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Featured Article

Peripheral complement interactions with amyloid β peptide in Alzheimer's disease: 2. Relationship to Aβ immunotherapy

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Abstract

Introduction: Our previous studies have shown that amyloid β peptide (Aβ) is subject to complement-mediated clearance from the peripheral circulation, and that this mechanism is deficient in Alzheimer's disease. The mechanism should be enhanced by Aβ antibodies that form immune complexes (ICs) with Aβ, and therefore may be relevant to current Aβ immunotherapy approaches.
Methods: Multidisciplinary methods were employed to demonstrate enhanced complement-mediated capture of Aβ antibody immune complexes compared with Aβ alone in both erythrocytes and THP1-derived macrophages.
Results: Aβ antibodies dramatically increased complement activation and opsonization of Aβ, followed by commensurately enhanced Aβ capture by human erythrocytes and macrophages. These in vitro findings were consistent with enhanced peripheral clearance of intravenously administered Aβ antibody immune complexes in nonhuman primates.
Discussion: Together with our previous results, showing significant Alzheimer's disease deficits in peripheral Aβ clearance, the present findings strongly suggest that peripheral mechanisms should not be ignored as contributors to the effects of Aβ immunotherapy.

Keywords: Alzheimer's disease; Amyloid β peptide; Aβ immunotherapy; Complement; Complement receptor 1; Macrophage; Erythrocyte; Blood; Human

1. Background

Over the last decade, the most visible strategy for the treatment of Alzheimer's disease (AD) has been amyloid β peptide (Aβ) immunotherapy (reviewed in [1]). Although the first efforts with Aβ immunotherapy failed to complete clinical trials [1], AD transgenic mice [2,3] and human AD patients [4,5] did show significantly reduced Aβ burden after treatment. Perhaps as a result, Aβ immunization approaches continue to be pursued [1].

A critical, unresolved issue with Aβ immunization is whether or not its presumed mechanism of action, enhanced glial clearance of brain Aβ (e.g., [6–8]), provides a sufficient explanation for its reported effects. For example, an Aβ antibody, m266, that did not react with brain Aβ deposits and appeared to have most if not all of its effect in the periphery, nonetheless reduced brain Aβ levels in a transgenic AD mouse model [9]. This antibody formed immune complexes (ICs) with Aβ in the peripheral circulation [10] and appeared to induce efflux of brain Aβ to plasma

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[9,11], leading to the "peripheral sink" hypothesis [9–11]. Moreover, the penetration of Aβ antibodies into the CNS remains open to debate. Levites et al. [12], for example, reported that only 1 fmol/mg of Aβ antibody could be detected in AD transgenic mouse brain after a 500-μg intraperitoneal injection. This brain concentration of antibody is nearly three orders of magnitude less than estimates of total brain Aβ in the mice [12]. Cerebrospinal fluid concentrations of bapineuzumab, a humanized monoclonal Aβ antibody, are also found to be, on a molar basis, approximately three orders of magnitude less than typical cerebrospinal fluid Aβ concentrations (reviewed in [13]).

The above considerations, of course, do not necessarily disallow direct CNS actions of Aβ immunotherapeutics. Golde [14], for example, has cogently argued that if endogenous antibodies can have material effects on the CNS, which is clearly the case [15,16], then exogenous antibodies should be able to do so as well. On the other hand, considering that only minute quantities of peripherally administered Aβ antibodies reach the CNS, whereas they are wholly and directly exposed to circulating Aβ, it is difficult to understand how interactions of Aβ antibodies with circulating Aβ can be ignored as at least a potential, additional mechanism of action for Aβ immunotherapy.

