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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 A $\beta$  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 A $\beta$ . Recent experiments have found that the peripheral (intraperitoneal) injection of A $\beta$  seeds induces A $\beta$  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 A $\beta$  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 hours, 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 A $\beta$ -rich brain extracts from human AD cases, a subset of intravenously delivered A $\beta$ -containing cells migrate to the brain. These experiments indicate that, in mouse models, circulating monocytes are potential vectors by which exogenously delivered, aggregated A $\beta$  travels from periphery to brain, and more generally support the hypothesis that macrophage-type cells can participate in the dissemination of proteopathic seeds.

### Keywords

A $\beta$ ; Alzheimer’s disease; amyloid; cerebral amyloid angiopathy; macrophage; prion

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## 1. Introduction

The aggregation of the beta-amyloid peptide (A $\beta$ ) in the brain is an early and integral event in the pathogenesis of Alzheimer's disease (Hardy and Selkoe, 2002; Holtzman et al., 2011). A $\beta$  normally exists in a structurally unfolded (intrinsically disordered) state, but in its pathogenic form, A $\beta$  becomes rich in  $\beta$ -sheet and induces the misfolding and subsequent self-assembly of other A $\beta$  molecules. These durable, prion-like multimeric seeds instigate the formation of senile plaques and cerebral  $\beta$ -amyloid angiopathy as monomeric A $\beta$  is recruited into the  $\beta$ -sheet-rich deposits (Jucker and Walker, 2013; Walker et al., 2006; Walker and LeVine, 2012). We and others have found that intracranial injections of A $\beta$  multimers seed Alzheimer-like pathology in A $\beta$ -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; Morales et al., 2012b; Rosen et al., 2012; Stohr et al., 2012; Stohr et al., 2014; Watts et al., 2011; Watts et al., 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; Clavaguera et al., 2014; Guo and Lee, 2011; Guo and Lee, 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 A $\beta$  multimers into the peritoneal cavity of APP-transgenic mice can seed A $\beta$  deposition in the brain, particularly in the form of cerebral  $\beta$ -amyloid angiopathy (Eisele et al., 2010; Eisele et al., 2014). While the evidence currently favors axonal transport as a key mode of lesion propagation within the nervous system, the mechanisms by which A $\beta$  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 A $\beta$  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 A $\beta$  may remain intact in macrophages for a long period of time (Frackowiak et al., 1992), but the evidence for the entry of A $\beta$ -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  $\beta$ -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 (Figure 1). The analysis showed 3 distinct populations based on the expression of specific markers: CD11b-high/F4/80-high; CD11b-intermediate/F4/80-negative; and CD11b-negative/F4/80-negative. Roughly 30 percent of the lavage consisted of large peritoneal macrophages (LPM), which are characterized by high expression of CD11b and F4/80 (Ghosn et al., 2010). The cell population characterized by intermediate CD11b and negative F4/80 expression was gated and investigated for Ly6G and Ly6C expression. The majority (94.3 percent) 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 CD11b-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 and exogenous macrophages had comparable patterns of distribution in the body. Our analysis then focused on the mice receiving exogenous macrophages. At both four days and four weeks post-infusion of cells, bead-laden phagocytes were primarily found in the spleen (Figure 2), liver, and kidney of the host mice, and (in much smaller quantities) in lung and blood (not shown). The spleen in particular contained more nanobeads than any other tissue, consistent with its role in the storage and deployment of monocytes (Swirski et al., 2009). In addition, a small number of bead-containing macrophages (<50 per 40 $\mu$ m section at 4 days and <10 per 40 $\mu$ m section at

4 weeks) were detected in grey matter structures and superficial cortex of the brain (not pictured).

### 2.3. A limited number of exogenous macrophages enter the intact brain

To quantify more precisely the exogenous macrophages that enter the intact brain in our paradigm, macrophages were elicited in the peritoneal cavity of CD45.1-expressing donor mice and then infused i.v. into CD45.2-expressing host mice. Twenty-four hours later, the host brains were gently homogenized and the blood vessels depleted from the homogenates to eliminate cells that might have remained within the vascular lumen following perfusion. For comparison, we also assessed the population of exogenous macrophages that had migrated to the spleen at the same time point. A small number of donor macrophages were detectable in the brain parenchyma 24 hours after i.v. infusion, and, as expected, many more donor cells had migrated to the spleen (Figure 3).

