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# Transport of cargo from periphery to brain by circulating monocytes

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ABSTRACT

The misfolding and aggregation of the Aβ peptide – a fundamental event in the pathogenesis of Alzheimer's disease – can be instigated in the brains of experimental animals by the intracranial infusion of brain extracts that are rich in aggregated Aβ. Recent experiments have found that the peripheral (intraperitoneal) injection of Aβ seeds induces Aβ deposition in the brains of APP-transgenic mice, largely in the form of cerebral amyloid angiopathy. Macrophage-type cells normally are involved in pathogen neutralization and antigen presentation, but under some circumstances, circulating monocytes have been found to act as vectors for the transport of pathogenic agents such as viruses and prions. The present study assessed the ability of peripheral monocytes to transport Aβ aggregates from the peritoneal cavity to the brain. Our initial experiments showed that intravenously delivered macrophages that had previously ingested fluorescent nanobeads as tracers migrate primarily to peripheral organs such as spleen and liver, but that a small number also reach the brain parenchyma. We next injected CD45.1-expressing monocytes from donor mice intravenously into CD45.2-expressing host mice; after 24 h, analysis by fluorescence-activated cell sorting (FACS) and histology confirmed that some CD45.1 monocytes enter the brain, particularly in the superficial cortex and around blood vessels. When the donor monocytes are first exposed to Aβ-rich brain extracts from human AD cases, a subset of intravenously delivered Aβ-containing cells migrate to the brain. These experiments indicate that, in mouse models, circulating monocytes are potential vectors by which exogenously delivered, aggregated Aβ travels from periphery to brain, and more generally support the hypothesis that macrophage-type cells can participate in the dissemination of proteopathic seeds.



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## 1. Introduction

The aggregation of the beta-amyloid peptide (Aβ) in the brain is an early and integral event in the pathogenesis of Alzheimer's disease (Hardy and Selkoe, 2002; Holtzman et al., 2011). Aβ normally exists in a structurally unfolded (intrinsically disordered) state, but in its pathogenic form, Aβ becomes rich in β-sheet and induces the misfolding and subsequent self-assembly of other Aβ molecules. These durable, prion-like multimeric seeds instigate the formation of senile plaques and cerebral β-amyloid angiopathy as monomeric Aβ is recruited into the β-sheet-rich deposits (Jucker and Walker, 2013; Walker et al., 2006; Walker and LeVine, 2012). We and others have found that intracranial injections of Aβ multimers seed Alzheimer-like pathology in Aβ-precursor protein (APP) transgenic rodent models (Duran-Aniotz et al., 2014; Eisele et al., 2009; Fritschi et al., 2014; Hamaguchi et al., 2012; Kane et al., 2000; Langer et al., 2011; Meyer-Luehmann et al., 2006; Morales et al., 2012a, 2012b; Rosen et al., 2012; Stohr et al., 2012, 2014; Watts et al., 2011, 2014).

Within the brain, the proteinaceous lesions that characterize Alzheimer's disease and other protein misfolding disorders appear to propagate among interconnected brain areas, suggestive of the dissemination of seeds by means of axonal transport (Boluda et al., 2015; Clavaguera et al., 2009, 2014; Guo and Lee, 2011, 2014; Hyman, 2014; Liu et al., 2012; Walker and LeVine, 2012; Walker et al., 2013; Ye et al., 2015). Other studies have shown that the injection of Aβ multimers into the peritoneal cavity of APP-transgenic mice can seed Aβ deposition in the brain, particularly in the form of cerebral β-amyloid angiopathy (Eisele et al., 2010, 2014). While the evidence currently favors axonal transport as a key mode of lesion propagation within the nervous system, the mechanisms by which Aβ seeds are transported from periphery to brain remain uncertain. The preponderance of amyloid angiopathy in the forebrain of intraperitoneally seeded APP23 transgenic mice (Eisele et al., 2010) suggests the possibility that the seeds reach the brain via the vasculature (Eisele et al., 2014). Furthermore, the presence of Aβ within circulating monocytes of these mice implicates these cells as possible vectors for the transport of seeds from periphery to brain (Eisele et al., 2014). The poor ability of microglia to degrade amyloid fibrils further supports the idea that aggregated Aβ may remain intact in macrophages for a long period of time (Frackowiak et al., 1992), but the evidence for the entry of Aβ-laden macrophages from the circulation into the brain remains indirect.

