# Molecular hallmarks of anti-chromatin antibodies associated with the lupus susceptibility locus, *Sle1*

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## ABSTRACT

Anti-nuclear antibodies constitute the hallmark of lupus. The NZM2410-derived *Sle1* lupus susceptibility interval on murine chromosome 1 breaches tolerance, leading to the emergence of anti-nuclear autoantibodies targeting nucleosomes. However, little is known about the molecular structure of the anti-nucleosome autoantibodies from this genetically simplified mouse model of lupus. In this study, the immunoglobulin heavy chain and light chain sequences of 50 anti-nuclear monoclonal antibodies derived from five B6.*Sle1^z* mice were compared to non-nuclear antibody controls. Compared to two different sets of non-nuclear antibodies, anti-nucleosome antibodies derived from B6.*Sle1^z* congenic mice exhibited a high degree of clonal expansion and three distinct sequence motifs in their heavy chains – cationic CDR3 stretches, non-anionic CDR2 regions, and an increased frequency of aspartate residues at H50, which together increased the likelihood of an antibody being chromatin-reactive by ~4-fold.

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## 1. Introduction

Anti-nuclear antibodies (ANAs) constitute an important hallmark of systemic lupus erythematosus, as extensively reviewed (Hahn, 1998; Kotzin, 1996; Pisetsky, 2000), though autoantibody-independent mechanisms leading to lupus nephritis have also been described (Chan et al., 1999; Lefkowith and Gilkeson, 1996; Liang et al., 2004; Shi et al., 2007; Waters et al., 2004). Comparative studies of ANAs with non-nuclear-antigen reactive Abs have highlighted several interesting molecular features, particularly in the immunoglobulin (Ig) heavy chains (HC), including the prominence of "R" residues in the CDR3 regions (Eilat and Anderson, 1994; Liang et al., 2004; Marion et al., 1992; Radic and Weigert, 1994; Chen et al., 2002), whose importance in facilitating DNA-reactivity has been unequivocally demonstrated through site-directed mutagenesis (Martin et al., 1994; Radic et al., 1993; Radic and Seal, 1997; Wloch et al., 1997). The evidence for distinct molecular signatures that distinguish the CDR2 regions of ANA HCs from those of non-ANAs has been less convincing. Nevertheless, sequence comparison studies and site-directed mutagenesis has helped demonstrate the potential importance of polarity at selected CDR2 positions in con-ferring or enhancing DNA-reactivity (Chen et al., 2002; Katz et al., 1994; Radic et al., 1993; Radic and Seal, 1997). In contrast to the HC, the light chains (LCs) of ANAs possess few molecular "signatures" that consistently light up across different datasets (Liang et al., 2003; Marion et al., 1992). This is in line with the prevailing notion that the HC may play the "dominant" role in dictating nuclear-antigen reactivity, while the LC may serve to modulate, or even veto this reactivity in the context of certain HC partners (Fitzsimons et al., 2000; Ibrahim et al., 1995; Li et al., 2001; Spatz et al., 1997).

As reviewed above, several previous studies have documented the sequence differences between anti-nuclear Abs and non-ANA controls. However, caution should be exercised in interpreting these data, for two important reasons. First, in most documented murine and human Ig repertoire studies, the lupus-afflicted subjects (or mice) and normal controls have had very different genetic backgrounds. Second, in both species, since lupus is polygenic in origin, one cannot attribute the observed repertoire differences to any single genetic event. To circumvent these two limitations, we elected to study the antibody repertoire in lupus using a genetically simplified mouse model-B6 mice rendered congenic for the NZM2410-derived lupus susceptibility interval, *Sle1^z* (Mohan et al., 1998; Morel et al., 1997).

Whereas B6 mice do not exhibit anti-nuclear autoantibodies, B6.*Sle1^z* congenics (which are on the same B6 genetic background) exhibit high titers of anti-nuclear autoantibodies, with preferential binding to nucleosomes and DNA/histone complexes (Mohan et al., 1998; Morel et al., 1997). A panel of anti-chromatin mAbs

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**Table 1**
Monoclonal anti-nuclear antibodies rescued from B6.*Sle1* mice.

