Characterization of Anti-Nuclear Antibody (ANA) Signatures in Murine Models of Lupus Using Genalyte Maverick Technology

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ABSTRACT

BACKGROUND/PURPOSE: Systemic lupus erythematosus (SLE) and lupus nephritis (LN) are autoimmune diseases characterized by circulating antibodies to nuclear self-antigens, including reactivities to double-stranded DNA, RNP and Sm. Preclinical mouse models exist that mimic aspects of human SLE/LN disease, and are used to study pathogenic mechanisms as well as to test responses to anti-inflammatory treatments. In clinical samples, autoantibody reactivity to nuclear antigens is heterogeneous, with individual patients exhibiting unique anti-nuclear antibody (ANA) signatures. We postulated that each distinct mouse model of lupus may also exhibit its own ANA signature, and that by identifying ANA reactivity profiles in preclinical lupus models, robust preclinical biomarker strategies and hypotheses for links with particular aspects of human disease may be devised.

METHODS: A Genalyte Maverick instrument was used to assess IgG reactivity to 13 clinically-relevant nuclear antigens including: SS-A 60, SS-A 52, SS-B, Sm, Sm/RNP, Scl-70, Jo-1, nucleosome, PCNA, Ku, Centromere A & B, Ribosomal P and dsDNA in a simultaneous manner. Analysis was performed on frozen plasma samples archived from several murine lupus models, including spontaneous NZBW-F1, IFNα-accelerated NZBW-F1, and spontaneous MRL-lpr. In some samples, both Maverick technology and in-house ELISA assays were used to assess ANA to dsDNA and Sm/RNP for cross-methodology validation.

RESULTS: Maverick analysis of nuclear antigen reactivities showed that each murine model had a distinct ANA signature. Spontaneous NZBW-F1 mice developed strong reactivity to dsDNA, with a lesser anti-RNP component. However, when NZBW-F1 disease was accelerated via injection of a non-replicative IFNα-inducing adenovirus, ANA reactivity was stronger to RNP nuclear antigen, with a lesser anti-dsDNA component. Both male and female MRL-lpr mice showed strong, age-dependent increases in multiple ANAs including reactivity to dsDNA, RNP, Sm, and nucleosome. Maverick assessment of ANA reactivities to dsDNA and Sm/RNP significantly correlated to titers generated by in-house ELISAs. Additional Maverick assessments of ANA from IFNα-accelerated NZBW-F1 mice treated prophylactically with mycophenolate mofetil (Cellcept), showed Cellcept significantly prevented anti-RNP autoantibody production, but only a trend was seen for decreases in anti-dsDNA autoantibody production.

CONCLUSIONS: These results show that each murine lupus model may exhibit its own unique ANA signature, and that Genalyte Maverick technology is a quick and useful methodology for identifying this signature via simultaneous assessment of several ANA from a single plasma sample. Furthermore, understanding ANA reactivity profiles in each model may help guide better preclinical biomarker design and will have impact on interpreting efficacy of anti-inflammatory treatments.

INTRODUCTION

- Presence of circulating autoantibodies, particularly anti-nuclear antibodies (ANA), is a commonly-used biomarker/mechanistic endpoint for preclinical lupus pharmacology studies. Current methods to determine ANA levels include custom-made ELISA assays which can take considerable hands-on time to prepare and run, which includes coating plates with antigen, blocking, and performing several incubation and wash steps with samples and detection reagents.
  - Most studies investigating ANA in preclinical lupus models measure only 1 or 2 ANA reactivities (ex. anti-dsDNA, anti-RNP) (citations). Limited time and resources, small sample volumes, and measure of ANA titer through plasma dilutions may limit the number of nuclear antigen reactivities from a preclinical sample.
  - Having the ability to measure multiple ANAs simultaneously from a small sample size (5µl) would provide a more detailed ANA signature and reduces time spent at bench to acquire such data.

OBJECTIVE

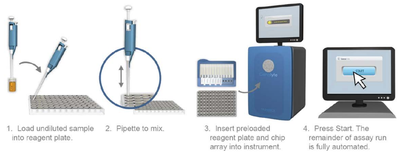
- To evaluate the utility of a novel, multiplexed ANA measurement technology (Genalyte Maverick) in preclinical murine models of lupus.