### 2.4. Exogenous macrophages containing A $\beta$ seeds enter the intact brain

Having established that macrophages can enter the brain from the bloodstream, even after ingestion of nanobeads, we next asked whether exogenous macrophages that had ingested A $\beta$ -rich brain extract also can enter the brain. First, to confirm the ingestion of human A $\beta$  by peritoneally elicited macrophages, wild-type mice received i.p. injections of AD brain extract containing ample aggregated A $\beta$ , and after 4 days the cells were harvested, plated on coverslips, and immunostained with antibody 6E10 to human-sequence A $\beta$ , which does not recognize murine A $\beta$  (K.S. et al., 1988). Confocal microscopy verified that macrophages ingest detectable quantities of the A $\beta$ -laden brain extract (Figure 4). CD45.1 macrophages containing human A $\beta$  were then infused i.v. into B6[CD45.2] host mice; after a 24 hour incubation period, immunohistochemistry revealed A $\beta$ -containing cells in the superficial neocortex and subarachnoid space (Figure 5). Additionally, some A $\beta$ -immunoreactive cells were localized around blood vessels (Figure 6), but we did not detect CD45.1 in these perivascular cells. The reason for the absence of the donor marker in perivascular cells is not clear, but suggests either that CD45.1 was undetectable or that the A $\beta$  may have been transferred from exogenous donor cells to host macrophages.

## 3. Discussion

Both circulating monocytes and microglial cells are, under the appropriate circumstances, involved in the immune response in the brain. These two types of innate immune cells are among the first to respond to pathological insults; they help to repair physical trauma, defend against pathogens, and remove debris such as the remnants of dead cells (Flannagan et al., 2009; Gregory and Devitt, 2004). While they share some common functions, monocytes and microglia have distinct origins (Epelman et al., 2014; Gautier et al., 2012; Hettinger et al., 2013; Murray et al., 2014) and they monitor different tissue domains within the brain. When alerted to a pathological event in the CNS, the resident microglia are responsible for initiating the immune response, which can include the recruitment of circulating monocytes (Gate et al., 2010). The role of peripheral monocytes that enter the brain remains somewhat ambiguous, in part because they can be difficult to distinguish from resident microglia (Prinz et al., 2011).

Circulating monocytes enter the brain under various pathological conditions, including immunosuppression (Bauer et al., 1995) and irradiation injury (Mildner et al., 2007). In addition, monocytes enter the brain when the blood-brain barrier is physically compromised, for example in cases of stroke (Schilling et al., 2003; Tanaka et al., 2003) or head injury (Szymdynger-Chodobska et al., 2012). Our study indicates that a small but consistent subset of exogenous circulating macrophages enter the intact, healthy brain, and this was so whether they contained nanobeads, A $\beta$ -rich brain extract, or no specific cargo.

Histological evidence that hematogenous monocytes can enter the brains of healthy mice was confirmed by FACS analysis. The brain samples for FACS underwent perfusion and vascular depletion to minimize the possibility that the exogenous cells detected by the cell sorter were within the blood vessels of the brain, rather than on the parenchymal (abluminal) side of the blood-brain barrier (Triguero et al., 1990). However, we cannot rule out the possibility that some of these cells were within the vasculature, for example as dislodged marginating cells (van Furth and Sluiter, 1986), or in areas lacking capillary endothelial tight junctions such as the circumventricular organs or choroid plexus. However, in our histological samples we did not detect exogenous cells in these regions, whereas they were evident in the brain parenchyma, indicating that some circulating cells do cross from blood to brain.

FACS analysis of cell types within the lavage sample demonstrated the presence of both macrophages (60%) and lymphocytes (30%). We believe that the macrophages are most likely to be responsible for the transport and dissemination of A $\beta$  seeds because they are the principal cell type that is specialized for the removal of dying/dead cells and cellular debris (Perry et al., 2010). Some evidence suggests that B-cells can be phagocytic in various disease states (Borrello and Phipps, 1996), and thus could contribute to the transport of A $\beta$  seeds. In contrast to seed dissemination, the presence of lymphocytes (which were primarily B-cells in the lavage sample) could result in the presentation of antigens to A $\beta$ . Macrophages also present antigen (Unanue, 1984), so it is important to consider the possibility that engagement of the adaptive immune response could eventually result in antibody production and a decrease in cerebral A $\beta$  deposition. Long-term studies of the impact of exogenous, A $\beta$ -containing immune cells will be needed to address this question.

