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Genetic manipulation of microglia during brain development and disease☆

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ABSTRACT

Microglia are unique cells in the central nervous system (CNS) and of particular importance for the development and homeostasis thereof. Recently, genetic manipulation of microglia *in vivo* has led to valuable insights about the origin of microglia and their behavior under steady-state conditions. Nevertheless, in pathological settings, their resting and surveillant nature can rapidly turn into either a beneficial or detrimental state significantly shaping disease courses. Therefore, it is tempting to manipulate these cells under pathological conditions in vivo and thereby decipher their contribution to the outcome of frequent neurological diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS) or amyotrophic lateral sclerosis (ALS). In this review, we will discuss which transgenic mouse models are currently available and can thus be used to genetically label microglia, to modulate their gene expression or to deplete them during development and under healthy conditions. Furthermore, the hallmarks of neurological disease models and how genetic manipulation of microglia will expand our knowledge about the underlying disease mechanisms will be discussed.
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1. Introduction

Microglia belong to the mononuclear phagocyte system (MPS). They are of early embryonic origin and closely related to other long-lived macrophages, e.g., Langerhans cells. Their longevity and self-maintaining character separates them from their close relatives in the blood, namely monocytes, which are short-lived and derived from hematopoietic stem cells in the bone marrow. Despite the fact that microglia are present in the CNS from early embryonic development onward actively surveilling and shaping their environment, periphery-derived myeloid cells, such as monocytes, come into play only under pathological CNS conditions. The close relationship between microglia, monocytes but also other macrophages in the body remains the most challenging issue in genetically manipulating microglia specifically *in vivo*, as they all share many myeloid markers like CD11b (*Itgam*), Lysozyme M (*Lyz2*), Iba1 (*Aif1*) and CX_3CR1 (*Cx3cr1*). The goal of this review is to discuss how transgenic mouse models can be used to target microglia during health and disease with special focus on Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and experimental autoimmune encephalomyelitis (EAE). We will discuss the most frequently applied transgenic mouse models in the context of their specificity and suitability as tools to investigate molecular pathways involved in CNS disorders.

2. Embryonic fate mapping—origin of microglia

Microglia can be detected for the first time during development around embryonic day (E) 9.5 when they enter the neuroectoderm [3]. They were thought to originate from erythromyeloid progenitors (EMP) in the yolk sac that were further characterized and separated into different subpopulations, which reflect distinct developmental stages [7]. In fact, we were able to show that microglia are derived from EMP in the yolk sac (YS) at E8.0 that express c-Kit and develop into immature macrophages at E9.0, which separate into an A1 and A2 population, respectively [75]. These developing macrophages are further subdivided according to their surface marker expression by down-regulating c-Kit and up-regulating CD45 in the A1 state that is followed by a subsequent up-regulation of the fractalkine receptor (CX_3CR1), the surface antigen F4/80 and colony-stimulating factor 1 receptor (CSF1R) [75]. Importantly, the maturation from the EMP to the A1 state is highly dependent on PU.1 (encoded by the *Spi1* gene), whereas the transition between A1 and A2 depends on the interferon-regulatory factor 8 (*Irf8*) [75]. Our results delineated findings by others using tamoxifen-inducible targeted labeling during embryonic development, so-called fate mapping studies [28,44,48,91,111].

In principle, most fate mapping and conditional knockout approaches took advantage of a Cre recombinase fused to a human

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estrogen receptor, which is mutated to reduce the affinity to endogenous estrogen but increase the affinity to tamoxifen instead. After the administration of tamoxifen, the fusion protein is released from the cytoplasm to translocate to the nucleus where it can target and finally excise the sequence between the so-called flanked loxP ("floxed") sites, e.g. a stop sequence preventing the transcription of the reporter yellow fluorescent protein (YFP) encoding region initiating the expression of YFP. The recombination event is irreversible, labeling both the targeted cells lifelong and their progeny, opening the possibility to follow these cells from development to adulthood. In the context of embryonic pulse labeling, the inducible Cre models are highly advantageous as they allow the induction of recombination only in a narrow time frame during development [44,48,111].

