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Potential neurotoxic inflammatory responses to Aβ vaccination in humans

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Summary. Studies in transgenic mouse models of Alzheimer's disease suggested the development of a vaccine that would induce the production of antibodies against amyloid-β (Aβ) peptide, which in turn would stimulate microglia to phagocytose and remove senile plaques. However, some patients in the human clinical trials developed symptoms of brain inflammation, demonstrated by lymphocyte infiltration and elevated protein levels. These parameters are indicative of a breakdown of the blood-brain-barrier and entry of T-cells into the brain. Aβ-specific activated T-helper cells have the potential to amplify the existing pro-inflammatory conditions that are present in the brains of Alzheimer's disease patients. Cytotoxic T-cells might even attack the amyloid precursor protein which is present on the surface of many cells, including neurons. Before undertaking further vaccination trials there is a need to re-assess the risks associated with Aβ vaccination and with the therapeutic containment of a neuroinflammatory response. These risks may not be justified in the light of recent studies which have shown the efficacy of conventional, low-risk treatments in slowing the progress of AD.

Keywords: Alzheimer's disease, vaccine, inflammation.

Elan Corporation has been testing a possible cure for Alzheimer's disease (AD) using a vaccine-directed approach against amyloid-β (Aβ), the presumed neurotoxic component of the neuritic plaques that deposit in the brains of AD patients. In phase I of the clinical trial the vaccine, AN-1792, was administered in a variety of dosage regimens to more than 100 patients with mild to moderate AD. The results indicated that AN-1792 was well tolerated and that a certain number of patients had developed a sufficient immunological response to AN-1792 to warrant initiation of phase 2 trials. However, in March 2002, Elan Corporation and Wyeth-Ayerst Laboratories halted their

phase 2A clinical trials after 15 out of 360 patients worldwide developed symptoms of central nervous system inflammation; two even suffered ischemic strokes (Steinberg, 2002). Elan's spokesperson, Ivan Lieberberg, claimed that the negative outcome of AN-1792 had not been anticipated and that the cause of the nervous system inflammation was unknown. Elan Corporation and Wyeth-Ayerst Laboratories recently announced their intention to test new vaccines against Aβ which they hope will avoid the inflammatory side-effects (Steinberg, 2002).

It may indeed be possible to avoid inflammatory side-effects with other vaccines once the cause of the inflammatory response to AN-1792 is known, but the list of suspects is long and much research will be needed to eliminate these possibilities. One of the difficulties confronting researchers is that AD patients already experience a chronic inflammatory process in the vicinity of their neuritic plaques (Aisen, 1996; McGeer and McGeer, 1997). Involvement of the immune system is evident from the presence of complement proteins of the classic pathway and by the activation of microglia, resulting in the release of pro-inflammatory cytokines and chemokines including IL-1, IL-6 and TNF-α as well as free radicals (Smith et al., 1996). Aβ fibrils can be modified non-enzymatically by endogenous sugars such as glucose, fructose and methylglyoxal to form "advanced glycation endproducts" (AGEs), that activate specific pro-inflammatory signal transduction pathways in which the receptor for AGEs (RAGE), and oxygen free radicals (as second messengers) are major players in intracellular signaling. Aβ and AGEs activate redox-sensitive transcription factors including NF-κB and AP-1 and lead to the subsequent upregulation of neurotoxic cytokines including IL-1, IL-6 and TNF-α (Neumann et al., 1999). The relevance of brain inflammation for AD is also demonstrated by the beneficial effects of non-steroidal anti-inflammatory drugs (NSAIDs) and anti-inflammatory antioxidants (McGeer and McGeer, 1998; Rogers et al., 1993; Hager et al., 2001). It is possible therefore, that the brain inflammation in patients vaccinated with AN-1792 indicates a "successful" response, in which antibodies have bound to neuritic plaques and have boosted the chronic inflammatory response into an "active" response facilitating the clearance of Aβ from the brain. In this scenario, the brain inflammation can be expected to subside once the plaque burden has decreased, and this should be accompanied by a stabilization, or even a recovery of cognitive function.

Other scenarios are less desirable. For example, the inflammation in the AN-1792 trials may have been due in part to activation of T-cells. Antibody production requires the activation of TH2 memory effector cells. In the elderly however, the predominant T-cell population are TH1 cells, which produce pro-inflammatory cytokines (eg. interferon-γ (IFN-γ) when stimulated by Aβ vaccination. IFN-γ in combination with Aβ, activate microglia, which secrete neurotoxic factors including TNF-α and nitric oxide (McMillian et al., 1995). In addition, the Aβ-specific T-cell line repertoire contains a high percentage of CD8-positive cytotoxic T-cells, which are capable of lysing neurons and astrocytes that present the Aβ sequence (Grubeck-Loebenstein et al., 2000). Thus any increase in the level of T-cell mediated neuroinflammation

caused by vaccination runs the risk of accelerating neuronal loss in AD patients who are already burdened with high levels of oxidative stress and inflammation (Halliday et al., 2000; Retz et al., 1998).