Taken together, our studies indicate that macrophages are able to take up and transport material from the periphery to the brain through the vasculature. In this light, the findings support the hypothesis that peripheral macrophages are a source of amyloidogenic A $\beta$  seeds that traffic to the brain from the peritoneal cavity in an experimental seeding model (Eisele et al., 2010; Eisele et al., 2014). The localization of cells adjacent to cerebral blood vessels further supports this conclusion, in that the A $\beta$  that deposits in the brains of peripherally seeded APP23 mice is mainly in the form of amyloid angiopathy (Eisele et al., 2010; Eisele et al., 2014).

In humans, there is currently no evidence that Alzheimer's disease can be induced by peripheral seeds (Irwin et al., 2013; Jucker and Walker, 2013). However, the fundamental molecular mechanisms by which proteopathic seeds arise and spread are unlikely to differ among species. At the cellular level, the role of macrophages – both central and peripheral –

in the deposition of A $\beta$  remains uncertain (Gate et al., 2010). Some studies have indicated that these cells can reduce the amount of A $\beta$  in the brain (Simard et al., 2006), whereas others indicate that they promote deposition (Akiyama et al., 2000). It is possible that both processes are at play, depending on the circumstances. For example, the removal of senile plaques in Alzheimer patients who had been immunized against A $\beta$  was associated with an increase in the amount of cerebral A $\beta$  angiopathy (Boche et al., 2008; Ferrer et al., 2004; Masliah et al., 2005; Nicoll et al., 2003), suggesting that A $\beta$  seeds were taken up and transported from the plaques to the vascular wall by macrophages (Boche et al., 2008; Masliah et al., 2005). Our model supports a general role of macrophages in the uptake and dissemination of A $\beta$  seeds; how the seeds exit the cells and stimulate A $\beta$  deposition in the vascular wall remains to be determined.

Another potential mechanism by which proteopathic seeds might reach the brain is axonal transport. In a preliminary experiment, we found that the intraperitoneal injection of the tracer fluorogold led to retrogradely labeled cells only in the dorsal motor nucleus of the vagus nerve (data not shown), an area that does not exhibit aggregated A $\beta$  in peripherally seeded models. Even so, we cannot yet rule out a role of neuronal transport in the translocation of A $\beta$  seeds from periphery to brain. Prions, for example, can reach the CNS from the periphery by neuronal transport (Bartz et al., 2005; McBride et al., 2001; Sigurdson et al., 2001; van Keulen et al., 1999). Recent experiments also indicate that  $\alpha$ -synuclein seeds are transported by the vagus nerve from the intestinal wall to the brain (Holmqvist et al., 2014). Within the CNS, cellular uptake and axonal transport mechanisms are implicated in the systematic spread of a number of pathogenic protein aggregates, including prions (Aguzzi, 2003; Borchelt et al., 1994; Buyukmihci et al., 1983; Liberski et al., 1990; Liberski et al., 2012; Scott et al., 1992), A $\beta$  (Hallbeck et al., 2013; Hamaguchi et al., 2012; Lee et al., 2010; Saper et al., 1987; Ye et al., 2015), tau (Braak and Del Tredici, 2011; Clavaguera et al., 2009; Holmes et al., 2014) and  $\alpha$ -synuclein (Angot et al., 2012; Desplats et al., 2009; Luk et al., 2012; Masuda-Suzukake et al., 2014).

In summary, our findings support the hypothesis that phagocytic cells are able to transport ingested cargo – including aggregated A $\beta$  – from the periphery to the brain in a mouse model. Macrophages thus appear to play contradictory roles in A $\beta$  deposition. On the one hand, they are predisposed to remove foreign substances and present antigen, but if they are unable to fully degrade the material, they may inadvertently disseminate it to other parts of the body, including the brain. By clarifying the involvement of macrophages in the trafficking of proteopathic seeds, we hope to pinpoint key mechanisms that can be targeted to slow this process within the brain.

## 4. Experimental procedures

### 4.1 Subjects

Non-transgenic C57BL/6 mice served as subjects. For the FACS/histology experiments, wild-type, inbred C57BL/6 mice expressing the hematopoietic cell marker CD45.2 (B6[CD45.2] mice) served as hosts, and congenic B6[CD45.1] mice (B6.SJL-Ptprca Pepcb/BoyJ; The Jackson Laboratory) served as donors. CD45 is a pan-leukocytic antigen that has two differentiable allelic forms in the two murine lines; as such, CD45.1-expressing cells



can be readily distinguished from CD45.2-expressing cells. Mice were housed in small groups under standard conditions at a temperature of 22°C and a 12 h light/dark cycle with *ad libitum* access to food and water. All experimental procedures were carried out in accordance with US federal guidelines, and were approved by the Emory Institutional Animal Care and Use Committee (IACUC).

