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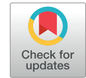
cDC1 are required for the initiation of collagen-induced arthritis



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A R T I C L E I N F O

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A B S T R A C T

Rheumatoid arthritis (RA) is chronic autoimmune disease which etiology remains unknown. Several cell types have been described to potentiate/aggravate the arthritic process however the initiating event in synovial inflammation is still elusive. Dendritic cells (DCs) are essential for the initiation of primary immune responses and thus we hypothesized that these cells might be crucial for RA induction. DCs are a heterogeneous population of cells comprising different subsets with distinct phenotype and function. Here we investigated which DC subset(s) is/are crucial for the initiation of the arthritic process.

We have previously demonstrated that Flt3−/− mice, with reduced DCs, were protected from collagen induced arthritis (CIA). Here we have shown that GM-CSF derived DCs in Flt3L−/− mice are functional but not sufficient to induce arthritis. Batf3$^{-/-}$ mice lacking both CD103^{+} and CD8α^{+} cDC1 were resistant to collagen induced arthritis (CIA), demonstrating that this DC subset is crucial for arthritis development. CEP-701 (a Flt3L inhibitor) treatment prevented CIA induction, and reduced dramatically the numbers CD103^{+} cDC1s present in the lymph nodes and synovium. Hence this study identified cDC1 as the main subset orchestrating the initiation of cell-mediated immunity in arthritis.

1. Introduction

Dendritic cells (DCs) are professional antigen-presenting cells that are specialized in the uptake of antigens and their transport from peripheral tissues to lymphoid organs. Due to their capacity to stimulate naive T cells, DCs have a central role in the initiation of primary immune responses and are considered promising tools and targets for immunotherapy. Abnormalities of DC homeostasis have been involved in the pathophysiology of various autoimmune diseases, including rheumatoid arthritis (RA) [1]. In RA, these cells infiltrate the site of inflammation, the synovium [2,3]. DCs can be identified and isolated from synovial fluid as well as tissue, and account for 5–7% of RA synovial fluid mononuclear cells (SFMCs). Synovial DCs show evidence of activation in vivo: upregulation of MHC, co-stimulatory molecules and RelB [4], expression of receptor activator of nuclear factor-κB (RANK) and its ligand (RANKL) [5], and increased production of pro-inflammatory cytokines (IL-1, IL-6 and TNF) when stimulated ex-vivo with Toll-like receptor (TLR) agonists [6].

The DC lineage is very heterogeneous and can be classified on the basis of ontogeny and function into two lineages: plasmacytoid DCs (pDCs) and conventional DCs (cDCs), the latter comprising cDC1 and cDC2 subpopulations [7,8]. Committed cDC progenitors restricted to only the cDC1 or the cDC2 lineage have recently been identified in mice [9,10] and in humans [11–13]. cDC1 are Batf3-dependent, cDC2 are Irf4-dependent, and pDCs are E2-2-dependent [8]. cDC1 cells express certain unique markers such as CD8α, CD103, and XCR1 in various

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tissues and are found both as lymphoid and non-lymphoid tissue cells, the latter of which can migrate via the lymph to draining lymph nodes to present tissue-derived antigens [14]. Functionally, cDC2 DCs prime $CD4^+$ T cells, while cDC1 primarily cross-present antigen to prime $CD8^+$ but also stimulate $CD4^+$ T cell help for cytotoxic T lymphocyte generation and B cell activation [15]. DC haematopoiesis is conserved between mammalian species and is distinct from monocyte development. Although monocytes can differentiate into DC, especially during inflammation, these are different from DC lineage cells. Flt3L and granulocyte macrophage-colony stimulating factor (GM-CSF) are important DC growth factors for steady-state conventional [16] and monocyte derived [17] DC generation, respectively.

It has been shown that pDCs play a role in suppressing arthritogenic autoimmunity [18] and that cDCs play a central role in driving arthritogenic autoimmunity and no other APC is sufficient for breach of self-tolerance [19]. Moreover, we have previously shown that Flt3L-signaling plays an important role in the induction of collagen-induced arthritis (CIA) since mice lacking this cytokine are protected from disease [20]**.** Although several studies reported the presence and importance of DCs at the site of inflammation in arthritis it is still unclear if these cells are required for disease induction and which DC subset(s) is/are involved in this process. Here we have sought to clarify the importance of Flt3L-dependent bona-fide DCs driving RA and which DC subset is required for the process.

2. Material and methods

Additional experimental details are provided in supplementary materials and methods.

2.1. Mice

$Flt3L^{-/-}$ mice, maintained on a C57BL/6 background, were a kind gift of Prof. S.E. Jacobsen (University of Oxford, Oxford, United Kingdom). Mice were crossed with WT C57BL/6 animals and 8–10 week old littermates were used for experiments. $Batf3^{-/-}$ mice were purchased from Jackson Laboratories. 8–10 weeks old male and female mice were used for the experiments. DBA1 mice were purchased from Charles River Laboratories. 10 weeks old mice were used for the experiments. The Institutional Animal Care and Use Committee of the Academic Medical Center approved all experiments.

2.2. DC migration in vivo

For the in vivo experiments 10 μg or 100 μg OVA-FITC labeled (Invitrogen) was injected in the base of the tail on the right and left sides of WT and $Flt3L^{-/-}$ mice and LNs were collected after 36 h. $FITC^+$ cells were quantified using the following markers: anti-CD11c (Pe-Cy7 eBioscience), anti-MHCII (APC-Cy7, Biolegend), anti-CD11b (Alexa700, eBioscience) and anti-CD103 (Percp-Cy5.5, Biolegend). Flow cytometry was performed using a FACS CANTO (Becton Dickinson) and analyzed with flow cytometry Analysis software (Tree Star).

2.3. BMDC uptake in vitro

For the in vitro uptake experiments bone marrow-derived DCs (BMDCs) were generated based on a modified protocol described previously [21]. BMDCs were pulsed with OVA-FITC (Invitrogen) for different time points and uptake was measured by FACS (FACS CANTO Becton Dickinson) and analyzed with flow cytometry Analysis software (Tree Star).

2.4. DC migration in vitro

As described before [22] ears were split into dorsal and ventral halves and floated split side down in 1 ml medium containing RPMI, 10% FCS, 50 μM β-mercaptoethanol and with or without CCL21 for 48 h. Emigrated non-adherent cells were stained with anti-CD11c (Pe-Cy7 eBioscience), anti-MHCII (APC-Cy7, Biolegend), anti-CD11b (Alexa700, eBioscience) and anti-CD103 (Percp-Cy5.5, Biolegend) and were quantified by FACS to determine the number and different subsets of emigrated DC from the skin to the culture medium. Acquisition was done using a FACS CANTO (Becton Dickinson) and data was analyzed with flow cytometry Analysis software (Tree Star).

