Original Contribution

IL-23 (Interleukin-23)–Producing Conventional Dendritic Cells Control the Detrimental IL-17 (Interleukin-17) Response in Stroke

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Background and Purpose—Inflammatory mechanisms can exacerbate ischemic tissue damage and worsen clinical outcome in patients with stroke. Both αβ and γδ T cells are established mediators of tissue damage in stroke, and the role of dendritic cells (DCs) in inducing the early events of T cell activation and differentiation in stroke is not well understood.

Methods—In a murine model of experimental stroke, we defined the immune phenotype of infiltrating DC subsets based on flow cytometry of surface markers, the expression of ontogenetic markers, and cytokine levels. We used conditional DC depletion, bone marrow chimeric mice, and IL-23 (interleukin-23) receptor-deficient mice to further explore the functional role of DCs.

Results—We show that the ischemic brain was rapidly infiltrated by IRF4$^+$/CD172a$^+$ conventional type 2 DCs and that conventional type 2 DCs were the most abundant subset in comparison with all other DC subsets. Twenty-four hours after ischemia onset, conventional type 2 DCs became the major source of IL-23, promoting neutrophil infiltration by induction of IL-17 (interleukin-17) in γδ T cells. Functionally, the depletion of CD11c$^+$ cells or the genetic disruption of the IL-23 signaling abrogated both IL-17 production in γδ T cells and neutrophil infiltration. Interruption of the IL-23/IL-17 cascade decreased infarct size and improved neurological outcome after stroke.

Conclusions—Our results suggest a central role for interferon regulatory factor 4-positive IL-23–producing conventional DCs in the IL-17–dependent secondary tissue damage in stroke.

Visual Overview—An online visual overview is available for this article. **(*Stroke*. 2018;49:00-00. DOI: 10.1161/STROKEAHA.117.019101.)**

Key Words: dendritic cells ■ inflammation ■ interleukin-17 ■ interleukin-23 ■ stroke

Ischemic stroke is the primary cause for sustained disability in the Western world. The initial ischemic brain damage leads to a robust activation of the immune system, which follows a pattern typical of sterile inflammation. Ischemic tissue hallmarks are the presence of IL-17 (interleukin-17)–positive γδ T cells, secondary neutrophil infiltration, the appearance of monocytes, and an upregulation of proinflammatory cytokines and chemokines.[1–3] Even though effector functions of γδ T cells have been mostly decoded, the triggering inflammatory events are largely unclear.[2,4]

Recently, it has been shown that apart from their role in classical concepts of antigen-dependent immune responses,[5] dendritic cells (DCs) are capable of locally shaping immune reactions independently of their migration to secondary lymphoid organs or antigen presentation.[6] Engagement of toll-like receptors on DCs by pathogen-associated molecular patterns or danger-associated molecular patterns lead to the rapid production of cytokines, which in turn can start a fast innate-like immune cascade. For example, both conventional DCs (cDCs) and

Received August 14, 2017; final revision received September 27, 2017; accepted October 16, 2017.

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Guest Editor for this article was Miguel Perez-Pinzon, PhD.

The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.117.019101/-/DC1.

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monocyte-derived DCs can produce IL-23 (interleukin-23) upon stimulation with TLR5 ligands.[7]

In cerebral ischemia, a significant amount of CD11c$^+$ cells appears early in the disease course.[8,9] However, the origin, phenotype and function of these cells are still unknown. Because of the exquisite ability of DCs to mount immune responses, these CD11c$^+$ cells could be of importance for the inflammatory response after stroke.

The identification and classification of these cells in the brain is of critical importance and has been recently debated.[10] In this study, we have used highly detailed multiparameter flow cytometry to identify brain-infiltrating DC subsets according to the classification recently proposed by Guilliams et al,[11] namely conventional type 1 DCs (cDC1s), conventional type 2 DCs (cDC2s), and plasmacytoid DCs. We further differentiated cDCs and plasmacytoid DCs from monocyte-derived DCs.

Using this approach, we identified in the ischemic hemisphere the presence of conventional CD172a$^+$/IRF4$^+$ type 2 DCs that were the major source of IL-23, which is essential for the expression of IL-17 in γδ T cells.

Materials and Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.

Animals

All animal experiments were approved by local animal care committees (Behörde für Lebensmittelsicherheit und Veterinärwesen Hamburg). We conducted the experiments according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (publication No. 83–123, revised 1996) and performed all procedures in accordance with the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments; http://www.nc3rs.org/ARRIVE). We randomized all mice and conducted transient middle cerebral artery occlusion (tMCAO) as described previously.[2] The detailed experimental description can be found in the online-only Data Supplement.

Analysis of Infarct Size by TTC Staining and Magnetic Resonance Imaging

We analyzed infarct size by vital staining using 2% (wt/vol) TTC (2,3,5-triphenyl-2-hydroxy-tetrazolium chloride) in phosphate buffer. Magnetic resonance imaging was performed on a dedicated 7T MR small animal imaging system (ClinScan; Bruker). Detailed experimental description can be found in the online-only Data Supplement.

Antibodies and Flow Cytometry

We performed flow cytometry for the analysis of cell types as described previously.[2] Detailed experimental description can be found in the online-only Data Supplement.

Cell Sorting, RNA Isolation, and Quantitative Real-Time Polymerase Chain Reaction

Immune cells were sorted using a BD fluorescence-activated cell sorting Aria IIIu, and quantitative real-time polymerase chain reaction was performed as described previously.[2] Detailed experimental description can be found in the online-only Data Supplement.

Immunohistochemistry and Immunofluorescence

We stained mouse brains with antibodies against Ly6G (1A8; Biolegend), GFAP (glial fibrillary acidic protein; Dako), Iba-1 (Wako), and laminin (1:100, Dako). Autoptic human brain sections were stained with antibodies against CD11c (Abcam, ab52632) and CD11b (Abcam, ab52478). Detailed experimental description can be found in the online-only Data Supplement.

Cell Transfer

We performed cell transfers as described previously.[2]

Bone Marrow Chimeras

Recipients were irradiated by whole-body irradiation (9 Gy) using a cesium-137 gamma irradiator (BIOBEAM 2000). Detailed experimental description can be found in the online-only Data Supplement.

Statistical Analysis

Statistical analyses were performed using the appropriate test indicated in the figure legends. Briefly, we used Student *t* test to compare infarct volumes, Mann–Whitney *U* test for the comparison of clinical scores, 1-way ANOVA for multiple comparisons with Bonferroni post hoc test, after validating the normal distribution of the data sets (Kolmogorov–Smirnov test), and 1-way ANOVA with Bonferroni post hoc test and unpaired Student *t* test for the comparison of ΔΔCt values between groups. *P* values <0.05 were considered statistically significant.