#### 4.2. Characterization of lavaged leukocytes by fluorescence-activated cell sorting (FACS)

To characterize the cell population that was induced in the mice, C57/BL6 mice received a 100µl intraperitoneal injection of thioglycolate on day 0, and intraperitoneal cells were collected by lavage on day 4 and gently centrifuged at 1000 x g for 5 min (Ray and Dittel, 2010). The supernatant was removed, the red blood cells lysed in diH<sub>2</sub>O, and the leukocytic fraction was re-sedimented at 1000 x g for 5 min and re-suspended in buffered physiological saline. This fraction was incubated with antibodies to CD11b and F4/80 (macrophage-specific antigens), Ly6G and Ly6C (transiently expressed on monocytes in the bone marrow), B220 (a pan B-cell marker) and CD3 (primarily expressed by T-cells) for 25 minutes on ice in CD16/CD32 mouse BN Fc Block (BD Pharmingen) (chromogens FITC and phycoerythrin [PE]). After washing, fixing buffer was added and the pellet was washed again. The pellet was resuspended in FACS buffer (PBS+ 2% FBS) and analyzed by FACS for expression of CD11b and F4/80 on double-positive cells. The cells were gated by size (forward scatter) and density (side scatter), and then plotted according to CD11b and F4/80 expression. The resulting population was further gated by level of expression. CD11b-intermediate and F4/80-negative expressing cells were gated and plotted according to Ly6G and Ly6C expression. CD11b-negative and F4/80-negative expressing cells were gated and plotted according to B220 and CD3 expression. In this way, the composition of cells in the lavage fraction can be quantified (see Results).

#### 4.3. Trafficking of macrophages bearing fluorescent nanobeads

Our first objective was to assess the systemic distribution of macrophages that had ingested fluorescent nanobeads as tracer cargo. Five wild-type C57/BL6 mice received intraperitoneal (i.p.) injections of red fluorescent nanobeads (SPHERO™ Fluorescent Nile Red Particles; 2 µl of 1% nanobeads [w/v], 0.53µm diameter, Spherotech, Lake Forest, IL, USA) along with 100µl of thioglycolate to elicit macrophages on day 0. On day 3, mice were given a second injection of nanobeads, and animals were then sacrificed on day 4. In one group of mice (n=5), the nanobead-containing macrophages were analyzed in the mice that received the nanobead injections, i.e., the tracked macrophages originated endogenously within the same mouse. In a separate group of mice (n=10), bead-laden macrophages were prepared in donor mice as described above, and then harvested and infused intravenously (i.v.) into host mice (exogenous macrophages).

Leukocytes were collected by lavage (Ray and Dittel, 2010) and gently centrifuged at 1000 x g for 5 min. The supernatant was removed, the red blood cells lysed in diH<sub>2</sub>O, and the leukocytic fraction re-sedimented at 1000 x g for 5 min, re-suspended in buffered physiological saline, and cells counted using a hemocytometer. The cells were assessed for viability in a test sample by trypan blue exclusion. In all cases, a minimum of 75% of the cells for infusion were viable. In each host mouse, 5×10<sup>6</sup> viable cells in 250µl physiological

saline were slowly infused intravenously via the tail vein. Following an incubation period of either 4 days or 4 weeks, the host mice were perfused with 200mL of cool (4–8°C) physiological saline and the following tissues collected: Brain, spleen, liver, lung, heart, kidney, pancreas, and blood.

#### **4.4. FACS analysis of CD45.1-expressing exogenous macrophages transferred to B6[CD45.2] host mice**

Our second objective was to determine the proportion of exogenous macrophages that enter the brain from the bloodstream following infusion of the cells into host mice. A total of 15 mice served as hosts in two separate experimental runs. Macrophages were elicited in the peritoneal cavity of B6[CD45.1] donor mice by i.p. injection of thioglycolate on Day 1 and Day 3, and the cells were collected by lavage on day 4 (Ray and Dittel, 2010), as described above. The CD45.1-bearing cells ( $5 \times 10^6$  cells/250 $\mu$ l) were then slowly infused i.v. into CD45.2-expressing host mice. 24 hours later, the host mice were transcardially perfused with 200mL of cool (4–8°C) physiological saline under deep Nembutal anesthesia. The whole brain and spleen were separately processed and the blood vessels depleted from the homogenates (D'Alessandro et al., 2013; Pertoft, 2000) as follows: The organs were homogenized over a 100 $\mu$ m mesh nylon sieve, lysed in collagenase IV (Worthington), and mononuclear cells (along with a small population (~10%) of other leukocytes; see Figure 1) were obtained by density-gradient centrifugation using Percoll (Amersham) solutions (40% and 70%). Cells were incubated with antibodies to CD45 (a pan-hematopoietic cell antigen) and CD45.1 for 25 minutes on ice in CD16/CD32 mouse BN Fc Block (BD Pharmingen) (chromogens FITC and phycoerythrin [PE]). After washing, fixing buffer was added and the pellet was washed again. The pellet was resuspended in FACS buffer (PBS + 2% FBS) and analyzed by FACS for expression of CD45.1 on CD11b/CD45 double-positive cells. The cells were gated by size (forward scatter) and density (side scatter), and then plotted according to CD45 and CD45.1 expression. In this way, the entry of exogenous macrophages from the donor mouse can be assessed quantitatively in the brain and spleen of the host mouse.

