GM-CSF Upregulated in Rheumatoid Arthritis Reverses Cognitive Impairment and Amyloidosis in Alzheimer Mice

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Supplementary Fig. 1. Significant variation of amyloid plaque load between mice. Standard fluorescent immunohistochemistry used 6E10/Alexa 488 and Hoechst nuclear stain (blue). Bright green spots indicate amyloid plaques. Pictures taken at 5X. Mouse numbers 148, 160, 171, and 211 received bilateral intracerebroventricular infusions of M-CSF and mice 164, 170, 176, and 177 received bilateral intracerebroventricular infusions of artificial cerebrospinal fluid.
Supplementary Fig. 2. Intrahippocampal injection of M-CSF (left hemisphere) and artificial cerebrospinal fluid (aCSF) (right hemisphere). A) The image is a montage of ~35 5X pictures and is representative of the effects seen from anterior hippocampus to posterior in all 4 M-CSF-injected mice. B) This photo shows enlargement of the M-CSF-injected left hemisphere, as seen following saline perfusion. Note the small bump at the site of injection (arrow). C) Image shows cyst or tumor-like growth formed in the needle track at the site of M-CSF injection. Cryosectioned at 14 µm and stained with 6E10/Alexa 488 and Hoechst. Picture taken at 20X.
Supplementary Fig. 3. Intrahippocampal injection of G-CSF (left hemispheres) and artificial cerebrospinal fluid (aCSF) (right hemispheres). 
A) Images are montages of ∼35 5X pictures each. Amyloid plaques indicated as white spots. Cryosectioned at 14 µm and stained with 6E10/Alexa546 and Hoechst nuclear stain. Sections numbered 1 through 6 and correspond with anterior to posterior. B) Amyloid plaques show a modest reduction of plaque in the left G-CSF-injected hemisphere. Error bars are ± Standard Error of the Mean. (Area, Integrated Density: p < 0.05).
Supplementary Fig. 4. Intrahippocampal injection of GMCSF (left hemispheres) and artificial cerebrospinal fluid (aCSF) (right hemispheres). Representative sections of each mouse proximal to injection site. Tissue sections stained with MabTech α-Aβ/Alexa 488. White spots indicate amyloid plaque immunolabelling. Images are montages of about 145 pictures taken at 10X. Figures 4A-C are from 14 µm frozen sections, and 4D is from a 5 µm paraffin-embedded section.
Supplementary Fig. 5. Quantification of reduced amyloid deposition in GM-CSF-injected left hemispheres versus artificial cerebrospinal fluid (aCSF)-injected right hemispheres. There were 5 montaged sections per mouse quantified. Each montaged section contained over 140 10X pictures and of these, 15-25 pictures per hemisphere were selected to quantify as described in Supplementary Figure 6 online. Each figure shows total or average values from the 5 sections/mouse with significance per individual mouse and significance over all 4 mice. Error bars are ± SEM: a,b) plaque areas; c,d) perimeter values; e) average feret diameters; and f) average integrated densities.
Supplementary Fig. 6. Microscopy and method of Image J analysis of amyloid deposition. Lateral rows of photomicrographs were taken of coronal sections in a manner that ensured minimal overlap between each photomicrograph and corresponding anatomical areas in each hemisphere. Hoechst nuclear staining was used as a tool to scroll through sections, allowing for minimal overlap between each photomicrograph, and then the image was taken with the appropriate fluorescence. After all photomicrographs of the section were taken, they were montaged into a full coronal picture of the section, with the outline of each photomicrograph displayed (Axiovision 4.7 Panorama Module software). Photomicrographs were then selected for analysis from each hemisphere (Fig. 6A). The histogram of each photomicrograph was analyzed and the resulting means and standard deviations were entered into a spreadsheet. One photomicrograph of the section was then thresholded to select the amyloid plaques. This thresholded value was used in the spreadsheet to normalize the threshold values of the other photomicrographs to the same standard deviation from their respective histogram mean (Fig. 6B). Each photomicrograph was then analyzed for Area, Perimeter, Feret Diameter, and Integrated Density values from their predetermined threshold values. Any threshold-selected data which came in contact with the edges of any photomicrograph were deleted via the Image J “Analyze Particles” dialogue box, so that overlap of photomicrographs did not allow quantification of any amyloid deposition more than once.

