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Adeno Associated Viral-mediated intraosseus labeling of bone marrow derived cells for CNS tracking

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Abstract

Inflammation, including microglial activation in the CNS, is an important hallmark in many neurodegenerative diseases. Microglial stimuli not only impact the brain microenvironment by production and release of cytokines and chemokines, but also influence the activity of bone marrow derived cells and blood born macrophage populations. In many diseases including brain disorders and spinal cord injury, researchers have tried to harbor the neuroprotective and repair properties of these subpopulations. Hematopoietic bone marrow derived cells (BMDCs) are of great interest, especially during gene therapy because certain hematopoietic cell subpopulations traffic to the sites of injury and inflammation. The aim of this study was to develop a method of labeling endogenous bone marrow derived cells through intraosseus impregnation of recombinant adeno-associated virus (rAAV) or lentivirus. We utilized rAAV serotype 9 (rAAV-9) or lentivirus for gene delivery of green fluorescence protein (GFP) to the mouse bone marrow cells. Flow cytometry showed that both viruses were able to efficiently transduce mouse bone marrow cells *in vivo*. However, the rAAV9–GFP viral construct transduced BMDCs more efficiently than the lentivirus (11.2% vs. 6.8%), as indicated by cellular GFP expression. We also demonstrate that GFP labeled cells correspond to bone marrow cells of myeloid origin using CD11b as a marker. Additionally, we characterized the ability of bone marrow derived, GFP labeled cells to extravasate into the brain parenchyma upon acute and subchronic neuroinflammatory stimuli in the mouse CNS. Viral mediated over expression of chemokine (C-C motif) ligand 2 (CCL2) or intracranial injection of lipopolysaccharide (LPS) recruited GFP labeled BMDCs from the periphery into the brain parenchyma compared to vehicle treated mice. Altogether our findings demonstrate a useful method of labeling endogenous BMDCs via viral transduction and the ability

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Conflict of interest

All authors declare no conflict of interest

to track subpopulations throughout the body following insult or injury. Alternatively, this method might find utility in delivering therapeutic genes for neuroinflammatory conditions.

Keywords

GFP labeling; recombinant adeno-associated virus serotype 9; bone marrow infection; monocyte; infiltration; inflammation

1. Introduction

Monocytes originate from hematopoietic stem cells in the bone marrow through several differentiation steps [1] and divide into subpopulations characterized by specific cell surface markers and chemokine receptor expression. Monocytes enter into the blood stream, and subsequently can extravasate and differentiate into tissue macrophages or dendritic cells following infection or inflammatory stimulation [2], [3]. They are important components of the innate immune system in maintenance of homeostasis, but also crucial in responding in inflammatory diseases. The ability of monocytes, or subsets, to traffic from bone marrow to blood and home to tissue has recently gained great interest for gene therapy in several diseases, including neurodegenerative disease. For instance, Richards et al. (2008) [4] showed that BMDCs transduced to express toll-like-receptor 2 via intraosseous injection of lentivirus (pLenti/GFP/TLR2) restricted amyloid-beta toxicity in an amyloid-depositing mouse. In another study using GFP positive/ CD11b positive monocytes from GFP transgenic mice, Lebson et al. (2010) [5] demonstrated that this monocyte population homed to the amyloid plaques in the brains of APP transgenic mice. Mice receiving monocytes transfected with an amyloid-degrading enzyme (neprilysin) halted amyloid deposition in this model [5]. Other approaches adapting lentivirus gene therapy [4, 6], total vs. partial body radiation [7], parabiosis in mice [8], or selective depletion of microglia in CNS [9], [10], [11] supported the idea that BMDCs cross the blood-brain barrier and can be used as therapeutic vehicles in CNS disorders [12], [13]. However, the use of BMDCs for cell therapy has initiated several questions, including graft versus host immune responses, the impact of radiation on the immune system, chemically depleting cells to extinguish certain cell populations, and the limitation of a single or repeated injection of monocytes into blood stream. In this study, we developed a rapid method in which we could apply targeted gene therapy to BMDCs and exploit their ability to traffic to the injured site or areas of inflammation. We compare the transduction efficiency of two viral vectors, lentivirus vs. rAAV serotype 9, in delivering the gene for green fluorescent protein (GFP) into the mouse femur to target BMDCs.