Results

DCs Infiltrate the Brain Parenchyma Early and Locate to the Perilesional Area

The number of CD11c$^+$ DCs was low in the healthy brain. However, after tMCAO, CD11c$^+$ DCs had entered the ischemic brain and settled already at 24 hours to the peri-infarct area and, more specifically, close to blood vessels (Figure 1A). Next, we examined by immunohistochemistry postmortem stroke tissue from patients who had died shortly after having a stroke (<24 hours; Figure 1B). While we rarely observed CD11c$^+$ cells in normal brain tissue, the CD11c expression increased in the penumbra area after stroke. The different expression patterns of CD11c and CD11b in postmortem stroke tissue indicate that the upregulation of CD11c is not only because of activation of CD11b$^+$ microglia but also because of a consequence of infiltrating CD11c$^+$ immune cells. To further analyze which DC subsets infiltrate the brain in our experimental stroke model, we used whole-brain fluorescence-activated cell sorting analysis. In a first step, DCs were classified as CD45^{high}CD11c$^+$MHCII$^+$Ly6G$^-$, thus excluding the CD45^{int}-expressing microglia and the Ly6G$^+$ neutrophils. Inside this population, we differentiated plasmacytoid DCs as B220$^+$, cDCs as B220$^-$Ly6C$^-$CD64$^-$F4/80^{low}, and monocyte-derived DCs as Ly6C^{int}F4/80^{int}SSC^{int}CD64^{int} (Figure 1C).[11] cDCs were the most prevalent DC subset at all time points. Already after 24 hours, we observed a significant increase to 4414±3329 cDCs per hemisphere when compared with 339±90 in sham-operated animals. cDCs reached maximum levels at day 3 with a subsequent decrease at day 7.

CD172a$^+$/*Irf4*-Expressing cDC2 Cells Are the Predominant Subpopulation of Brain-Infiltrating cDCs

In nonlymphoid tissues, cDCs can further be differentiated into XCR1$^+$/IRF8$^+$ cDC1s and CD172a$^+$/IRF4$^+$ cDC2s.[12] Flow cytometric analysis revealed that the vast majority of infiltrating cDCs was XCR1$^-$/CD172a$^+$ cDC2s at all investigated time points (Figure 2A). We also observed CD172a$^-$/XCR1$^+$ cDC1s,

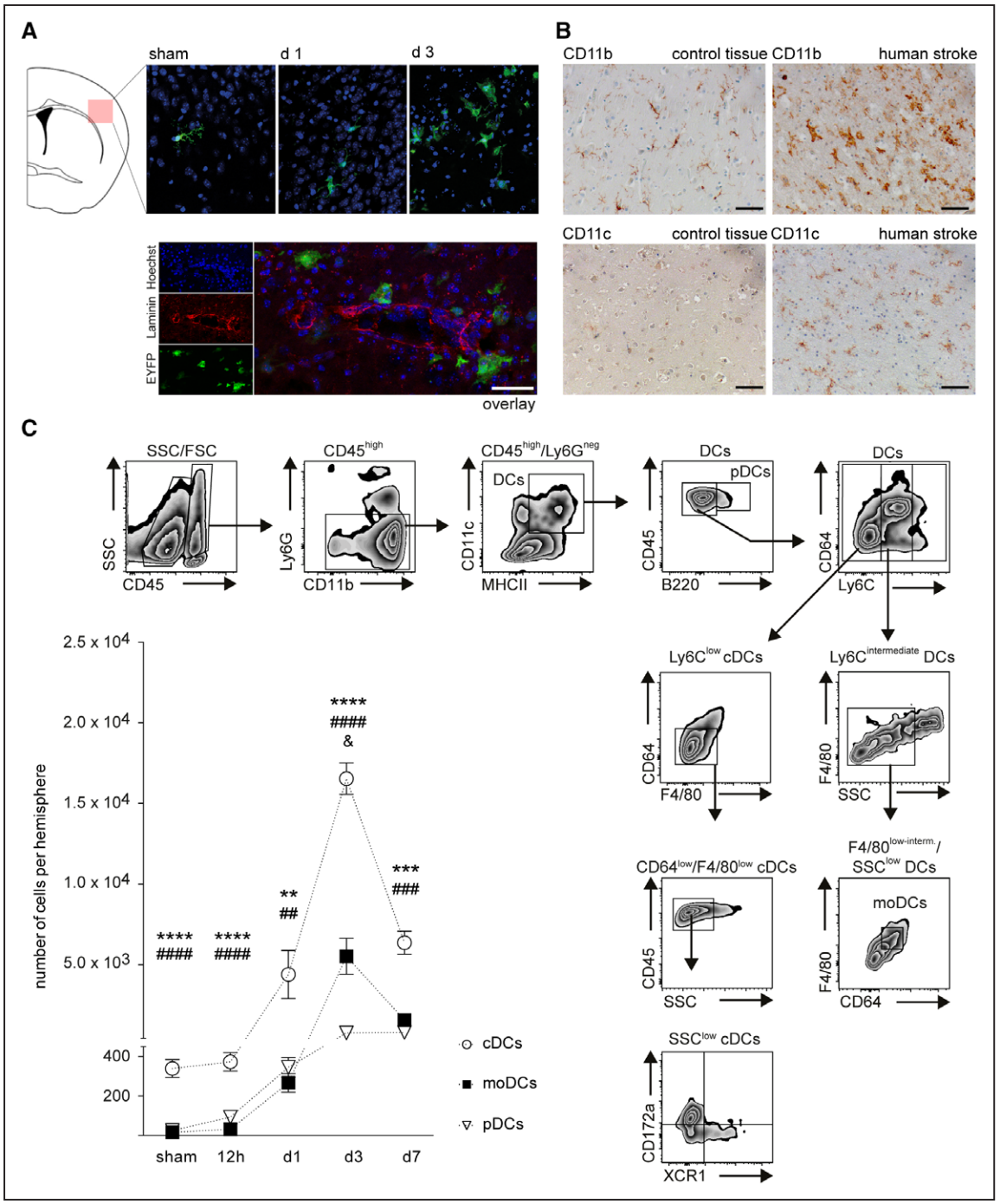

Figure 1. Dendritic cells (DCs) infiltrate the ischemic hemisphere and locate to the penumbra area. **A**, Visualization of EYFP (enhanced yellow fluorescent protein)-positive DCs in the penumbra of ischemic hemispheres of *Cd11c*-EYFP mice 1 and 3 days after transient middle cerebral artery occlusion (scale bars, 20 μm), and histological staining for laminin in ischemic hemispheres of *CD11c*-EYFP-mice at day 3 (scale bar, 0.7 mm). **B**, Immunohistochemical analysis of immune cells in ischemic human brain tissue 24 hours after stroke and unaffected brain tissue. Analysis was performed to detect CD11b and CD11c (scale bars, 30 μm). **C**, Flow cytometry of brain-infiltrating DC subsets. Gating strategy to identify plasmacytoid DCs (pDCs), monocyte-derived DCs (moDCs), and conventional DCs (cDCs) after staining for B220, CD11b, CD11c, CD45, CD64, CD172a, F4/80, Ly6C, Ly6G, MHCII, and XCR1. For absolute quantification, TrueCount tubes were used. **C**, The graphs show mean±SD of 4 to 6 animals per group, in 3 to 5 independent experiments for each time point. Statistical significances analyzed by 1-way ANOVA with Bonferroni post hoc test. $^{**}P<0.01$. FSC indicates forward scatter; and SSC, side scatter. *cDCs vs moDCs, #cDCs vs pDCs, and moDCs vs pDCs.