2.5. Competitive migration in vivo

BMDCs from WT and $Flt3L^{-/-}$ mice were generated according to the protocol described above. BMDCs (10^6 cells/ml) were labeled with the following fluorescent dyes: CFSE (2.5 mM; Molecular Probes) or with DDAO (2.5 mM; Molecular Probes) for 7 min at 37 °C. A 1:1 ratio of 10^6 CFSE-labeled WT BMDCs and 10^6 DDAO-labeled $Flt3L^{-/-}$ BMDCs was injected into the right (R) footpad of WT or $Flt3L^{-/-}$ recipients. To exclude any effect of the labelling dyes WT DDAO and Flt3L−/− BMDC were injected into the left (L) footpad. CFSE and DDAO-labeled DC mixture was injected in a volume of 30 μl into the footpad of WT or $Flt3L^{-/-}$ recipients**.** After 36 h LNs were collected and both $DDAO^+$ and $CFSE^+$ populations were quantified by FACS.

2.6. Capacity of WT and $Flt3L^{-/-}$ BMDCs to induce proliferation of OT-I and OT-II cells in vivo and in vitro

OT-I and OT-II T cells were isolated from pooled LNs and spleen of OT-I or OT-II mice kept on a Rag-22/2 3 B6 (CD45.1) background using a $CD8^+$ and $CD4^+$ T cell-positive isolation kit (Dynal, Invitrogen), respectively. For in vivo studies, 4×10^6 CFSE-labeled OT-I and OT-II T cells were adoptively transferred intravenously (i.v.) into WT or $Flt3L^{-/-}$ mice. After 24 h mice were challenged with 10 μg OVA (Sigma) intradermally (i.d) or intraperitoneally (i.p). After 3 days T cell proliferation was measured by flow cytometry as a loss of CFSE staining. For the in vitro studies, WT or $Flt3L^{-/-}$ BMDCs were cultured with CFSE-labeled OT-I and OT-II T cells in a 1:4 ratio and in the presence of OVA. After 3 days of culture, T cell proliferation was measured by FACS. The percentages and numbers of divisions of OT-I and OT-II T cells that have proliferated were determined.

2.7. CIA in WT, $Flt3L^{-/-}$ and $Batf3^{-/-}$ animals

$Flt3L^{-/-}$, WT and $Batf3^{-/-}$ mice were immunized as previously described [23] and were inspected three times a week for signs of arthritis by two independent observers. All mice were sacrificed on day 43 after CIA induction. Blood, LNs and paws were harvested for analysis. Single-cell suspensions were obtained and after erythrocyte lysis (red blood cell lysis buffer, 2 min at RT; Sigma), cells were stained with the indicated fluorochrome-conjugated antibodies for surface markers and intracellular cytokines. LN cells were stained using the following markers: anti-TNF (APC, eBioscience), anti-IL-2 (APC, eBioscience), anti-IL-17 (Alexa 488, eBioscience), anti-IFNγ (PercP Cy5.5, eBioscience), anti-IL10 (PE, eBioscience), anti-GM-CSF (PE, eBioscience), anti-B220 (PerCP, eBioscience), anti-CD19 (Alexa 700, eBioscience), anti-GL7 (biotin, eBioscience), anti-IgD (PE, BD Pharmingen), anti-CD38 (FITC, eBioscience), anti-CD95 (APC, eBioscience) and streptavidin (PE-Cy7, eBioscience). Synovial cells were isolated and stained using antibodies against CD11c (PE-Cy7, eBioscience), MHCII (APC-Cy7, eBioscience), CD11b (Alexa 700) and CD103 (FITC, eBioscience). Serum levels of antibodies against chicken collagen type II (cCII) were measured by ELISA. Further details are described in supplementary methods.

2.8. CEP-701 treatment

Ten-week-old male DBA1 mice (Charles River) were immunized to induce CIA as previously described [23]. Mice were treated