rAAV is a widely used viral vector, with an increasing number of clinical trials each year using it. rAAV has a number of advantages, including lack of pathogenicity, low immunogenicity, long term gene expression, and episomal persistence of the transgene, in addition to well established viral production protocols. Furthermore there are a number of recombinant serotypes available that confer different cellular tropisms. The episomal persistence has a unique advantage for transducing dividing cells when a more transient expression of the delivered transgene is wanted or required. This would reduce any long-

term deleterious effects of transgene expression. However, integration by lentivirus has the advantage of permanent transduction of infected cells, although at the risk of insertion into an oncogene. In our studies, we observed that both viral vectors could transduce BMDCs in vivo. However, the rAAV9 transduced a higher percentage of cells than lentivirus. Further, we demonstrate that these labeled BMDCs infiltrated the brain following either lipopolysaccharide (LPS) injections or rAAV-mediated CCL2 overexpression in the CNS.

2. Material and methods

2.1 Animals and Virus Preparation

Fourteen month old C576/BL6 mice (n=12) were housed and treated according to institutional and National Institutes of Health standards. Recombinant adeno-associated serotype 9 virus (rAAV-9) was generated using pAAV9 and pXX6 in HEK293 cells as described previously [14]. Both GFP (UF11 plasmid, ATCC #MBA-331) and CCL2 (GI: 141803162) were cloned into the rAAV vector under the control of the hybrid CMV chicken beta-actin promoter. CCL2 was cloned using PCR and cDNA generated from a mouse using the primers gagaccgggtccaccatgcaggctcctgtcatgcttc and gaggctagcctagttcactgtcacactggcactcc. Clones were confirmed by DNA sequencing prior to use and virus titers were determined by dot blot as described previously [15]. AAV viral titer of 4.5×10^{13} vector genomes (vg)/ml were used in the study. Lentivirus expressing GFP ($>1 \times 10^{13}$ TU/ml) was purchased from Cell Biolabs, LVT-300.

2.3 Intraosseous injections

Mice were anesthetized with isoflurane, and both knees were flexed with support behind each knee. Hair was shaved around the joint area and 70% alcohol and iodine was used to clean the area. A 1 ml syringe with a 25 (5/8 length) gauge needle was inserted into the intrafemoral space by gentle twisting and pressure between the condyles at the top of the femur between the tibia and femur joint. The 25 (5/8 length) gauge needle and cap was left in place and the 1ml syringe was gently removed. A (25 μ l 1702 RN) Hamilton syringe with a 32G needle (7803-04, 32 Gauge RN 2" point size 4, referred to as bone marrow needles) was inserted into the plastic cap opening and threaded through the needle opening of the 25 (5/8 length) gauge needle (fig. 1). The 32 G bone marrow needle was marked at 3.5 cm from the tip to indicate the length at which to discontinue insertion. Five microliters of solution (AAV-GFP (4.5×10^{13} vg/ml, Lenti-GFP $>1 \times 10^{13}$ TU/ml Cell Biolabs, LVT-300) was slowly injected by free hand into the shaft of the femur using the 25 μ l Hamilton syringe and slowly removed to limit backflow. The 25 (5/8 length) gauge needle was gently removed and mice were monitored and allowed to recover. At the time of intraosseous (i.o.) injections and 48hr after, all mice received 10 mg/kg ketoprofen subcutaneously.

