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## The role of differently activated macrophages in axonal damage during multiple sclerosis

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# CHAPTER

# 7

## **Diverging effects of classically (M1) and alternatively (M2) activated macrophages on neurons**

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*Manuscript in preparation*

**Abstract:**

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) characterized by demyelinating lesions associated with axonal damage and axonal loss. Macrophages play a prominent role in MS and participate in both axonal damage and repair. These opposing functions may be exerted by distinct macrophage subsets. The two most extreme subsets are classically activated (CA/M1) pro-inflammatory macrophages, induced *in vitro* by 2 day exposure to LPS and IFN- $\gamma$ , and alternatively activated (AA/M2) anti-inflammatory macrophages, generated by 2 day exposure to IL-4 and IL-10. The aim of this study was to determine the effects of these differently activated macrophages on neuronal integrity *in vitro*.

Our results confirm the neurotoxic effect of CA macrophages, which are activated to produce reactive oxygen species (ROS), nitric oxide (NO) and other neurotoxic mediators. By alternative activation of macrophages, no neurotoxicity is observed either in co-culture or by conditioned media. Furthermore, we show for the first time that AA macrophages express higher levels of brain derived neurotrophic factor (BDNF), nerve growth factor (NGF) and platelet derived growth factor (PDGF) mRNA compared to CA macrophages.

In conclusion, CA macrophages were found to be toxic to neurons *in vitro*, while AA macrophages were not. During repair, AA macrophages could be beneficial, due to the production of growth factors. In MS lesions this could indicate that AA macrophages induce axonal repair, while CA macrophages produce bystander damage. Skewing the macrophage phenotype could therefore be an interesting therapeutical target.

### Introduction

The major pathological hallmarks of MS are multiple demyelinated lesions containing perivascular infiltrates and astrogliosis. The lesions are also associated with axonal damage and loss<sup>1</sup>. Axonal loss is the major correlate for clinical disability occurring in patients<sup>2,3</sup>. Axonal loss and injury take place both early and later in the disease course and is present both in acute and chronic lesions<sup>4-6</sup>. The degree of inflammation correlates with axonal loss and injury<sup>7</sup>.

Inflammatory infiltrates in MS lesions contain large amounts of macrophages. Several studies have found links between macrophages and axonal damage. In experimental autoimmune encephalomyelitis (EAE), an animal model for MS, it has been shown that elimination of infiltrating macrophages reduced both clinical signs and axonal damage<sup>8</sup>. Furthermore, during spinal cord injury (SCI), elimination of infiltrating macrophages increased axonal repair and functional outcome<sup>9,10</sup>. Clinical signs of EAE were significantly enhanced when the macrophage inhibitory signal of CD200/CD200R between neurons and macrophages was blocked<sup>11</sup>. This suggests a direct link between increased macrophage activity and aggravated disease course in EAE. Finally, macrophages are able to secrete a plethora of neurotoxic substances, such as matrix metalloproteinases<sup>12</sup>, reactive oxygen species (ROS)<sup>13</sup> and nitric oxide (NO)<sup>14</sup>. These studies all indicate that macrophages are detrimental.

However the role of macrophages is more complex. Several studies have shown that activated macrophages can actually be beneficial during CNS repair. During SCI macrophages can create a growth-permissive environment in which axonal regeneration can take place<sup>15,16</sup>. In MS lesions, activated macrophages can be observed in areas with increased growth-associated protein-43 (GAP-43) expression<sup>17</sup>. *In vitro* AA macrophage conditioned medium has been found to promote axonal outgrowth<sup>18,18</sup>. Furthermore, macrophages are beneficial because they remove myelin debris, which is growth inhibiting for axons<sup>19,20</sup>, they release growth factors<sup>21,22</sup> and they support axonal repair<sup>23-25</sup>.

