Development of autoreactive transitional B cells induced by apoptotic Ag stimulation

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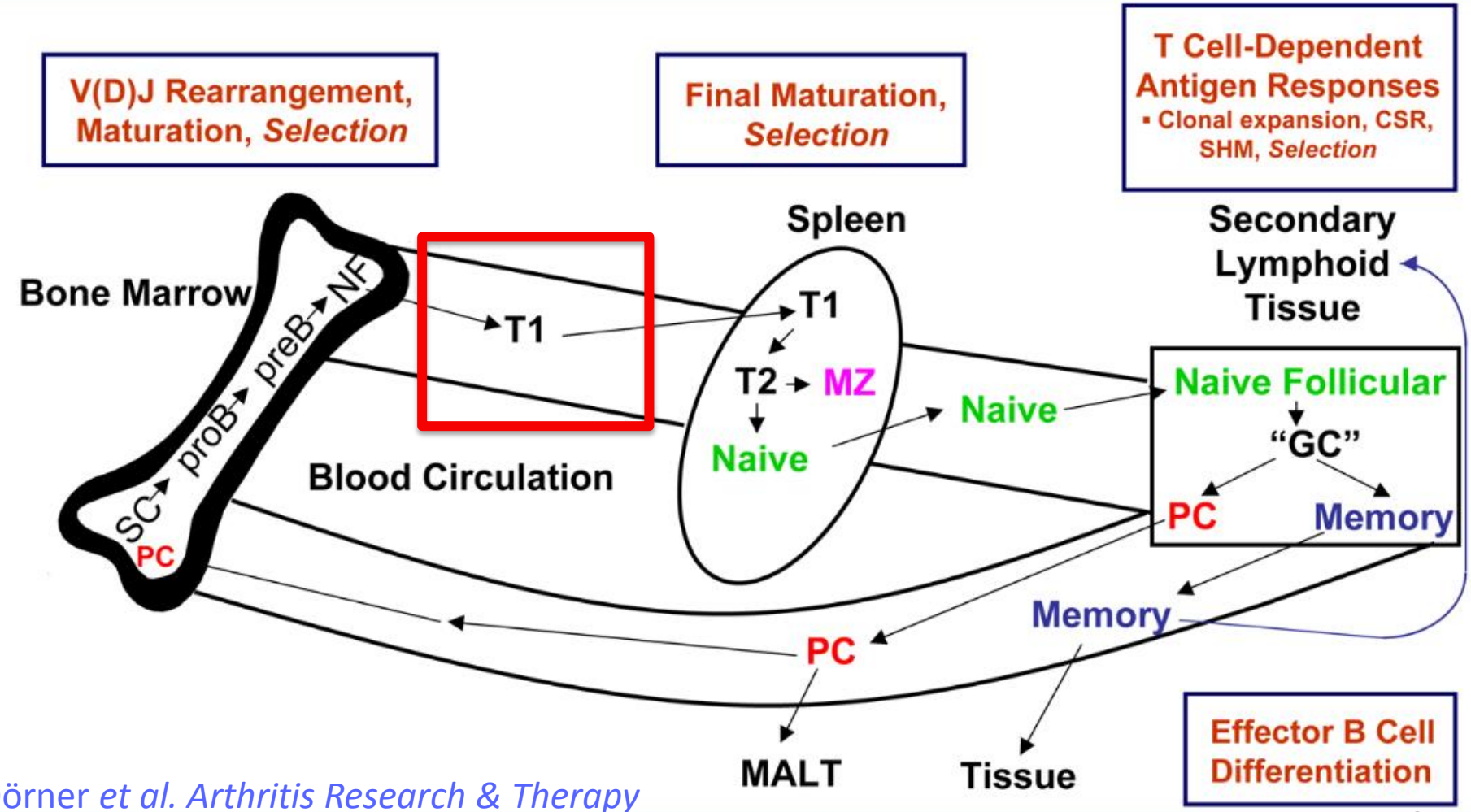
February 26, 2015

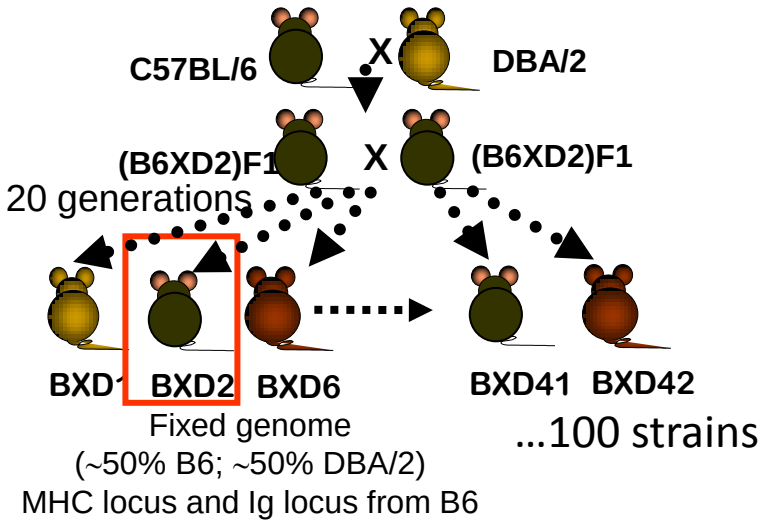
Research In Progress

Hypothesis: AC stimulation (and other factors eg. IL-17R signaling) of transitional B cells promotes development of autoreactive T1 B cells



