Brief Communication:
Immunotargeting of Apolipoprotein E in Amyloid: An Initial Trial in Mice

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Abstract. Apolipoprotein E is commonly present in systemic amyloid deposits. To investigate the possibility of using apolipoprotein E immunotargeting in the diagnosis and treatment of amyloidosis, we examined whether anti-apolipoprotein E monoclonal antibody was bound to murine amyloid deposits in vivo and whether it influenced amyloidogenesis. This study utilized a radiolabeled monoclonal antibody specific to human apolipoprotein E fragments and human apolipoprotein E-knock-in mice, in which AA amyloidosis was induced. Accumulation of the injected radiolabeled antibody was significantly higher in the organs of amyloidotic mice than in those of non-amyloidotic mice. Plasma clearance of the antibody did not differ between the amyloidotic and non-amyloidotic mice. The antibody was given to mice during amyloid induction but failed to prevent amyloidogenesis. The results of this initial study are encouraging, but considerable improvement is necessary, particularly in regard to development of a high affinity antibody.

Keywords: amyloid, apolipoprotein E, immunotargeting, reactive amyloidosis

Introduction
Systemic amyloidosis is a disease entity where insoluble fibrillar aggregates accumulate in the extracellular spaces of organs [1]. Amyloidosis is classified clinically on the basis of the type of proteins that constitute the amyloid fibrils. The fibrils consist of principal proteins that are specific to each disease type and common constituents that are present in all types. The latter include serum amyloid P component (SAP) and apolipoprotein E (ApoE) [2,3].

Amyloidosis is generally diagnosed on the basis of histological examination, a procedure that has proven largely satisfactory. However, histological analysis has limitations; it is invasive and cannot be used to evaluate the systemic distribution of amyloid deposits. Moreover the biopsy sample does not always contain the amyloid deposits. To circumvent these problems, scintigraphy utilizing labeled SAP, which has high affinity for all amyloid deposits, has been developed and introduced into clinical practice [4]. The usefulness of the procedure has been demonstrated [5]; however, it has not been widely adopted because of a lack of commercial availability.

In the present study, we focus on ApoE, another common constituent of amyloid. We developed an anti-ApoE monoclonal antibody that recognizes the ApoE portion generated by degradation of amyloid deposits [6,7]. The development of an ApoE knock-in mouse [8] made it possible to utilize this antibody in vivo. To provide a foundation for future diagnostic and therapeutic applications, in this pilot study we investigated whether the injected antibody accumulates in the organs of mice with reactive amyloidosis and whether the antibody can prevent amyloidogenesis.
Materials and Methods

Induction of amyloidosis. A human ApoE knock-in mouse was developed by one of the authors, as previously described [8]. There were 3 substrains, one for each of the 3 isoforms of human ApoE (E2, E3, E4). For amyloidosis induction, 15- to 20-wk-old female mice were used. Amyloid enhancing factor (AEF) was prepared according to a previous report [9] and administered ip. This was followed by ip injections of 0.5 ml of Freund’s complete adjuvant weekly, for three times. The presence of amyloid deposits was confirmed by histological examinations of the organs. This protocol consistently yielded severe amyloid deposition in the spleen, liver, kidneys, and intestine of the susceptible murine substrains.

Antibody. A previously established murine monoclonal antibody, clone YK-2 (mouse IgG1) [6,7], fractionated into IgG by protein G affinity chromatography, was used. The epitope of this antibody is a portion around residue 220 of ApoE [7]. The antibody was used as a whole IgG molecule or as a F(ab)2 fragment prepared by treatment with pepsin.

Antibody accumulation in amyloidotic organs. The antibody was iodinated with Na125I using the conventional chloramine T method. On average, activity was 6,000 cpm/ng of labeled antibodies. Amyloidotic and non-induced (control) mice were given 0.3 mg of labeled antibody iv via the tail vein. At 24 or 72 hr later, the mice were sacrificed by exsanguination, after which the spleen, liver, kidneys and small intestine were removed. Pieces of the organs were weighed and radioactivity was assessed using a gamma scintillation counter. Results were expressed as a percentage of the injected activity per g (wet wt) of tissue.

Plasma clearance. Plasma clearance of the injected antibody was determined in a manner similar to the accumulation study. At the indicated time points, blood was drawn retro-orbitally under anesthesia and radioactivity counting was performed.

Prevention trial. To explore whether the antibody prevents experimental amyloidosis, amyloidosis was induced by administration of AEF, as above, and subsequent subcutaneous administration of 0.3 ml of 2% silver nitrate. The antibody (2 mg per mouse) was given ip at day 1 and day 2. At day 4, the spleen was removed and amyloid deposition was examined histologically.

Statistical analysis. Data were analyzed using the paired Student’s t-test and Statview software.

Results

Induction of amyloidosis. Reactive amyloidosis was successfully induced in human ApoE2 knock-in mice but not in ApoE3 or ApoE4 knock-in mice. Serum ApoE concentration was measured in the non-induced state in the mice. The serum level of human ApoE was 1-2 mg/dl in ApoE2 knock-in mice; it was undetectable (<0.03 mg/dl) in the other 2 substrains.

