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Graphical Abstract

Highlights
- Human microglia renew at a median rate of 28% per year
- Microglial cells are on average 4.2 years old
- Most of the microglia population (>96%) is renewed throughout life

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In Brief
Taking advantage of the decreasing level of atmospheric $^{14}$C since the Cold War, Réu et al. show that human microglia, unlike most other hematopoietic lineages, slowly turn over at a yearly median rate of 28%. The absence of a large quiescent subpopulation indicates that most microglia will renew throughout life.
The Lifespan and Turnover of Microglia in the Human Brain

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SUMMARY

The hematopoietic system seeds the CNS with microglial progenitor cells during the fetal period, but the subsequent cell generation dynamics and maintenance of this population have been poorly understood. We report that microglia, unlike most other hematopoietic lineages, renew slowly at a median rate of 28% per year, and some microglia last for more than two decades. Furthermore, we find no evidence for the existence of a substantial population of quiescent long-lived cells, meaning that the microglia population in the human brain is sustained by continuous slow turnover throughout adult life.

RESULTS

We analyzed the frontal and occipital cortices from two subjects (17 and 41 years old) who had received IdU (5-Iodo-2-deoxyuridine) as a radiosensitizer for cancer treatment (Table S1). On average, 0.8% of Iba1+ parenchymal microglia in the cortex were IdU+ after 4 days (donor 1) or 10 days (donor 2) of IdU administration (Figures 1A and 1B; Table S1). Accounting for the labeling period, it averages at 0.14% labeling per day (Figure 1C). These observations do, however, come with the following caveats: (1) the sample size is small and additional samples are not available; and (2) the subjects studied are not healthy individuals, and thus may exhibit aberrant turnover of different populations of cells. Still, our observations provide us with a general estimate of what to expect in our downstream analysis based on retrospective 14C measurements.

Followed CD11b magnetic bead selection, we isolated by fluorescence-activated cell sorting (FACS) CD45+CD11b+ microglia from the adult human postmortem cerebral cortex in order to perform retrospective 14C dating (Figures 2A–2C) (Olah et al., 2012). We used CD20, a B cell marker, to evaluate the presence of blood-borne cells in dissociated cortical preparations. The percentage of B cells in brain samples prior to MACS purification (Figure 2D) is very low relative to the circulation.
often cells need to divide within an individual of a given age in or-
2010). In simple terms, this is accomplished by calculating how
shots of cell dynamics for each individual donor (Bernard et al.,
is replaced at a fixed rate, it is possible to obtain robust snap-
ure 3C). Using a homogeneous turnover model in which each cell
it is possible to calculate the average cell age of the sample (Fig-
Based on the year of collection and date of birth of the sample,
we performed retrospective birth dating of microglia isolated
population (Spalding et al., 2005)( Figure 3A). Using this strategy,
we generated cell age distributions stochastically, showing that
we are one where the majority of the population (>96%) is renewed and we have no evidence to support the existence of a significant subpopulation of quiescent very long-lived cells (Figure 3D). We have employed a conservative mathematical model, with few assumptions, which does not preclude that an extended dataset or other variables could explain the heterogeneity observed.

Cells that became postmitotic soon after birth have a unique
14C signature, corresponding to the atmospheric level at that moment in time. This allowed us to directly investigate the possible existence of a non-dividing subpopulation, using a heterogeneous turnover model (Figure 3D). The model that best fits our data are one where the majority of the population (>96%) is renewed and we have no evidence to support the existence of a significant subpopulation of quiescent very long-lived cells (Figure 3D). We have employed a conservative mathematical model, with few assumptions, which does not preclude that an extended dataset or other variables could explain the heterogeneity observed.

Based on the average cell age and cell division rate observed, we generated cell age distributions stochastically, showing that within an individual there is a wide range of microglia ages (Figure 3E). Simply because not all cells are dividing at the same time, some are quite young due to recent cell division and others can be more than 20 years old (Figure 3E).

The lower rate of microglia renewal compared with most other immune cells is probably a manifestation of the immune-privileged status of the CNS (Figure 3F) (Busch et al., 2007; Macallan et al., 2005). On the other hand, in comparison with other cells of

Figure 1. IdU Incorporation
(A) Confocal image with orthogonal projections, from human cortex, revealing microglia positive for Iba1.
(B) Co-staining of Iba1 and the thymidine analog IdU.
(C) Percentage of microglia incorporating IdU per day (mean ± SD). Each data point represents a glass slide. Nuclei are labeled with antibodies to histone H3. Scale bars, 10 μm.
the CNS, microglia show a high exchange rate (Figure 3F) (Spalding et al., 2005, 2013; Yeung et al., 2014). Thus, a constant basal renewal is likely necessary for the maintenance of a cohort of young and healthy microglial cells.

