Characterizing Disease Progression in Preclinical Systemic Lupus Erythematosus Using Multimodality In Vivo Ultrasound and Fluorescent Imaging

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1 Abstract

Systemic Lupus Erythematosus (SLE) in humans is a complex, multiorgan, systemic autoimmune disorder that can cause acute or chronic inflammation of multiple organ systems, involving autoantibody production, lymphoid activation/hyperplasia, splenomegaly, and nephritis. There are multiple mouse models that capture some of the important hallmarks of the human disease, and the MRL Mice Fas-lpr (MRL/lpr) mouse is one of the most commonly used, developing lymphoproliferation by 12-16 weeks of age, as well as progressive proteinuria and renal disease, nephropathy, and skin lesions, typically dying around 20 weeks of age. As fluorescent (FL) imaging using injectable NIR fluorescent probes has the potential for the sensitive detection of biological changes associated with disease, we selected a panel of probes for monitoring lupus progression in MRL/lpr mice. We used IVISense™ fluorescent probes (Cat K 680 FAST [CK680], Transreceptor Receptor 750 [TF750], IVIS image receptor 750 [IVIS]), Pan Cathepsin 680 [PC680], Cat 750 FAST [CT750], and Vascular 680 [VAS680] (Revitl) to detect, visualize, and quantify biological changes in various organ systems noninvasively in living MRL/lpr mice as compared to normal control mice. Furthermore, ultrasound imaging of the kidney and spleen was also acquired on the Vega (Revitl) to characterize size and density changes associated with disease progression. MRL/lpr and control mice were assessed at 12, 14, 16, 18, and 20 weeks for increased levels of proteinuria and lymphoproliferation.

2 Murine Spontaneous Lupus Protocol

MRL Mice Fas-lpr (MRL/lpr) and related AKR mice (Charles River Labs) were monitored longitudinally at 14, 16, 18, and 20 weeks by regular NIR Fluorescence imaging using 6 imaging probes as biomarkers of inflammation, progressive activation hyperplasia, and vascular changes. At each imaging time, a small number of mice were selected for ex vivo tissue assessment of fluorescent signal in a range of organ tissues, providing access to difficult to detect tissues as well as improving sensitivity of quantification. P-value: Student t-test against control (p<0.05, *p<0.01, **p<0.001).

3 Lupus General Metrics

- Proteinuria
- Lymph Node Weight

4 In Vivo Probe Screening at 18 Weeks of Lupus

<table>
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<tr>
<th>Probe</th>
<th>Spectral</th>
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<td>IR750</td>
<td>PC680</td>
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5 Vega Ultrasound of Kidneys and Spleen: Method

Kidney and spleen size measurements were acquired by ultrasound (Vega, Revitl) using the linear transducer array B-M mode. Arrows were placed either spotty (kidney) or left side down (spleen). Spleen length was measured along the longest axis and width at the mid-point of the spleen. (B) Kidney width was determined from the base of renal vein to the apex of the capsule in the opposite direction. Volumes were estimated using the formula 0.5 x L x W x H.

6 Ultrasound of Kidneys and Spleen in Lupus

7 Lupus Treatment: Rapamycin inhibition of mTOR

8 FL Imaging of Rapamycin Lupus Treatment

9 Ultrasound assessment of Rapamycin Efficacy

10 Summary

The present studies provide evidence for the utility of fluorescence and ultrasound using the IVISense fluorescent imaging system for the detection and quantification of spontaneous lupus in MRL Mps Fas-lpr/mice. IVISense Cath K and Transreceptor NFR fluorescent imaging probes detected changes in liver, kidney, spleen, and/or lymph nodes associated with disease progression. Ultrasound imaging provided non-invasive means for assessing pathological changes in spleen and kidney size. Early treatment of mice (starting at 12 weeks of age), effectively reduced early signs of proteinuria in addition to preventing glomerular changes to the spleen and kidney as assessed by ultrasound. Fluorescent imaging with the cathemora Ch-shuttle probe, in particular, efficiently detected a decrease in tissue fluorescence to levels near normal mouse controls.

In conclusion, fluorescent imaging of relevant biomarkers and ultrasound measurements of spleen size and kidney size can be performed quickly and easily to provide robust measurements of lupus progression and treatment efficacy.