METHODS

GENALYTE MAVERICK INSTRUMENT & METHODOLOGY

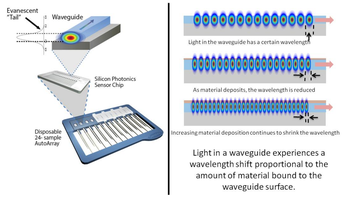
- Genalyte Maverick technology is a detection system used for multiplexed binding assays. Needed to run assays: Maverick, reagent plate, chip array. Each biosensor on the chip is coated with a distinct antigen or antibody which enables multiplexing.
- Infrared laser generates light in a broad wavelength range, which traverses a dedicated linear waveguide for each biosensor. The biosensor "resonates" at a particular wavelength, trapping the light.
- Antibodies or proteins binding to antigens on the biosensor cause a shift in ring resonance trapping a different wavelength. Resonance shift is monitored by the Maverick instrument and results are calculated by Genalyte software.

Schematic of Maverick Protocol

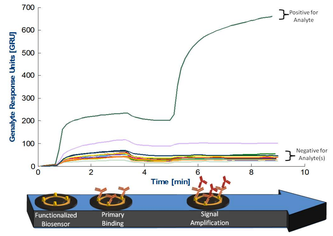


Principles of Maverick Technology

Silicon Photonics – How it Works



Real Time Monitoring



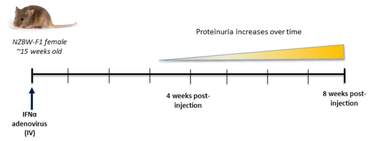
METHODS (CONTINUED)

Definitions of nuclear antigens assessed:

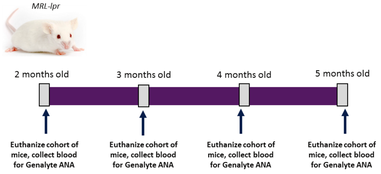
- **RNP =** Ribonucleoprotein; is an extractable nuclear antigen (ENA)
- **Sm =** Smith antigen; is an ENA
- **dsDNA** = Double-stranded DNA
- **Nucleosome** = Fundamental subunit of chromatin (DNA wrapped around histones)
- **Ribo-P Po** = Ribosomal P protein
- **SS-A 60** = Sjögren's-syndrome-related antigen A "Ro", 60 kD; is an ENA
- **SS-A 52** = Sjögren's-syndrome-related antigen A "Ro", 52 kD; is an ENA
- **PCNA** = Proliferating cell nuclear antigen
- **Jo-1** = Histidyl tRNA synthetase; is an ENA
- **Scl-70** = Topoisomerase 1, a 100-kD nuclear and nucleolar enzyme ; is an ENA
- **Ku** = Nuclear protein that binds to DNA double-strand break ends (DNA repair)
- **CenP A&B** = Centromere proteins A&B, facilitate centromere formation
- **SS-B** = Sjögren syndrome type B antigen "La"; is an ENA

PRECLINICAL LUPUS MODELS EVALUATED FOR ANA:

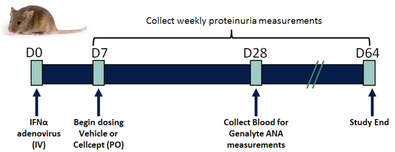
- NZBW-F1 female, IFNα-accelerated; evaluated at 2-4 months of age:



- NZBW-F1 female, spontaneous ; evaluated at ~6 months of age.
- MRL-MpJ parent strain (non-lupus); evaluated at 2 months of age.
- MRL-*lpr* male & female, spontaneous ; evaluated at 2-5 months of age:



- NZBW-F1 female, IFNα-accelerated, treated prophylactically with Vehicle or Cellcept (mycophenolate mofetil) ; evaluated at 5-6 months of age:

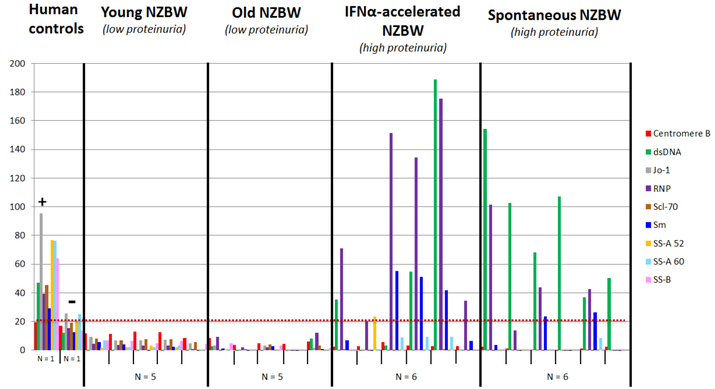


STATISTICAL METHODS

- All statistical analyses were performed using Graphpad Prism5.
- Correlations were done using 2-tailed analyses. Proteinuria plotted as Survival analysis and analyzed by Log-rank (Mantel-Cox) Test. ANA measures were analyzed by One-way ANOVA with Dunnett's post-test.