2.4 Bone marrow cell isolation

Briefly, mice receiving intraosseous injection or ubiquitously expressing GFP mice were overdosed with SomnaSol ® and their femurs and tibias were removed aseptically. Femur and tibia marrow cavities were flushed with RPMI 1640 media containing fetal bovine serum (FBS) and HEPES, pH 7.4, using a 25 gauge needle. Single-cell suspensions were prepared by repeat pipetting and the cell preparations were passed through a 70 μ m nylon

mesh to remove cell debris. Red blood cells were removed by using 3 mL of RBC lysis buffer and incubated at room temperature for 5 min prior to adding cold PBS. Cells were centrifuged, washed twice in RPMI 1640, and total bone marrow cells were counted using a hemocytometer. Fifty to seventy million bone marrow cells from the injected mice were suspended in 0.2 ml of PBS+0.5% BSA and analyzed for GFP positive expression using cytochemistry and flow cytometry analysis (described below).

Positive control GFP+ CD11b+ bone marrow cells were isolated from mice ubiquitously expressing GFP [C57BL/6-Tg(UBC-GFP)30Scha/J (stock #004353)] using Miltenyi Biotec's LS columns and MidiMacs magnet following the manufacturer's instructions as previously described [5]. Briefly, 50-70 million bone marrow cells from GFP transgenic mice were suspended in 0.2 ml of PBS+0.5% BSA and incubated for 15 min together with CD11b antibody conjugated to magnetic microbeads at 4°C (Miltenyi Biotec, Ca, US). The cell suspension was applied to the supplied column in a magnetic field and the CD11b+ fraction separated from the unlabeled cells by washing three times with 3 ml of buffer. The column was separated from the magnet and CD11b+ cells were collected. The purity of immunomagnetically separated cells was measured using flow cytometry.

2.5 Intracranial animal Injections

The injection procedures were performed using the convection enhanced delivery method (CED) as described previously [14]. Briefly, mice at 14 month old age were anesthetized with 1.5% isoflurane in 1% oxygen and secured into a stereotactic apparatus. Each group received unilateral intracranial injections of 2 µl rAAV9-CCL2 (7×10^{12} vg/ml; n=4; 7 days after i.o injection), 2 µl LPS (2.5 mg/ml; n=4; 21 days after i.o injection) or 2 µl 0.9% saline in the right hippocampus. The coordinates of injection were anteroposterior, -2.7 mm; lateral -2.7 mm, vertical -3.0 mm from bregma. A microsyringe injector and controller (Stoelting, IL, US) were used to inject 2 µl of virus at a constant rate of 2.5 µl/min. An additional 1 min was allowed before the needle was raised slowly and the scalp incision was closed. Mice were euthanized 24 days after intracranial rAAV9-CCL2 injections and 3 days after intracranial LPS injections, including saline controls at each time point. A list of reagents used is summarized in Table 1.

2.6 Staining of bone marrow cells for cytochemistry and flow cytometry analysis

Bone marrow from CCL2, LPS- or saline- injected mice were collected as described above and prepared for GFP immunocytochemistry/ flow cytometry analysis. Approximately 50×10^4 cells/animal in suspension were incubated with fluorescein isothiocyanate (FITC)-labeled antibody to GFP (1 mg/ml) (Abcam, Ca, US), or with isotype-matched control antibody, for 30 min on ice. Gating for GFP intensity was determined based on CD11b +/GFP+ bone marrow population and the isotype IgG antibody stain. GFP fluorescence intensity was measured using Accuri flow cytometer and software.

BMDCs were fixed in 4% paraformaldehyde (PFA) for 24h at 4°C. An aliquot of 50×10^4 cells/animal was rinsed two times with PBS and then pelleted onto a slide using a Cytospin 4 at 1200 rpm for 10 min at room temperature (Thermo Scientific, MA, USA). Slides were incubated with anti-chicken GFP primary antibody overnight at 4°C (AbCam, CA, US), then

incubated in an anti-chicken biotinylated secondary antibody (2 h) (VectorLabs, CA, USA) followed by amplification in ABC streptavidin-peroxidase (VectorLabs, CA, USA). Peroxidase reactions consist of 1.4 mM diaminobenzidine with 0.03% hydrogen peroxide in PBS for 5 min. Additionally, cells were also visualized by GFP fluorescence with Vectashield Hardset Mounting Medium (VectorLabs, CA, USA).

