Autoimmunity

Anti-nucleosome antibodies: specific markers for SLE

by Dr J. Gosink

Autoantibodies against nucleosomes (ANuA) are known to be sensitive markers for systemic lupus erythematosus (SLE), but until now their clinical relevance seemed to be limited because sera from patients with progressive systemic sclerosis (PSS) also showed positive reactions with conventional ELISA test systems. This article describes a new ANuA ELISA based on a novel nucleosome preparation, which consists of mononucleosomes free of histone H1, non-histone proteins such as Scl-70, and chromatin DNA fragments. Studies with this second generation ELISA demonstrated that ANuA are highly and specifically associated with SLE but not with PSS.

Systemic lupus erythematosus (SLE) is a multi-systemic, inflammatory autoimmune disorder of connective tissue, which predominantly involves the skin, joints, kidneys and serosal membranes. SLE is characterised by the presence of a multitude of different autoantibodies in patients’ sera directed against components of the cell nucleus, cytoplasm, cell membranes and other autoantigens. Anti-nucleosome antibodies (ANuA) are among the anti-nuclear antibodies found in SLE patients, along with specific antibodies against double-stranded DNA (dsDNA), Sm and others [1].

The term “nucleosome” defines a unit of chromatin which consists of 146 base pairs of DNA wrapped around a protein core [Figure 1]. The protein core is an octamer consisting of two molecules of each of the histones H2A, H2B, H3 and H4. Neighbouring nucleosomes are joined together by linker DNA, which is associated with histone H1 outside the nucleosome core. Anti-dsDNA and anti-histone antibodies belong to the nucleosome family as do anti-nucleosome-specific antibodies, since nucleosomes share several common epitopes with dsDNA and histones. Nucleosome-specific antibodies do not react with the individual components of nucleosomes, i.e. DNA and histones, but recognise conformational epitopes resulting from interactions between DNA and histones.

Although nucleosomes are among the most important autoantigens in SLE and the prevalence of ANuA in sera from SLE patients is high [2], the diagnostic use of this parameter has been greatly limited until now, since with conventional ANuA test systems many patients with progressive systemic sclerosis (PSS) also demonstrated significant positive reactions. It was even mistakenly assumed that ANuA were associated with both diseases.

PSS patients, like SLE patients, exhibit a number of anti-nuclear antibodies, of which antibodies against various DNA-associated proteins, for example centromere proteins and Scl-70, are characteristic for PSS [3]. Anti-centromere antibodies are pathogenomonic markers for the limited form (CREST syndrome) of PSS, while antibodies against Scl-70 occur in up to 75% of cases of PSS and are associated with the diffuse, proximal form of PSS.

Various procedures for the preparation of nucleosomes have been described. The most common of these involves digesting nuclei from calf thymus with nuclease S7, which causes internucleosomal cleavage. However, nuclease S7 digestion often results in di- or oligonucleosomes or submononucleosome particles. One of the best established methods for separating mononucleosomes from oligomers is sucrose gradient centrifugation [4]. Various preparations were additionally treated with NaCl solution (end concentration 0.55 M) and further purified by discontinuous sucrose gradient centrifugation with 10, 30 and 50% (w/v) sucrose. The nucleosome-containing fraction was then characterised by gel electrophoresis and Western blot analysis and demonstrated to be free of histone H1, Scl-70, other non-histone proteins, chromatin and DNA fragments [Figure 2].

Experimental procedure
Two nucleosome preparations were produced. For the conventional preparation (1st generation nucleosomes) nuclei from calf thymus were isolated and digested with nuclease S7. For the new nucleosome preparation (2nd generation nucleosomes) nucleosomes prepared in the same way were additionally treated with NaCl solution (end concentration 0.55 M). The two nucleosome preparations were used as target antigens in 1st and 2nd generation ELISAs. Sera from 515 patients with different rheumatic diseases such as SLE, PSS, Sjögren’s syndrome (SS) and polymyositis (PM), as well as sera from 204 healthy blood donors, were tested with these ELISAs.

