Microglial phagocytosis of giant aggregates of β-amyloid

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Abstract

Aggregates of lyophilized synthetic β-amyloid peptide 1-42 (Aβ) can reach sizes up to a hundred of micrometers, are remarkably stable in aqueous medium and exhibit properties that have been previously characterized by us and that are partially similar to those of senile plaque core of Alzheimer’s disease. In this work we investigate the phagocytosis of such giant aggregates of Aβ (GAAbs) stained with thioflavin T (TTh-T) by ameboid microglia isolated from developing embryonic or postnatal rat brains, and compare it to the phagocytosis of fluorescent latex beads under modulation of diffusible astrocytic factors, which influence the morphology of the microglia. Small latex beads (70 μm in diameter) are phagocytosed by microglia in both, round and ramified morphologies. In round morphologies, microglia phagocytose small GAAbs, but attack also GAAbs too large to be engulfed. First indications for digestion of GAAbs by microglia are presented.

Introduction

GAAbs can be studied directly with standard light microscopic techniques even under extreme solvent conditions. Utilization of GAAbs for understanding the phagocytosis and clearance of senile plaque cores of Alzheimer’s disease by microglia have not be possible. Thioflavin T fluorescence indicates the presence of aggregated structures. In general the stained GAAbs are also apparent GAAb presence could be observed. This might be indication for transport of aggregated material of small sizes to different regions of the cell and therefore for GAAb clearance. Further completely or partially destained GAAbs can be observed. Although most GAAbs are stained some unstained aggregates unfortunately can also be found outside of the cells. Therefore destained GAAbs inside of the cells at the moment can only be seen as indication for processes on the aggregates. Further research will be necessary to provide unequivocal prove that the destaining really occurs inside of the cell. Interestingly these GAAbs, although destained, show structural identical features of other GAAbs in the phase contrast images and not any indication that digestion might be at work at them. Phagocytosis and clearance of senile plaque cores of Alzheimer’s disease by microglia have been reported (DeWitt et al., 1998), and therefore it would be very surprising if microglia clearance of GAAbs should not be possible.

Phagocytic activity

Fluorescent latex beads (Sigma, 1 μm, polystyrene, amine-modified), fluorescent RED demonstrate normal phagocytic activity even of microglia deprived of astrocytic factors on ramification. Changes in GAAb staining might be interpretable as the result of GAAb cleavage processes, although in most cases no obvious structural changes in GAAb morphology have been observable. Further investigations will be necessary to clarify, if GAAb clearance really takes place.

Conclusions

• The phagocytosis of giant aggregates of β-amyloid (GAAbs) could be directly imaged in time series. It showed that:
  • Free microglia attach to the surface in 10-20 minutes
  • Microglia can stand unfavorable conditions (phys. temperature) for at least 30-45 minutes
  • Microglia can accumulate large quantities of GAAbs
  • Changes in GAAb staining might be interpretable as the result of GAAb clearing processes, although in most cases no obvious structural changes in GAAb morphology have been observable. Further investigations will be necessary to clarify, if GAAb clearance really takes place.

• Ramified morphology of the microglia was observed even over time, but could not clearly be linked to modulation by diffusible astrocytic factors on ramification as reported in the character (Vanier et al., 1998) could not be reproduced by us.

Microbial GAAbs phagocytosis – the process

Microglial phagocytosis of TTh stained GAAbs over time.

Microglia are shown at different times after the addition of mechanically crushed and previously TTh stained GAAbs. Notice the number of destained phagocytosed GAAbs and the increasing amounts of phagocytosed GAAbs.

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Non-phagocytosable GAAbs

GAAbs too large to be engulfed directly are nevertheless attacked by microglia.

GAAbs destaining. First indication for microglial digestion of GAAbs?

Thioflavin T fluorescence indicates the presence of aggregated structures. In general the stained GAAbs are also directly visible in the phase contrast images. But in certain cases, fluorescence in cellular regions not related to direct contact with the glass coverslip may give the impression of destaining. This effect is most likely due to the distribution of different regions of the cell and therefore for GAAb clearance. Further completely or partially destained GAAbs can often be observed. Although most GAAbs are stained some unstained aggregates unfortunately can also be found outside of the cells. Therefore destained GAAbs inside of the cells at the moment can only be seen as an indication for processes on the aggregates. Further research will be necessary to provide unequivocal proof that the destaining really occurs inside of the cell. Interestingly these GAAbs, although destained, show structural identical features of other GAAbs in the phase contrast images and not any indication that digestion might be at work at them. Phagocytosis and clearance of senile plaque cores of Alzheimer’s disease by microglia have been reported (DeWitt et al., 1998), and therefore it would be very surprising if microglia clearance of GAAbs should not be possible.

Microglial ramification

Ramification of microglia has been observed as a function of age of the culture. The image at the right shows the in vitro network formed by a 3 days old microglial network. The presence of astrocytic factors on ramification is required in the character (Vanier et al., 1998) could not be reproduced by us.

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