2.7 Image analysis

Immunohistochemistry/immunocytochemistry stains were digitally scanned with the Mirax Scan (Zeiss, Gottingen, Germany) slide scanner and quantified using Mirax Viewer image software. For immunofluorescence, slides were imaged using a Zeiss AxioVision Imager Z1 microscope, and processed using AxioVision 4.8 image software (Zeiss, Gottingen, Germany).

2.8 Statistical Analysis

Statistical analysis was performed using 1-way ANOVA using Stat View software version 5.0 (SAS Institute Inc, Cary NC, US). Graphs were generated using GraphPad Prism 4.0 (La Jolla, CA, US).

3. Results and Discussion

3.1 Delivery of virus-GFP to bone marrow cells

We identified a method for *in vivo* labeling of endogenous bone marrow cells. For that reason we utilized and compared rAAV9 virus to lentivirus as a vehicle for delivering GFP cDNA to the mouse bone marrow cells. In Figure 1, a 1 mL syringe with a 5/8 “ 25 gauge guide needle was inserted into the intrafemoral space between the condyles at the top of the femur between the tibia and femur joint (Figure.1, panel 2). The 25 gauge needle and cap was left in place and the 1 mL syringe was gently removed. A twenty- five microliter Hamilton syringe with point size 4 needle (referred to as a bone marrow needle) was inserted into the plastic cap opening and threaded through the needle opening of the 25 gauge needle (Figure 1, panel 1). The bone marrow needle was marked at 3.5 cm from the tip to indicate the length at which to discontinue insertion. Five microliters of rAAV-GFP (4.5×10^{13} vg/ml) or Lentivirus-GFP solution (1×10^{13} TU/ml) was slowly injected by free hand into the shaft of the femur. The 25 μ l Hamilton syringe was slowly removed to limit backflow (depicted by blue dye in Figure 1, panel 3). The 25 gauge needle was gently removed and mice were monitored during recovery from anesthesia.

3.2 Characterization of GFP-labeled bone marrow cells

Published studies from our group and others have often used bone marrow cells isolated from mice ubiquitously overexpressing GFP or using of GFP-labeled bone marrow chimeras using total body irradiation [5, 15, 16]. However, methods to label endogenous myeloid cells might prove advantageous over whole body irradiation and bone marrow grafts as a way to trace the delivery of therapeutic genes. Studies using lentivirus GFP have been reported for *in vivo* bone marrow transduction, however transduction efficiency was not indicated [4]. Here we identify the efficiency of AAV versus lentivirus to transduce and express GFP as a reporter gene into the BMDCs.

To determine this, we harvested the bone marrow transduced by each vector and utilized flow cytometry to measure total and GFP labeled cells. The gate for GFP expressing cells was determined by using bone marrow cells isolated from a GFP mouse with ubiquitous GFP expression. The total bone marrow population was positively selected for monocytes with CD11b- conjugated magnetic beads and further sorted by granularity (size scattered area, SSC-A) vs. size of the cells (forward scattered area, FSC-A) of the CD11b cells (Figure 2A). Furthermore, the CD11b⁺ population gated in P1 (94% enriched population) was plotted against GFP intensity. As shown in the histogram of panel A, 100% of these cells expressed GFP in the GFP transgenic mouse. We established that cells with GFP intensity higher than the arbitrary value of 10^4 were considered as GFP positive cells in this study (Figure. 2A, center and far right panels). Previously, we have shown that this population primarily contains monocytes [5, 15]. Next, we harvested total bone marrow of animals injected with rAAV-9 GFP and stained for GFP expression. For the total population 44.5 % of the cells that fell within P1 gate maintained the same SSC-A/FSC-A properties as CD11b⁺ cells (Figure 2B, left panel). Furthermore, we demonstrated that 11.2 % of the cells were transduced by rAAV-9 and expressed GFP based on the set arbitrary value of 10^4 (Figure 2B, center panel). The histogram in Fig 2B right panel represent the GFP⁺ population compared to the non- GFP expressing cells. We performed the same analyses with lentivirus-GFP transduced cells (Figure 2C). We found that the same yield of cells populated the P1 gate (40%), however only 6.8% of these cells expressed GFP. The bone marrow cells in P1 gate maintain the same SSC/FSC-A properties, suggesting that they are of myeloid origin (compared to Figure 2A).

