Microglia Stem Cell Therapy

Abstract

Cognitive functions of the brain decline with age. The resulting disability leads to a loss of quality of life but also of productivity and puts a severe strain on social care systems. There are no existing therapies and only hypotheses concerning their cause.

On of the protective cell types in the brain are called microglia and they loose function with age. Our project aim is therefore to replace the non-functional microglia with new and young cells. We will therefore produce these cells in the laboratory and use a stem cell source. We will test the hypothesis in an animal model transplanting the microglia into the blood. The first step is to detect cells in the mice and than we will determine the effect of the cells.

The actual effect of young microglia in the brain is then assessed by measuring microglia activity, proliferation of neuroprogenitors and plaque density in the brain. A reduction in plaque density of Alzheimer mice would be a first proof that the transplanted microglia are performing their expected function. It is also known that in the aged brain microglia activity is up regulated and that after a removal of junk, for example in brain lesions, the activity of microglia settles down. A little while later neurogenesis starts and the brain regenerates. We will, therefore measure microglia activity and neurogenesis.

Introduction

What are microglia: Microglia comprise about 10% of the cell population of the brain and form the main first immune defense of the CNS. They are phagocytic, cytotoxic, present antigens and they can promote repair after injury (Simard 2007).

Microglia in the brain display normally a quiescent state in which phagocytosis, immune response and migration are downregulated and the microglia show a ramified form with long processes (Davalos 2005; Nimmerjahn 2005).

Microglia react to lesions by proliferation. They migrate actively towards amyloid-β plaques in 1-2 days (Meyer-Luehmann 2008) and take on an amoeboid morphology (Kreutzberg 1996; Stence 2001)

Microglia are thought to originate from a special progenitor cell during embryogenesis and that they are replenished during life by myeloid progenitors migrating from the bone marrow which invade the brain and mature to microglia (Lawson 1992; Rezaie 2002; Chan 2007; Simard 2007). In fact it has been shown that bone marrow cells can be differentiated to microglia (Davoust 2006).

What role do microglia play in age: The incidence of Alzheimer and autoimmune diseases in age has been hypothesized to result from non-removed debris (Streit 2006). The common hypothesis points to a disregulation of old microglia and holds that overreactive and

constantly inflamed microglia might damage neuronal tissue (McGeer 1995). Newer findings suggest that microglia associated with tau pathologies are senescent instead of reactive and unable to perform function (Streit 2009). Microglia loose their ability to recognize and digest debris during age (Stolzing 2006). However studies involving complete microglia ablation for short periods did not result in increased plaque formation or neuritic dystrophy (Grathwohl 2009)

Replenishment of microglia during lifetime has been suggested to occur locally in the brain and by invasion of bone marrow progenitor cells (Streit 2006). Bone marrow transplantation has routinely resulted in bone marrow derived microglia in the brain (Rodriguez 2007) and intravenously injected hematopoietic stem cells have migrated to the brain, differentiated into microglia and reduced infarct. Newer findings however show the necessity of irradiation to obtain sufficient numbers of invading cells (Ajami 2007; Mildner 2007). This was suspected to be due to irradiation either raising the permeability of the blood brain barrier or to provide signals strongly enhancing proliferation. This complements the view that a possible replenishment throughout age both by local proliferation and by invasion of progenitors do not suffice to prevent the slow deterioration of microglia cell population and function in age (Stolzing 2006; Streit 2006; Flanary 2007; Sierra 2007; Sawada 2008).

Prospects and pitfalls of a microglia therapy: Both the hypothesis of disregulated, constantly inflamed microglia and the hypothesis of microglia senescence and non sufficient replenishment suggest the introduction of functional microglia as one possible solution.

The hypothesis of bystander damage by microglia (McGeer 1995) shows that the formation of massive plaques might only be a much later appearing symptom of the functional loss of the microglia population. In this view the actual Alzheimer disease starts already with the accumulation of relatively small amounts that act chronically inflammatory and is at the time of massive plaque formation already long at work. While dysfunctional microglia can not easily be removed from the brain the introduction of functional microglia might remove the causes for inflammation.

The hypothesis of microglia senescence and the association of dysfunctional, senescent microglia with plaques call even more readily for a cell therapy of microglia in age. In view of both hypotheses, a treatment of the symptoms, for example through down regulation of the inflammatory response, does not remove the underlying causes of aging. Even a degradation of plaques if not properly digested by dysfunctional microglia will not enhance Alzheimer condition. Therefore function of microglia has to be restored. Experiments on the influence of irradiation in bone marrow transplantation have shown that invasion of cells in absence of irradiation is low (Ajami 2007; Mildner 2007). Studies of non ablated, syngeneic conditions

have however shown that massive amounts of cells are necessary to archive comparable levels of chimerism in bone marrow (Ramshaw 1995). This points to a competition for stem cells niches which is alleviated by irradiation (Colvin 2004). In age, on the other hand, the permeability of the blood brain barrier increases which has also been linked to Alzheimer (Farrall 2009; Valle 2009).

