletion; these mice also offer Cre-dependent gene ablation, albeit not with the same efficiency as in *Tg(Itgax-cre)1-1Reiz* mice<sup>23</sup>, and noninvasive whole-body bioluminescence imaging.

## METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturemethods/>.

Note: Supplementary information is available on the Nature Methods website.

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## AUTHOR CONTRIBUTIONS

A.P.T., N.G. and C.K. conceived the study and wrote the manuscript. A.P.T. and C.H. performed most experiments. C.O., C.L., S.Y. and N.G. performed individual experiments. S.Y., G.J.H. and D.R.E. provided crucial ideas or essential reagents. A.P.T., C.H., C.O., C.L., S.Y., G.J.H., D.R.E., N.G. and C.K. discussed and interpreted results.

## COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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## ONLINE METHODS

**Mice and reagents.** All mice were bred and kept under specific pathogen-free conditions at the central animal facilities of the Bonn Medical Faculty, except *CD11c.DTR* mice in the BALB/c background, which were maintained in the animal facilities of the Weizmann Institute, Rehovot, Israel, and eGFP-reporter RA/EG mice<sup>22</sup>, which were maintained and crossed to *CD11c.LuciDTR* mice at the animal facilities of the German Cancer Research Center, Heidelberg, Germany. All lines had been backcrossed more than 10 times to the C57BL/6 or BALB/c background. For dendritic cell depletion, transgenic mice were injected intraperitoneally with 8 ng or 40 ng per gram body weight diphtheria toxin (Sigma-Aldrich). *CD11c.LuciDTR* mice did not tolerate more than five daily injections of 8 ng per gram body weight diphtheria toxin (*CD11c.DTR* mice died after the second injection, *CD11c.DOG* mice did not die). PMNs were depleted by injecting 1 mg of 1A8 antibody from BioXcell. Neutralizing antibodies to CXCL1 (clone 124014, 0.25 µg per gram body weight) and CXCL2 (AF-452-NA, 0.1 µg per gram body weight) were from R&D Systems. Animal experiments had been approved by a local and by a governmental animal ethics reviewing board.

**Generation of *CD11c.LuciDTR* mice.** *CD11c.LuciDTR* BAC transgenic mice (*B6.Cg-Tg(Itgax-EGFP,-CRE,-DTR,-LUC)2Gjh/Crl*) were generated by genetic recombineering as previously described<sup>32,33</sup>. Briefly, a cDNA construct encoding eGFP, Cre recombinase, human DTR and click beetle green 99 (CBG99) luciferase was generated using standard protocols. Coding sequences were separated by the self-cleaving 2A sequence from porcine teschovirus-1, which yields stoichiometric production of the individual proteins<sup>34</sup>. The construct was introduced at the start codon of the *Itgax* gene in the BAC RPCI-24-7812 (BACPAC Resource Center, Children's Hospital Oakland, California, USA) using *Escherichia coli* EL250 (ref. 32), linearized using NotI and injected into pronuclei of fertilized C57BL/6N oocytes. Genotyping was carried out by PCR from genomic DNA of tail biopsies using the following primers for DTR: 5'-GCCACCATGAAGCTGCTGCC G-3' and 5'-TGAGTGGGAATTAGTCATGCC-3'. *CD11c.LuciDTR* mice are available from N.G.

**Bioluminescence imaging.** Bioluminescence imaging was performed as previously described<sup>33</sup>. Briefly, mice were anesthetized with isoflurane and injected intraperitoneally with 4.5 mg D-luciferin (SynChem). Five minutes later mice were imaged for 2 min at 37 °C using an IVIS 100 imaging system with LiveImage 2.50 software (Xenogen). Light output was quantified as photons per second per square centimeter.