3.3 Immunocyto/histochemistry analysis of GFP expressing cells

To visualize BMDCs, 50,000 cells were pelleted onto slides using a cytospin. We performed immunocytochemistry/immunofluorescence on the CD11b positively selected, GFP⁺ expressing cells to determine the expression levels of GFP throughout the cells (Figure 3A). We found that the GFP expression levels of AAV9-GFP infected cells were characterized as dim (arrow) to high (arrowhead) intensity for GFP (Figure 3B). The lentivirus infected cells were also positively stained for GFP and also displayed various expression levels of GFP (Figure 3C). Quantification of the number of GFP positively stained bone marrow cells showed no statistical significance between the two experimental groups (Figure. 3D). IgG stained bone marrow cells served as the isotype control in this experiment.

3.4 Infiltration of bone marrow cells in the CNS

To investigate whether bone marrow cells transduced with GFP remained functional and extravasated into the brain during acute injury, we used either LPS [17] or viral mediated delivery of CCL2 as inflammatory stimuli. Several reports reveal a critical role of CCL2-CCR2 signaling in promoting monocyte emigration from bone marrow to blood. CCR2 signaling contributes to monocyte homeostasis even during non-inflammatory conditions [18]. CCL2 is a chemoattractant molecule that can promote infiltration of monocytes to sites of inflammation including to the brain as demonstrated by our group and others [15, 19-21]. Therefore, we used intracranial injections of rAAV9 virus to over express CCL2 in the mouse hippocampus. For the intraosseous injections, we chose the rAAV9-GFP construct over the lentivirus-GFP for two reasons: 1) the 1.6 fold higher BMDCs transduction efficacy

over the lentivirus (Figure 2 and 3). Prior work demonstrated CNS infiltration of lentivirus labeled BMDCs but the rAAV ability of labeling these cells has not yet been demonstrated [4]. 2) In fact, rAAV's episomal persistence could have greater potential for clinical use due to its limited activation of oncogenes. Mice received i.o injections of rAAV9-GFP and either intracranial LPS, rAAV9-CCL2 or saline for a period of 3 days (LPS/ saline) or 3 weeks (rAAV-CCL2/ saline), respectively (Figure 4A). Three weeks after rAAV-CCL2 expression, GFP labeled BMDCs were observed in the hippocampus compared to intracranial vehicle (saline; Figure 4B). Cells were primarily found in the brain parenchyma close to the injection site in dentate gyrus (DG)/hilus and in perivascular areas and meninges. These infiltrated cells were phenotypically identified as mononuclear and expressed dim to high intensity of GFP staining, similar to that observed from *in vitro* cytochemical staining and characterization (Figure 3). Higher magnification of the GFP+ infiltrating cells in DG is shown in Figure 4, panel B7-8. In contrast, mice injected with saline showed no infiltrating BDMCs in any section examined throughout the brain (Figure 4, panel B1-4, C1-3).

LPS injections into the mouse hippocampus were also able to induce migration of rAAV9-GFP labeled BDMC (Figure 4, panel C4-6). Immunohistochemical analysis of the brain revealed GFP+ cells close to the lateral ventricles and perivascular blood vessels. These cells morphologically resemble those stained *in vitro* and the cells recruited by rAAV-CCL2. Overall, we observed less GFP+ cells in the parenchyma of mice following LPS than induced by AAV-mediated CCL2 expression (images not shown). This may simply be due to higher levels of chemokines present in the rAAV9-CCL2 treated animals compared to the levels generated from a single bolus LPS injection. Furthermore, the continued three-week expression of CCL2 from viral delivery may account for higher and more consistent numbers of GFP+ BMDCs found in the mouse CNS.