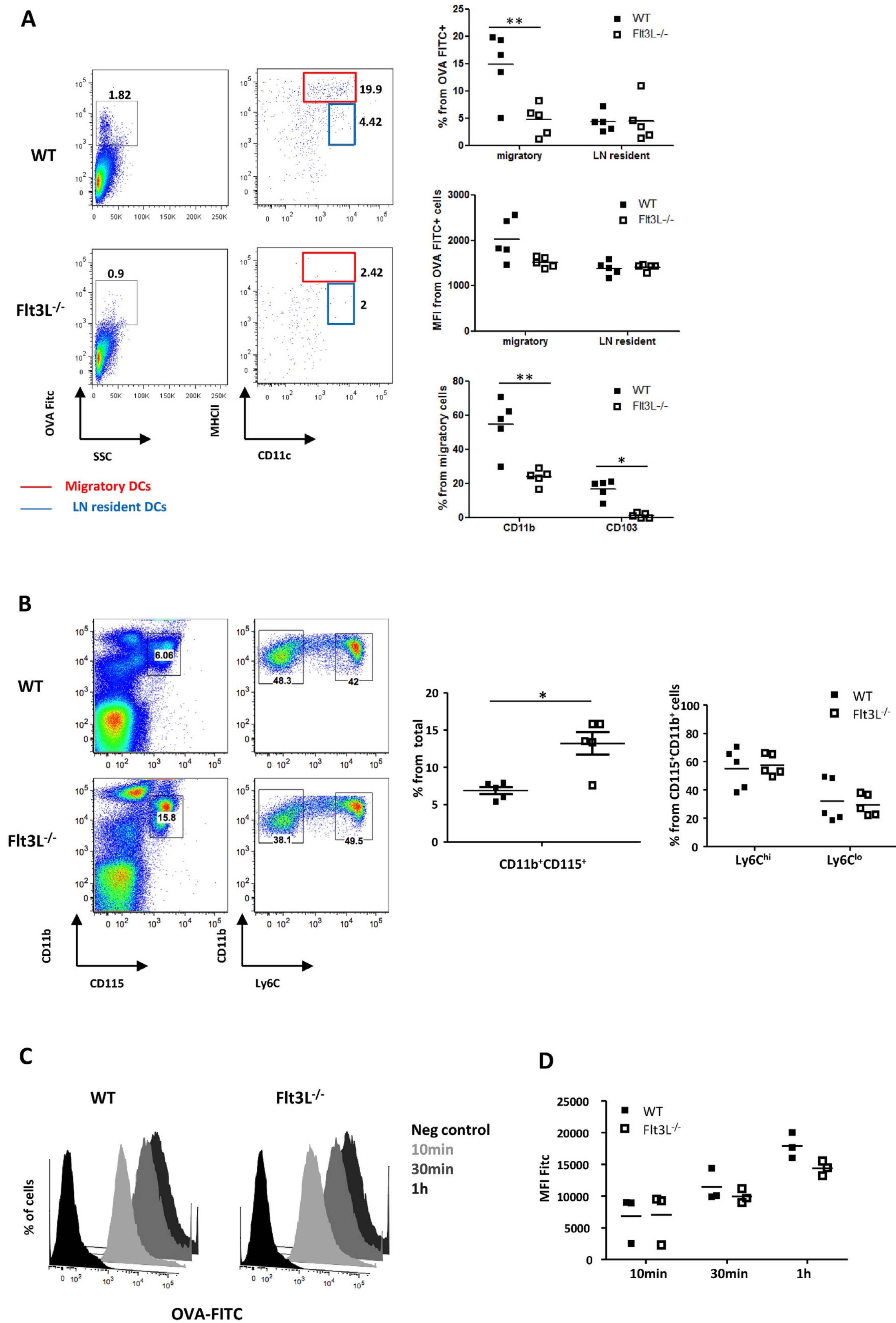

Fig. 1. Reduced antigen availability in the LNs of Flt3L$^{-/-}$ compared to WT mice. (A) DCs were divided in migratory and resident based on the expression of CD11C and MHCII. (upper graph) Percentage of migratory (CD11c^{+}MHCII^{hi}) and LN resident (CD11c^{+}MHCII^{int}) OVA-FITC^{+} DCs WT and Flt3L$^{-/-}$ LNs. (middle graph) Mean fluorescent intensity (MFI) of migratory and LN resident FITC^{+} DCs and (bottom graph) percentage of migratory CD103^{+} and CD11b^{+} DC populations from migratory OVA-FITC^{+} DCs. (B) Percentage of circulating CD11b^{+}CD115^{+} monocytes and Ly6C^{hi}, Ly6C^{lo} populations in WT and Flt3L$^{-/-}$ mice. (C) In vitro uptake capacity of WT and Flt3L$^{-/-}$ BMDC after OVA-FITC administration. Data is shown as percentage of cells. (D) MFI quantification of WT and Flt3L$^{-/-}$ FITC^{+} BMDCs after OVA-FITC administration. Data represent three independent experiments with three to six mice per group. Mean ± SEM is shown. *$p < 0.05$.

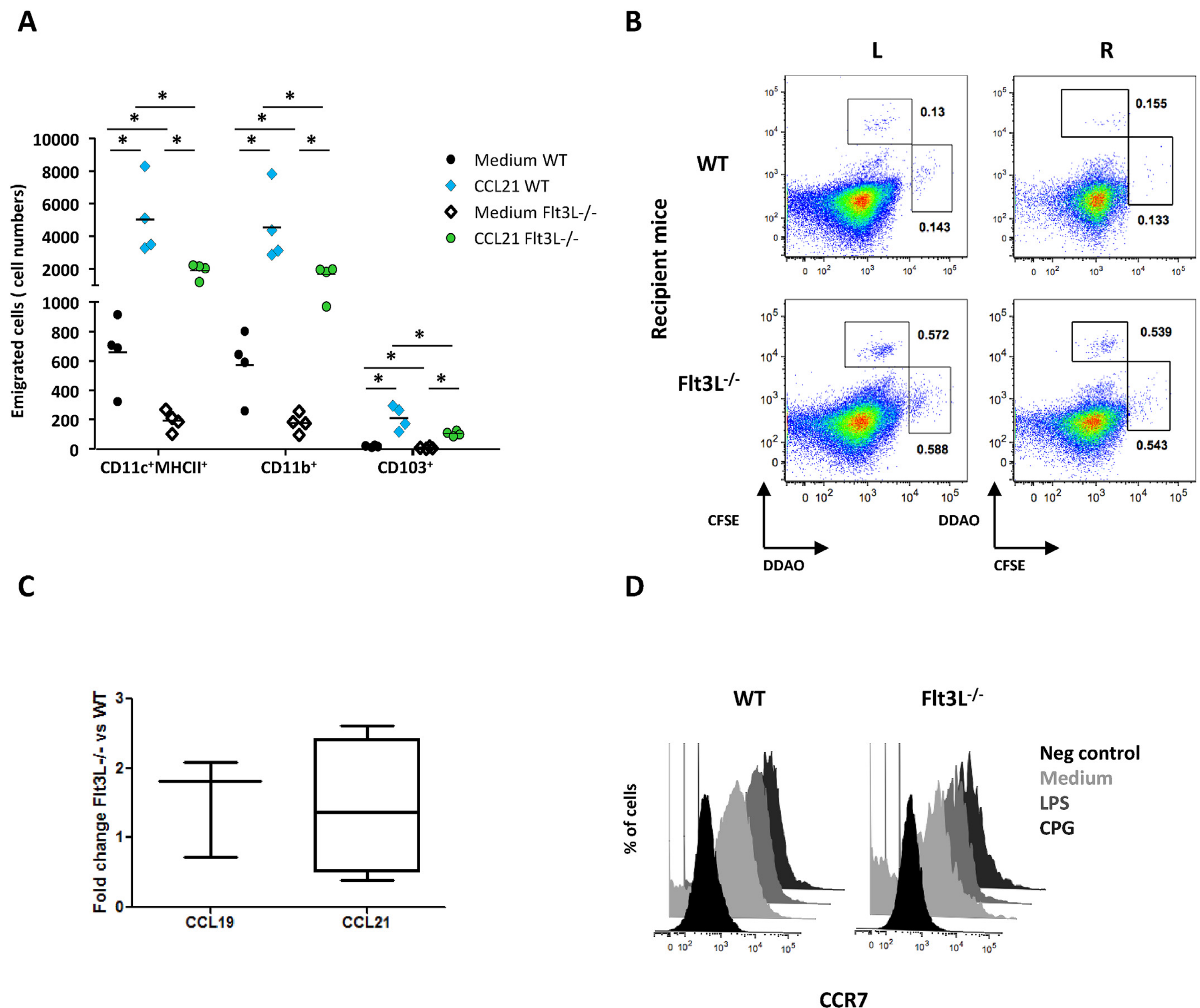

Fig. 2. Migratory functions are not altered in Flt3L$^{-/-}$ mice compared to WT mice. (A) In vitro migratory capacity of skin explant dermal Flt3L$^{-/-}$ and WT DC populations. (B) In vivo migratory capacity of WT and Flt3L$^{-/-}$ BMDCs. (C) mRNA expression of CCL19 and CCL21 in skin explants from WT and Flt3L$^{-/-}$ mice. (D) WT and Flt3L$^{-/-}$ BMDCs CCR7 expression. Data represent at least two (D) or three (A-C) independent experiments with three to four mice per group. Mean ± SEM is shown. *$p < 0.05$.

intraperitoneally with CEP-701 (20 mg/kg, LC laboratories) or vehicle solution every 12 h for 15 consecutive days. LNs and paws were collected for further examination. Single-cell suspensions were obtained and stained using the following fluorochrome-conjugated antibodies: anti-CD11c (PE-Cy7, eBioscience), anti-MHCII (APC-Cy7, eBioscience), anti-CD11b (Alexa 700), anti-CD103 (FITC, eBioscience), anti-CD8 (eFluor780, eBioscience), anti-CD3 (Alexa 700, eBioscience) and anti-CD45 (PE, eBioscience).

2.9. Statistical analysis

The difference between two groups was calculated with the Mann-Whitney U test. If two or more groups were compared, Kruskal-Wallis test was used (GraphPad Prism version 5.0; GraphPad, San Diego, CA). $p < 0.05$ was considered significant.

3. Results

3.1. Reduced antigen availability in Flt3L$^{-/-}$ draining LNs upon immunization

CIA is initiated in mice by intradermal injection of bovine or chicken type II collagen in Freund's complete adjuvant (CFA) emulsion. A booster injection with collagen alone or in combination with CFA, administered intraperitoneally or intradermally, is followed by arthritis development [23] where innate and adaptive immune responses play an important role. We have previously shown that Flt3L$^{-/-}$ mice were protected from CIA development, showing reduced T cell activation [20].