**DISCUSSION**

Administering nucleotide analogs for a short time period introduces a bias to label the cells with the highest proliferation rate within a potentially heterogeneous population. Also, labeled cells that continue to proliferate after the labeling period will give rise to additional positively labeled cells that lead to overestimations of cell proliferation (Neese et al., 2002). Nevertheless, samples of human brain labeled with nucleotide analogs are very valuable, not only due to their rarity but also as a confirmatory tool of in vivo renewal in humans (Eriksson et al., 1998; Ernst et al., 2014; Yeung et al., 2014).

Analysis of the integration of atmospheric $^{14}$C, derived from nuclear bomb tests, in genomic DNA is cumulative and gives a more comprehensive view of cell age and cell division history (Spalding et al., 2005), and it is possible to analyze tissue from subjects without previous serious illness. There is little exchange of carbon atoms in genomic DNA in non-dividing cells, and the effect of DNA repair and methylation is well below the detection limit of this retrospective birth dating strategy (Bergmann et al., 2012; Ernst et al., 2014; Spalding et al., 2005), even after, for example, stroke, where there is a substantial increase in DNA damage and repair (Huttner et al., 2014).

Most immune cells do not live longer than a few days or weeks (Busch et al., 2007; Macallan et al., 2005), making microglia one of the slowest dividing immune cells described to date. An extreme exception is plasma cells in the intestine, where the subset with the slowest renewal rate has a median age of 22 years (Landsverk et al., 2017). The turnover rate of microglia in humans is substantially lower than in mice. There is a much higher exchange of oligodendrocytes in mice compared with humans (Yeung et al., 2014), and it is possible that clearance of myelin and cell debris calls for a higher exchange rate of microglia in mice.

Based on Ki-67 staining, a recent study estimated that, at any given moment, 2% of microglia are proliferating in the
Different models for distribution of data on the atmospheric $^{14}$C curve considering different frequencies of dividing cells. The model that best fits the data are

Representation of the average age of microglia in each individual and linear regression (black line). Thus, the birth date of the cell population can be read off the x axis.

Considering the turnover rate, the average cell age, and the fact that all microglial cells do not divide simultaneously, we created a stochastic cell age distribution model. Our model shows that within an individual there is a distribution of cells of different ages, with some having recently renewed and others not.

In conclusion, $^{14}$C analyses reveal that microglia as a whole have a turnover rate of 0.08% a day, a low turnover rate in comparison with other immune cells (granulocytes, monocytes, and naive B cells) but a high turnover rate relative to other CNS cells (neurons in the dentate gyrus, oligodendrocytes, and cortical neurons).

**EXPERIMENTAL PROCEDURES**

**Tissue Collection**

Neocortical tissue was obtained from donors admitted for autopsy at the Department of Forensic Medicine in Stockholm from 2014 to 2016, after informed consent from the relatives. The ethical permit for this study was granted by the Regional Ethics Committee of Stockholm (2010/313-31/3).

IdU Quantification

The sections were immersed in xylene to remove the paraffin, and the tissue was rehydrated in descending ethanol series. Triton X-100 (0.2%) was used to permeabilize the tissue, and antigen retrieval was performed in 0.05% citraconic acid solution (pH 7.4) for 20 min in a domestic steamer. The sections were left for 20 min at room temperature and then immersed in 2.0 N HCl for 40 min. The slides were blocked (10% donkey normal serum in PBS with 0.2% Triton X-100) at room temperature for 1 hr. Following incubation with the primary antibodies (1:10 mouse anti-BrdU, BD347580; 1:100 goat anti-Histone H3, Abcam 12079; 1:100 rabbit anti-Iba1, Wako 019-19741), the sections were incubated with the secondary antibodies (1:200 donkey anti-mouse Cy3, Jackson ImmunoResearch 715-165-150; 1:200 donkey anti-goat A647, Blodcentralen, Karolinska University Hospital.}

N. Buffy coats were obtained from anonymous regular blood donors at NIH. Buffy coats were obtained from the National Heart, Lung and Blood Institute, NIH. Buffy coats were obtained from anonymous regular blood donors at Blodcentralen, Karolinska University Hospital.
Jackson ImmunoResearch 705-605-147: 1:200 donkey anti-rabbit A488, Jackson ImmunoResearch 711-545-152) and inspected in an LSM 700 (Carl Zeiss) confocal microscope. The percentage of IdU+ microglia in each slide was found based on the total number of Ibα+ cells and the number of Ibα1+ IdU+ cells. By dividing the percentage of labeled cells by the number of labeling days (4 for donor 1 and 10 for donor 2), we calculated the daily labeling. The raw data and the calculations are in Table S1.