As a component of the innate immune system, macrophages normally serve to phagocytose and degrade exogenous pathogens such as microbes, and they also present antigen to cells of the adaptive immune system (Alberts et al., 2002). However, in some instances macrophages have been found to ingest and disseminate pathogens intact (Ferreira et al., 2010; Johnson et al., 2010; Kirby et al., 2009; Tanaka et al., 2012), thereby contributing to the disease process. In the brain, microglia are resident macrophages that originate from the yolk sac early in embryogenesis and replenish themselves by self-replication (Prinz et al., 2011). Particularly in disease states, circulating (hematogenous) monocytes can differentiate into macrophages in the brain, where they become part of local cellular networks (Mildner et al., 2007; Priller et al., 2001). Studies in which the blood–brain barrier is disrupted by irradiation demonstrate an increased infiltration of circulating monocytes into the brain (Mildner et al., 2007). Furthermore, disease models of multiple sclerosis show that hematogenous monocytes enter the brain and are responsible for stripping myelin from axons (Lampert, 1978). Activation of circulating monocytes in an Alzheimer's disease model resulted in the increased phagocytosis of cerebral β-amyloid, thereby reducing the number of senile plaques (Shaftel et al., 2007; Town et al., 2008). Thus, in disease states it is clear that circulating monocytes are able to gain access to the brain, but it is thought that few, if any, circulating monocytes cross the intact blood–brain barrier to enter the healthy brain (Kroll and Neuwelt, 1998; Prinz et al., 2011; Zhang and Pardridge, 2001). In the present study, we tested the hypothesis that monocytes are able to phagocytose and convey cargo from the peritoneal cavity to the brain in healthy mice. We found that limited numbers of these cells can enter the brain parenchyma, and thus could act as vectors for the transport of proteopathic seeds.

## 2. Results

### 2.1. *Characterization of lavage cell-types by FACS*

To characterize the cells collected by lavage, FACS analysis was performed using antibodies specific for macrophages and lymphocytes (Fig. 1). The analysis showed 3 distinct populations based on the expression of specific markers: CDllb-high/F4/80-high; CDllb-intermediate/F4/80-negative; and CDllb-negative/F4/80-negative. Roughly 30% of the lavage consisted of large peritoneal macrophages (LPM), which are characterized by high expression of CDllb and F4/80 (Ghosn et al., 2010). The cell population characterized by intermediate CDllb and negative F4/80 expression was gated and investigated for Ly6G and Ly6C expression. The majority (94.3%) of the cells were Ly6G-negative and Ly6C-negative, indicating that these cells are small peritoneal macrophages (Ghosn et al., 2010; Gordon and Taylor, 2005; Rose et al., 2012). The CDllb-negative/F4/80negative cells were gated and investigated for expression of B220 (B-cells) and CD3 (T-cells) (Rodig et al., 2005). Of this subpopulation, 80.5 percent of the cells were B-cells and 15.7 percent were T-cells. Overall, this analysis indicates that the lavage cells are ~60% macrophages (LPM and SPM) and ~30% lymphocytes (most of which are B-cells).

### 2.2. *Systemic distribution of labeled macrophages*

To determine the general distribution of macrophages that had previously ingested fluorescent nanobeads, we assessed nanobead-labeled cells histologically in the brain and systemic organs. Host mice received either i.p. injections of nanobeads (ingested by endogenous macrophages), or i.v. injections of exogenous, nanobead-laden macrophages harvested from the peritoneal cavity of donor mice. In both groups, the systemic distribution of macrophages was similar, i.e., both endogenous