Genetic deletion of the Flt3 [24] or Flt3l [25] genes in mice decreases the numbers of DCs [25,26]), confirming their importance in DC homeostasis. To understand whether decreased DCs numbers leads to reduced antigen availability in the draining LNs in Flt3L$^{-/-}$ mice, WT and Flt3L$^{-/-}$ mice were immunized with FITC-labeled OVA. We observed a significant reduction in the percentage of FITC^{+} DCs in the LNs of

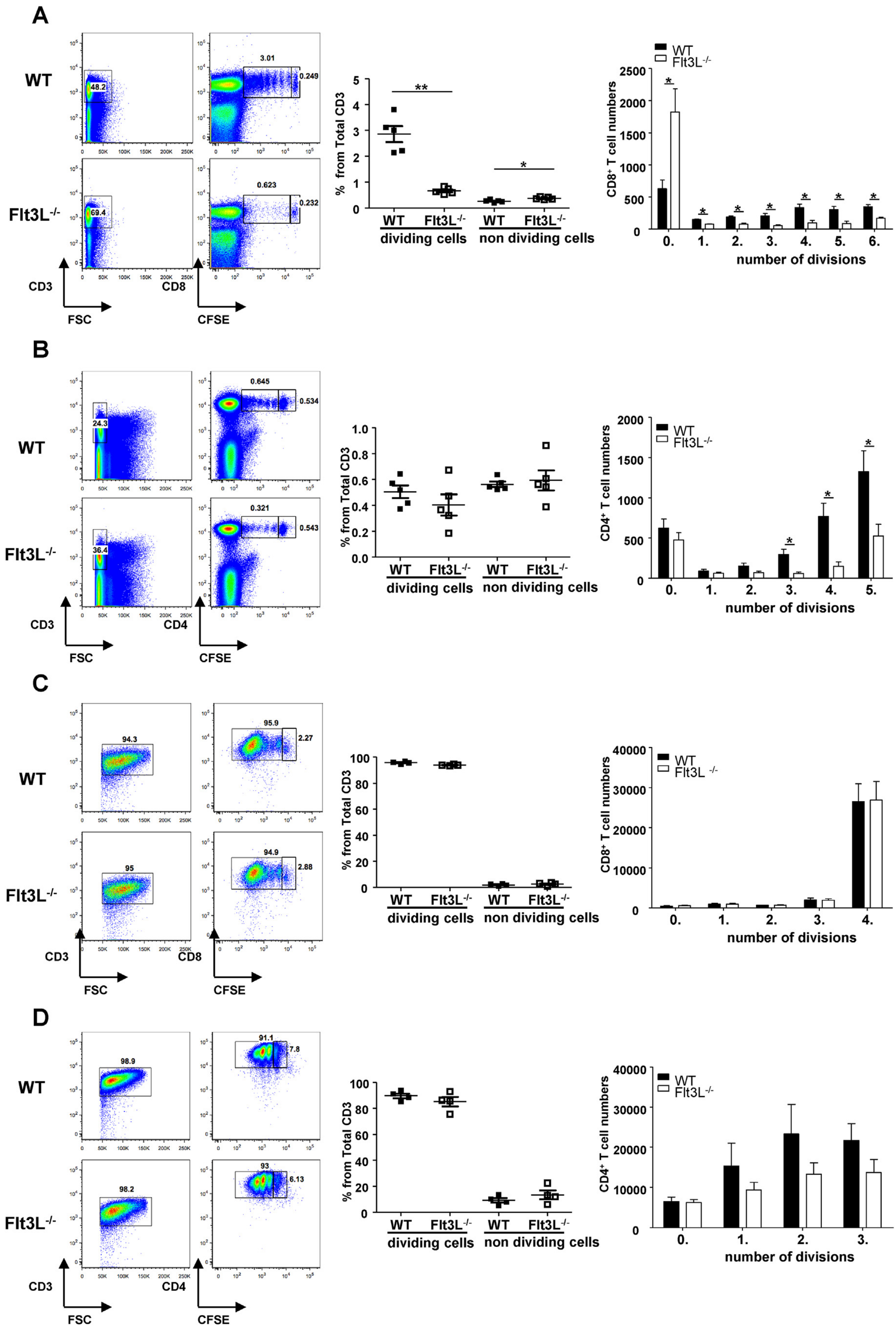

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Fig. 3. WT and Flt3L$^{-/-}$ DC capacity to induce OVA-specific CD8^{+} and CD4^{+} T cell proliferation in vivo and in vitro. (A) (Left graph) Representative plot showing in vivo proliferation of CFSE-labeled OVA-specific CD8^{+} T cells in the LNs of intradermally injected mice after 3 days. (Middle graph) Percentage of CFSE-labeled OVA-specific CD8^{+} T cells dividing and non-dividing cells. (Right graph) Number of CFSE-labeled OVA-specific CD8^{+} T cells per division. (B) (Left graph) Representative plot showing in vivo proliferation of CFSE-labeled OVA-specific CD4^{+} T cells in the LNs of intraperitoneally injected mice after 3 days. (Middle graph) Percentage of CFSE-labeled OVA-specific CD4^{+} T dividing and non-dividing cells. (Right graph) Number of CFSE-labeled OVA-specific CD4^{+} T cells per division. (C) (Left graph) Representative plot showing in vitro proliferation of CFSE-labeled OVA-specific CD8^{+} T cells after 3 day co-culture with WT or Flt3L$^{-/-}$ BMDCs. (Middle graph) Percentage of CFSE-labeled OVA-specific CD8^{+} T dividing and non-dividing cells. (Right graph) Number of CFSE-labeled OVA-specific CD8^{+} T cells per division. (D) (Left graph) Representative plot showing in vitro proliferation of CFSE-labeled OVA-specific CD4^{+} T cells after 3 day co-culture with WT or Flt3L$^{-/-}$ BMDCs. (Middle graph) Percentage of CFSE-labeled OVA-specific CD4^{+} T dividing and non-dividing cells. (Right graph) Number of CFSE-labeled OVA-specific CD4^{+} T cells per division. Data represent two independent experiments with five mice per group. Mean ± SEM is shown. *$p < 0.05$; **$p < 0.01$.

Flt3L$^{-/-}$ mice compared to WT (Fig. 1A, p = 0.002 upper graph). No difference in the mean fluorescence intensity (MFI) of FITC^{+} DCs that migrated to the LNs was found (Fig. 1A, middle graph) suggesting that WT and Flt3$^{-/-}$ DCs have similar uptake capacity. Analysis of OVA-FITC^{+} DCs revealed a reduction in both CD11b^{+} and CD103^{+} DC migratory populations Flt3L$^{-/-}$ mice (Fig. 1A, lower graph, p = 0.02 and p = 0.04 respectively).