Together these data indicate that rAAV-transduced BDMCs can be used to label endogenous bone marrow populations that infiltrate the CNS following neuroinflammatory stimuli. To our knowledge this is the first evidence to demonstrate the extravasation of rAAV9-GFP transduced BMDCs into the CNS parenchyma during neuroinflammatory stimuli. Therefore, rAAV might serve as a useful tool for BMDCs transduction approaches used as combination gene and cell therapy for inflammatory conditions.

5. Conclusions

The present study used rAAV9-mediated gene delivery to BMDCs to track BMDC CNS infiltration following neuroinflammation. We found that rAAV9-mediated transduction of endogenous BMDCs via intra-osseous injection was sufficient to label CD11b cells and that these cells were able to infiltrate the CNS after an intracranial LPS injection or viral vector-mediated CCL2 expression. It should be noted that we used rAAV serotype 9 in this study, but other serotypes may offer different cellular tropism or degrees of transduction of BMDCs. Wild type and capsid mutants of rAAV6 have recently been shown to transduce dendritic cells and hematopoietic stem cells [22] [23]. Further studies are warranted to determine which AAV serotypes are most efficient at transducing different populations of BMDCs. Although not addressed here, the consideration of monocyte specific promoters could also be examined to limit off target expression of transgenes in other tissues. One

limitation of rAAV mediated gene therapy remains the size to the gene insert, with a maximum packaging size of approximately 5 Kb. However we find these data very promising as an effective method of *in vivo* transduction of BMDCs.

Our study demonstrates the utility of using viral vectors for transducing BMDCs *in vivo* with the reporter GFP transgene (or a future therapeutic gene); circumventing the use of *ex vivo* transfections or transfer of BMDCs from a donor animal into lethally irradiated hosts. Hematopoietic stem cells have been used as a gene delivery vehicle in severe combined immunodeficiency (SCID) and adrenoleukodystrophy (ALD) and have been successfully translated into clinical trials [24, 25]. Infiltration of peripheral myeloid derived cells into the brain parenchyma following the signals from amyloid plaques has been shown in AD models [4, 5, 7, 11]. Furthermore, these authors have been shown that transfected BMDCs *ex vivo* with therapeutic genes were capable of removing amyloid depositions [5] and rescue behavior impairment of transgenic mice [10]. Hence, the transduction of bone marrow derived immune cells utilizing the method described here may offer a different therapeutic strategy for numerous disease purposes.

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References

1. Iwasaki H, Akashi K. Myeloid lineage commitment from the hematopoietic stem cell. *Immunity*. 2007; 26(6):726–40. [PubMed: 17582345]
2. Auffray C, Sieweke MH, Geissmann F. Blood monocytes: development, heterogeneity, and relationship with dendritic cells. *Annu Rev Immunol*. 2009; 27:669–92. [PubMed: 19132917]
3. Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity*. 2003; 19(1):71–82. [PubMed: 12871640]
4. Richard KL, et al. Toll-like receptor 2 acts as a natural innate immune receptor to clear amyloid beta 1-42 and delay the cognitive decline in a mouse model of Alzheimer's disease. *J Neurosci*. 2008; 28(22):5784–93. [PubMed: 18509040]
5. Lebson L, et al. Trafficking CD11b-positive blood cells deliver therapeutic genes to the brain of amyloid-depositing transgenic mice. *J Neurosci*. 2010; 30(29):9651–8. [PubMed: 20660248]
6. Spencer B, et al. Long-term neprilysin gene transfer is associated with reduced levels of intracellular Aβeta and behavioral improvement in APP transgenic mice. *BMC Neurosci*. 2008; 9:109. [PubMed: 19014502]
7. Malm TM, et al. Bone-marrow-derived cells contribute to the recruitment of microglial cells in response to beta-amyloid deposition in APP/PS1 double transgenic Alzheimer mice. *Neurobiol Dis*. 2005; 18(1):134–42. [PubMed: 15649704]
8. Villeda SA, et al. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. *Nat Med*. 2014; 20(6):659–63. [PubMed: 24793238]
9. Varvel NH, et al. Microglial repopulation model reveals a robust homeostatic process for replacing CNS myeloid cells. *Proc Natl Acad Sci U S A*. 2012; 109(44):18150–5. [PubMed: 23071306]
10. Elmore MR, et al. Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. *Neuron*. 2014; 82(2):380–97. [PubMed: 24742461]
11. Simard AR, et al. Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron*. 2006; 49(4):489–502. [PubMed: 16476660]
12. Hess DC, et al. Do bone marrow cells generate neurons? *Arch Neurol*. 2004; 61(4):483–5. [PubMed: 15096394]