To further characterize the cells and tissues in this experiment, induced intraperitoneal cells were harvested from donor mice on day 4 and plated in EMEM (Eagle's Minimum Essential Medium). To promote growth we used 10ng/ml of macrophage colony-stimulating factor (M-CSF). Murine macrophages were isolated according to the protocol of Fortier and Falk (Fortier and Falk, 2001). The cells were maintained for 3 days before they were dried on slides and processed for immunocytochemistry. For histology, the brains of B6[CD45.2] host mice were sectioned at 40 $\mu$ m thickness (as described below) and stained with antibodies 6E10 to human A $\beta$ , laminin (blood vessels), and CD45.1 (transferred hematopoietic cells (see section 4.6 below for antibody details). Tissues were counterstained with a fluorescent nuclear marker (4',6-diamidino-2-phenylindole [DAPI]).

#### **4.5. Trafficking of macrophages bearing A $\beta$ -rich brain extract**

As a third objective, we sought to assess the fate of macrophages that had specifically ingested A $\beta$ -rich brain extract to determine whether these cells can subsequently enter the host brain. The extract was prepared as described previously (Kane et al., 2000; Meyer-



Luehmann et al., 2006). In brief, neocortical tissue from a histopathologically verified AD case was homogenized at 10% (w/v) in PBS at 4°C, followed by brief sonication, also at 4°C. The homogenate was centrifuged at 3,000×g for 5 minutes at 4°C, and the supernatant was aliquoted and immediately frozen at –80°C until use. Donor B6[CD45.1] mice received i.p. injections of either thioglycolate alone (resulting cells infused into 6 host mice) or thioglycolate and 250µl of 10% AD brain extract (resulting cells infused into 9 host mice) on day 0 and day 3, and were sacrificed on day 4. Macrophages were collected by lavage, processed, counted, and slowly infused into B6[CD45.2] host mice as described above. After 24 hours, the host mice were perfused with cool (4–8°C) physiological saline and the brain and systemic organs collected for analysis.

#### 4.6. Immunohistochemistry

All tissues were immersion-fixed in buffered, 4% de-polymerized paraformaldehyde for 24 hours followed by cryoprotection in buffered 30% sucrose. The tissues were frozen, sectioned at 40µm thickness on a Leica CM3050 S cryostat, and mounted onto slides. Lavage samples of cells were dried onto slides and fixed with methanol. Selected specimens were immunostained with the following antibodies: 6E10 (1:5,000) (Covance), a mouse monoclonal antibody to human-sequence Aβ with an epitope at residues 3–8; rabbit polyclonal antibodies R361 and R398 (both at 1:15,000) (courtesy of Dr. Pankaj Mehta of the Institute for Basic Research on Developmental Disabilities, Staten Island, NY) to Aβ32–40 and Aβ33–42, respectively; and antibodies to CD45 (1:5000), CD45.1(1:1000) (BD Pharmingen), and laminin (1:1000) (Abcam ab11575). Specimens were washed in PBS, permeabilized using PBST (PBS and 0.2% Tween), and blocked for 1 h in 2% serum in PBST. Primary antibodies were added to PBST containing 2% serum and incubated with the cells or tissues overnight at 4°C while gently rocking. Secondary antibodies were added to PBST containing 2% serum and incubated with the specimens for 1.5 h at room temperature. In addition, some samples were counterstained with DAPI or hematoxylin. Cells and tissues were examined with a Leica DMLB microscope or an Olympus FV1000/TIRF inverted confocal microscope.