Circulating monocytes can play an important role during inflammation by differentiating locally into mo-DCs, under the control of GM-CSF [27], and migrating to the LN carrying antigen [28]. In the steady-state condition there was an increase in the percentage of CD115^{+}CD11b^{+} monocytes in the blood of Flt3L$^{-/-}$ mice compared to WT (Fig. 1B, p = 0.03, right graph), and no difference in the "inflammatory" Ly6C^{hi} and "patrolling" Ly6C^{lo} monocytes frequencies was observed (Fig. 1B, left graph). Despite a small increase in Flt3L$^{-/-}$ circulating monocytes frequency no differences in total monocyte cell numbers [25,28] and mo-DC numbers and function [29] comparing to WT have been reported.

To examine the uptake capacity of mo-DCs in Flt3L$^{-/-}$ mice, we used GM–CSF–derived BMDCs as a model for Flt3-independent DCs. GM-CSF BMDCs were pulsed with FITC-labeled OVA and antigen uptake was measured by FACS. There were no differences in antigen uptake in Flt3L$^{-/-}$ BMDCs compared to WT BMDCs, in percentage of uptake (Fig. 1C) or in the amount of antigen per cell basis (MFI, Fig. 1D).

3.2. Mo-DCs in Flt3L$^{-/-}$ mice do not display defects in migratory capacity

To test the mobilization capacity of DCs present in Flt3$^{-/-}$ mice in vitro, skin explants were floated on culture medium alone or supplemented with CCL21, a chemokine that regulates cell trafficking [30]. As expected, the number of total DCs (CD11c^{+}MHCII^{+}) emigrated into the culture medium, with or without CCL21, was reduced in Flt3L$^{-/-}$ mice compared to WT (Fig. 2A, p = 0.028). However, despite reduced total cell emigration in Flt3L$^{-/-}$ mice we observed an increase of migration when the culture medium was CCL21 supplemented, indicating that these cells were responsive to this chemokine. As previously reported, we observed reductions in skin CD103^{+} and CD11b^{+} DC populations (Fig. 2A, p = 0.028).

In order to study in detail Flt3L-independent mo-DC migration in vivo we performed a competition assay using GM-CSF derived BMDCs. As shown in Fig. 2B, no difference was found in the amount of migrated GM-CSF derived DCs from WT or Flt3L$^{-/-}$ mice in the LNs of recipient mice. Importantly, there was also no difference in the migratory capacity of Flt3L$^{-/-}$ BMDCs compared to WT BMDCs when transferred into a WT or Flt3L$^{-/-}$ recipient mouse showing that the basal levels of chemokines that regulate cell trafficking are similar between Flt3L$^{-/-}$ and WT mice. This observation was confirmed by gene expression analysis of CCL21 and CCL19 in skin explants where no differences were observed between Flt3L$^{-/-}$ and WT mice (Fig. 2C). CCR7 is involved in steady-state and inflammation-induced LN migration of DCs via afferent lymphatics [31]. Flt3L$^{-/-}$ BMDCs expressed CCR7 and TLR ligands LPS and CpG upregulated its expression in the same manner as WT BMDCs (Fig. 2D). Taken together, this data suggests that the reduced antigen availability in Flt3L−/− LNs after immunization resulted from a decrease in DC numbers.

3.3. Flt3L$^{-/-}$ mice have reduced (in vivo) antigen-specific T cell proliferation but no intrinsic defects in antigen presentation

To study the outcome of reduced Flt3L-dependent DCs for the initiation of T cell responses, we made use of OVA TCR transgenic mice. CD8^{+} OVA-specific T cells (OT-I) or CD4^{+} OVA-specific T cells (OT-II) were adoptively transferred into WT or Flt3L$^{-/-}$ mice. After 24 h, mice were challenged with OVA and CFA intradermally, and 3 days later draining LNs and spleen were collected and OVA-specific T cell responses were analyzed. Importantly, we observed an almost complete abrogation in the proliferation of OT-I cells in the lymph nodes after intradermal OVA challenge in Flt3L$^{-/-}$ compared to WT mice (both for percentage of proliferating cells and number of divisions; Fig. 3A, p = 0.007, middle graph).

No differences were observed in the percentage of proliferating OT-II cells (Fig. 3B, middle graph), but there was a reduction in the number of divisions each CD4^{+}T cell underwent in Flt3L$^{-/-}$ compared to WT mice (Fig. 3B, right graph). A similar result was observed when analyzing the spleen (Supplementary Figure 1).

Next, we addressed whether Flt3L$^{-/-}$ mo-DCs had any defect in antigen presentation. LPS-activated GM-CSF derived BMDCs from WT or Flt3L$^{-/-}$ mice were cultured with OT-I or OT-II T cells in the presence of OVA and after 3 days OVA-specific T cell responses was assessed. WT and Flt3L$^{-/-}$ BMDCs could equally induce T cell proliferation, indicating that Flt3L$^{-/-}$ mo-DCs do not display intrinsic defects on antigen presentation capacity (Fig. 3C and D. A qPCR array for 84 known genes related with antigen uptake, processing, migration, presentation, cytokine and chemokine production was performed in WT and Flt3L$^{-/-}$ BMDCs and no major differences were observed (Supplementary Figure 2 and 3 and S2). Overall T cell responses are diminished in Flt3L$^{-/-}$ mice and in particular CD8^{+} T cell proliferation is severely impaired due to reduced Flt3L dependent-DCs.

3.4. Batf3$^{-/-}$ mice are protected from CIA

In order to clarify the importance of cDC1 for CIA induction we made use of Batf3$^{-/-}$ mice. Mice lacking the transcription factor Batf3 have a defect in the development of CD8α^{+} and CD103^{+}CD11b^{-} cDC1 [32] and impaired CD8^{+} T cell responses [33,34]. Batf3$^{-/-}$ were protected from CIA induction (Fig. 4A, p = 0.02) and histologically showed a trend for reduced synovial infiltration (Fig. 4B, p = 0.053) and significant reduced cartilage damage (Fig. 4B, p = 0.03) compared to WT. LN analysis showed a reduction in the amount of TNF- and IFN-γ-producing CD8^{+} T cells in Batf3$^{-/-}$ mice compared to WT (Fig. 5A, p = 0.033 and p = 0.016 respectively, right graph). There was no difference in CD4^{+} T cell cytokine production (Fig. 5A, left graph) and in the amount of FoxP3^{+} regulatory T cells (Supplementary Figure 4) in Batf3$^{-/-}$ LNs compared to WT. We reported a trend towards lower total B cell numbers in Batf3$^{-/-}$ mice compared to WT (Fig. 5B, left graph). Importantly a reduction of germinal center B cells and CII-specific antibody responses in Batf3$^{-/-}$ LNs was observed (Fig. 5B, p = 0.016 and 5C, IgG1 p = 0.016 and IgG2a p = 0.04, right graph). In addition, synovial CD103^{+} DCs were specifically reduced in Batf3$^{-/-}$ mice compared to WT (Fig. 5D, p = 0.01). We showed a reduction of neutrophil infiltration in the synovium of Flt3L$^{-/-}$ and Batf3$^{-/-}$ mice compared to arthritic WT mice (Supplementary