We have explored specific mechanisms by which Aβ/Aβ antibody immune complexes (Aβ ICs) formed in blood in the course of Aβ immunization might enhance clearance of Aβ through enhanced interactions with the complement system. These studies were informed by the fact that major pathways for peripheral pathogen clearance in primates hinge on complement receptor 1 (CR1) [17], single nucleotide polymorphisms in which have been consistently identified as a significant risk factor for AD [18–22]. Compared with Aβ alone, we found that the presence of Aβ antibodies in the fluid phase dramatically increased virtually all steps in the major pathways for peripheral pathogen clearance in primates including complement activation, formation of complement-opsonized complexes that are ligands for CR1, and peripheral capture and disposal of Aβ through CR1-mediated erythrocyte and macrophage mechanisms. Consistent with these in vitro results, clearance of Aβ from plasma and erythrocyte compartments in vivo was also robustly enhanced in nonhuman primates intravenously (IV) inoculated with Aβ ICs. Although, as noted, these findings do not disallow CNS actions of Aβ immunotherapy, they do strongly suggest that peripheral effects should be considered as well—particularly because peripheral strategies might avoid the CNS adverse effects that have been encountered in previous AD immunotherapy trials [1,4,5].

2. Methods

2.1. Subjects

Human erythrocytes for the various experiments were obtained from study investigators under an Institutional Review Board–approved protocol. Two male cynomolgus macaque monkeys (25 years old, 7.0-kg weight and 28 years old, 6.5-kg weight) received intravenous injections of Aβ, and blood samples were taken from them at various intervals (see the following sections). These studies were performed under an Institutional Animal Care and Use Committee–approved protocol.

2.2. Preparation of Aβ

Lyophilized human synthetic Aβ(1–42) (GenScript, Piscataway, NJ) or FITC-conjugated Aβ(1–42) (Bachem, Torrance, CA) was resuspended in sterile 100% DMSO (Sigma, St. Louis, MO) at 10 mg/ml, diluted to 2 mg/ml in sterile ddH_2O, and then brought to 1 mg/ml in sterile 100 mM Tris, pH 7.4. The suspension was incubated overnight at room temperature (RT), in the dark, with shaking at 450 rpm. The resulting 1 mg/ml stock solution was then diluted with 100-mM Tris to achieve the concentrations employed in the experiments. Western blots of Aβ solutions prepared in this manner showed the presence of Aβ aggregates at multiple molecular weights, an important point because the monomeric form of Aβ poorly activates complement, if at all, whereas Aβ aggregates are relatively potent activators [23].

2.3. Serum complement activation

Various concentrations of Aβ prepared in 100 mM Tris, as previously mentioned, were incubated with either Aβ antibody (4G8; Biolegend, San Diego, CA) or PBS, pH 7.2, after which the solutions were mixed with normal human serum (NHS) (CompTech, Tyler, TX) for 30 minutes at 37°C. NHS plus 10 mM EDTA (Amresco, Solon, OH) (final concentration in the serum) was employed as a control. C3a production, one of several standard measures of complement activation, was assayed by ELISA (Affymetrix, Santa Clara, CA, #BHS2089) following the manufacturer's protocol.

2.4. iC3b Western blots and densitometry

NHS was incubated with 300-μg/ml Aβ alone or Aβ ICs for 30 minutes at 37°C to permit complement activation, generation of complement opsonins, and their covalent binding to Aβ. To form Aβ ICs, a 9:1 molar ratio of Aβ:4G8 antibody was employed, as this ratio gave optimal complement activation (see Section 3.1). As a control to block complement activation and opsonization, 10-mM EDTA was added to NHS before incubation with Aβ or Aβ ICs. The solutions were run under reducing/denaturing conditions on SDS-PAGE 4–15% mini-PROTEAN TGX gels (BioRad, Hercules, CA, #146-1086), transferred to PVDF membranes (BioRad, #170-4156), blotted with a biotinylated (Thermo Fisher Scientific, Waltham, MA, #21326) iC3b antibody (Quidel, San Diego, CA), and imaged on an Odyssey Imaging System (LI-COR, Lincoln, NE). To control for any effects of