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Amyloid beta immunization worsens iron deposits in the choroid plexus and cerebral microbleeds

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ABSTRACT

Anti-amyloid beta (Aβ) immunotherapy provides potential benefits in Alzheimer's disease patients. Nevertheless, strategies based on $A\beta_{1-42}$ peptide induced encephalomyelitis and possible microhemorrhages. These outcomes were not expected from studies performed in rodents. It is critical to determine if other animal models better predict side effects of immunotherapies. Mouse lemur primates can develop amyloidosis with aging. Here we used old lemurs to study immunotherapy based on $A\beta_{1-42}$ or Aβ-derivative ($K6A\beta_{1-30}$). We followed anti-$A\beta_{40}$ immunoglobulin G and M responses and Aβ levels in plasma. In vivo magnetic resonance imaging and histology were used to evaluate amyloidosis, neuroinflammation, vasogenic edema, microhemorrhages, and brain iron deposits. The animals responded mainly to the $A\beta_{1-42}$ immunogen. This treatment induced immune response and increased Aβ levels in plasma and also microhemorrhages and iron deposits in the choroid plexus. A complementary study of untreated lemurs showed iron accumulation in the choroid plexus with normal aging. Worsening of iron accumulation is thus a potential side effect of Aβ-immunization at prodromal stages of Alzheimer's disease, and should be monitored in clinical trials.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease that is the most common cause of dementia. Anti-amyloid beta (Aβ) immunotherapies aim to reduce the Aβ lesions that are critical for the pathogenesis of this disease (Hardy and Selkoe, 2002). They can be dissociated into: (1) active immunotherapies during which Aβ or Aβ derivative proteins are injected to activate the immune system and elicit anti-Aβ antibodies; or (2) passive immunotherapies that rely on the administration of anti-Aβ antibodies. The initial evaluation of these therapies in transgenic mouse models of β-amyloidosis, was based on active strategy with $A\beta_{1-42}$ peptides in Freund's adjuvant. The outcome was a reduction of Aβ plaques (Schenk et al., 1999) and a stabilization of cognitive performance in these models (Janus et al., 2000; Morgan et al., 2000). These successes led to a first clinical trial based on administration of synthetic $A\beta_{1-42}$ peptide associated with the QS21 adjuvant (AN1792) in patients with clinical criteria for a diagnosis of AD. This trial decreased Aβ load (Ferrer et al., 2004; Masliah et al., 2005; Nicoll et al., 2003), reduced some but not all (Holmes et al., 2008) of the neuronal alterations that characterize AD (Boche et al., 2010; Serrano-Pozo et al., 2010),

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and provided some cognitive benefits in certain patients (Gilman et al., 2005; Hock et al., 2003). However, this first clinical trial induced meningoencephalomyelitis in some individuals (Orgogozo et al., 2003). This alteration was attributed to cytotoxic T cells and/or autoimmune reactions to AN1792. Other possible side effects of immunotherapies such as severe cerebral amyloid angiopathy (CAA) and microhemorrhages have also been reported during this trial (Ferrer et al., 2004; Uro-Coste et al., 2010). Also, in most patients without meningoencephalomyelitis from the AN1792 trial, cognitive outcomes were not modified by the therapy (Holmes et al., 2008). Since this first trial, several clinical trials have been initiated using either active or passive immunotherapies (see Aisen and Vellas, 2013 and Mangialasche et al., 2010 for reviews). They provided interesting results such as a reduction of amyloid load (Rinne et al., 2010), but no significant improvement of cognitive outcomes (Aisen and Vellas, 2013). They also reported side effects such as microhemorrhages and vasogenic edemas (Sperling et al., 2011), although the latter lesion seems to occur mainly during passive immunotherapy and not in active immunotherapy. The side effects that can be detected in vivo using magnetic resonance imaging (MRI) in humans have been called "amyloid imaging-related abnormalities" (Sperling et al., 2011).

After these outcomes, several points became obvious for further trials. First, new trials should be administered in prodromal stages of the disease. Second, approaches based on active immunotherapy should selectively target B-cell epitopes leading to humoral (Th2) immunity and antibody production without stimulating T cells to avoid neuroinflammation and toxicity. This can be done by selecting appropriate adjuvants and vaccines. For example, the alum adjuvant might be better than Freund's adjuvant because it promotes humoral immunity (Asuni et al., 2006; Cribbs et al., 2003). Regarding the vaccines, several developments tried to reduce or eliminate the midregion and C-terminal part of Aβ because it contains T-cell epitopes and retains the 2 major immunogenic sites of Aβ peptides (i.e., the 1–11 and 22–28 residues) (Cribbs et al., 2003; Jameson and Wolf, 1988). For example, some approaches were based on the use of the $A\beta_{1-6}$ (Wiessner et al., 2011), $A\beta_{1-15}$ (Ghochikyan et al., 2006; Muhs et al., 2007), $A\beta_{1-15}$ derivatives (Maier et al., 2006), $A\beta_{1-16}$ (Muhs et al., 2007), or $A\beta_{1-28}$ (Petrushina et al., 2008) peptides. In a previous work, we designed the $K6A\beta_{1-30}$, a nonfibrillogenic, nontoxic Aβ homologous peptide which has 6 lysines on the N-terminus to increase immunogenicity and enhance solubility. This modification, in addition to removal of the C-terminal amino acids of Aβ, also reduces its propensity to form β-sheets. This immunogen elicited an antibody response similar to $A\beta_{1-42}$ in mice which resulted in a comparable therapeutic efficacy (Sigurdsson et al., 2001). Third, outcomes of the first trial also highlighted the need to test anti-Aβ vaccines in non-transgenic animal models to better predict their efficiency and potential side effects. For example, Lemere et al. (2004) and Gandy et al. (2004) evaluated immunotherapy with $A\beta_{1-42}$ in Freund's adjuvant in old Caribbean Vervets and Rhesus Macaques, respectively. They showed that immunized primates generated anti-Aβ antibodies. Plasmatic Aβ levels were elevated in the immunized animals although, unlike control animals, they had no plaque deposition in the brain.

