Evaluating Fifteen Complement Proteins as Potential Biomarkers of Alzheimer’s Disease Using MILLIPLEX® MAP Human Complement Panels

Introduction

The complement system consists of a large number of plasma proteins that assist, or complement, the ability of phagocytic cells and antibodies to clear pathogens. Once stimulated, the end result of the cascade is a massive amplification of response and activation of the membrane attack complex, which forms a transmembrane pore causing osmotic lysis of target cells.

Because of the potential to be extremely damaging to host tissues, complement system activation must be tightly controlled. The complement system is thought to play a key role in many diseases with an immune component, such as asthma or sepsis and in many autoimmune diseases, including systemic lupus erythematosus (SLE), inflammatory bowel disease, rheumatoid arthritis and multiple sclerosis. It is also becoming increasingly associated with neurological disease, such as Alzheimer’s Disease and conditions such as spinal cord injuries.

The ability to assess levels of multiple complement proteins simultaneously in patient CSF, serum or plasma to determine “complement profiles” of disease would be of great value. Until now, researchers could assay only individual complement components using ELISAs. These require large amounts of sample and time, as well as increased expense. However, using the Luminex® xMAP® technology, we have developed two MILLIPLEX® MAP Human Complement panels, based on dilution factors, to determine levels of 15 key complement proteins simultaneously in serum or plasma. Panel 1 (Cat. No. HCMP1MAP-19K) measures C2, C4b, C5, C5a and Factor D, mannose-binding lectin and Factor I, while Panel 2 (Cat. No. HCMP2MAP-19K) measures C1q, C3, C3b/iC3b, C4, Factor B, Factor H and properdin.

Methods

Luminex® 200™ system. This is a compact unit consisting of an analyzer, a computer, and software (Luminex® Corporation, Austin, TX). Microspheres. Magnetic microsphere beads were purchased from Luminex® Corp. Each set of beads is distinguished by different ratios of two internal dyes yielding a unique fluorescent signature to each bead set. Capture antibodies were covalently coupled to the carboxylate-modified magnetic microsphere beads.

Immunoassay Protocol. The multiplex assay was performed in a 96-well plate. The detailed procedure is as follows: wet the plate with 150 µL wash buffer for 10 min and decant. Add 25 µL standards or samples, 25 µL beads, 25 µL assay buffer and incubate at 4°C. Wash the beads three times then add 50 µL biotinylated detection Ab cocktail and incubate at RT for 1 hour. Add 50 µL Streptavidin-Phycoerythrin and further incubate at RT for 30 min. Lastly, wash beads three times, add 150 µL sheath fluid and read on Luminex® instrumentation.

Human Complement Panels Characteristics (Representative Data)

<table>
<thead>
<tr>
<th>Complement Panel 1</th>
<th>Standard Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2</td>
<td>C4b</td>
</tr>
<tr>
<td>Mannose-binding lectin</td>
<td>Factor I</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Complement Panel 2</th>
<th>Standard Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q</td>
<td>C3</td>
</tr>
<tr>
<td>Factor B</td>
<td>Factor H</td>
</tr>
</tbody>
</table>

Results

Samples Tested:
- Normal, Mild Cognitive Impairment (MCI) and Alzheimer’s Disease (AD) CSF samples and AD serum and plasma samples were from commercial sources.
- SLE and juvenile idiopathic arthritis (JIA) samples were kindly provided by Dr. Terry Moore, Saint Louis University, Saint Louis, MO.
- Sepsis samples were from Discovery Life Sciences Inc. Los Osos, CA.
- Healthy control serum and plasma samples were from Bioreclamation LLC, Westbury, NY.

Sample Dilution:
- CSF samples for Panel 1 were tested at 10-fold dilution for C2, C4b, C5, C5a and Factor D, and at 100-fold for Factor D.
- CSF samples for Panel 2 were tested at 100-fold dilution for C1q, C3b/iC3b, C4, Factor B and Factor D, and at 500-fold dilution for C3 and Factor B.
- Serum/Plasma samples for Panel 1 were tested at 200-fold dilution, as per protocol instructions.
- Serum/Plasma samples for Panel 2 were tested at 40,000-fold dilution, as per protocol instructions.

Previous Study

The MILLIPLEX® MAP Human Complement Panels have been developed to provide researchers with a distinctive tool to accurately and efficiently determine serum/plasma and CSF levels of 15 key complement components.

Summary

Sample factors were measured in CSF of AD, MCI and control patients, and in the serum of AD and healthy control individuals. Complement Factors H and D were identified as potential biomarkers with CSF concentrations significantly elevated in AD vs. MCI patient samples. In serum samples, Factor D trended higher in AD samples than in healthy control samples but was not significant. Factor C3b/C3b and Properdin were significantly higher in healthy control serum samples.

In previous studies measuring complement factors in the serum of SLE, JIA, sepsis patients and healthy individuals, the autoimmune diseases SLE and JIA had unique complement factor profiles compared to sepsis patient and healthy control samples.

The MILLIPLEX® MAP Human Complement Panels will save time and money, while reducing the amount of sample required, compared to individual complement component measurements by ELISA.

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

Robert Keith, Danielle Pepin, and Qiang Xiao
Millipore Sigma, St. Louis, Missouri 63103