**Urinary tract infection model.** UPEC strain 536 (ref. 20) were grown for 5 h at 37 °C in LB medium. Bacteria were collected by centrifugation at 1,200g for 10 min and resuspended in 2 ml LB medium to a final concentration of  $1 \times 10^{10}$  CFU per ml. Female mice of 8 to 12 weeks of age were anesthetized with Avertin (40 mg 2,2,2-tri bromoethanol (Sigma-Aldrich) dissolved in 1 ml *tert*-amyl alcohol (0.01 ml g<sup>-1</sup> body weight intraperitoneally)) and were infected by transurethral instillation of  $1 \times 10^9$  UPEC using a flexible polyethylene catheter (outer diameter 0.6 mm; BD) coated

with Instillagel (Farco Pharma). Three hours later the procedure was repeated to induce pyelonephritis. Before analysis, mice were perfused with sterile PBS. The number of ascended bacteria was quantified by scoring CFU after overnight culture of kidney collagenase digest or of homogenates prepared in PBS with an Ultra Turrax at 37 °C on CPS ID plates (Biomérieux).

**Isolation of phagocytes from the kidney.** A previously described protocol was used<sup>7</sup>. In brief, kidneys were sliced with a scalpel into 6 similar pieces and digested for 25 min at 37 °C with 0.5 mg ml<sup>-1</sup> collagenase and 100 µg ml<sup>-1</sup> DNase I in RPMI 1640 medium (Invitrogen) containing 10% heat-inactivated FCS (PAA Laboratories), 20 mM HEPES (Carl Roth), 0.1% β-mercaptoethanol, 2 mM L-glutamine and antibiotics (Sigma-Aldrich). After digestion kidneys were mashed and incubated for an additional 15 min at 37 °C. Single-cell suspensions were filtered through a 100 µm nylon mesh and washed with PBS lacking Ca<sup>2+</sup> and Mg<sup>2+</sup> but containing 0.1% heat-inactivated FCS and 0.5% sodium azide for surface-marker analysis.

**Analysis of phagocytes by flow cytometry.** Single cell suspensions were generated by digestion with 1 mg/ml collagenase IV (Sigma) and 50 U ml<sup>-1</sup> DNaseI (Sigma) in PBS-3% FCS for 30 min at 37 °C under mild stirring. Cells were incubated with clone 2.4G2 culture supernatant to block Fc receptors. The following labeled antibodies from Pharmingen or eBiosciences were used at 1:200 dilution for staining of  $1 \times 10^6$  cell: Ly6G-FITC (clone 1A8), B220-PE (RA3-6B2), CD4-PE (GK1.5), CD8-APC and CD8-PerCP-Cy5.5 (53-6.7), Ly6C-PerCP-Cy5.5 (HK1.4), CD11b-PE, CD11b-APC (M1/70), Gr-1-PerCP-Cy5.5 (RB6-8C5) and CD11c-FITC and CD11c-APC (HL-3), MHC II-FITC and MHC II-PE (N418), CD19-Alexa Fluor 488 and CD19-Alexa Fluor 647 (6d5), CD3-FITC and CD3-APC (145-2C11), NK1.1-APC (PK136), CD138-biotin (281-2), streptavidin-APC and streptavidin-PE-Cy7. F4/80-PE (CI:A3-1), PDCA-1-biotin (927) and CD45-APC-Cy7 and CD45-PacificBlue (30-F11) were from BioLegend. Ki67 and Alexa Fluor 647 carboxylic acid were from Invitrogen. Kidney dendritic cells were defined as CD11c<sup>+</sup> CD11b<sup>+</sup> F4/80<sup>+</sup>, macrophages as CD11c<sup>-</sup> CD11b<sup>hi</sup> F4/80<sup>+</sup>, mature PMN as F4/80<sup>-</sup> CD11b<sup>hi</sup> Gr1<sup>hi</sup> cells and PMN precursors as F4/80<sup>-</sup> CD11b<sup>hi</sup> Gr1<sup>lo</sup> cells as previously described<sup>7,25</sup>. Dead cells were excluded with Hoechst 33342 stain and for intracellular staining the LIFE/DEAD fixable violet dead cell stain kit (Invitrogen) was used.

**Statistical analysis.** Results are expressed as mean ± s.d. Comparisons were made using Mann-Whitney or ANOVA test in combination with Bonferroni's post-test for comparing two or more groups, respectively, using Prism 5 software (GraphPad Software).

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