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**Highlights**

Exogenous macrophages can enter the brains of healthy host mice after taking up A $\beta$

Exogenous macrophages are found in the superficial neocortex and near blood vessels

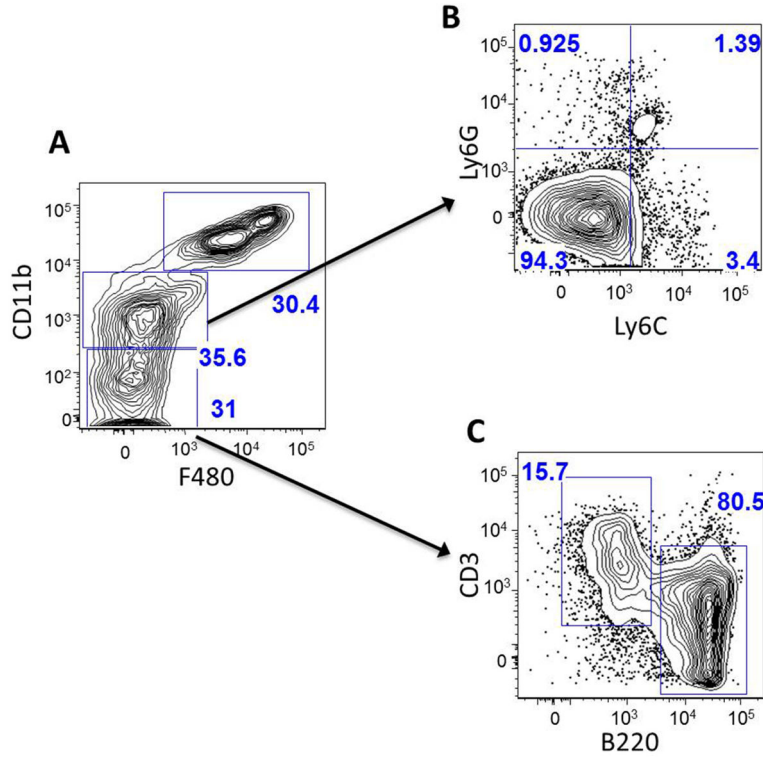
Macrophages may act as vectors for the spread of proteopathic seeds

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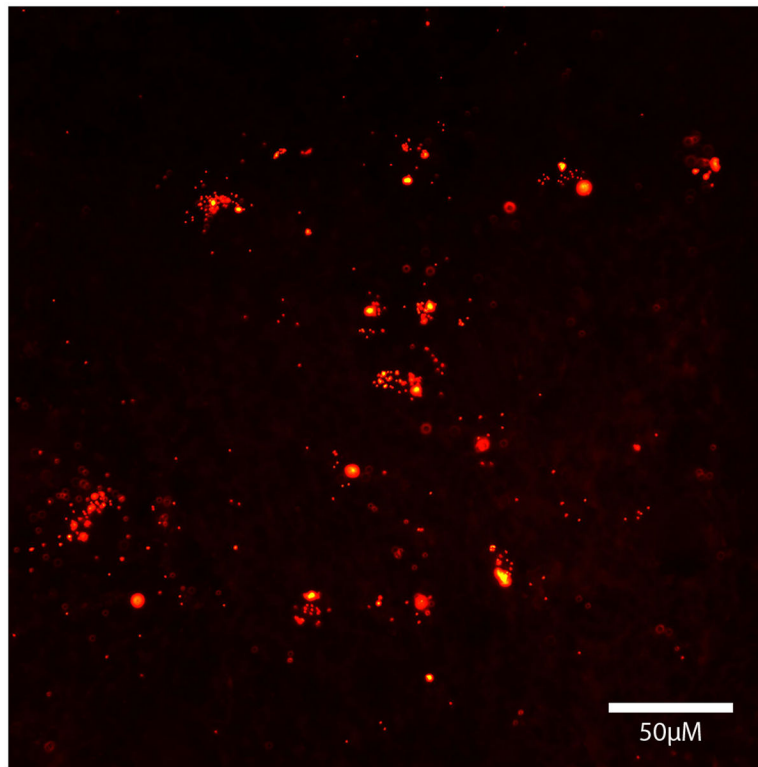
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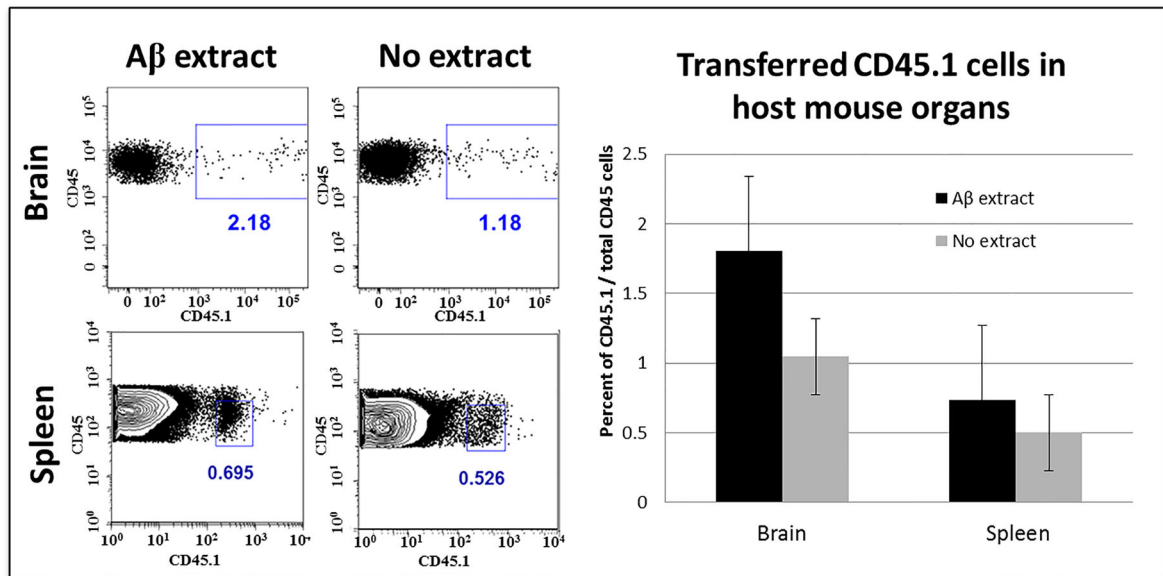