13. Simard AR, Rivest S. Neuroprotective properties of the innate immune system and bone marrow stem cells in Alzheimer's disease. *Mol Psychiatry*. 2006; 11(4):327–35. [PubMed: 16491130]
14. Carty N, et al. Intracranial injection of AAV expressing NEP but not IDE reduces amyloid pathology in APP+PS1 transgenic mice. *PLoS One*. 2013; 8(3):e59626. [PubMed: 23555730]
15. Selenica ML, et al. Diverse activation of microglia by chemokine (C-C motif) ligand 2 overexpression in brain. *J Neuroinflammation*. 2013; 10:86. [PubMed: 23866683]
16. Simard AR, Rivest S. Bone marrow stem cells have the ability to populate the entire central nervous system into fully differentiated parenchymal microglia. *FASEB J*. 2004; 18(9):998–1000. [PubMed: 15084516]
17. Lacroix S, Feinstein D, Rivest S. The bacterial endotoxin lipopolysaccharide has the ability to target the brain in upregulating its membrane CD14 receptor within specific cellular populations. *Brain Pathol*. 1998; 8(4):625–40. [PubMed: 9804372]
18. Serbina NV, Pamer EG. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol*. 2006; 7(3):311–7. [PubMed: 16462739]
19. Fuentes ME, et al. Controlled recruitment of monocytes and macrophages to specific organs through transgenic expression of monocyte chemoattractant protein-1. *J Immunol*. 1995; 155(12): 5769–76. [PubMed: 7499865]
20. Toft-Hansen H, et al. Metalloproteinases control brain inflammation induced by pertussis toxin in mice overexpressing the chemokine CCL2 in the central nervous system. *J Immunol*. 2006; 177(10):7242–9. [PubMed: 17082642]
21. Schellenberg AE, et al. Blood-brain barrier disruption in CCL2 transgenic mice during pertussis toxin-induced brain inflammation. *Fluids Barriers CNS*. 2012; 9(1):10. [PubMed: 22546091]
22. Song L, et al. Optimizing the transduction efficiency of capsid-modified AAV6 serotype vectors in primary human hematopoietic stem cells in vitro and in a xenograft mouse model in vivo. *Cytherapy*. 2013; 15(8):986–98. [PubMed: 23830234]
23. Pandya J, et al. Rationally designed capsid and transgene cassette of AAV6 vectors for dendritic cell-based cancer immunotherapy. *Immunol Cell Biol*. 2014; 92(2):116–23. [PubMed: 24217810]
24. Nienhuis A. The growing clinical impact of gene therapy. *Mol Ther*. 2008; 16(6):995–6. [PubMed: 18500234]
25. Cartier N, et al. Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. *Science*. 2009; 326(5954):818–23. [PubMed: 19892975]

Figure 1.