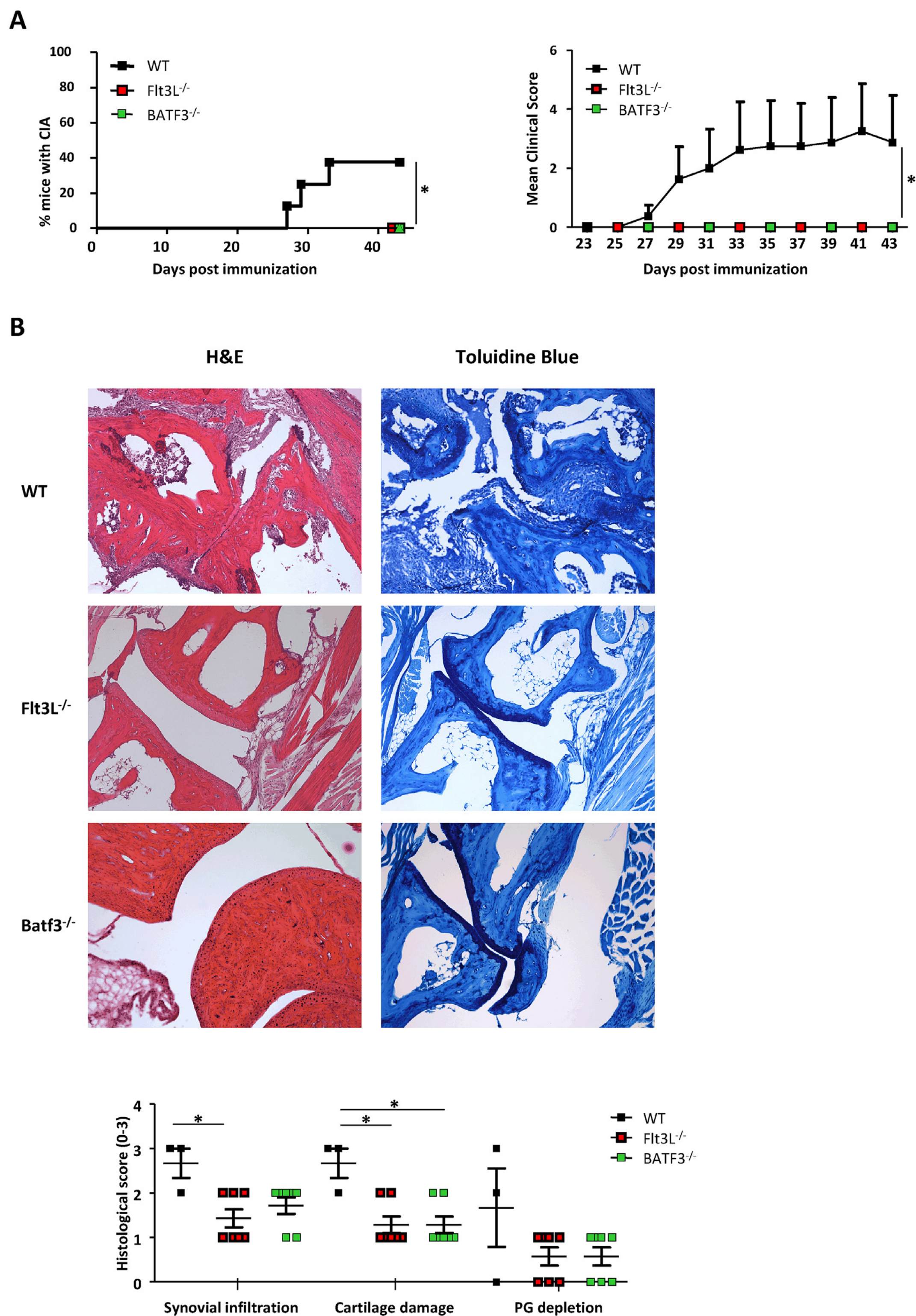

Fig. 4. Batf3$^{-/-}$ mice are protected from CIA. Clinical responses at the acute phase of the disease. (A) Incidence and severity of CIA in WT, Flt3L$^{-/-}$ and Batf3$^{-/-}$ mice. (B) Histological analysis of the knee of WT, Batf3$^{-/-}$ and Flt3L$^{-/-}$ mice. Data obtained at day 43 after first immunization, WT and Flt3L−/− eight mice per group, Batf3−/− seven mice per group. *p < 0.05.

Figure 5, p = 0.01). Batf3$^{-/-}$ mice, lacking cDC1 were protected from CIA, showing reduced T cell activation and antibody production. This indicates that cDC1 are necessary for the initiation of cellular and humoral responses in CIA.

3.5. cDC1 depletion by Flt3 inhibitor CEP-701 protects mice from CIA

In order to access the therapeutic potential of the Flt3 inhibitor CEP-701 in preventing CIA development, CIA was induced in DBA1 mice and before showing clinical signs of disease mice were treated with CEP-701. We observed that CEP701-treated mice did not develop CIA over time