**Fig. 1. Characterization of lavaged leukocytes by FACS analysis**

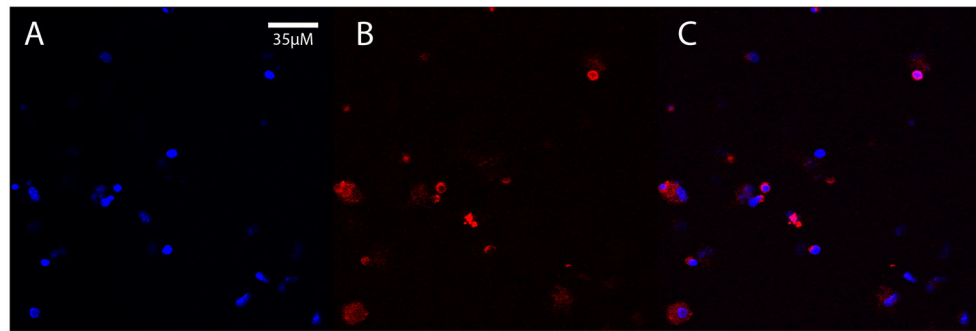
**A.** Whole lavage sample. Upper right gate (30.4%) represents CD11b-high & F4/80-high cells, which are large peritoneal macrophages (LPM). The middle gate (35.6%) represents CD11b-intermediate and F4/80-negative cells. This fraction of cells was further probed for expression of Ly6G and Ly6C (**B**). 94.3% of these cells were Ly6G-negative and Ly6C-negative, indicating that they are small peritoneal macrophages (SPM). The bottom gate of panel A denotes CD11b-negative and F4/80-negative cells. This fraction was further probed for markers of B-cells (B220) and T-cells (CD3) (**C**). 80.5 percent of these cells were B-cells and 15.7% were T-cells. Overall, this analysis indicates that the lavage cells consist of ~60% macrophages (LPM and SPM), ~30% lymphocytes (most of which are B-cells), and 10% other cells.