which exhibited delayed infiltration kinetics. To validate that the XCR1$^-$/CD172a$^+$ cDCs found in the brain belong to the cDC2 subset, we relied on expression levels of C-C motif chemokine receptor 2 (CCR2) and ontogenetic markers. As reported previously,[13] we also found that cDC2s had a CCR2^{high} profile, whereas microglia showed significantly lower CCR2 levels (Figure 2B). We next analyzed the expression of established transcription factors in fluorescence-activated cell-sorted

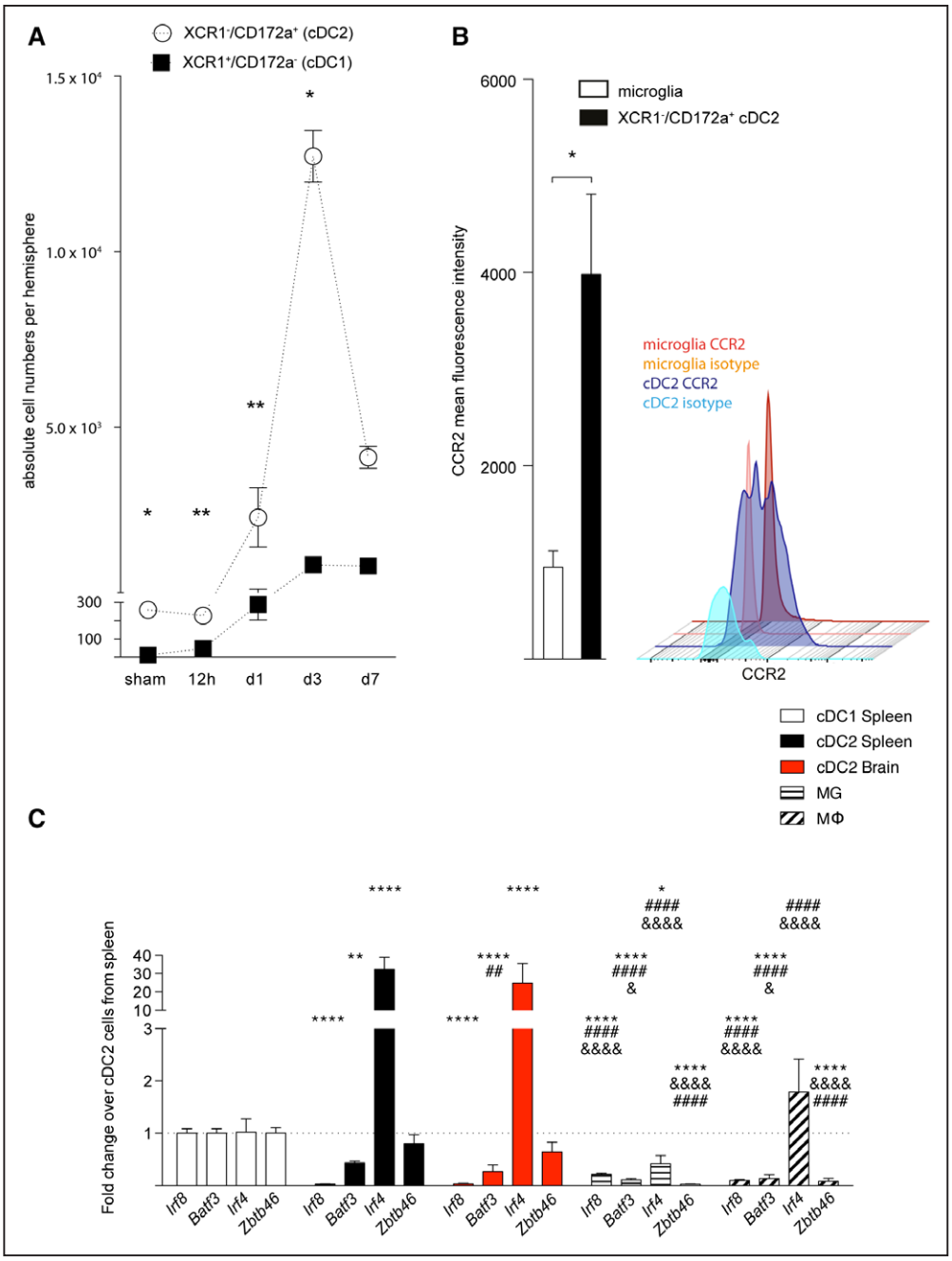

Figure 2. Classification of infiltrating dendritic cell subsets by immunophenotyping and analysis of ontogenetic markers. **A**, Absolute numbers of XCR1⁺ cDC1s and CD172a⁺ conventional type 2 DCs (cDC2s) in ischemic hemispheres after transient middle cerebral artery occlusion (tMCAO). Cell counts were determined by flow cytometric analysis of central nervous system-infiltrating cells after Percoll density centrifugation. For absolute quantification, TrueCount tubes were used. **B**, Flow cytometric analysis of CCR2 level on central nervous system-infiltrating CD172a⁺ cDC2s and microglia 24 hours after tMCAO. **C**, Relative gene expression of *Irf4*, *Irf8*, *Batf3*, and *Zbtb46* in central nervous system-infiltrating and splenic CD45^{high}/CD11b⁺/CD11c⁺/MHCII⁺/CD172a⁺/Ly6c⁻/Ly6g⁻/F4/80⁻/XCR1⁻ cDC2, CD45^{intermed}/CD11b⁺ microglia and central nervous system-infiltrating CD45^{high}/CD11b⁺/CD11c⁻/MHCII⁻/Ly6g⁻/F4/80⁺ macrophages purified 24 hours after tMCAO by fluorescence-activated cell sorting. Expression levels were normalized to corresponding levels of splenic cDC1s. The graphs show mean±SEM of 5 to 8 animals per group, in 5 to 7 independent experiments for each time point. Statistical significances analyzed by (**A**) 2-way ANOVA with Bonferroni post hoc test, (**B**) Student *t* test, and (**C**) 1-way ANOVA with Bonferroni post hoc test. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001. *vs cDC1 spleen, #vs cDC2 spleen, and vs cDC2 brain. MΦ indicates macrophages; and MG, microglia.

cDC2s from ischemic hemispheres at 12 and 24 hours after tMCAO. After normalization to corresponding levels in splenic cDC1s, we observed high levels for *Irf4* in combination with low levels for *Irf8* and *Batf3* in CD172a⁺ cDC2s derived from ischemic brains and spleens (Figure 2C; Figures I and II in the online-only Data Supplement). Furthermore, the expression of the DC-specific gene *Zbtb46* was maintained in cDC2s. Overall, high levels for *Irf4*, low levels for *Irf8* and *Batf3*, and expression of CD172a and CCR2 are consistent with the established profile of cDC2s. Most importantly, microglia and

macrophages extracted from same ischemic hemispheres displayed substantially different gene expression profiles. The results demonstrate the abundance of the newly described cDC2 cell population in the ischemic brain tissue.