The diverging effects of macrophages may be caused by the fact that macrophages are not one homogeneous cell population, but form a highly plastic cell population ready to respond to various stimuli in the environment. Macrophages are able to change their physiology and phenotype in response to many signals, creating different subsets of macrophages with diverse functions in homeostasis, immune response and tissue repair<sup>26-28</sup>. The two most studied and extreme phenotypes of macrophages are: classically activated (CA/M1) macrophages and alternatively activated (AA/M2) macrophages<sup>26,29,30</sup>. Due to the secretion of high amounts of pro-inflammatory cytokines<sup>31</sup> and oxygen and nitrogen radicals<sup>13</sup>, CA macrophages are effective in tumor killing and host defense against pathogens. However, in course of action, they can induce bystander damage. AA macrophages are considered to be anti-inflammatory due to the expression of anti-inflammatory cytokines<sup>29</sup>. Furthermore, AA macrophages are involved in repair processes such as angiogenesis<sup>32</sup> and extracellular matrix remodeling<sup>33</sup>.

In tissues outside the CNS the diverging effects of macrophages on damage and repair are investigated extensively. In this study we determined whether CA



and AA macrophages have diverging effects on neurons. We hypothesize that CA macrophages are harmful to neurons, while AA macrophages have neuroprotective effects.

### Materials and Methods

#### *Animals*


For neuronal cultures of neonatal cortex, timed pregnant C57BL/6 mice were bred in the animal facility. Adult C57BL/6 (Charles River, Maastricht, the Netherlands) mice were used for isolation of bone marrow.

All the experiments described in this paper were performed according to guidelines of the local University Committee on Animal Welfare, which follow the European Communities Council Directive (86/609/EEC).

#### *Primary neuronal cultures*

Primary neuronal cultures were prepared as described<sup>34</sup>. In brief, gestational day 19 mice were used. The skull and meninges were removed, the cortex isolated and divided into smaller fragments. The fragments were incubated with trypsin (1%; Sigma-Aldrich, Zwijndrecht, the Netherlands) in HBSS (Invitrogen, Breda, the Netherlands) and 0.1 mM DNase (Sigma-Aldrich) for 15 min at 37°. After washing, a single cell suspension was generated by 5 times trituration with a fire-polished Pasteurs pipette. The cells were brought to a concentration of  $0.25 \times 10^6$  cells/ml in complete neuron medium (containing Neurobasal medium (Invitrogen), 2% B27 supplement (Invitrogen), 1% glutamax (Invitrogen) and 0.1% gentamycin (Invitrogen)). The cells were plated on LabTek chamberslides (Nunc, Uden the Netherlands) or 96-wells plates (Greiner Bio-One, Alphen a/d Rijn, the Netherlands) coated overnight with poly-L-lysine (Sigma-Aldrich).

#### *Bone marrow derived macrophages*



Bone marrow derived macrophages were produced as described previously<sup>35</sup>. Bone marrow was flushed from the femurs and tibias of C57BL/6 mice. The cells were cultured for 7-10 days in complete macrophage medium (DMEM (Invitrogen) supplemented with 10% fetal calf serum (FCS, VWR, Amsterdam, the Netherlands), 15% conditioned medium from macrophage-colony stimulating factor-secreting L929 fibroblasts (ECACC, Sigma-Aldrich) and 2% penicillin/streptomycin-glutamine (Invitrogen)). After 7-10 days in culture the adherent cells had matured into macrophages (aprox. 98% pure). The cells were washed, to remove non-adherent cells, and used for the experiments.

To generate the CA phenotype macrophages were exposed for two days to  $5 \times 10^3$  U/ml IFN- $\gamma$  (U-Cytech, Utrecht, the Netherlands) and 10 ng/ml *Escherichia coli* LPS (026:B6; Sigma-Aldrich) in fresh culture medium. The AA phenotype was induced by two day exposure to 10 ng/ml IL-4 (Invitrogen, Breda, the Netherlands)<sup>36</sup> and 10 ng/ml IL-10 in the culture medium. Control macrophages were not exposed to these factors. In order to determine the phenotype of the macrophages, production of NO was measured using the Griess assay and FACS analysis was

performed. For the FACS analysis the macrophages were washed twice and the first antibody was added (for list see Table 1) for one h. After washing, the macrophages were exposed to the secondary antibody for one h. The macrophages were analysed using flow cytometry (FACS Calibur, Becton Dickinson, Erembodegem, Belgium) combined with Cellquest Pro software (Becton Dickinson). Omission of the primary antibody was included as negative control.