Figure 1. Structure of a nucleosome: dsDNA and histone proteins (H1, H2A, H2B, H3, H4).

Figure 2. 2nd generation nucleosomes are free from: Top left: DNA fragments, TAE agarose gel analysis. Top right: non-histone proteins, SDS-PAGE analysis. Bottom left: histone H1, Western blot analysis. Bottom right: Scl-70, Western blot analysis.
Results
In the ELISA based on 1st generation nucleosomes, 62% of the sera from SLE patients but also a high percentage (52%) of the sera from PSS patients showed a positive reaction. Using 2nd generation nucleosomes ANuA were detected with the same sensitivity (58%) in SLE patients, but remarkably the sera from PSS patients were all negative [Table 1, Figure 3]. Sera from patients with other rheumatic diseases as well as from healthy blood donors were also negative with the 2nd generation ELISA, with the exception of one Sjögren’s syndrome serum. Therefore, the specificity of the 2nd generation ELISA for SLE with respect to all serum panels, including PSS, was 99.7%. These results were confirmed in an independent study involving a large cohort (432 patients with rheumatic or other diseases and 127 blood donors) [5], in which ANuA were detected with a sensitivity of 54% and a specificity of 95-99% using the 2nd generation ELISA. In order to investigate whether the positive reactions of PSS sera were due to contamination of the 1st generation nucleosome preparation with Scl-70, sera were tested for anti-Scl-70 antibodies using a Farr assay. The Farr test showed that sera from PSS patients were positive for anti-Scl-70, whereas the 2nd generation preparation consisted of pure nucleosomes. The reactivities of PSS sera with the 1st generation ELISA are false positives due to contamination of the conventional nucleosome preparation with Scl-70.

Figure 3. Specific detection of ANuA in SLE with 2nd generation nucleosomes (demonstrated using immunoblot test strips coated with 1st or 2nd generation nucleosomes).

ANuA were detected in a higher percentage (56%) of sera than the classic marker anti-dsDNA antibodies (34%). Moreover, 18% of the sera were positive for ANuA but negative for both anti-dsDNA and anti-histone antibodies. This finding was confirmed in an independent study [5], in which ANuA (determined using the EUROIMMUN 2nd generation ELISA) were detected in 16% of SLE sera in the absence of anti-dsDNA antibodies (determined by a Farr assay). By testing for the two SLE-specific antibodies (anti-dsDNA and anti-nucleosome) in parallel, the diagnostic hit rate could be increased significantly to 59%.

Summary
It has been assumed up until now that anti-nucleosome antibodies (ANuA) are associated with progressive systemic sclerosis (PSS) as well as systemic lupus erythematosus (SLE). This study shows that the observed reactivities of PSS sera in conventional anti-nucleosome ELISA are actually due to contamination of traditional nucleosome preparations with Scl-70. A new nucleosome preparation technique based on sucrose gradient centrifugation yielded nucleosomes that were demonstrably free of Scl-70 and other non-histone contaminants. This nucleosome preparation (2nd generation) is the basis for a new anti-nucleosome ELISA. The determination of ANuA with this ELISA greatly enhances SLE diagnostics, and ANuA can now be regarded as a highly specific (99.7%) and sensitive (56-58%) marker for SLE.

The author
J. Gosink, Ph.D.,
Product Manager,
EUROIMMUN AG, Luebeck, Germany,
Fax +49 451 5855 591

References

Table 1. Prevalence of ANuA in various autoimmune diseases.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>n</th>
<th>Anti-nucleosome ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st generation</td>
</tr>
<tr>
<td>SLE</td>
<td>295</td>
<td>62%</td>
</tr>
<tr>
<td>PSS</td>
<td>119</td>
<td>52%</td>
</tr>
<tr>
<td>Sjögren’s syndrome</td>
<td>48</td>
<td>4%</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>53</td>
<td>2%</td>
</tr>
<tr>
<td>Blood donors</td>
<td>204</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 4. Percentage of positive reactivities in 89 SLE patients using anti-nucleosome, anti-dsDNA and anti-histone ELISA systems.