Depletion of CD11c⁺ Cells in the *Cd11c.DOG* Model Is Protective in Stroke

Next, we used the *Cd11c.DOG* mouse model, in which a DTR (diphtheria toxin receptor) is expressed under the CD11c promoter.[14] The *Cd11c.DOG* model is widely used to deplete conventional CD11c⁺ DCs via injection of diphtheria toxin (DTX).[15] To control potential toxic effects of DTX, we treated heterozygous *Cd11c.DOG* mice and littermate controls with DTX. Daily injections of 8 ng/g body weight DTX started 24 hours before the tMCAO procedure[14] and resulted in a significant reduction of splenic and brain-infiltrating DCs (Figure 3A and 3B). Interestingly, DTX-treated *Cd11c.DOG* mice showed significantly reduced infarct sizes, milder neurological symptoms, and reduced mortality compared with DTX-treated littermate controls (Figure 3C and 3D).

CD11c⁺ Cells Induce Neutrophil Infiltration Into Ischemic Hemispheres Via Induction of IL-17 in γδ T Cells

Analysis of the immune cell infiltration in ischemic brains of *Cd11c.DOG* mice revealed significant reduction in neutrophil infiltration at days 1 and 3. Absolute numbers of other infiltrating leukocyte populations were not significantly affected (Figure 4A and 4B; Figure IIIA in the online-only Data Supplement). In fact, we saw an increase in neutrophils in

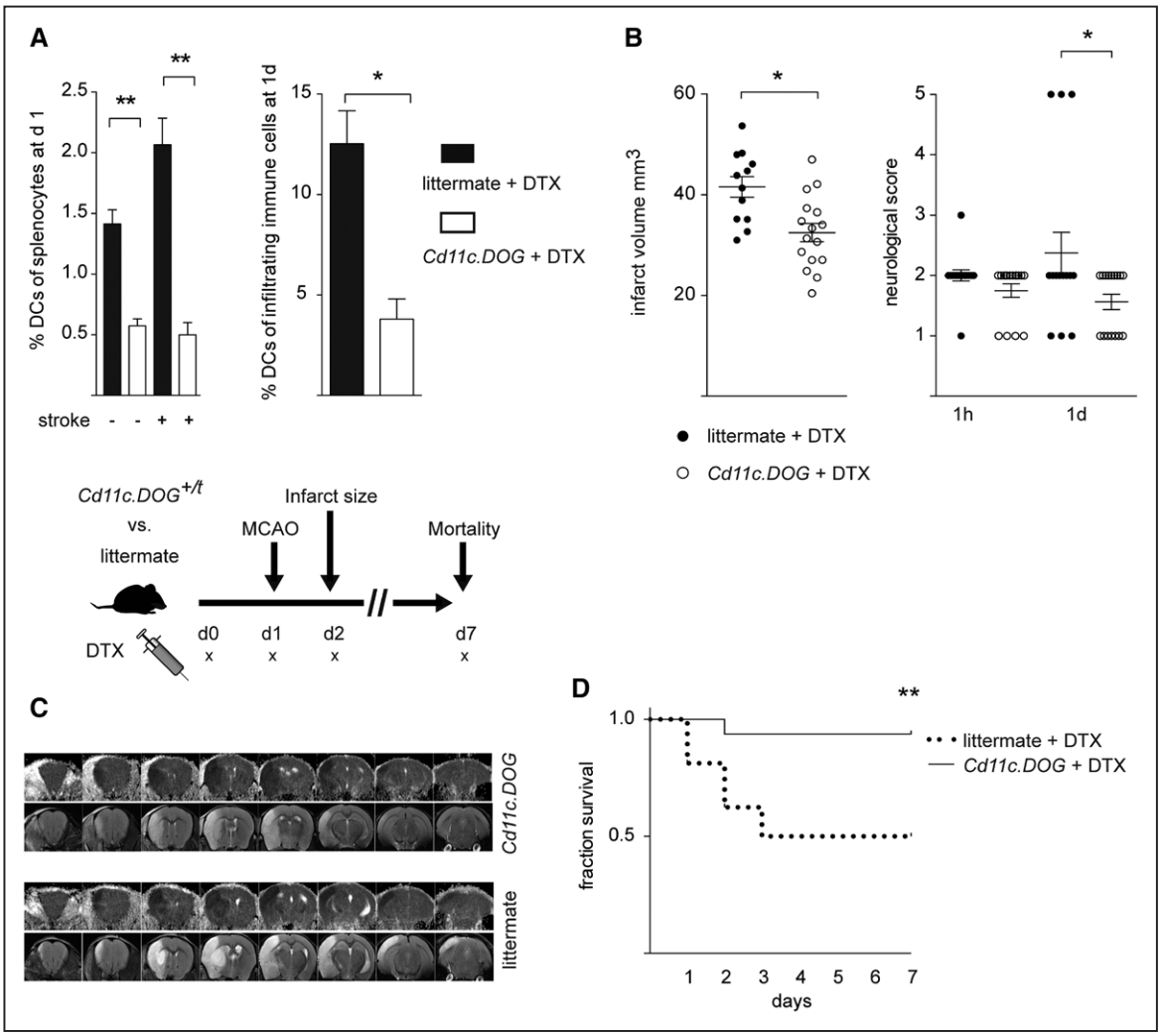

Figure 3. Depletion of CD11c⁺ cells in *Cd11c.DOG* mice is protective in a murine model of ischemic stroke. **A**, Depletion of CD11c⁺ cells in *Cd11c.DOG* mice after continuous daily administration of 8 ng/g body weight diphtheria toxin (DTX). Graph shows the percentages of splenic CD11c⁺/MHCII⁺ dendritic cells (DCs) of total living splenocytes in *Cd11c.DOG* mice compared with littermate controls 3 days after DTX administration. Infiltration of CD11c⁺/MHCII⁺ cells into ischemic hemispheres in *Cd11c.DOG* mice and littermate controls 1 day after transient middle cerebral artery occlusion (tMCAO). Daily administration of DTX was started 1 day before tMCAO. Cell counts were performed by flow cytometric analysis of splenocytes and central nervous system-infiltrating cells after staining for CD11b, CD11c, CD45, Ly6G, and MHCII. Graphs show the mean±SD of 9 to 12 animals per group from 3 to 4 independent experiments. **B** and **C**, Magnetic resonance imaging was used to quantify (representative T2 image) infarct volume at day 1 after tMCAO (**left**) in littermate controls and *Cd11c.DOG* mice after daily DTX administration. Neurological scores were performed 1 hour and 1 day (**right**) after middle cerebral artery occlusion (MCAO). Data are presented as mean±SD of 12 littermate controls and 16 *Cd11c.DOG* animals. Statistical significances analyzed by (**A** and **B**) Student *t* test, Mann–Whitney *U* test (neurological scores), and (**D**) χ^2 test (survival rate). **P*<0.05, ***P*<0.01.