Primary antibody	Subtype	Dilution	Secondary antibody	Dilution
<b>F4/80</b> (Serotec, Oxford, UK)	IgG	1:500	Anti-mouse Alexa 488	1:400
<b>MAC-1</b> (Serotec, Oxford, UK)	IgG	1:500	Anti-rabbit Alexa-488	1:400
<b>MR</b> biotinylated (Biolegend, Uithoorn, the Netherlands)	IgG	1:100	Streptavidin-Alexa 488	1:400

**Table 1: Primary antibodies and dilutions used in FACS analysis of macrophages.**

The conditioned medium from control (Co-CM), CA (CA-CM) and AA (AA-CM) macrophages was harvested after the induction of the phenotype, at day 2.

Macrophages were harvested by 15 min incubation with lidocaine (4 mg/ml in PBS) at 37°C. Afterwards the macrophages were washed by centrifugation (5 min at 170 g).

#### *Exposure of neuronal cultures to differently activated macrophages*

After 2 days of culturing in chamberslides the neuronal cultures were exposed to differently activated macrophages. The macrophages were harvested as described above and brought to a concentration of  $0.2 \times 10^6$  cells/ml in a mixture of complete neurobasal medium and macrophage medium (3:1). The neurobasal medium from the neuronal cultures was replaced by 250  $\mu$ l of the macrophage suspension. The neurons were incubated with the macrophages for 4 h at 37°C. The cells were then fixed in 4% paraformaldehyde for 30 min, washed three times with PBS and stored at 4°C until immunohistochemistry.

For staining, fixed neurons in chamberslides were washed twice with PBS and incubated with normal goat serum for one h. Afterwards, the neurons were incubated overnight at 4°C with a mouse-anti-mouse- $\beta$ -tubulin (1:200, Covance, Uden, the Netherlands) in PBS containing 0.1% Triton. After washing, the neurons were incubated for one h with goat-anti-mouse IgG-Alexa 488 (1:400, Sigma-Aldrich) and rhodamine (1:300, Sigma-Aldrich) in PBS containing 0.1% Triton. To visualize the nuclei, the cells were counterstained with Hoechst (Invitrogen). The sections were embedded in mounting medium. Omission of the primary antibody was included as negative control.

For quantification of the number of neurons and neurites, at least 8 random positions were analysed per well from 3 to 5 separate experiments. Images were taken on a Leica DM6000 (Leica LAS AF software, Leica Microsystems, Bensheim, Germany). The number of neurons present in every microscopic field was counted using the image analysis program AnalySIS software (Soft Imaging System GmbH, Münster, Germany).



### *Exposure of neuronal cultures to conditioned medium from differently activated macrophages*

To determine the effect of conditioned medium of the differentially activated macrophages on neuronal integrity, primary neurons were cultured in a 96-wells plate. After 7 days in culture, the medium was removed from the neurons and either conditioned medium from differently activated macrophages (AA-CM, CA-CM or CO-CM) or fresh neurobasal medium was added for 24 hs. A methyl tetrazolium (MTT) assay was used to determine mitochondrial activity, as a measure for neuronal vitality. Neurons were exposed to 0.5 mg/ml MTT (Sigma-Aldrich) for one h. A 85% dimethyl sulfoxide (DMSO) and 15% 0.1M glycine solution was added and the neurons were gently shaken for 5 min for complete dissolution to take place. The resulting solution was transferred to a new 96-wells plate and absorbance was measured at 540 nm on a Benchmark microplate reader (Bio-Rad laboratories, Veenendaal, the Netherlands). All experimental conditions were performed in triplicate.