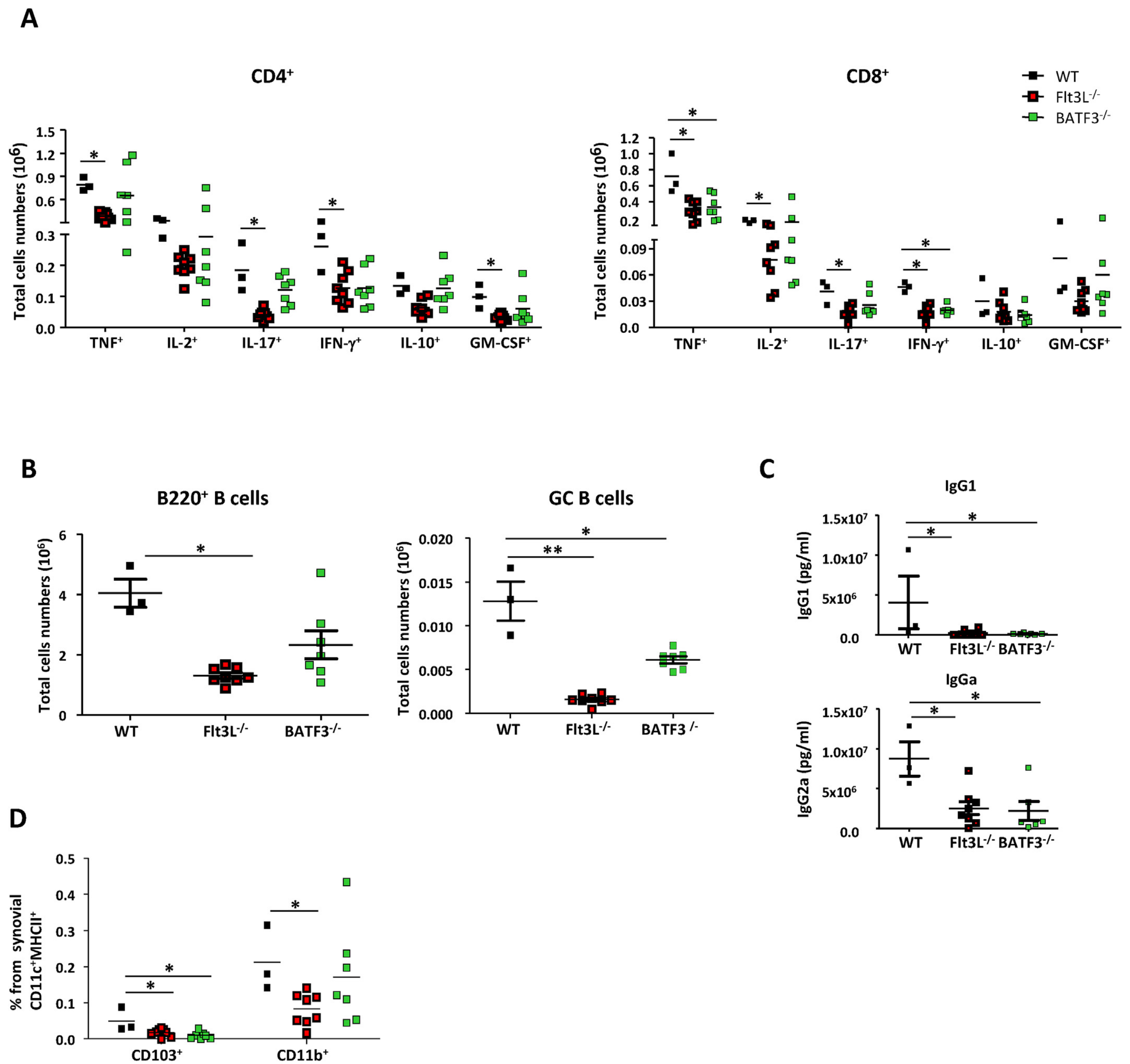

Fig. 5. Batf3$^{-/-}$ mice are protected from CIA. Cellular responses at the acute phase of the disease. (A) CD4^{+} and CD8^{+} T cell cytokine production in WT, Batf3$^{-/-}$ and Flt3L$^{-/-}$ LNs. (B) Total B cell numbers and total germinal center B cells in WT, Batf3$^{-/-}$ and Flt3L$^{-/-}$ LNs. (C) CII-specific antibody responses in WT, Batf3$^{-/-}$ and Flt3L$^{-/-}$ serum. (D) Synovial CD11b^{+} and CD103^{+} DCs. Data obtained at day 43 after first immunization, WT and Flt3L$^{-/-}$ eight mice per group, Batf3$^{-/-}$ seven mice per group. Mean ± SEM is shown. *$p < 0.05$.

(Fig. 6A, p = 0.02). Both CD103^{+} and CD8α^{+} DCs were severely reduced in CEP-701-treated mice compared to vehicle-treated mice (Fig. 6B, p = 0.003). Two different DC populations were present in arthritic synovium of vehicle-treated mice based on the expression of CD103 and CD11b phenotypical markers. Interestingly, CEP-701 treatment led to a dramatic and specific reduction of synovial CD103^{+} DCs compared to vehicle-treated mice (Fig. 6C, p = 0.01) while the CD11b^{+} DC subset remained unchanged. A significant reduction in the percentage of synovial CD3^{+} T cells was observed in CEP-701-treated mice compared to vehicle-treated mice (Fig. 6D, p = 0.01). Importantly, the number of synovial T cells correlated with disease severity (Fig. 6D, p = 0.003; $r^2 = 0.32$). CEP701 treated mice were protected from CIA, showing a dramatic reduction of cDC1 populations suggesting that these cells are crucial for disease induction.

4. Discussion

Although DCs in arthritis are the object of longstanding and considerable research, studies on DCs as inducers of autoimmunity are more recent and less numerous [35]. Here we emphasized the role of Flt3-dependent DC cells driving arthritis and identified cDC1 as required for that process. These findings provide a more precise developmental and functional picture of which DC subset is required for arthritis induction in mice that is very different from prior analyses that were based on total deletion of DCs (either pDCs [18] or cDCs [19]). We demonstrated that cDC1 present antigen in the context of murine inflammatory