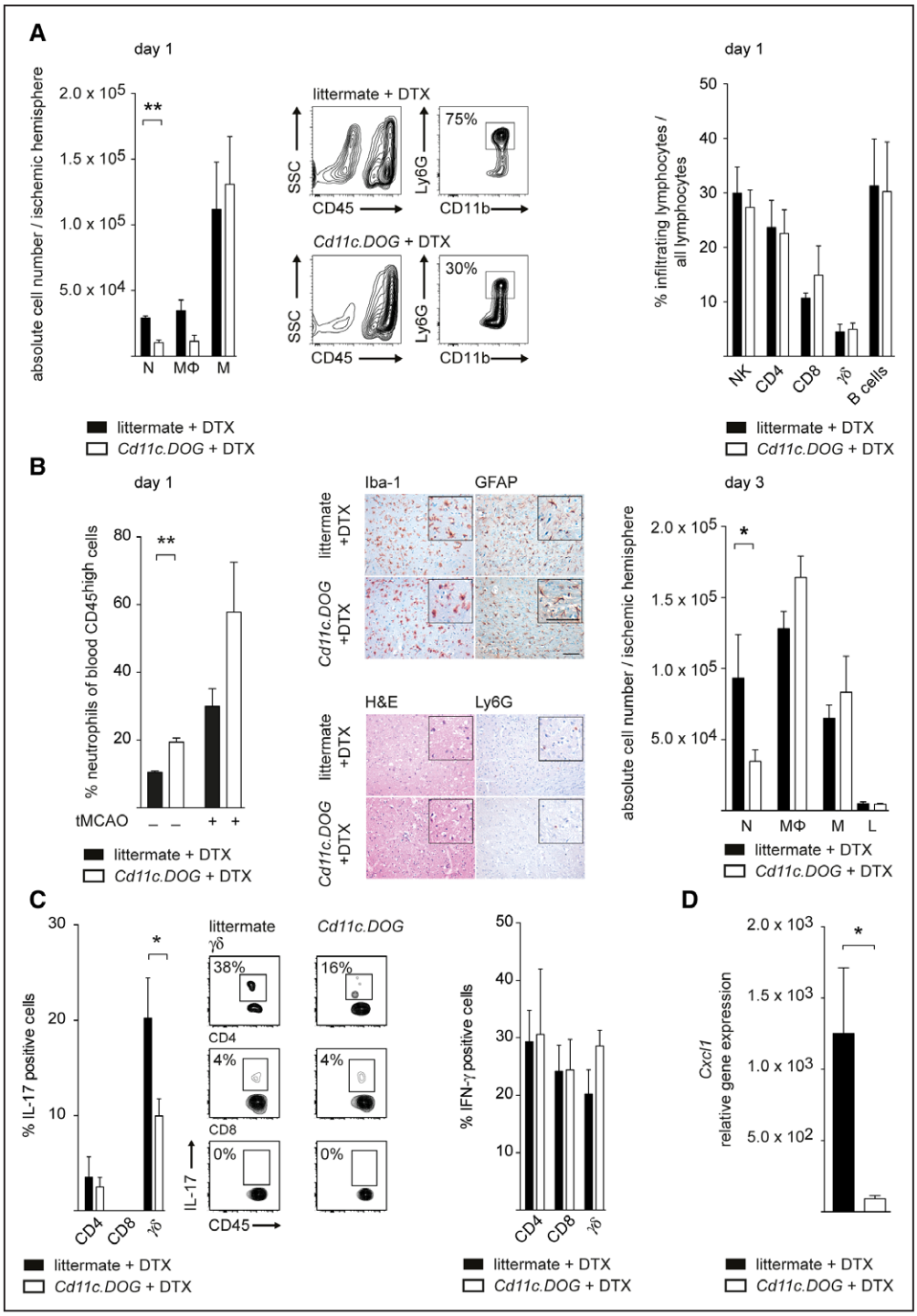

Figure 4. Depletion of CD11c$^+$ cells alters the migration of neutrophils and the production of IL-17 (interleukin-17) by γδ T cells. **A**, Absolute numbers of neutrophils (n; CD45^{high}/Ly6G$^+$/CD11b$^+$), macrophages (mΦ; CD45^{high}/Ly6G$^-$/CD11b$^+$), microglia (m; CD45^{intermediate}/CD11b$^+$), and frequency of CD4$^+$, CD8$^+$, γδ T cells, NK cells, and B cells in ischemic hemispheres of diphtheria toxin (DTX)-treated littermate controls and *Cd11c.DOG* mice 1 day after middle cerebral artery occlusion (MCAO). **B**, Frequency of peripheral blood neutrophils after DTX administration in littermate controls and *CD11c.DOG* mice 1 day after MCAO or sham surgery, immunohistochemical staining of macrophages/microglia (Iba-1), astrocytes (GFAP [glial fibrillary acidic protein]), and neutrophils (Ly6G) in DTX-treated littermate controls and *CD11c.DOG* mice 1 day after MCAO (scale bar, 50 μm), and absolute numbers of neutrophils (N), macrophages (MΦ), microglia (M), and lymphocytes (L) 3 days after MCAO. **C**, Flow cytometric analysis of IL-17 (interleukin-17) and IFN-γ (interferon-γ) produced by CD4$^+$, CD8$^+$, and γδ T cells isolated from ischemic hemispheres at day 1 after stroke induction. **A**–**C**, Cell counts were determined by flow cytometric analysis. **D**, Relative gene expression of *Cxcl1* in ischemic hemispheres of DTX-treated littermate controls and *Cd11c.DOG* mice 24 hours after transient middle cerebral artery occlusion (tMCAO). **A**–**D**, Daily DTX administration of 8 ng/g body weight DTX was started 1 day before MCAO or sham surgery. Data show the mean±SD of 9 to 12 animals per group, analyzed in 3 to 4 (**A**–**C**) or 6 (**D**) independent experiments. Statistical significances were analyzed by Student *t* test in all cases. **P*<0.05, ***P*<0.01. SSC indicates side scatter.

the peripheral blood of *Cd11c.DOG* mice treated with DTX (Figure 4B), which further underscores the dysfunctional neutrophil migration into the brain. We and others have previously shown that the recruitment of neutrophils into ischemic hemispheres depends on the IL-17A production by γδ T cells, which in turn induces C-X-C motif chemokine ligand 1 production by astrocytes.[2] After depletion of CD11c$^+$ cells, we observed a significant decrease of IL-17 levels in brain-infiltrating γδ T cells on days 1 and 3, which was paralleled by increased IFN-γ (interferon-γ) levels in γδ T cells on day 3 (Figure 4C; Figure IIIB in the online-only Data Supplement), whereas cytokine levels in infiltrating αβ T cells were not altered. Consistent with the reduction in IL-17, we detected significantly decreased *Cxcl1* levels in DTX-treated *Cd11c.DOG* mice (Figure 4D).