| Mice | mAb[1] | Isotype | Nucleosome | dsDNA | ssDNA | Histone |
|------|--------|---------|------------|-------|-------|---------|
| B6.*Sle1*[z] #1 | 1A1G12[a] | IgG2a | ++ | − | + | − |
| B6.*Sle1*[z] #2 | 1BD2[b] | IgG2a | ++ | ++ | ++ | − |
| B6.*Sle1*[z] #3 | 1CA4[c] | IgG2a | ++ | + | ++ | |
| | 1CB3 | IgG2a | ++ | − | − | − |
| | 1CD3 | IgG2a | + | − | − | ++ |
| | 1CE6 | IgM | ++ | − | − | − |
| | 1CE7 | IgG2a | ++ | + | − | − |
| B6.*Sle1*[z] #4 | 1DB2 | IgG2b | ++ | − | − | − |
| | 1DC1[e] | IgM | ++ | − | − | − |
| | 1DC3 | IgM | + | + | + | + |
| | 1DC5 | IgM | ++ | ++ | − | − |
| | 1DC7[f] | IgM | ++ | + | − | + |
| | 1DC9 | IgG2a | + | + | ++ | ++ |
| | 1DE1 | IgM | | + | ++ | − |
| | 1DE4 | IgG2b | ++ | + | − | − |
| | 1DG5[d] | IgG2a | ++ | + | + | ++ |
| B6.*Sle1*[z] #5 | 1EA9 | IgG2a | (−/+) | + | + | + |
| | 1EA10 | IgG1 | ++ | − | − | − |
| | 1EB2 | IgG2b | − | − | + | − |
| | 1EB5 | IgM | ++ | − | − | + |
| | 1EC1 | IgM | ++ | ++ | + | + |
| | 1ED6 | IgM | ++ | − | − | + |
| | 1ED7 | IgG2a | | − | − | + |
| | 1ED9 | IgG2a | − | − | ++ | − |
| | 1EE5[g] | IgG2a | ++ | − | − | + |
| | 1EG4 | IgM | (−/+) | + | + | + |
| | 1EG8 | IgG2a | ++ | + | + | + |
| | 1EH1 | IgG2a | − | − | + | + |
| | 1EH2 | IgM | − | + | ++ | − |
| | 1EH4 | IgM | | − | + | ++ |
| | 1EH5 | IgM | − | − | ++ | − |
| | 1EJ5 | IgM | + | − | ++ | + |

[1] Listed are anti-nuclear mAbs generated from 5 B6.*Sle1*[z] mice. Clones with two or more members (as determined by shared HC CDR3 regions) are indicated with alphabets in superscripts, and are represented by the most mutated clone (in order to capture as much of the mutational information as possible). Clonal sizes are as follows: a – 5 (i.e., 5 mAbs were clonally related); b – 4; c – 2; d – 5; e – 2; f – 2; g – 5. More detailed CDR sequence information is presented in Tables 2 and 3. The "+" nomenclature used to describe the strength of nuclear antigen binding is detailed in Section 2. Comparison of the usage frequencies of HC and LC to those noted among non-ANAs is summarized in Fig. 1.

were generated from this strain and examined for antigen specificity and sequence structure. In contrast to their LC sequences, ANA HC sequences from B6.*Sle1*[z]-derived mAbs exhibited three distinct sequence motifs – cationic CDR3 stretches, non-anionic CDR2 regions, and an increased frequency of aspartate residues at H50. Together, these three motifs increased the likelihood of an antibody being chromatin-reactive by ~4-fold.

## 2. Materials and methods

### 2.1. Mice

B6.*Sle1*[z] are C57BL/6 (B6) mice rendered congenic homozygotes for NZM2410-derived *Sle1*[z], a 37 centimorgan lupus susceptibility interval on chromosome 1, with termini at *D1MIT101* and *D1MIT155* (Morel et al., 1997). These mice are strongly seropositive for anti-chromatin and anti-histone/DNA Abs, but weakly positive for anti-dsDNA Abs (Mohan et al., 1998), while the B6 controls were seronegative for these specificities. Mice used for studies were 6–9 mo-old females, housed in a specific pathogen free colony at UT Southwestern Medical Center Department of Animal Resources.

### 2.2. Hybridoma studies

Spleens removed aseptically from 6–9 mo-old, anti-chromatin seropositive B6.*Sle1*[z] mice were fused to the SP2/0 fusion partner and plated as described (Liang et al., 2004). Single-colony wells that were secreting antibodies (IgM or IgG) were sub-cloned twice,

to ensure clonality, as described (Liang et al., 2004). Hybridoma supernatants were purified using ammonium sulfate precipitation and Protein A chromatography, quantitated using a Coomassie PLUS assay kit (Pierce, Rockford, IL), isotyped using ELISA, adjusted to a concentration of 1–10 μg/ml and tested for anti-nuclear reactivity by ELISA, as described (Liang et al., 2004; Mohan et al., 1998). For the binding strengths shown in Table 1, ODs in the respective antigen-specific ELISAs were mapped onto a semi-quantitative scale, by normalizing against the total Ig level in each sample, as described (Liang et al., 2004). On this scale, "+", and "++" indicate that the antigen-specific OD values registered by the respective mAbs were 0.2–0.5, or >0.5-fold higher, respectively, relative to the corresponding OD values recorded for "total Ig", assayed in parallel.

### 2.3. Antibody sequence analysis

Sequences were aligned using OMIGA 3.0 (Oxford Molecular, Oxford, UK), blasted against public databases of mouse Ig sequences (http://www.ncbi.nlm.nih.gov/igblast), assigned to their respective germline origins as described (Gu et al., 1991; Haines et al., 2001), and deposited into Genbank (accession numbers AY436820–AY436914). The control databases of non-ANA sequences described in this study represent recently assembled collections of non-ANA HC and LC sequences drawn from the Genbank (Liang et al., 2003; Sedrak et al., 2003). Importantly, these abridged databases had no clonal replicates, and no two Abs shared the same antigen specificity. For the statistical comparisons, multi-member clones were represented by one member each so as to minimize