We have worked extensively on the aging and functional loss of the microglia cell population (Stolzing 2006). I optimized differentiation of microglia from bone marrow and showed - to my knowledge for the first time - the actual functional capacity of bone marrow derived microglia (In progress of being published). Furthermore I could show that my bone marrow derived microglia invade brain slices *in vitro*. Based on the hypothesis that the functional loss of microglia in age is responsible for Alzheimer we propose to use our microglia derived from bone marrow in a cell based therapy of Alzheimer. We propose to introduce our differentiated microglia intravenously. Migration to the brain is tracked by sex based RTPCR. These functional microglia might then remove and digest the inflammatory debris in the brain, therefore remove the root cause of inflammation of aged microglia. Over a longer follow up period a down regulation of inflammation and onset of neurogenesis might take place. To assess such effects numbers, density and activation states of microglia, numbers and densities of neuronal progenitor cells and number, densities and size of plaques are measured using stereology.

Work plan outline

Part 1: Stem Cell Transplantation

Transplantation of 10^7 microglia differentiated from bone marrow stem cells into APP/PS1 Alzheimer mice and aged controls. Mice are sacrificed after a period of 30 days.

Part 2: Cell tracking

Tracking of microglia migration to the brain using RT-PCR based detection of male DNA in female hosts. RT-PCR detection of male DNA in female hosts allows to quantify the amount of transplanted cells in these tissues.

Part 3: Assessment of treatment effects

Analysis of the different brain regions (hippocampus, telencephalon, cerebellum) towards the distribution of amyloid plaques (marker used: thioflavin T), frequency of microglia (Iba-1), activity/proinflammatory of microglia (co-localization of Iba-1 and MHCII, versus IL-6) and frequency of neuronal precursor cells (co-localisation of DCX and KI67). To obtain absolute densities of cells and plaques we will use stereology based cell counting.

Therapy scheme

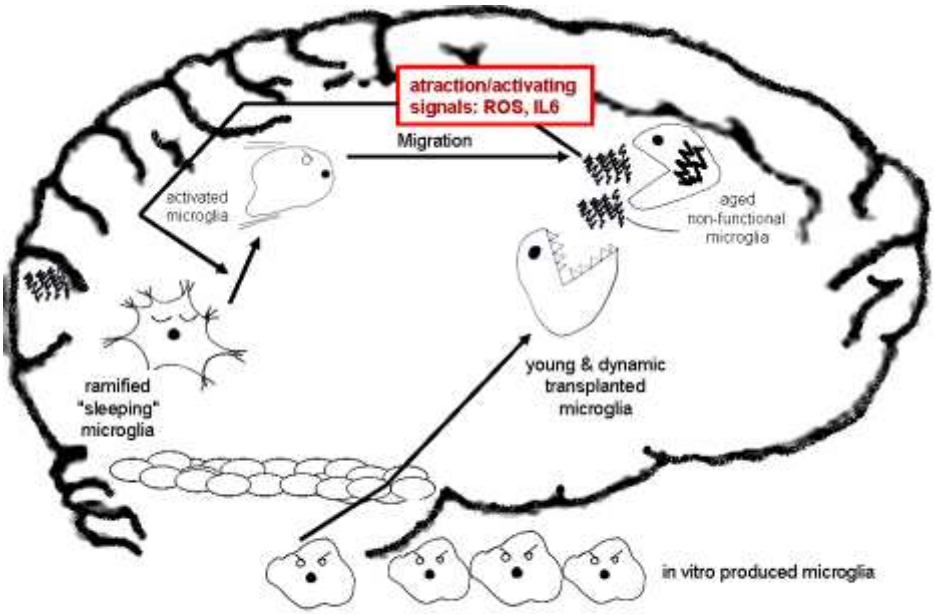


Fig. 1: The in vitro produced microglia are being injected into mice, they migrate to the brain and cross the blood-brain barrier. There they can migrate towards amyloid/protein plaques and digest them. This will hopefully decrease the permanent activation of the aged microglia, which can no longer degrade any plaques. These aged microglia are sometimes filled with protein aggregates which they can no longer degrade. The cycle of microglia activation and recruitment of brain resident microglia might be stopped, leading to a reduction of inflammation.