Infiltrating IL-17$^+$ γδ T Cells Express the IL-23R

A potential regulator of IL-17 production in T cells is IL-23.[16] To address the role of IL-23 in stroke, we analyzed a knock-in reporter mouse, in which an IRES GFP (internal ribosomal entry site green fluorescent protein) cassette was introduced in the endogenous IL-23R (IL-23 receptor) gene locus.[17] In heterozygous mice, IL-23R–expressing cells can be visualized by their GFP expression (*Il23r.gfp.KI*). When bred as homozygotes (*Il23r*$^{-/-}$), the deletion of the IL-23R abrogates their responsiveness to IL-23. In our model, 3 days after tMCAO, we observed that ≈80% of the brain-infiltrating IL-17A$^+$ γδ T cells coexpressed the IL-23R (Figure 5A).

Il23r$^{-/-}$ Mice Have a Defective IL-17 Response in γδ T Cells and Are Protected From Ischemic Stroke

Neutrophil infiltration, frequency of IL-17–producing γδ T cells, *Cxcl1* expression, and infarct sizes were significantly decreased in *Il23r*$^{-/-}$ mice after tMCAO (Figure 5A through 5C). In contrast, infiltration of macrophages, DCs and T-cell subpopulations, expansion of microglia, IFN-γ production in αβ T cells, and mortality rate were not affected by the IL-23R deficiency (Figure 5A and 5B; Figure IVB and IVC in the online-only Data Supplement). To underline the importance of the IL-23R on T cells, we transferred unfractionated T cells from *Il23r*$^{+/+}$ or *Il23r*$^{-/-}$ mice to T cell–deficient mice (*Rag1*$^{-/-}$ mice). Three days after stroke, neutrophil infiltration into ischemic hemispheres was significantly reduced after transfer of *Il23r*$^{-/-}$ T cells compared with *Il23r*$^{+/+}$ T cells (Figure 5D).

IL-23 Is Rapidly Induced in Brain-Infiltrating cDC2 Cells

To test whether cDC2s released IL-23, we measured levels of *Il23p19* and *Tnf* transcripts in cDC2s, macrophages, and microglia, sorted from ischemic hemispheres and in splenic cDC2s by quantitative real-time polymerase chain reaction (gating strategy in Figure I in the online-only Data Supplement). After normalization to levels in microglia, we found that *Il23p19* transcripts were significantly upregulated in cDC2s in comparison with infiltrating macrophages and resident microglia at 24 hours, whereas *Tnf* levels were reduced in cDC2s (Figure 6A). Comparing the *Il23p19* levels from brain-derived cDC2s with splenic cDC2s, we found a 70-fold increase in cDC2s derived from ischemic hemispheres (Figure 6B). In congruence, we found reduced expression levels of *Il23p19* transcripts in ischemic hemispheres of *CD11c.DOG* mice after depletion of CD11c$^+$ cells (Figure 6C). To confirm that infiltrating cDC2s and not microglia are the main source of IL-23, we generated bone marrow chimeric mice. Wild-type animals reconstituted with *Il23p19*$^{-/-}$ bone marrow showed a significantly reduced neutrophil infiltration into ischemic hemispheres (Figure 6D), underscoring the essential expression of IL-23 in peripheral immune cells.

Discussion

In this study, we identify IRF4$^+$/CD172a$^+$ DCs (cDC2s) as the predominant DC subset in the ischemic brain, and major source of IL-23, which in turn promotes IL-17 production in γδ T cells and the subsequent recruitment of neutrophils to the ischemic hemisphere. Thus, cDC2s orchestrate the early phase of poststroke inflammation. Disruption of the IL-23–IL-17 pathway, either by depletion of CD11c$^+$ cells or by genetic ablation of the IL-23R, resulted in a significantly reduced frequency of IL-17–producing γδ T cells in ischemic brains and improved the neurological outcome after stroke. Moreover, our analysis of human pathological tissue revealed that DCs are present in human stroke tissue.

DCs had already been described as part of the infiltrating immune cells in stroke, but their phenotype and immunologic function remained elusive. These CD11c$^+$ cells were primarily described as CD45^{high}/CD11b$^+$/CD8α$^-$ and located both in direct proximity of the stroke but also in remote areas.[8] According to the new DC nomenclature proposed by Guilliams et al,[11] we can now classify the infiltrating DCs in stroke based on their phenotype, function, and lineage-specific transcription markers. Applying this classification, we found that CD172a$^+$ cDC2s were the predominant subpopulation already at 24 hours after stroke, outnumbering monocyte-derived DCs, plasmacytoid DCs, and cDC1s. We further confirmed the identity of the cDC2s based on high expression levels of the developmental transcription factors *Irf4* and *Zbtb46* in combination with low levels of *Irf8* and *Batf3*.[18,19]

The observation of an early protection from ischemic stroke after depletion of CD11c$^+$ cells is in line with a central role of these rapidly infiltrating cDC2s in the initiation of sterile inflammation. We found that the cDC2s served as an essential early source of IL-23. The subsequent IL-23–dependent immune response is similar to the initiation of inflammation during infection.[20] In short, IL-23 drives and sustains IL-17 production in γδ T cells, which in turn triggers mechanisms for neutrophil recruitment. γδ T cells are lymphocytes, which can be rapidly activated in a T cell receptor-independent manner. Interestingly, others and we have shown that a specific subpopulation of γδ T cells, so called natural IL-17–producing γδ T cells, is responsible for the rapid IL-17 production in models of stroke, infection, and autoimmunity.[20,21] These IL-17$^+$ γδ T cells also carry the IL-23R and, therefore, can be activated by IL-23.[20] In other organ systems, *Irf4*-expressing cDC2 cells are also specialized in driving IL-17 responses. These cells are known to induce IL-17 responses in the lung and the intestine.[22] However, our finding that the depletion of CD11c$^+$ cells improved survival, whereas the genetic ablation of the IL-23R did not reveal significant effects on mortality, indicates that CD11c$^+$ cells exert their detrimental function not only through IL-23 but also other mechanisms.