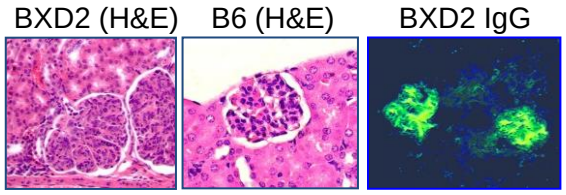
Dörner *et al. Arthritis Research & Therapy* 2009 **11**:247 doi:10.1186/ar2780

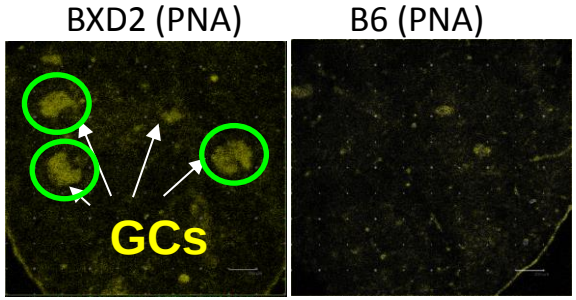
BXD2 autoAb development follows similar pattern



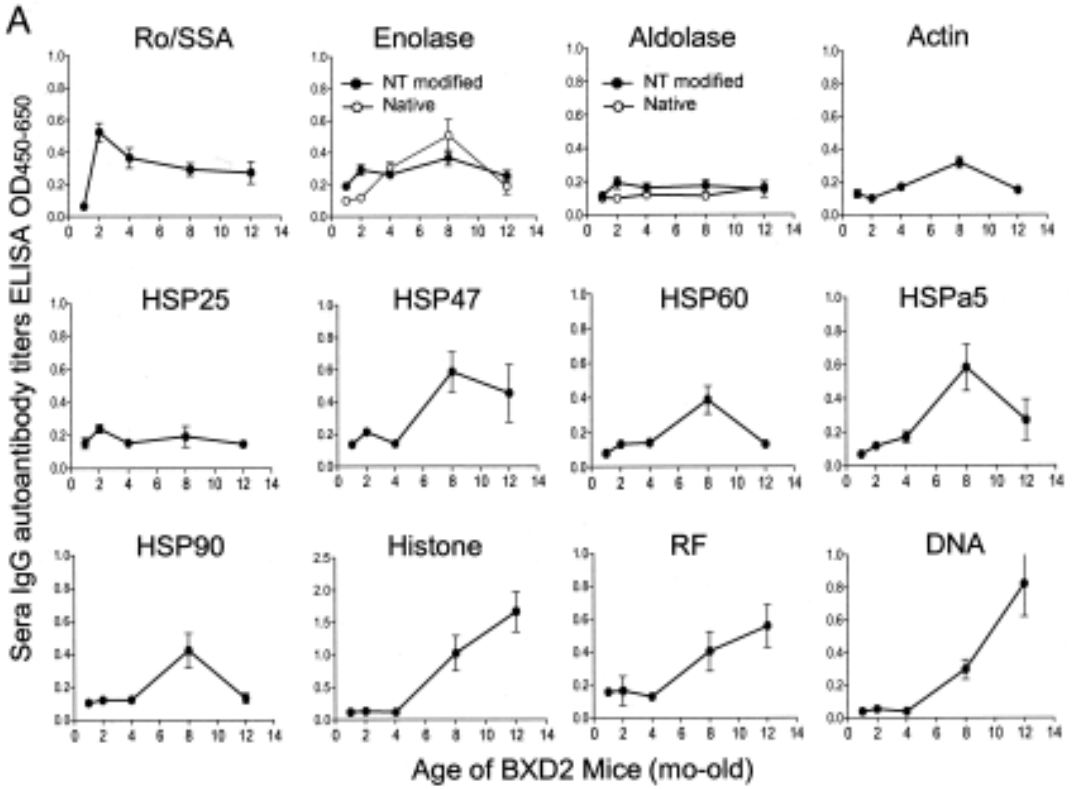
Fixed genome
(~50% B6; ~50% DBA/2)
MHC locus and Ig locus from B6

…100 strains





Spleen GC staining using PNA



Mountz et al, Scan J Immunol 2004
Hsu et. al. Arthritis Rheum. 2006
Hsu et al, J Immunol 2007

La and Ro cluster in apoptotic blebs

J Exp Med. 1994 Apr 1;179(4):1317-30.