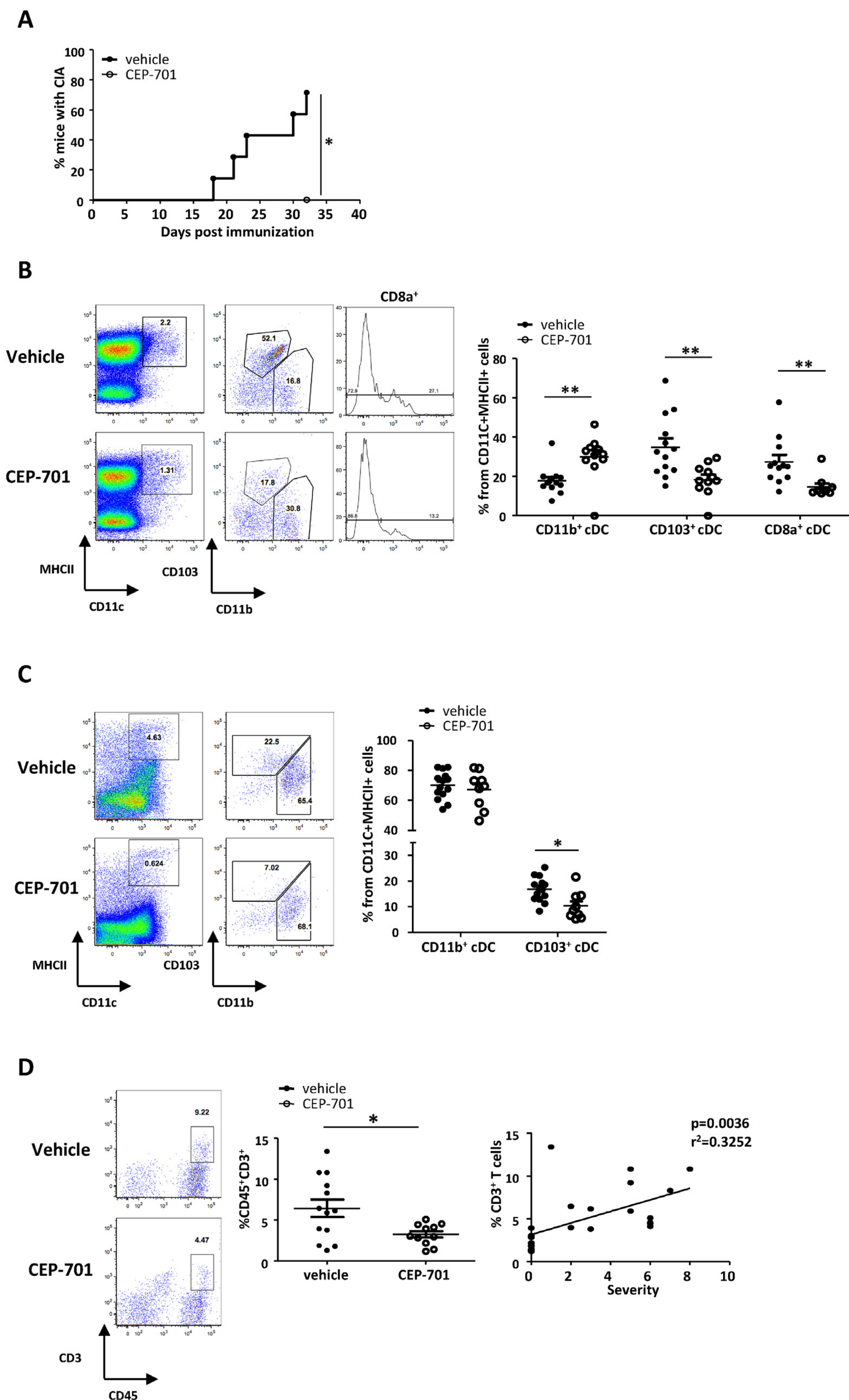

Fig. 6. Flt3 inhibitor CEP-701 treatment reduces CD103$^+$ DCs in LNs and synovium of arthritic mice. (A) CIA incidence after CEP-701 treatment. (B) CD103$^+$, CD11b$^+$ and CD8α$^+$ DC populations in LNs of CEP-701-treated mice. (C) CD103$^+$, CD11b$^+$ and CD8α$^+$ DC populations in the synovium of CEP-701-treated mice. (D) CD45$^+$ CD3$^+$ T cells in the synovium of CEP-701-treated mice. Correlation between the percentage of synovial CD3$^+$ T cells and disease severity. Data obtained at day 43 after first immunization, treated group eleven mice per group, non-treated group thirteen mice per group. Mean ± SEM is shown. *$p < 0.05$.

arthritis and that development of autoreactivity in this model is crucially dependent on their presence. Since cDC1 are required for initiating $CD8^+$ T cell responses [33], this study also points to a previously unappreciated role of $CD8^+$ T cells for the development of arthritis. Data on the role of $CD8^+$ T cells in autoimmune diseases have been scarce, especially when compared to research on $CD4^+$ T cells [36]. Although the pathogenesis of CIA is not fully understood, evidence supports a central role for T cell activation in the initiation and persistence of the chronic autoimmune response [37,38]. This concept is supported by the demonstration that the strongest genetic risk for RA is conferred by the HLA locus [39]. In juvenile idiopathic arthritis, despite HLA class II predominantly determines disease risk [40], a clear association of the HLAA*02:01 locus with disease severity is evident, whereas HLAA*01:01-positive individuals seem to be protected from disease [41,42]. The evidence that specific HLA class I molecules have an association with susceptibility to autoimmune disease supports the idea that $CD8^+$ T cells have a role in autoimmunity.

Additionally, peripheral blood of patients with early RA (disease duration <1 year), show an increase in the absolute number of $CD8^+$ T cells compared to healthy controls [43]. On the other hand, the number of $CD8^+$ T cells was reduced in patients in remission compared with both healthy controls and patients with active disease [44]. $CD8^+$ T-cell populations are also expanded in the synovial fluid of patients with RA, JIA and PsA [45–47]. Additionally, $CD8^+$ T cells have been implicated in the formation of ectopic germinal centers in rheumatoid synovitis [48]. These structures are observed in nearly 50% of RA patients, and support ongoing production of class-switched autoantibodies [49] thus suggesting an important role in the disease process.

In this study we have demonstrated that absence of cDC1 in Flt3L$^{-/-}$, Batf3$^{-/-}$ and CEP-701-treated mice led to CIA protection with reduced proliferating T cells in LNs and synovium and reduced B cell activation. Importantly, T cell responses in Flt3L$^{-/-}$ mice were reduced in vivo, and in particular $CD8^+$ T cell proliferation was dramatically diminished after intradermal administration of antigen. These data support the importance of cDC1in the initiation of $CD8^+$ T cell responses [50].

It has been reported that Batf3$^{-/-}$ mice have a constitutive absence of cDC1 [32]. We observed that Flt3L$^{-/-}$ mice resistance to CIA is comparable to that of Batf3$^{-/-}$ mice. These data suggest that cDC1 are likely to be of profound importance in the arthritic process and that inflammatory monocyte-derived DCs under the control of GM-CSF appear not to be sufficient for T cell activation and CIA induction. Under inflammatory settings, cDC1 development in Batf3−/− can be compensated by the induction in DC precursors of the paralog genes Batf and Batf2. Further studies using these mice in arthritic models are necessary to evaluate the contribution of cDC1 and CD8 T cell responses for disease severity [9,51]. We showed that CEP-701 administration before clinical signs of disease prevented CIA induction by targeting $CD103^+$ and $CD8\alpha^+$ cDC1. The present study is the first, to our knowledge, that identifies the presence of migratory $CD11b^+$ and $CD103^+$ DC populations in the CIA synovium. Specifically, a reduction in the percentage of $CD103^+$ DCs and in the percentage of T cells present in the synovium (which correlated with CIA disease severity) was observed in mice treated with CEP-701. Since CEP-701 specifically targeted $CD103^+$ and $CD8\alpha^+$ DCs and proved to be beneficial in preventing CIA induction we reason that using this compound might be a good therapeutic strategy in individuals at risk to develop arthritis. This data is also in accordance with the observation that minor subset of Batf3-dependent APCs in islets of Langerhans was shown to be essential for the development of autoimmune diabetes [52] where they actively contribute for the initiation of the autoimmune process. Additionally, it was also reported that $CD103^+CD11b^-$ DC subset is sufficient to cause and amplify graft-versus-host disease, as exemplified in Irf4-deficient bone marrow chimeras [53].

Studies in humans are more scarce due to the small numbers of $CD141^+$ DC in blood and the unique challenge of isolating these cells from tissues but, importantly, a significant enrichment of human cDC1, $CD141^+$ DCs, in the inflamed synovial join has been shown [54]. Synovial $CD141^+$ DC–T cell interactions had the ability to further activate synovial fibroblasts, inducing adhesive and invasive pathogenic mechanisms, suggesting that these cells might play a role in the inflammatory process [54].Peripheral blood $CD141^+$ DC, but not pDC, frequency was also inversely correlated with the interferon signature in early RA patients and may be a hitherto unappreciated source of type I IFN [55]. Overall, this suggests that $CD141^+$ DCs, despite their relatively small numbers when compared with other cellular subsets, may actively contribute to disease pathogenesis. Further studies in humans are required in order to elucidate the specific role of the cDC1 subset driving/maintaining autoimmunity.

5. Conclusion

This study demonstrates that cDC1 present Ag in the context of murine inflammatory arthritis and that development of autoreactivity in this model is crucially dependent on their presence. cDC1 induced $CD8^+$ T cell cytokine production and/or B cell antibody production are of importance for the induction of CIA. Targeting this DC subset might be of interest and valuable in individuals at risk of developing autoimmune disorders.

Credit author statement

MIR, SG, KAR, SEJ, PPT and MCL: Conceptualization and design, MIR, BH, SA and MCL: Methodology, MIR, MCL: Data curation, PPT, MCL: Supervision, MIR: Writing - original draft, MIR, SA, BH, SA, KAR, SEJ, PPT, MCL: Writing-reviewing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtauto.2020.100066.

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