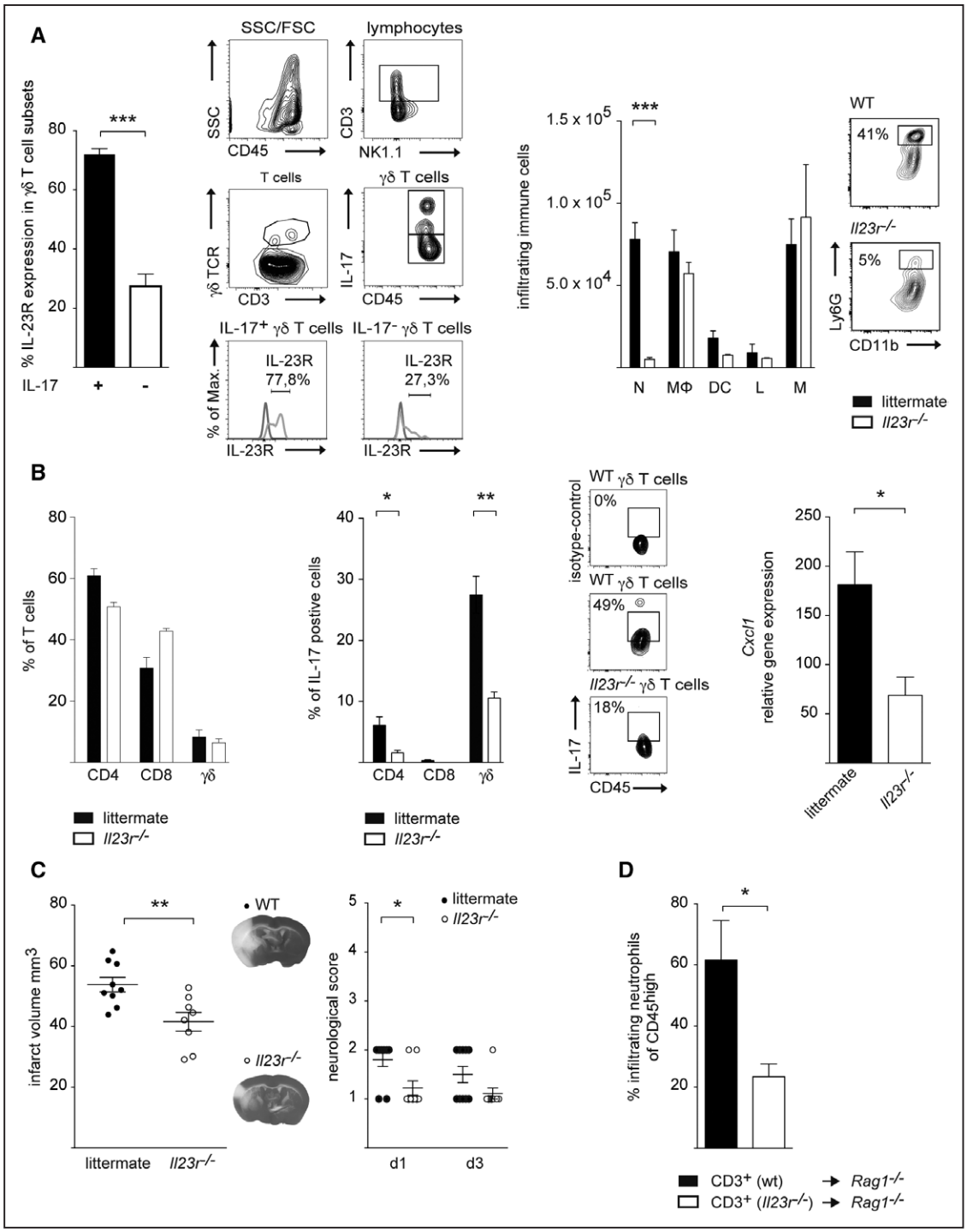

Figure 5. Disruption of IL-23 (interleukin-23) signaling is protective in ischemic stroke. **A**, Flow cytometric analysis of the IL-23R (IL-23 receptor) expression in IL-17$^+$ and IL-17$^-$ γδ T-cell subsets isolated from ischemic hemispheres of *Il23r.gfp.KI* mice, and flow cytometric analysis of absolute numbers of neutrophils (N), macrophages (MΦ), dendritic cells (DC), lymphocytes (L), and microglia (M) isolated from ischemic hemispheres of littermate controls and *Il23r*$^{-/-}$ mice 3 days after transient middle cerebral artery occlusion (tMCAO). Lymphocytes from *Il23r.gfp.KI* mice were stained for CD45, CD3, NK1.1, and γδ T cell receptor followed by an intracellular staining for IL-17. Histograms show expression of IL-23R in IL-17–positive and negative γδ T cells of 1 representative experiment at day 3. central nervous system-infiltrating cells were stained for CD45, Ly6G, CD11b, CD11b, MHCII, B220, CD3, NK1.1, CD4, CD8, and γδ T cell receptor. **B**, Frequency of CD4$^+$, CD8$^+$, and γδ T cells, flow cytometric analysis of IL-17 produced by CD4$^+$, CD8$^+$, and γδ T cells isolated from ischemic hemispheres of littermate controls and *Il23r*$^{-/-}$ mice 3 days after tMCAO, and relative gene expression of *Cxcl1* in ischemic hemispheres of littermate controls and *Il23r*$^{-/-}$ mice 24 hours after tMCAO. **C**, Triphenyltetrazolium chloride (TTC) staining for evaluation of infarct volume at day 3 (**left**), and neurological scores at days 1 and 3 (**right**) of littermate controls and *Il23r*$^{-/-}$ mice after middle cerebral artery occlusion (MCAO). Data are presented as mean±SD of 8 littermate controls and 8 *Il23r*$^{-/-}$ animals. **D**, Percentage of neutrophils in central nervous system-infiltrating cells of *Rag1*$^{-/-}$ mice reconstituted 1 hour before stroke induction with 1×10^7 CD3$^+$ cells isolated from wild-type (WT) or *Il23r*$^{-/-}$ mice. **A**, **B**, and **D**, Data show the mean±SD of 4 to 8 animals per group, analyzed in 3 to 4 independent experiments. Statistical significances were analyzed by Student *t* test (**A**–**D**) and Mann–Whitney *U* test for neurological scores (**C**). **P*<0.05, ***P*<0.01, ****P*<0.001. FSC indicates forward scatter; SSC, side scatter.