Within the Image J “Analyze Particles” dialogue box, the Outlines mask was also selected to visually confirm that the plaques were quantified accurately (Fig. 6C). This Outline mask also numbered each plaque, which was used for selecting and eliminating artifact from the results. The results from each individual photomicrograph were copied into a 2nd separate spreadsheet, such that the data were separated into their respective hemispheres (CSF-treated versus artificial cerebrospinal fluid-treated). After all photomicrographs from a coronal section had been analyzed and data entered into the spreadsheet, the Area and Perimeter values were totaled and averaged. The Feret Diameter and Integrated Density values were also averaged. The overall data from each section (sums and averages) were then entered into a 3rd spreadsheet. A total of 5 sections per mouse, anterior to posterior, were analyzed and entered into this 3rd spreadsheet. A total of 4 mice per intrahippocampal-injected CSF were analyzed. Significance per mouse and over all 4 mice per CSF was determined by paired Students t test with p values < 0.5 considered significant. Supplementary Fig. 6A. Coronal section taken at 10X, showing demarcation of individual photomicrographs to be analyzed for amyloid deposition.
Supplementary Fig. 6B. Thresholding of one photomicrograph and using its threshold value to find the threshold value for all other photomicrographs within the coronal section at the same standard deviation from the mean.
Supplementary Fig. 6C. Example of the Oultines mask and underlying ImageJ spreadsheet data.
Supplementary Fig. 7. Behavioral Tasks.

Radial Arm Water Maze. For the RAWM task of spatial working memory, an aluminum insert was placed into a 100cm circular pool to create 6 radially-distributed swim arms emanating from a central circular swim area. An assortment of 2-D and 3-D visual cues surrounded the pool. The number of errors prior to locating which one of the 6 swim arms contained a submerged escape platform (9 cm diameter) was determined for 5 trials/day. There was a 30-min time delay between the 4th trial (T4; final acquisition trial) and 5th trial (T5; memory retention trial). The platform location was changed daily to a different arm, with different start arms for each of the 5 trials semi-randomly selected from the remaining 5 swim arms. During each trial (60 s maximum), the mouse was returned to that trial’s start arm upon swimming into an incorrect arm and the latency time required to locate the submerged platform was recorded. If the mouse did not find the platform within a 60-s trial, it was guided to the platform for a 30-s stay. The numbers of errors and escape latency during trials 4 and 5 are both considered indices of working memory and are temporally similar to standard registration/recall testing of specific items used clinically in evaluating AD patients.

Cognitive Interference Task. This task was designed to mimic, measure-for-measure, a cognitive interference task recently utilized clinically to discriminate between normal aged, MCI, and AD patients. The task involves two RAWM set-ups in two different rooms, with two sets of visual cues different from those utilized in standard RAWM testing. The task requires animals to remember a set of visual cues (in RAWM-A), so that following interference with a different set of cues (in RAWM-B), the initial set of cues can be recalled to successfully solve the RAWM task. Five behavioral measures were examined: A1-A3 (Composite three-trial recall score from first 3 trials performed in RAWM-A), B (proactive interference measure attained from a single trial in RAWM-B), A4 (retroactive interference measure attained during a single trial in RAWM-A), and A5 (delayed-recall measure attained from a single trial in RAWM-A following a 20-min delay between A4 and A5). As with the standard RAWM task, this interference task involves the platform location being changed daily to a different arm for both RAWM set-ups. For A1 and B trials, the animal is initially allowed one minute to find the platform on their own before being guided to the platform. Then the actual trial is performed in each case. As with the standard RAWM task, animals were given 60 s to find the escape platform per trial, with the number of errors and escape latency recorded per trial.