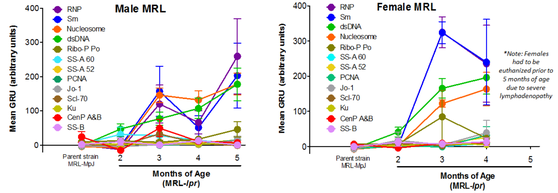
RESULTS

Figure 1. ANA signatures in proteinuric and non-proteinuric NZBW mice



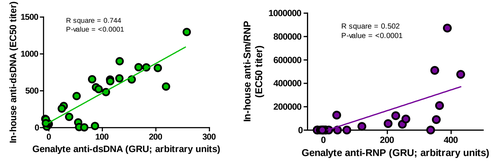
- Regardless of age, non-proteinuric NZBW-F1 mice do not exhibit ANA production
- Interferon-accelerated NZBW-F1 proteinuric mice exhibit reactivity to RNP, dsDNA, and Sm, with highest reactivity to RNP
- Spontaneous aged proteinuric NZBW-F1 mice exhibit reactivity to RNP and dsDNA, with highest reactivity to dsDNA

Figure 2. ANA signature in lupus-prone male and female MRL-*lpr* mice



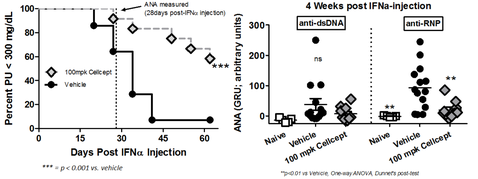
- Over time, MRL-lpr mice exhibit marked increases in autoantibody production to several autoantigens, including RNP, Sm, Nucleosome, and dsDNA. Females show more rapid onset and overall higher levels of anti-RNP and anti-Sm.
- No reactivity to other ANAs evaluated (SS-A 60, SS-A 52, SS-B, Scl-70, Jo-1, PCNA, Ku, and ribosomal P).

Figure 3. Genalyte ANA measurements correlate with traditional anti-dsDNA ELISA.



- Strong correlations were seen between in-house ELISA anti-dsDNA and Genalyte GRU anti-dsDNA.
- Moderate correlations seen between in-house ELISA anti-Sm/RNP and Genalyte GRU RNP.

Figure 4. Maverick ANA assessment of plasma from lupus-prone IFNα-accelerated NZBW-F1 mice treated prophylactically with Cellcept highlights robust response to therapy when evaluating anti-RNP reactivity.



- As predicted in Figure 1, IFNα accelerated NZBW mice show more robust ANA response to RNP antigen than dsDNA.
- Prophylactic treatment with Cellcept significantly prevented autoantibody production to RNP. Only a trend was seen for anti-dsDNA.

CONCLUSIONS

- Genalyte Maverick ANA evaluation allows for robust and high-throughput assessment of ANAs from several preclinical murine models of lupus.
  - Maverick ANA measurements correlate with traditional in-house ELISA measurements (anti-dsDNA & anti-RNP)
- Each preclinical model appears to exhibit a unique pattern of ANA reactivity:
  - IFNα-accelerated NZBW shows strongest RNP reactivity
  - Spontaneous NZBW shows strongest dsDNA reactivity
  - Male and female MRL-*lpr* exhibit strong reactivity to RNP, Sm, nucleosome, and dsDNA. Females show earlier onset and more robust reactivity to RNP and Sm than males.
- When evaluating efficacy of anti-inflammatory reagent (Cellcept) in the IFNα accelerated NZBW lupus model, assessing changes in the most highly-produced ANA in that model guided clearer interpretations of disease induction and drug efficacy:
  - Mid-way through study, Vehicle-treated mice show a significant increase in anti-RNP compared to naïve mice, but only a trend seen for anti-dsDNA.
  - Prophylactic dosing of Cellcept caused a significant inhibition of anti-RNP production, with only a trend seen with anti-dsDNA.
- **In preclinical models that exhibit multiple anti-nuclear antibody reactivities, simultaneous assessment of several ANA may be warranted. Understanding ANA reactivity profiles in each model may help guide a more meaningful preclinical biomarker strategy and may have impact on evaluating efficacy of anti-inflammatory treatments.**

ACKNOWLEDGEMENTS

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DISCLOSURES

All authors are employees of AbbVie. The design, study conduct, and financial support for this research was provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

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