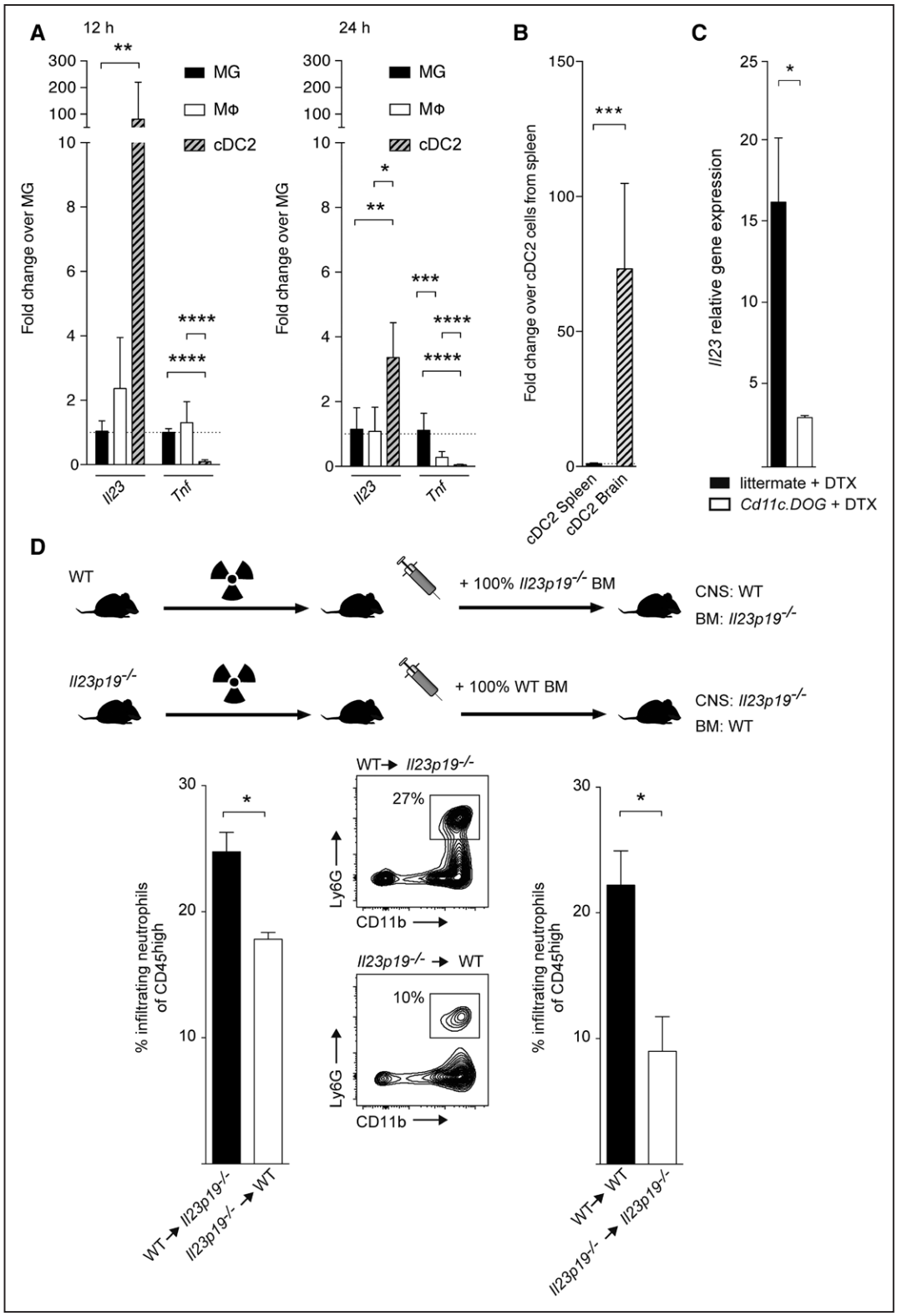

Figure 6. IL-23 (interleukin-23) is rapidly induced in brain-infiltrating conventional type 2 DCs (cDC2) cells. **A**, Relative gene expression of *Il23* and *Tnf* in infiltrating cDC2s ($CD45^{high}/CD11b^{+}/CD11c^{+}/MHCII^{+}/CD172a^{+}/Ly6c^{-}/Ly6g^{-}/F4/80^{-}/XCR1^{-}$) and macrophages/monocytes ($CD45^{high}/CD11b^{+}/F4/80^{+}/CD11c^{-}/Ly6g^{-}$) purified from ischemic hemispheres 12 and 24 hours after middle cerebral artery occlusion (MCAO) by fluorescence-activated cell sorting. Expression levels were normalized to corresponding levels in (**A**) microglia ($CD45^{intermediate}/CD11b^{+}$) or (**B**) splenic cDC2s. The graphs show the mean±SD of 6 to 8 independent experiments for each time point. **C**, Relative gene expression of *Il23* in the stroked hemispheres of diphtheria toxin (DTX)-treated *CD11c.DOG* mice and littermate controls 24 hours after transient middle cerebral artery occlusion. Daily administration of DTX was started 1 day before MCAO (n=6 mice). **D**, Frequency of neutrophils in ischemic hemispheres of bone marrow chimeras at day 1 after MCAO (n=6 mice). Statistical significances were analyzed by (**A**) 1-way ANOVA with Bonferroni post hoc test and (**B**–**D**) Student *t* test. **P*<0.05, ***P*<0.01, ****P*<0.001. MΦ indicates macrophages; and MG, microglia.

Previously, infiltrating macrophages were believed to be the main source of IL-23 in stroke.[4] This apparent discrepancy can be explained by the gating strategy applied for the fluorescence-activated cell sorting of macrophages and microglia in previous studies.[4] Macrophages were identified by gating on CD45^{high}/CD11b^{+} cells—a strategy that included IL-23–producing cDC2s. Furthermore, based on our chimera experiments, we can now attribute the IL-23/IL-17–mediated effects to radiation-sensitive peripheral immune cells.

Throughout the study, we focused on the role of cDC2s because of their rapid infiltration kinetics and significantly increased abundance in comparison with all other DC subsets. Nevertheless, we also observed cDC1s with delayed infiltration kinetics. Even though the immunologic function of the cDC1 cells is unclear, a regulatory role for XCR1^{+}/CD172a^{-} cDC1s can be speculated. Recent experimental data suggest that CD103^{+} DCs from the small intestine can shape the IL-17 response in T cells. In a model of experimental stroke, alterations of the intestinal flora led to a decrease in IL-17–positive γδ T cells in the ischemic brain. These changes could be traced back to an increase of CD103^{+} DCs in the gut. The CD103^{+} are likely to belong to the cDC1 subset, which we found in ischemic hemisphere at later stages.[23]

In conclusion, our study provides further insights into the inflammatory cytokine and immune cell networks of post-stroke inflammation and suggests that rapidly infiltrating IRF4^{+}/CD172a^{+} cDC2s are an essential source of IL-23. These findings indicate that cDC2s are critical for the initiation of the detrimental IL-17–driven innate immune response in stroke.

Acknowledgments

We thank Ellen Orthey and Anika Ruhl for excellent technical assistance and Natalio Garbi for providing *Cd11c.DOG* mice.

Sources of Funding

This work was supported by grants from the European Research Area Network/nEUROSurv (Dr Magnus) and the Hermann und Lilly Schilling-Foundation for Medical Research (Dr Magnus). M. Gallizioli was supported by the European Community (FP7-PEOPLE-2013-ITN-n°07962). Dr Korn was supported by the Deutsche Forschungsgemeinschaft (SFB 1054 and TR 128), by the European Research Council (CoG 647215 to Dr Korn), and by the German Ministry of Education and Research (Kompetenznetz Multiple Sklerose, T-BinNMO).

Disclosures

None.

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