Autoantigens Targeted in Systemic Lupus Erythematosus Are Clustered in Two Populations of Surface Structures on Apoptotic Keratinocytes

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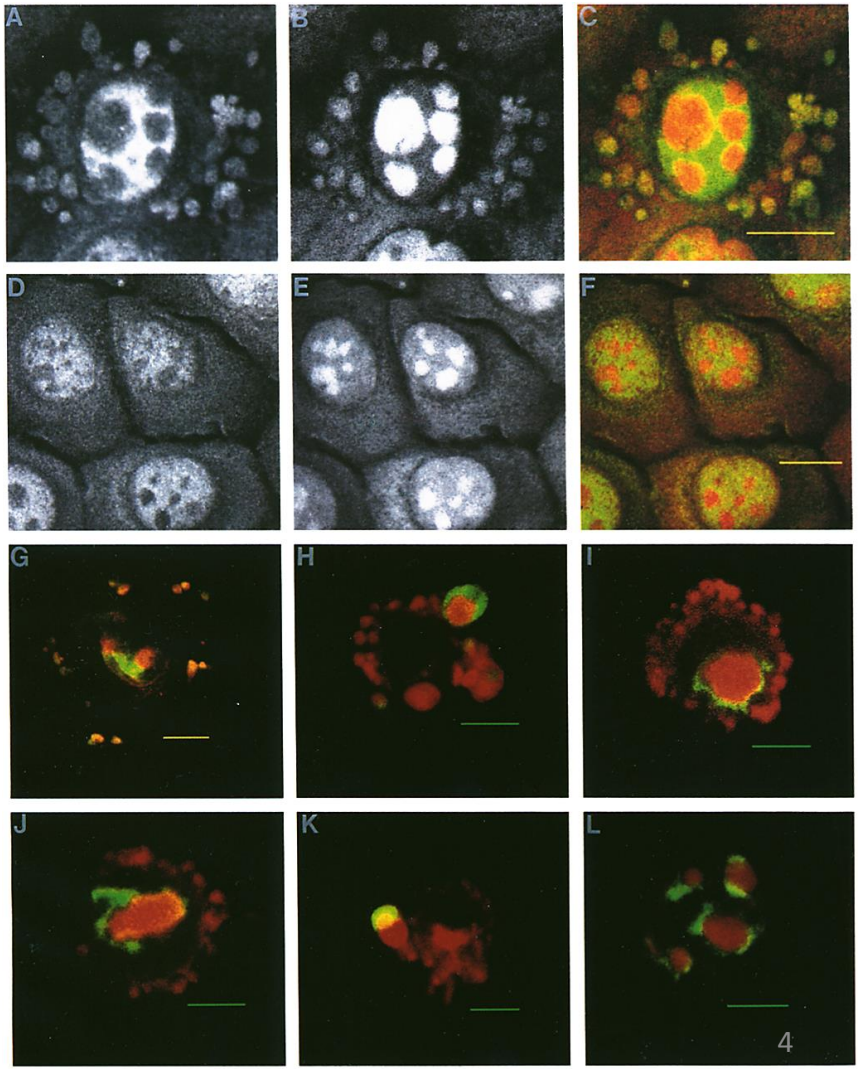
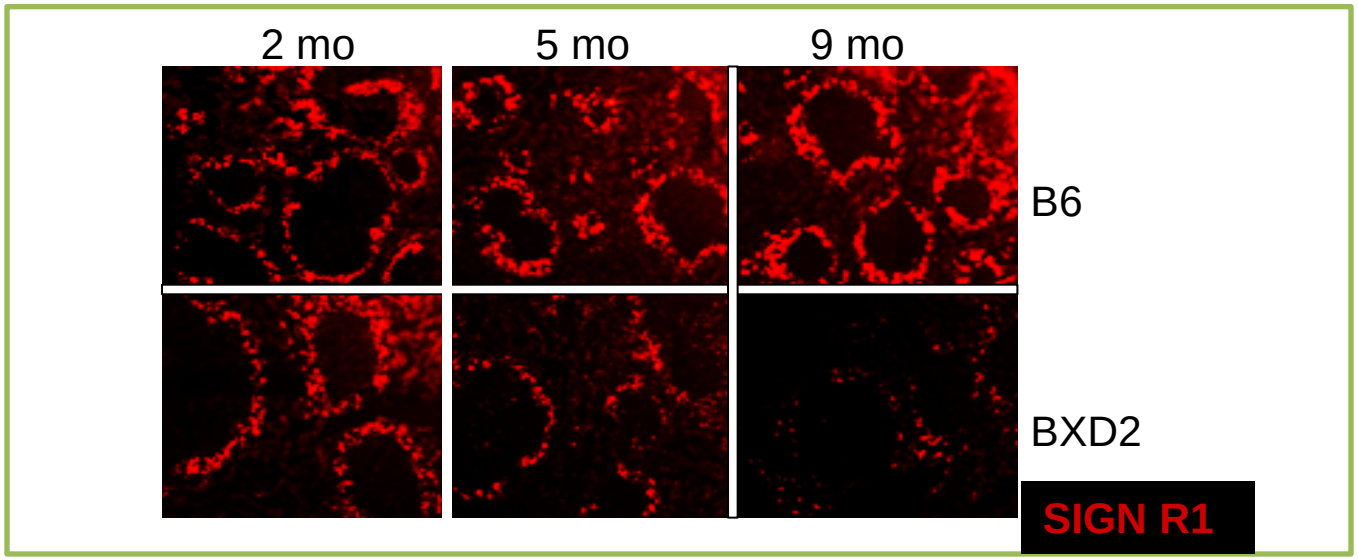
Summary