To generate damaged primary neurons, the neurons were cultured for 7 days in a 96-wells plate and then they were exposed to 0.02 mM vincristine (Sigma-Aldrich) for 1.5 hr. Vincristine binds to tubulin, preventing microtubule assembly. Afterwards, the neurons were washed and conditioned medium of the differently activated macrophages was added. The neurons were incubated with either conditioned medium or neurobasal medium for 24 h. An MTT assay was used to determine vitality at the end of the experiment.

### *cDNA synthesis*

RNA was isolated using TRIzol (Invitrogen) as described by the manufacturer. After 2 days *in vitro*, macrophages were washed with phosphate buffered saline (PBS) and one ml of TRIzol was added to 2 million cells. Phase Lock Gel-Heavy tubes (Invitrogen) were pre-spun briefly (1500 g for 30 seconds). The cell lysate in TRIzol was added to the Phase Lock Gel-Heavy tubes and incubated for 5 min prior to addition of 0.2 ml chloroform. The samples were centrifuged at 12,000 g for 15 min. The aqueous phase was transferred to a fresh tube and 0.5 ml isopropanol was added to precipitate the RNA. After shaking vigorously, the samples were incubated for 10 min at room temperature. The samples were centrifuged for 30 min at 12,000 g and 4°C. The supernatant was removed and the pellet was washed in 75% ethanol by vortexing. The samples were centrifuged for 15 min at 12,000 g and 4°C. The supernatant was removed and the pellet left to dry. Afterwards the pellet was dissolved in prewarmed RNase free water.

The cDNA synthesis was performed using reverse transcriptase system (Promega, Leiden, The Netherlands) as indicated by the manufacturer. Per sample 6 µl 25 mM MgCl<sub>2</sub>, 3 µl Reverse transcriptase buffer, 2 µl deoxyribonucleotides, 0.4 µl RNAsin, 0.4 µl AMV reverse transcriptase, 0.6 µl random hexamer primers and one µg of RNA was added. The total volume was brought to 20 µl using RNase free water. The samples were incubated for 10 min at RT and a further 15 min at 42°C. The final step is 5 min at 99°C.

<b>BDNF</b>	Forward: TGGAAGCCTGAATGAATGGAC
	Reverse: CAGGAACCCAGGAGAGTAACCA
<b>NGF</b>	Forward: GCTGGATGGCATGCTGGACC
	Reverse: GCTATGCACCTCACTGCGGC
<b>PDGFc</b>	Forward: ATTGGACCTGCTCAACAATGC
	Reverse: TAGAGGCTGTCCAAGTCCACCT
<b>PDGFd</b>	Forward: GCTGAAGCTGACCAATGCAGT
	Reverse: GCATGTGCAGGACTTCCAGTT
<b>HPRT</b>	Forward: CCTAAGATGAGCGCAAGTTGAA
	Reverse: CCACAGGACTAGAACACCTGCTAA
<b>B-actin</b>	Forward: TGACAGGATGCAGAAGGAGATTACT
	Reverse: AGCCACCGATCCACACAGA

Table 2: Sequence of primers used for qPCR

### *Real-time quantitative PCR*

Real-time quantitative-transcription-PCR using a LightCycler (Roche, Almere, the Netherlands) was performed in order to assess target gene mRNA expression. The detection system used was SYBR GREEN as described by the manufacturer. Target gene expression levels were corrected for GAPDH mRNA levels. The primer pairs used are listed in Table 2.

### *Western blot*

After stimulation of the macrophages, the cells were lysed. Using SDS-polyacrylamide gel electrophoresis proteins were separated. Subsequently they were blotted onto nitrocellulose membranes, which were blocked using 5% low fat milk powder and incubated with anti-BDNF antibody in PBS (1:100) overnight. After washing, the membrane was exposed to the secondary goat-anti-rabbit IRDye (LI-Cor, Homburg, Germany) for one h. Subsequently the membrane was washed and fluorescence was measured on an Odyssey infrared imager (LI-Cor).