**Tissue Dissociation**

After careful removal of the meninges and all visible blood vessels, the tissue was cut into small pieces and thoroughly rinsed with PBS. The tissue was then homogenized in media A (1x HBSS, 150 mM HEPES, 2 mM EDTA, 5% BSA) with 2 U/mL papain (Worthington) and 10 U/mL DnaseI (Roche) at 37°C for 1.5 h. The homogenized tissue was mix with 3 volumes of sucrose media I (PBS, 0.7 M sucrose, 2 mM EDTA) and centrifuged for 20 min at 1,000 g. The pellet was then suspended in sucrose media II (PBS, 0.9 M sucrose, 2 mM EDTA) and centrifuged for 25 min at 800 x g. Finally the pellet was resuspended in blocking solution (PBS, 0.1% FBS, 2 mM EDTA) and filtered through a 40 μm cell strainer.

**Cell Isolation**

The cell suspension was incubated for 5 min with human Fc-gamma receptor (FcγR)-binding inhibitor (1:100; eBioscience) and for 30 min with CD11b antibody-conjugated microbeads (1:25, 130-093-634; Miltenyi Biotec). The magnetic isolation was performed according to the manufacturer. The samples were next incubated for 20 min with PE-CD11b (1:20, clone ICRF44; BioLegend) and Alexa 647 CD45 (1:20, clone H303; BioLegend), and finally FACS sorted in an Influx flow cytometer (BD Biosciences). Blood cells were isolated from buffy coats by density gradient (Lymphoprep). Peripheral blood mononuclear cells (PBMCs) were positively selected with CD11b antibody-conjugated microbeads (1:25, 130-093-634; Miltenyi Biotec) according to the manufacturer.

**DNA Isolation**

In order to prevent carbon contaminations, we performed the DNA isolation in a clean room (ISO8). The extraction protocol was modified from Miller et al. (1988). The glassware was preheated for 4 h at 450°C. 1 mL of lysis buffer (100 mM Tris [pH 8.0], 200 mM NaCl, 1% SDS, and 5 mM EDTA) and 12 μL of Proteinase K (40 mg/ml) were added to the sorted cells and incubated at 65°C overnight. The samples were further incubated at 65°C for 1 hr after 6 μL of RNase cocktail (Ambion) was added. 600 μL of NaCl (5 M) was added to the sample; then it was vortexed for 30 s. The solution was spun down at 13,000 rpm for 6 min. The supernatant containing the DNA was transferred to a 12 mL glass vial. Ethanol 95% (6 mL) was added and the glass vial was manually agitated. The DNA precipitate was washed three times in DNA washing buffer (70% ethanol [v/v] and 0.5 M NaCl), dried at 65°C overnight, and resuspended in 0.5 mL DNase/RNase free water (GIBCO/Invitrogen). The DNA purity and concentration were verified by UV spectroscopy (NanoDrop).

**Accelerator Mass Spectrometry**

DNA samples suspended in 0.5 mL of water were lyophilized to dryness in a vacuum centrifuged at 2,000 rpm for 2 hr. To convert the samples into graphite, we added excess CuO to each dry sample, and the quartz tubes were evacuated for 1 hr. The graphite was then cut into small pieces and thoroughly rinsed with PBS. The tissue was then homogenized in media A (1x HBSS, 150 mM HEPES, 2 mM EDTA, 5% BSA) with 2 U/mL papain (Worthington) and 10 U/mL DnaseI (Roche) at 37°C for 1.5 h. The homogenized tissue was mix with 3 volumes of sucrose media I (PBS, 0.7 M sucrose, 2 mM EDTA) and centrifuged for 20 min at 1,000 g. The pellet was then suspended in sucrose media II (PBS, 0.9 M sucrose, 2 mM EDTA) and centrifuged for 25 min at 800 x g. Finally the pellet was resuspended in blocking solution (PBS, 0.1% FBS, 2 mM EDTA) and filtered through a 40 μm cell strainer.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2017.07.004.

**AUTHOR CONTRIBUTIONS**

P.R. and J.F. designed the study. P.R. performed most of the experiments. A.K. performed the IdU analyses. S.B. did all of the mathematical analyses. J.E.M. performed additional experiments. M.S. and G.P. did the AMS measurements. K.A. and H.D. collected and classified the samples for 13C, S.P. and J.T. collected and classified the IdU samples. P.R., J.E.M., and J.F. wrote the manuscript.

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