A further complication relates to a difficulty in being able to limit the inflammatory response to the insoluble Aβ in plaques while sparing soluble Aβ monomers, the parent protein [amyloid precursor protein (APP)] and APP fragments. Such selectivity is not easy to achieve because soluble and insoluble Aβ and APP share the same amino acid sequence. The soluble form of Aβ is present in all human brains throughout life, and while its function is unknown its ubiquity strongly suggests that it contributes to normal brain function. Soluble APP fragments are known to have trophic properties, and APP plays a key role in assisting neuronal recovery and repair following brain injury (Masters and Beyreuther, 1997; Selkoe et al., 1996). Since most of the Aβ epitope is located at the extracellular side of APP, there is a risk that antibodies to Aβ might recognize the native APP protein at the cell surface. Binding of antibodies to the cell surface could lead to complement activation and subsequent opsonisation. An auto-immune response to APP will increase the degree of injury sustained by brain cells while simultaneously reducing the brain's capacity for recovery and repair. If the cases of nervous system inflammation in the AN-1792 trials involve such a response, other patients in these trials who have a lower titer of antibodies may develop the same symptoms and associated brain damage over a longer period of time.

An important feature of the insoluble Aβ in plaques is that it exclusively forms β-pleated sheets. By immunising patients with this conformation of Aβ, it might be possible to generate antibodies that cannot recognise soluble Aβ or APP. This approach, which reminds us of Paul Ehrlich's (incorrect) "Instruction theory" of antibody specificity, is not certain of success because it is not known how antigen-presenting cells process Aβ fibrils and whether the B-cell response can be limited to antibodies that exclusively recognize the β-sheet conformation of fibrillar Aβ. Advocates of this approach have noted that experiments in animals have demonstrated that it is possible to limit the auto-immune response to the production of antibodies that mark fibrillar plaques for receptor-mediated phagocytosis. Such a process appears to occur in transgenic mice that overexpress mutated human APP. These mice develop Aβ plaques and associated memory impairment; vaccination with human Aβ causes antibodies to target the plaques, which are then removed by phagocytes and memory is restored (Arendash et al., 2001; Games et al., 2000; Schenk et al., 1999) There is however, an important difference between these mice and humans. The antibody response in mice is elicited against the human form of Aβ, which has an amino acid sequence that is different from the mouse form. This allows the production of high titer antibodies against the non-functional human Aβ (Dickey et al., 2001), while limiting the humoral- and cell-mediated response against the endogenous mouse Aβ sequence. Therefore, the autoimmune response in mice is less likely to run the risk of incurring collateral damage by targeting functionally important APP and soluble Aβ.