### *Statistical analysis*

Statistics were performed in SPSS (15.0.0, Chicago, USA). All data are expressed as means  $\pm$  SEM of 3 to 4 separate experiments performed in duplicate or triplicate. The data were analyzed using a one-way ANOVA with Bonferoni correction and a p-value of  $<0.05$  was considered significant.

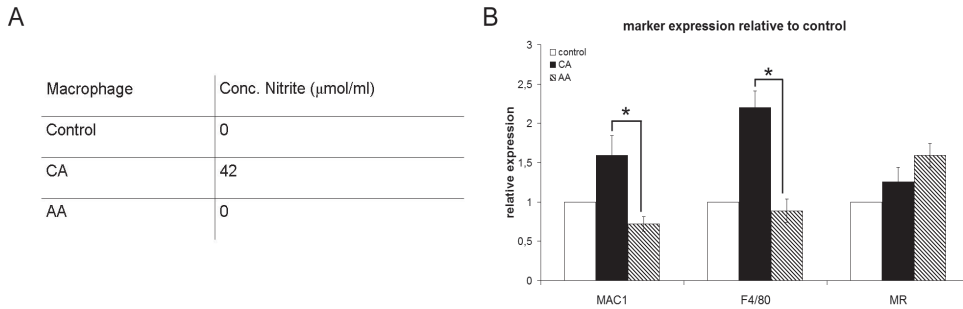
## **Results**

### *Macrophage phenotype*

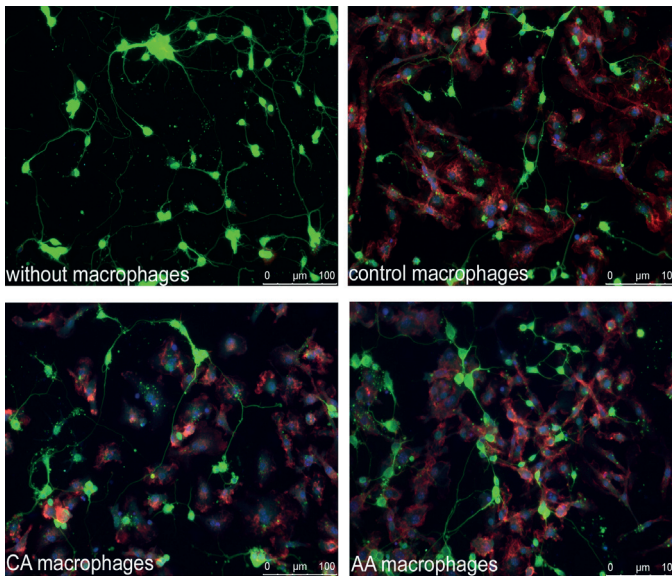
To confirm the phenotype of the differentially activated macrophages, the NO production was measured, as a marker for the CA phenotype. CA macrophages



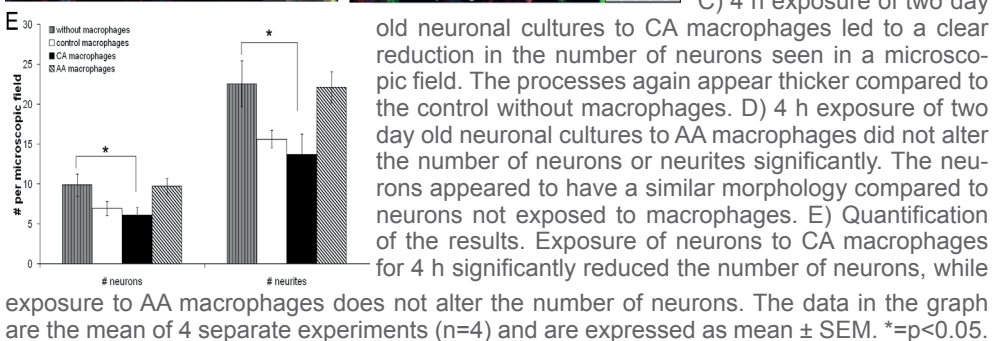




**Figure 1: Macrophage phenotype.** Macrophages were stimulated *in vitro* with either IFN- $\gamma$  and LPS, to induce the CA phenotype, or IL-4 and IL-10, to induce the AA phenotype, for 48h. Representative data are shown.  $*=p<0.05$ . A) NO production is significantly higher in CA macrophages compared to AA or control macrophages. B) FACS analysis of CA and AA macrophages. MAC-1 and F4/80 are consistently and significantly upregulated on CA macrophages. MR is consistently expressed higher on AA macrophages compared to CA macrophages, but this upregulation is not significant.