The first genetic study to decipher microglia origin used pulse labeling of timed pregnancies [44]. The runt-related transcription factor 1 (*Runx1*) promotor was applied to express the Cre recombinase fused to two mutated estrogen-receptors (MER) at the N- and C-terminus (Mer-Cre-Mer) [108]. Interestingly, *Runx1* was also shown to be important for the transition of amoeboid microglia into their ramified appearance at postnatal stages and is additionally expressed in adult microglia after injury [137]. The expression of *Runx1* is tightly regulated over time and at different sites of hematopoiesis during development. Runx1 is highly important for the initiation of the definitive hematopoietic system that takes place in the aorto-gonado-mesonephros region (AGM) and in the fetal liver (FL) during embryonic development [108]. The fetal liver is colonized by YS macrophages but also by hematopoietic stem cells (HSC) from the AGM. The AGM lasts until E12.5, while definitive hematopoiesis begins around the same time in the FL. Before birth, hematopoietic cells in the FL start to colonize the bone marrow finally giving rise to all hematopoietic lineages in the adult (Fig. 1) [26,47]. Despite the partial labeling of the cells of the definitive hematopoiesis, the *Runx1*$^{\text{Mer-Cre-Mer}}$ mouse model crossed to *Rosa26*-stop-EYFP reporter mice (*R26*-YFP) proved to be a suitable tool to target embryonic microglia precursors before E7.5 in the YS [44]. Importantly, peripheral blood cells such as monocytes were only marginally labeled at this stage. At later stages, the labeling of microglia continuously decreased while labeling of monocytes and lung macrophages peaked at E8.5 to E9.5 [44] (Fig. 1).

The CSF1R, encoded by the c-*fms* gene, is an important pro-survival factor that is commonly expressed by macrophages and essential for proliferation and differentiation [56,98]. The receptor has two ligands, CSF1 and interleukin (IL)-34, which have non-overlapping expression pattern and function in the CNS and other compartments of the body [81,92,122,124]. Mice lacking the CSF1 ligand, so-called *Csf1*$^{\text{op/op}}$ mice, suffer from osteopetrosis that is characterized by malformation of the bones due to the absence of osteoclasts [127]. Interestingly, *Csf1*$^{\text{op/op}}$ mice have reduced numbers of microglia, whereas the CSF1R knockout virtually lacks microglia and is lethal after several weeks of age [36,44,92]. The fact that microglia and their precursors strongly rely on this receptor makes it tempting to use for genetic targeting. A reporter line expressing enhanced green fluorescent protein (GFP) under the control of a promotor element of the c-*fms* gene revealed that GFP-positive cells could be detected as early as E9.5 in the embryonic yolk sac and other tissues at E13.5 [109]. In line with this finding, immature microglia were visualized using the *Iba1*-eGFP model in the CNS from E11.5 onward [61]. Besides the *Csf1r*-GFP reporter line, also called "MacGreen" mouse, two Cre models exist. First, the *Csf1r*$^{\text{iCre}}$ harbors a constitutive active Cre whereas the *Csf1r*$^{\text{Mer-Cre-Mer}}$ line can be induced upon tamoxifen application [29,102]. Like the GFP reporter, both the constitutive and inducible mouse model control the expression of the iCre/Mer-Cre-Mer protein via a promotor element of the c-*fms* gene, randomly inserted in the genome [29,102].