Time plan

Project part	**6 months**					
	1. month	2. month	3. month	4. month	5. month	6. month
Transplantation						
Cell tracking						
Stereology			Iba-1 MHC II	amyloid MHC II	amyloid IL-6	DCX Ki-67

Milestones

The project is modular structured. The minimal unit is the isolation and differentiation of the microglia, the transplantation and the tracking of the cells. After this the histology would follow in 4 steps.

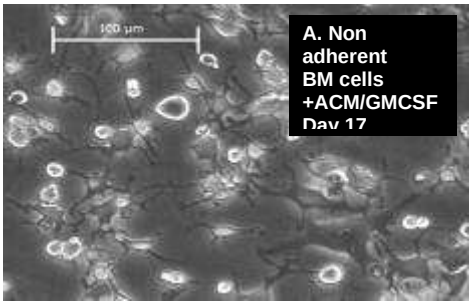
Methods

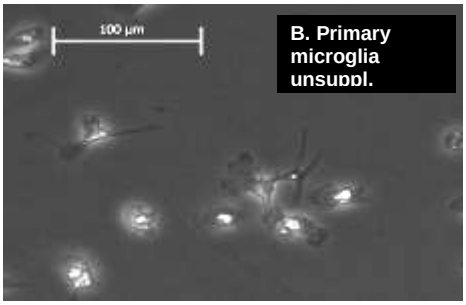
Part 1: Cell transplantation

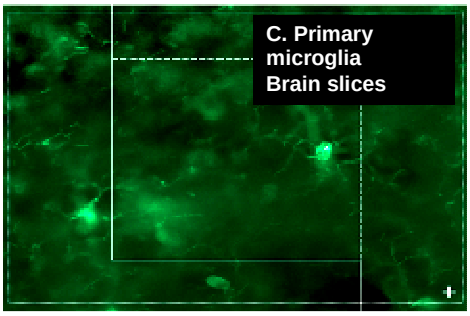
Animals: As Alzheimer model and transplant host we use Alzheimer APP/PS1 mice. As aged controls C57BL/6 mice are used. As bone marrow donors for microglia differentiation we use 2-3 month old C57BL/6 mice.

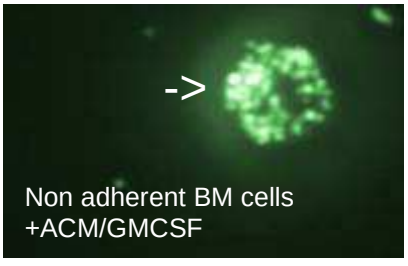
Cell differentiation: Bone marrow cells are differentiated to microglia using a combination of selective adhesion and cytokine supplementation (to be published).

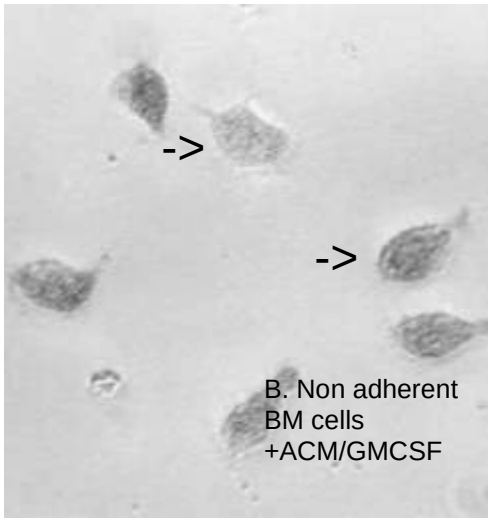
Briefly, 10^7 BM cells are cultured for 11 days in a 90mm petri dish in DMEM/10% FCS. After 11 days, non adherent cells from 2 petri dishes are flushed off and cultured for additional 6 days in a 60mm petri dish in DMEM/10% FCS, 50% ACM, 20ng/ml GM-CSF.











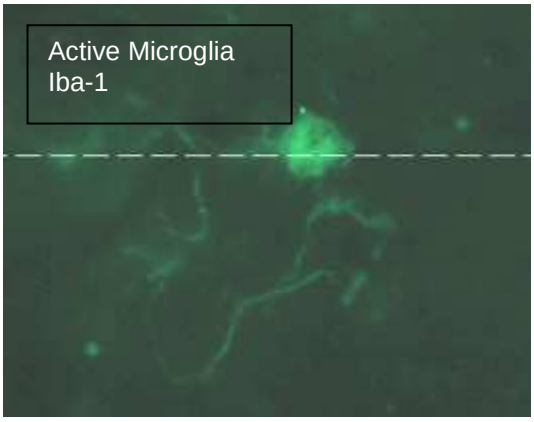
Transplantation: 10^7 BM derived microglia are harvested from differentiation culture and injected into the tail vein of Alzheimer (APP/PS1), mature (12 months old) and aged (18 months old) C57BL/6 control mice. Additionally non transplanted Alzheimer mice and aged control mice are compared. For injection Vasculon IV cannulas (BD) are used. Mice are sacrificed 7 days or 30 days after microglia transplantation. The animals are perfused and brains are cut with a CM-3050S cryostat (Leica) to obtain 40µm thick brain slices.