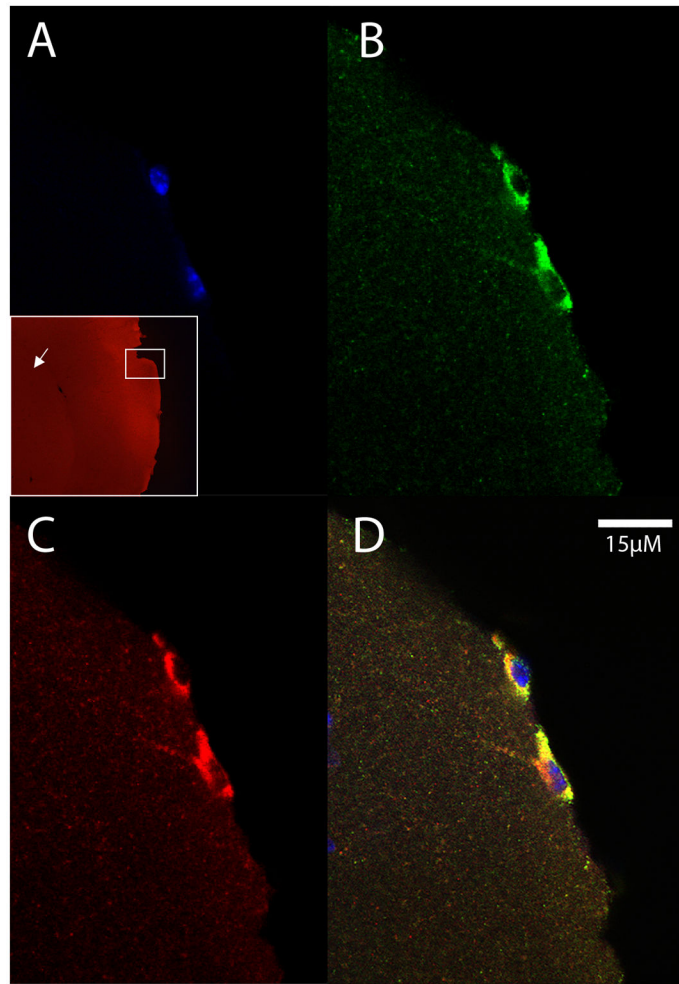
**Fig. 2.** Fluorescent nanobeads (red) in the spleen of a host mouse 4 weeks after i.v. injection of bead-laden macrophages from a donor mouse.



**Fig. 3.** FACS analysis of intravenously transferred CD45.1 cells in the brain and spleen of a B6[CD45.2] host mouse. Cells were gated for CD45 immunoreactivity (all hematopoietic cells) and CD45.1 (exogenous donor cells). Exposure of the donor cells to Aβ-rich AD brain extract in the i.p. injectate did not significantly influence the relative quantity of transferred cells that entered the brain or spleen.

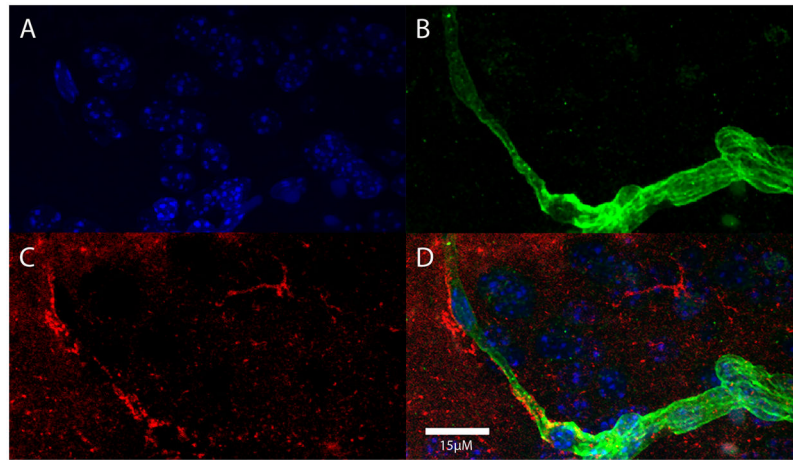


**Fig. 4.** Macrophages harvested from the peritoneal cavity following i.p. injection of AD brain extract contain immunodetectable human A $\beta$ . **A)** Cell nuclei stained with DAPI (blue); **B)** A $\beta$  immunofluorescence (red, antibody 6E10); **C)** merged images.



**Fig. 5.** Exogenous macrophages from a CD45.1-expressing donor mouse in the superficial neocortex of a CD45.2-expressing host mouse. The cells were exposed to A $\beta$ -rich AD brain extract in the peritoneal cavity of donor mice, collected by lavage, and injected intravenously into the host mouse 24 hours prior to sacrifice. **A)** DAPI nuclear stain (blue) with low magnification inset showing the location of the cells (white box) in the superficial neocortex (arrow marks the striatum); **B)** CD45.1 (green); **C)** A $\beta$  (red); **D)** merged images.





**Fig. 6.** A $\beta$  immunoreactivity (red) adjacent to a laminin-immunoreactive blood vessel (green) in the neocortex of a CD45.2-expressing host mouse 24 hours after an i.v. infusion of exogenous macrophages. In this case, the A $\beta$ -immunoreactive cells were not demonstrably immunopositive for the donor antigen (CD45.1). **A)** DAPI nuclear stain (blue); **B)** laminin (green); **C)** A $\beta$  (red); **D)** merged images.