**Figure 2: Neuronal cultures after 4 hs of exposure to macrophages.** Neuronal cultures were stained for  $\beta$ -tubulin (green) and actin (red) using immunofluorescence. Blue: nuclear staining (Hoechst). Representative images are shown. A) In two day old neuronal cultures not exposed to macrophages many neurons could be observed with long thin processes contacting each other. B) Exposure of two day old neuronal cultures to control macrophages led to a reduction in the number of neurons. The processes of the neurons appear thicker. C) 4 h exposure of two day

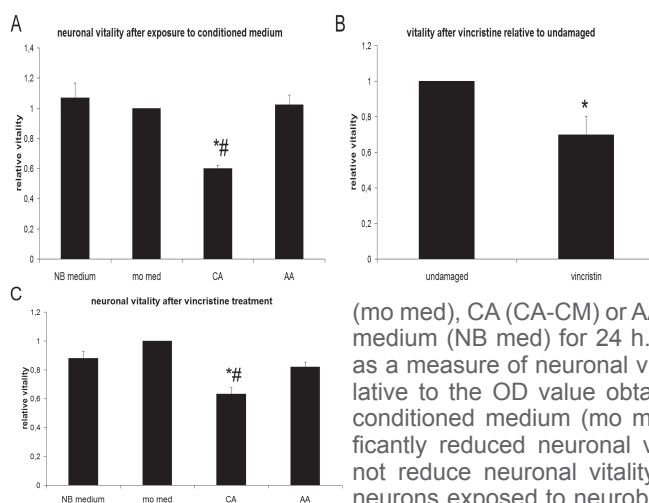


## Effects of CA and AA macrophages on neurons

were found to produce significant amounts of NO, while AA and control macrophages did not produce detectable amounts of NO (Figure 1A). Using FACS analysis, expression of F4/80, Mac-1 and MR was determined. MR is a well described marker for the AA phenotype<sup>36</sup>. Mac-1 expression was higher in macrophages stimulated with IFN- $\gamma$  and LPS<sup>37</sup>. The immunoreactivity for Mac-1 and F4/80 was significantly upregulated on CA macrophages, while MR immunoreactivity was slightly but consistently upregulated on AA macrophages (Figure 1B).

### Effect of CA and AA macrophages on neuronal cultures

To investigate whether the activation phenotype of the macrophages had an effect on neuronal integrity, primary cortical neurons were cultured in the presence or absence of CA and AA macrophages. After 4 h of exposure to control macrophages the number of neurons was slightly, though not significantly, reduced (Figure 2). Exposure to CA macrophages led to a significant ( $p < 0.05$ ) decrease in neuronal cell number. Exposure to AA macrophages did not lead to any significant decrease in the number of neurons compared to the control without macrophages. The number of neurons was significantly higher after exposure to AA macrophages compared to CA macrophages.