Overview of the technique used for intra-osseous injection into bone marrow intraosseus needle placement, shown in the images, provides an efficient route for administering either rAAV9- or lentivirus GFP. The dissected leg is shown for visualization. The areas indicated by rectangles and/or numbers are magnified in the insets. [1] A 25 gauge syringe needle was inserted through the bone's hard cortex and into the soft marrow interior from the distal anterior aspect of the femur. [2] A Hamilton syringe (cat # 7653, 10 µl) with a 33 gauge needle was inserted into the 25 gauge needle and used to deliver the virus. Panel [3] shows 5µl of blue dye filling the femur cavity.

Figure 2.

Flow cytometry analysis of transduction of bone marrow derived cells (BMDCs) with AAV9-GFP or Lenti-virus GFP compared with GFP transgenic mice. [A] CD11b BMDCs from transgenic mice with ubiquitous GFP expression were positively selected using CD11b magnetic antibody beads. Other mice received 5ul via intra-osseous injection of either [B] AAV9-GFP or [C] Lentivirus-GFP. Total bone marrow was collected and analyzed by flow cytometry. On each panel, the left most graph displays the population of cells with the size characteristic of monocytes. Cells were gated on the CD11b/ GFP⁺ population. The center graph displays intensity of GFP staining in the population. The right most graphs in each panel depict the frequency of GFP expression in the population. Panel [A] shows that 100% of CD11b enriched cells were GFP⁺ in GFP transgenic mice, as expected. [B] AAV9-GFP and [C] Lentivirus-GFP infected 11.2 % and 6.8% of the gated population respectively (mean $\pm$ SEM; n=4).

Figure 3.

Transduction of bone marrow derived cells with AAV9-GFP or Lentivirus GFP. CD11b positively selected BMDCs from GFP transgenic mice [A] or mice that received intraosseous (i.o.) injection of 5ul of either [B] AAV9-GFP or [C] Lenti-GFP. Three weeks post i.o., total bone marrow was collected, cytopspined onto slides and analyzed by immuno/cytochemistry. [A-C] shows immunocytochemistry (brown) and immunofluorescent (green) bone marrow stains of GFP for [A] CD11b+/GFP+ mice, [B] AAV9-GFP, and [C] Lenti-GFP bone marrow cells.

Figure 4.

CNS Infiltration of transduced BMDC following LPS or CCL2 overexpression. [A] Schematic presentation of experimental design. All mice received i.o. injections with rAAV9-GFP. One week post i.o., some mice received intracranial AAV9-CCL2 or saline into the hippocampus (HPC) and were euthanized after 3 weeks. Other mice received intracranial injection of LPS or saline 3 weeks post i.o. with euthanasia 3 days later. [B, C] Micrographs show positive immunostaining (black) of GFP+ cells in the HPC after AAV-CCL2 [B.6-8] or LPS [C.4-6], but not after saline [B.1-4, C.1-3] injections. GFP+ cells were present in the hemisphere receiving AAV-CCL2 or LPS injections (ipsilateral), but not on the contralateral hemisphere [B.5, C.4]. The area depicted by rectangles in B.2, B.5, C.1 and C.4 are magnified in B.3-4; B.7-8, C.2-3 and C.5-6, respectively. These micrographs show GFP+ cells in the parenchyma of dentate gyrus (DG), hilus [B.3, B.7] and accumulation in the vasculature [B.4, B.8] after AAV-CCL2. After LPS, GFP+ cells were found mostly in blood vessels [C.2, C.5] and the ventricular space of the third ventricle [C.3, C.6]. Asterisks (*) indicate the injection sites.

Table 1

List of Reagents

Reagent	Purpose	Cat #	Company
UF11	plasmid	#MBA-331	ATCC
CCL2	plasmid	na	in house
GFP Lentivirus	in vivo transfection	#LVT-300	Cell Biolabs
Hamilton syringe	intraosses and cerebral injections	#1702 RN	Hamilton
32G needle	bone marrow needle	#7803-04 RN	Hamilton
CD11b magnetic beads	isolation of CD11b+ bone marrow cells	#130-093-634	Miltenyi Biotec
VectaShield harset Mounting Medium	coverslipping media	#H-1400	VectorLabs