Here, we investigated immunotherapy based on $A\beta_{1-42}$ or Aβ-derivatives administered with alum adjuvant in old mouse lemurs. In this small primate (100 g), 5% to 20% of aged animals develop Aβ amyloidosis (Languille et al., 2012; Mestre-Frances et al., 2000). A previous study in young animals, comparing $A\beta_{1-42}$ and Aβ-derivatives, has shown that immunization promotes antibody response against $A\beta_{1-40}$ and $A\beta_{1-42}$ and increases plasmatic Aβ load (Trouche et al., 2009). Here, we studied animals without amyloid plaques or with a very small extracellular amyloid load, but presenting with intracellular and vascular amyloid deposits. We show that $A\beta_{1-42}$ immunization increases plasmatic Aβ levels, and also microhemorrhages and iron deposition in the choroid plexus (CP) of aged animals including in Aβ-plaque free animals. The latter effect is a new potential side effect of anti-Aβ treatment administered at the prodromal stage of the disease.

2. Methods

2.1. Animals

Our study evaluated the effects of immunotherapy and aging in mouse lemurs. First, the immunotherapy study was performed in 20 animals aged 4.1 to 6.4 years: a first cohort of 8 animals (5.9 ± 0.1 years) were treated with $A\beta_{1-42}$ ($n = 4$) or with $K6A\beta_{1-30}$ ($n = 4$) vaccines and were followed-up using MRI and biochemical parameters (antibody titers, Aβ levels in plasma) during 10 months (Fig. 1); a second cohort of 12 animals (4.7 ± 0.2 years) were followed with the same protocol but treated with $K6A\beta_{1-30}$ ($n = 6$) or with the adjuvant alone ($n = 6$). The brains of these 20 animals were then histologically evaluated. Second, the aging study was performed in 28 non-treated mouse lemurs aged 1.6 to 6.4 years (young adults, $n = 9$; 1.9 ± 0.2 years; middle-aged, $n = 11$; 4.5 ± 0.1 years; and old, $n = 8$; 5.9 ± 0.1 years) that were studied using MRI in vivo. All the animals were born and raised in a laboratory breeding colony at Montpellier, France. Animal care was in accordance with institutional guidelines and the animal protocol was approved by the local ethics committee (authorization #CEEA-LR-1002).

2.2. Peptides

The peptides used for the immunization were synthesized using the solid-phase technique at the Keck Foundation at Yale University, as previously described in Asuni et al. (2006) and Sigurdsson et al. (2004).

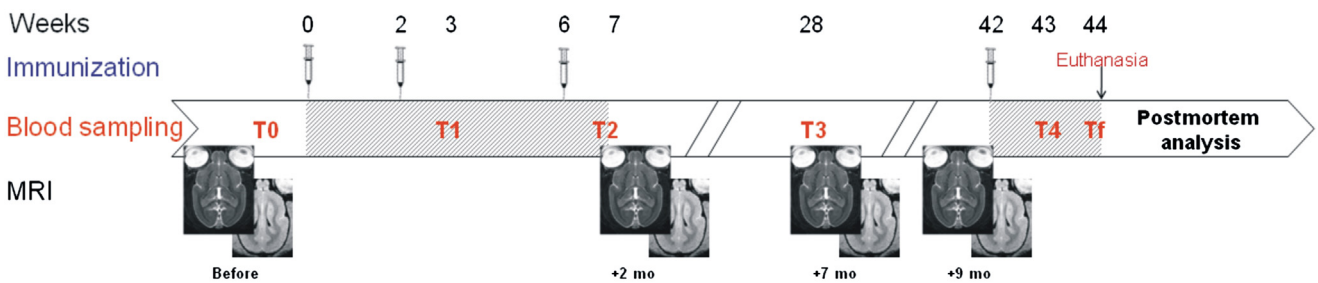

Fig. 1. Diagram depicting the timeline of the immunizations, bleeds for measurements of antibody response and amyloid beta levels, and magnetic resonance imaging sessions. Hatched areas correspond to immunization and second phase of immunization.