### Figure 3: Effect of exposure of neuronal cultures to conditioned media of differently activated macrophages.

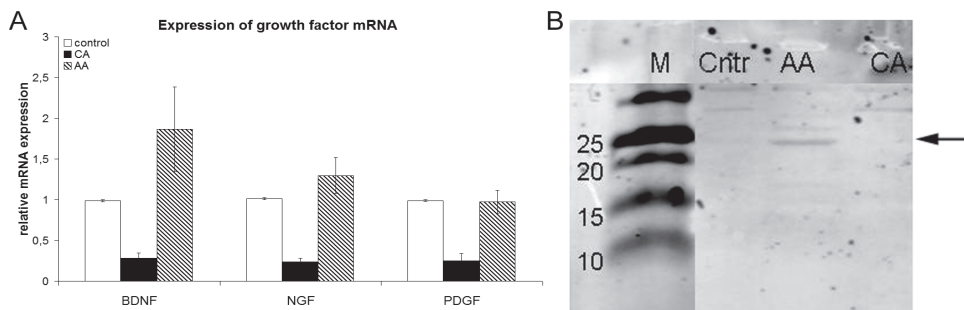
The data are expressed as the mean of 5 separate experiments ( $n=5$ ). A) Seven day old neuronal cultures were exposed to conditioned media of the differently activated macrophages (control (no med), CA (CA-CM) or AA (AA-CM) or complete neuronal medium (NB med) for 24 h. An MTT assay was performed, as a measure of neuronal vitality. Vitality was expressed relative to the OD value obtained using control macrophage conditioned medium (no med). Exposure to CA-CM significantly reduced neuronal vitality. Exposure to AA-CM did not reduce neuronal vitality. \*= significantly different from neurons exposed to neurobasal medium ( $p < 0.05$ ). # = significantly different from neurons exposed to AA-CM. B) Seven day old neuronal cultures were damaged by exposure to vincristine for 1.5h. After 24h neuronal vitality was assessed using an MTT assay. Vitality was expressed relative to the OD value obtained using neurobasal control medium. Exposure to vincristine significantly reduced neuronal vitality. \*= significantly different from neurons exposed to neurobasal medium alone. C) Seven day old neuronal cultures were damaged by exposure to vincristine for 1.5h. After the induction of damage by vincristine, the cultures were exposed to conditioned media of differently activated macrophages or complete neuronal medium for 24 h and an MTT assay was performed. Vitality was expressed relative to the OD value obtained using control macrophage conditioned medium. Exposure to CA-CM reduced vitality even further. Exposure to AA-CM led to a significantly higher vitality compared to CA-CM. \*= significantly different from neurons exposed to neurobasal medium ( $p < 0.05$ ). # = significantly different from neurons exposed to AA-CM.



### Effect of CA and AA conditioned medium on neuronal cultures

Next, we assessed whether differentially activated macrophages secrete factors that affect neuronal vitality, by exposing the neurons to conditioned media of the differently activated macrophages for 24h. Afterwards an MTT assay was performed to establish mitochondrial activity as a parameter for neuronal vitality. Changing the medium from neurobasal complete to CA-CM in the neuronal cultures led to a significant decrease in vitality. Exposure to AA-CM did not lead to a decrease in neuronal vitality (Figure 3A), since vitality was comparable to neuronal cultures exposed to complete neuronal medium.

To investigate whether AA-CM or CA-CM could contribute to neuronal repair, neuronal cultures were damaged using vincristine and exposed to either control, Co-CM, AA-CM or CA-CM for 24 h. After incubation with vincristine for 1.5 h, neuronal vitality was significantly reduced compared to control neurobasal medium (Figure 3B). Exposure to CA-CM significantly decreased neuronal vitality further compared to neurons exposed to vincristine and neurobasal medium (Figure 3C). Exposure to AA-CM resulted in a similar vitality compared to the neurobasal medium. Exposure to AA-CM for 24 h after vincristine led to a significantly higher vitality compared to CA-CM.



**Figure 4: Expression of PDGF, BDNF and NGF mRNA and BDNF protein.** A) The expression of growth factor mRNA was determined in 3 separate experiments. The data are expressed relative to control macrophages. A similar pattern emerged in all experiments. The expression of PDGF, BDNF and NGF mRNA was increased in AA macrophages compared to CA macrophages. B) BDNF protein was expressed at a higher level in AA macrophages compared to both CA and control macrophages.

### Expression of growth factors by CA and AA macrophages

Due to the activation of the arginase pathway and other downstream effects, AA macrophages can secrete growth factors<sup>38</sup>. Therefore, we determined the expression of nerve growth factor (NGF), platelet derived growth factor (PDGF) and brain derived neurotrophic growth factor (BDNF) in the differentially activated macrophages.