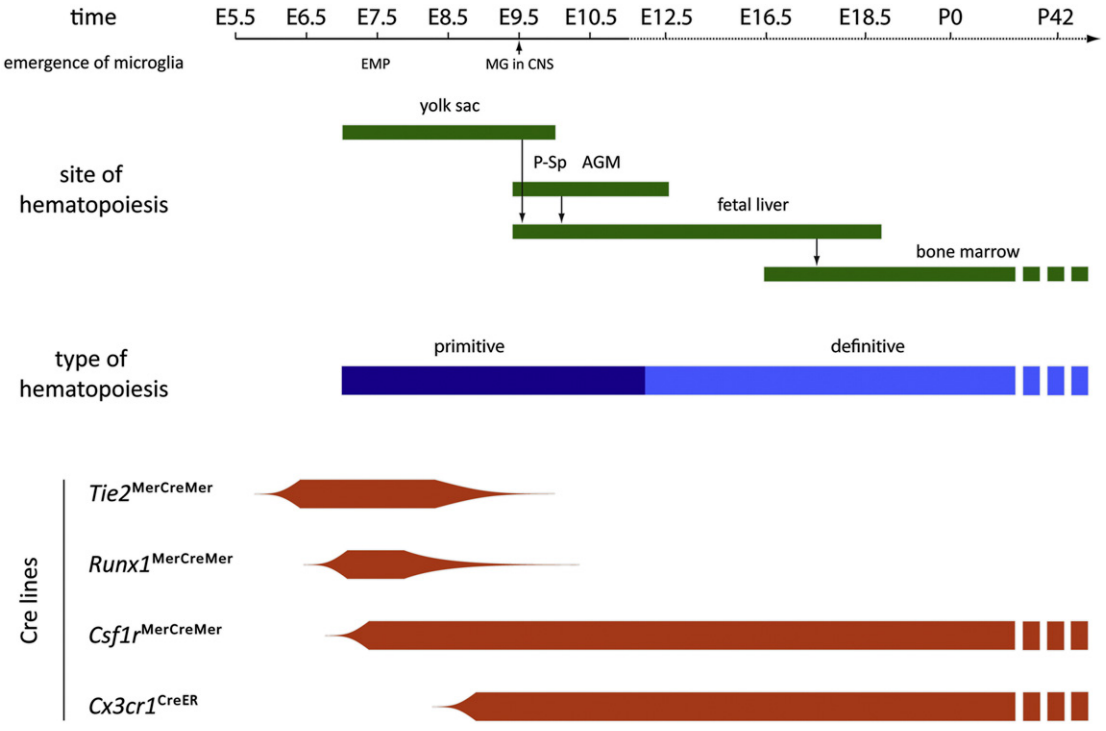

Fig. 1. Time course of hematopoiesis in mouse, the appearance of the EMP as microglia precursor in the yolk sac, microglia in the brain and activity of selected Cre-driver lines to target microglia during development. Hematopoietic cells can be detected first in blood islands in the yolk sac from embryonic day (E) 7.5 onward. These cells have both erythroid and myeloid differentiation potential; therefore, they are called erythromyeloid progenitors (EMP). EMP-derived microglia precursors colonize the neuroectoderm at E9.5 (MG → CNS). At the same time, the para-aortic splanchnopleura (p-Sp) emerges and subsequently develops into the aorto-gonado-mesonephros (AGM) region. The fetal liver (FL) is colonized as early as E9.5 by yolk sac-derived EMP and later on by hematopoietic stem cells (HSC) from the p-Sp/AGM around E10. At E12.5, the type of hematopoiesis switches from primitive (dark blue) to definitive hematopoiesis (light blue) in the FL. Before birth, fetal liver HSC settle down in the bone marrow. It has been shown that *Tie2*$^{\text{Mer-Cre-Mer}}$ highly labels microglia precursors when pulsed at E6.5 and E7.5 but only marginally at later stages [48]. The earliest time point to label microglia precursors using the *Runx1*$^{\text{Mer-Cre-Mer}}$ was E6.5-E7.0 that peaked around E7.25-E7.5 while the labeling was virtually absent at E10.5 [44]. Pulse labeling with *Csf1r*$^{\text{Mer-Cre-Mer}}$ targeted microglia precursors from E7.5 onward [28,35,48,111]. *Cx3cr1*$^{\text{CreER}}$ mice can be used from E9 onward when the expression is initiated in the A2 population.