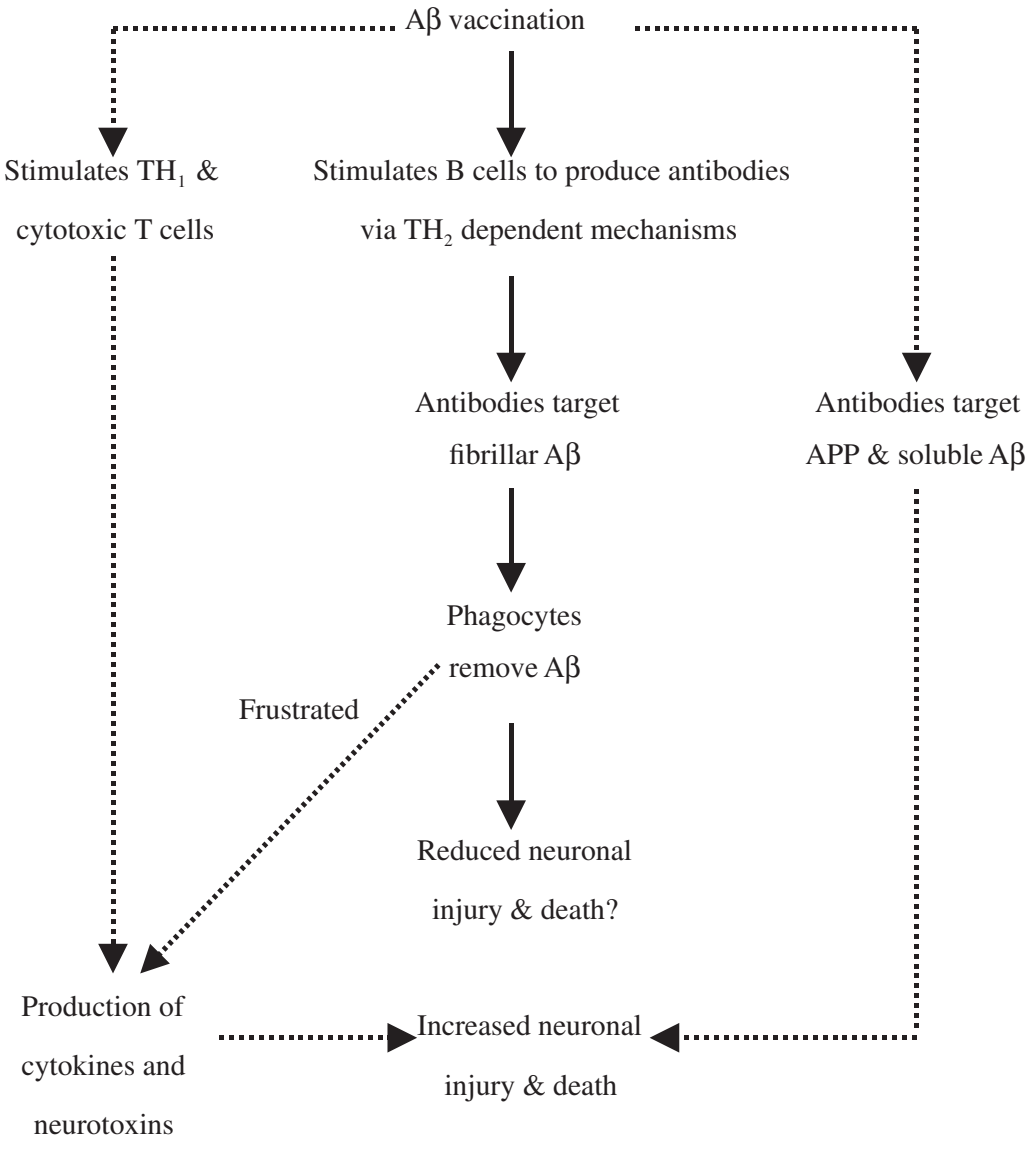

Fig. 1. Potential immunological outcomes of Aβ vaccination. Solid arrows indicate the optimal outcome, while the dotted lines indicate routes that lead towards a undesirable and neurotoxic inflammatory response

A second important difference between transgenic mice and humans relates to the conformation of Aβ in plaques. It is doubtful whether Aβ plaques can be as easily removed from the brains of AD patients as they can from transgenic mice. Plaques in AD brains are characterised by a high degree of covalent protein crosslinks, including those formed by dityrosines or AGEs (Wong et al., 2001). These crosslinks make proteins highly resistant to degradation and limit their solubility under physiological conditions. For example, plaques from APP transgenic mice can be dissolved in phosphate buffer, whereas Aβ plaques from AD brains require harsh solvents such as formic

acid (Kalback et al., 2002). If plaques cannot be phagocytozed and cleared, chronic activation of microglia and perhaps also astrocytes ("frustrated phagocytosis") will occur, involving the sustained release of free radicals and neurotoxic cytokines (Schubert et al., 1998).

Ivan Lieberburg, Elan's chief scientific and medical officer, revealed more data about immunological parameters in the affected patients. Whereas antibody titers did not correlate with the presence or severity of symptoms, elevated protein and lymphocyte levels, but no viruses or bacteria were found in the CSF. These findings are supportive of the hypothesis that activated T-cells might have entered the brain, as is thought to occur in multiple sclerosis (Steinberg, 2002).

Clinical outcomes of other autoimmune disorders such as multiple sclerosis and systemic lupus erythematosus lead us to expect that an autoimmune response against Aβ will be long-lasting and possibly irreversible. Treatment of inflammation of the central nervous system arising from such an autoimmune response may require an aggressive and sustained treatment with immunosuppressants and corticosteroids, which often have negative side-effects (Graham, 1994; Veenstra et al., 1999). Elderly patients tolerate these drugs less well than younger people, and display a higher mortality from secondary complications such as infection (particularly pneumonia) and hypertension (Husain et al., 2001; Meier-Kriesche and Kaplan, 2001).

The preceding overview indicates that neuroinflammation is an expected outcome of successful Aβ vaccination in humans. It has also shown that several routes lead towards a non-specific and neurotoxic inflammatory response (Fig. 1). These routes include the activation of cytotoxic T-cells, the stimulation of frustrated phagocytosis, and the generation of an antibody-directed response to functionally important proteins such as APP and soluble Aβ. In view of the risks associated with Aβ vaccination and with the therapeutic containment of a neuroinflammatory response, there is a need to question whether further vaccination trials can be justified. A decision on this matter needs to be made in the light of recent studies that have shown the efficacy of conventional, low-risk treatments in slowing the progress of AD. These include NMDA antagonists such as memantine (Kornhuber et al., 1994), free radical scavengers such as Gingko biloba, N-acetylcysteine and lipoic acid (Adair et al., 2001; Hager et al., 2001; Stoll et al., 1996), carbonyl scavengers such as tenilsetam (Ihl et al., 1989), NSAIDs such as indomethacin (Rogers et al., 1993) and statins such as lovastatin and pravastatin (Wolozin et al., 2000).

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