In 3 separate experiments a similar pattern emerged. The BDNF, PDGF and NGF mRNA levels were all higher in AA macrophages than in CA macrophages (Figure 4A). In line with these data the level of BDNF protein was found to be in-

creased in AA macrophages compared to CA macrophages in Western blot (Figure 4B).

### Discussion

In this study we investigated the effects of CA and AA macrophages on neuronal integrity. In the CNS, both macrophages and microglia, the resident macrophages of the brain, have beneficial and detrimental effects on neuronal integrity<sup>39</sup>. Here we investigated whether these effects were linked to an activational phenotype.

First of all, we observed that CA macrophages were neurotoxic in co-culture and secreted factors in CA-CM that were toxic. This is in line with our hypothesis, previous reports<sup>18;40;41</sup> and the many studies that have described the neurotoxic effects of macrophage products such as ROS, NO, glutamate and pro-inflammatory cytokines<sup>42</sup>, which are specifically produced by CA macrophages<sup>26;29</sup>. In CA-CM ROS and NO will probably not play a major role, since these are short lived substrates. In conditioned medium from macrophages stimulated by LPS and TNF- $\alpha$  glutamate was found to be responsible for neurotoxicity<sup>43</sup>, indicating that glutamate might play an important role in the neurotoxicity of CA macrophages. Glutamate could play an important role in neurotoxicity during MS<sup>44-46</sup>.

In contrast to CA macrophages, AA macrophages were not neurotoxic. Alternative activation of macrophages did not induce any of the damaging effects observed with CA macrophages. Both co-culture and AA-CM did not reduce neuronal integrity and vitality. Similar results were observed for microglia<sup>47;48</sup>, which possess an M2 phenotype<sup>49</sup>. Likewise, exposure to dexamethasone, a glucocorticoid, reduced the neurotoxic effects of macrophages treated with LPS<sup>41</sup> and glucocorticoids are an alternative way to induce the AA phenotype<sup>26</sup>.

We observed that after the induction of axonal damage, using vincristine<sup>50</sup>, neuronal vitality decreased significantly. The addition of CA-CM led to a further decrease in neuronal vitality, while addition of AA-CM did not. Indirect evidence supports our findings. Microglial conditioned medium was able to protect cerebellar granule neurons from 6-hydroxydopamine-induced neurotoxicity<sup>47</sup>, and glutamate excitotoxicity<sup>51</sup>.

Recently, an interesting paper addressed the diverging effects of CA/M1 and AA/M2 macrophages on neuronal integrity<sup>18</sup>. Kigerl *et al.*<sup>18</sup> showed, using conditioned media of differently activated macrophages, that conditioned medium from M1 macrophages was toxic, while conditioned medium from M2 macrophages was not and could even induce axon growth across a gradient of inhibitory substrate. The diverging effects of the conditioned media that we observed is in line with this finding. However, we extended these findings, in that we also looked at the effect of direct co-culture, we determined the effect of the conditioned media on damaged neurons and determined growth factor expression.

In our study we showed for the first time that alternative activation of macrophages induces the production of nerve growth factors such as PDGF, BDNF and NGF. During neurodegeneration, the production of nerve growth factors by AA macrophages may be beneficial. For example, BDNF and NGF induce neuronal rege-



neration after dorsal root transection<sup>52</sup>. PDGF expression could also be beneficial during MS, since it promotes neuronal differentiation<sup>53</sup> and has neuroprotective effects in cortical neurons<sup>54,55</sup>. In MS lesions macrophages are a potential source of BDNF<sup>56,57</sup>. It would be interesting to study the production of nerve growth factors by foamy macrophages in MS lesions, since these foamy macrophages have been shown to possess characteristics of an AA macrophage phenotype<sup>58</sup>.

In conclusion, our data indicate that CA macrophages are neurotoxic in MS lesions and promote axonal degeneration. In contrast, AA macrophages may contribute to axonal repair due to the expression of nerve growth factors. Shifting the balance from a CA to an AA activation state may therefore offer a promising strategy for therapy.

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