Accumulation of injected antibody in amyloidotic organs. The antibody was first used in its intact IgG form. When examined at 24 hr after injection,

<table>
<thead>
<tr>
<th>Organ</th>
<th>Distribution of 125I (% dose/g tissue) after whole IgG administration</th>
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<tbody>
<tr>
<td></td>
<td>Amyloidotic mice</td>
<td>Control mice</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24 hr (n=6)</td>
<td>24 hr (n=7)</td>
<td>72 hr (n=6)</td>
</tr>
<tr>
<td>Blood</td>
<td>14.3±1.2</td>
<td>10.0±1.6</td>
<td>13.1±1.8</td>
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<tr>
<td>Spleen</td>
<td>9.9±1.8</td>
<td>6.9±1.3</td>
<td>1.7±0.3</td>
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<tr>
<td>Liver</td>
<td>7.0±1.4</td>
<td>5.6±1.5**</td>
<td>3.7±0.7</td>
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<tr>
<td>Kidney</td>
<td>9.1±1.2**</td>
<td>7.2±1.4**</td>
<td>4.1±1.0</td>
</tr>
<tr>
<td>Intestine</td>
<td>3.6±0.6**</td>
<td>2.5±0.7*</td>
<td>1.4±0.6</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Organ</th>
<th>Distribution of 125I (% dose/g tissue) after F(ab)2 IgG administration</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Amyloidotic mice</td>
<td>Control mice</td>
</tr>
<tr>
<td></td>
<td>24 hr (n=4)</td>
<td>24 hr (n=3)</td>
</tr>
<tr>
<td>Blood</td>
<td>2.90±1.8</td>
<td>2.41±0.12</td>
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<tr>
<td>Spleen</td>
<td>2.10±0.32*</td>
<td>0.11±0.01</td>
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<tr>
<td>Liver</td>
<td>1.85±0.28**</td>
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</tr>
<tr>
<td>Kidney</td>
<td>5.11±2.52**</td>
<td>0.97±0.80</td>
</tr>
<tr>
<td>Intestine</td>
<td>0.81±0.15**</td>
<td>0.06±0.00</td>
</tr>
</tbody>
</table>

* p <0.01 vs controls; ** p <0.05 vs controls.
radioactivity was significantly higher in spleen (p <0.01), kidney (p <0.05), and intestine (p <0.05) of amyloidotic mice than in the respective organs of control mice (Table 1). At 72 hr, the difference in radioactivity between the 2 groups was also significant (p <0.05) in liver. The use of F(ab)₂ antibody effectively reduced the background radioactivity, an effect that could be seen in blood radioactivity. Under these conditions, the difference between the groups was significant in spleen (p <0.01), liver (p <0.05), and kidney (p <0.05 (Table 1). In general, the difference was greatest in spleen.

**Plasma clearance of injected antibody.** In plasma, the injected antibody ranged from 50-60% of the initial level at 3.5 hr, 27-32% at 24 hr, and 12-20% at 72 hr. There was no difference in the clearance rate between the amyloidotic (n=2) and control (n=2) mice (Fig. 1).

**Prevention trial.** The induction protocol resulted in mild deposition of amyloid in the white pulp of spleen. The antibody administration was performed in 3 mice; however, they all showed splenic amyloid deposits that were indistinguishable from those in the corresponding control mice (n=5).

**Discussion**

We previously conducted an experiment utilizing a protocol similar to the present study [9]. In that study, a monoclonal antibody to murine serum amyloid A (SAA), a precursor of AA-amyloid, was used. The injected antibody was confirmed to bind to the amyloid deposits. Although that study indicated that our approach might be useful, we were concerned that the antibody might react with circulating SAA and affect the biological function of SAA. The antibody used in the present study has some advantages. First, the antibody recognizes the neo-epitope in ApoE generated by degradation, but reacts little with intact ApoE [7]. Second, since ApoE co-exists in amyloid deposits regardless of type, the system can be applied to any type of amyloidosis. Finally, development of the human ApoE knock-in mouse facilitated the present research.

Reactive amyloidosis was induced in the ApoE2 knock-in mice but not in the ApoE3 or ApoE4 knock-in mice. The ApoE2 strain produced serum ApoE at levels similar to those seen in humans. This appears to support previous research indicating that ApoE plays a role in amyloidogenesis [10].

The present study revealed that injected YK-2 monoclonal antibody accumulated in amyloidotic organs, and that the difference in accumulation between the amyloidotic and control mice was most marked in the spleen, among the organs examined. The spleen is the organ where the deposition occurs first, and this may explain the higher level of accumulation noted there. The relatively poor results in the liver may be due to high blood flow, which results in a high background. Although wash-out effects were greater at 72 hr than at 24 hr, the difference in accumulation between amyloidotic and control animals was not prominent. In order to further reduce the high background, the F(ab)_2 form of the antibody was used. Although the background was reduced because of accelerated plasma clearance, the magnitude of accumulation was not substantially improved. These results suggest that bound antibodies might also part from deposits at an early phase, probably due to their relatively low affinity. This may explain the negative results in clearance studies.
As previously reported in the anti-SAA protocol [11] and the SAP protocol [12], substances with high affinity should rapidly disappear from circulation and be incorporated into the amyloid deposits. The possible low affinity of the present antibody may also explain the negative results of the prevention trial. Preventive use of antibodies to amyloid substances may require not only high affinity, but also high specificity to structures in which amyloid is present [13].

In conclusion, an ApoE immunotargeting strategy may be possible. However, under the present experimental conditions, the low affinity of the antibody renders in vivo imaging difficult because of high background and weak accumulation. Refinement of this procedure will be necessary before its therapeutic potential can be assessed.

Acknowledgement

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References