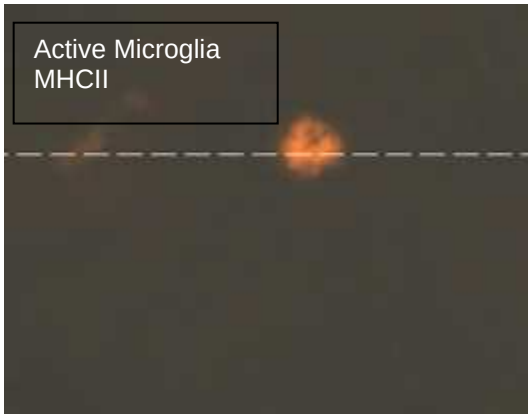
Part 2: Cell tracking

Cell tracking: Cells are tracked using RTPCR based on primers amplifying fragments on the Y chromosome. Donor cells are taken from male mice and transplanted into female mice. DNA is isolated from tissue of the transplanted mice using Trifast (Peqlab). To detect possible Y DNA in the tissues, RTPCR (Light Cycler 480 Roche) is run to amplify an 81bp fragment of the Y chromosome. Mixed male/female DNA is used as standard to quantify the Y copy number.

Part 3: Assessment of treatment effects

Stereology: Brains of transplanted Alzheimer and control mice are perfused and cut into 40 µm slices using a cryostat. A region is defined by the confinement of the hippocampus through the cornu ammonis and dentate gyrus. Microglia numbers, activation state and numbers of neuronal precursors are counted with Stereo Investigator (MBF Bioscience) in every 8^{th} brain slice using the optical fractionator method. From this data a stereological estimate of whole cell numbers, densities and volumes is made. Slices are stained for Iba-1 (microglia marker) and MHCII (activated microglia if colocalized with Iba-1). For measurement of neuronal precursor numbers DCX and Ki-67 are used as markers.





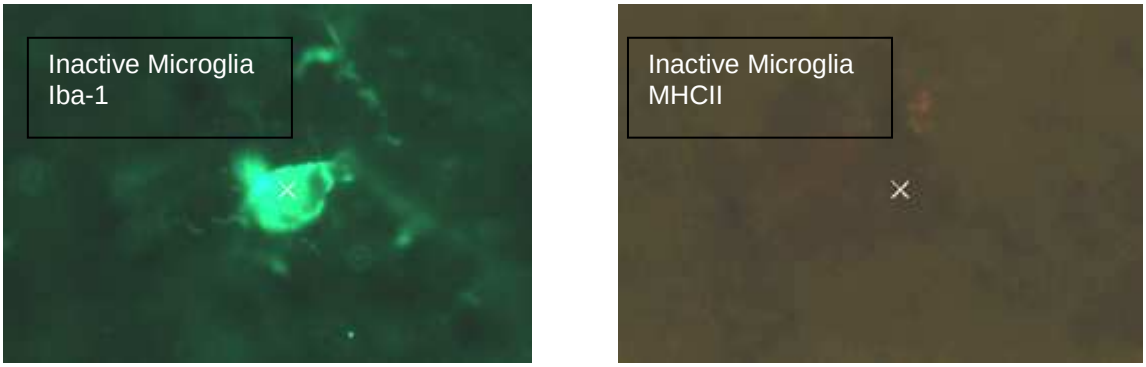


Fig. 3: Measurement of microglia activation. Both active and inactive microglia are positive for Iba-1 (green). Active microglia show a round morphology and are positive for MHCII.

Amyloid plaques: Amyloid plaques in Alzheimer mice are marked with thioflavin T. Plaques are counted in every 8th slice of the defined hippocampus region and absolute numbers and density estimated. For size measurement plaques are randomly chosen and measured with the stereological nucleator method (Stereo Investigator, MBF Bioscience).

Budget

Graduate student	$ 6,000 (1 month = 1,000)
Animal costs	$ 2,000
Cell culture media & cytokines	$ 1,000
DNA extraction & PCR	$ 1,000
Antibodies & histology	$ 2,000 (500 per step)
Summary	**$ 12,000**

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