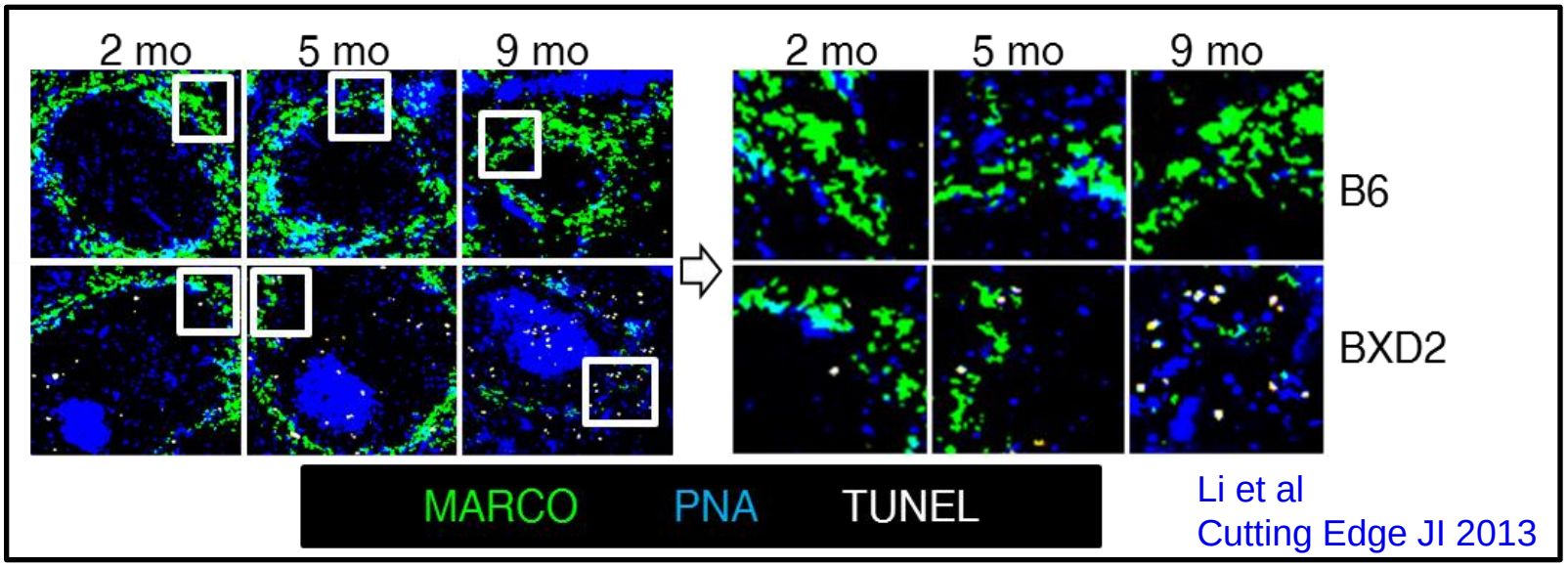
Systemic lupus erythematosus is a multisystem autoimmune disease in which the autoantibody response targets a variety of autoantigens of diverse subcellular location. We show here that these autoantigens are clustered in two distinct populations of blebs at the surface of apoptotic cells. The population of smaller blebs contains fragmented endoplasmic reticulum (ER) and ribosomes, as well as the ribonucleoprotein, Ro. The larger blebs (apoptotic bodies) contain nucleosomal DNA, Ro, La, and the small nuclear ribonucleoproteins. These autoantigen clusters have in common their proximity to the ER and nuclear membranes, sites of increased generation of reactive oxygen species in apoptotic cells. Oxidative modification at these sites may be a mechanism that unites this diverse group of molecules together as autoantigens.



What drives BXD2 autoAb formation to the ubiquitous AC?

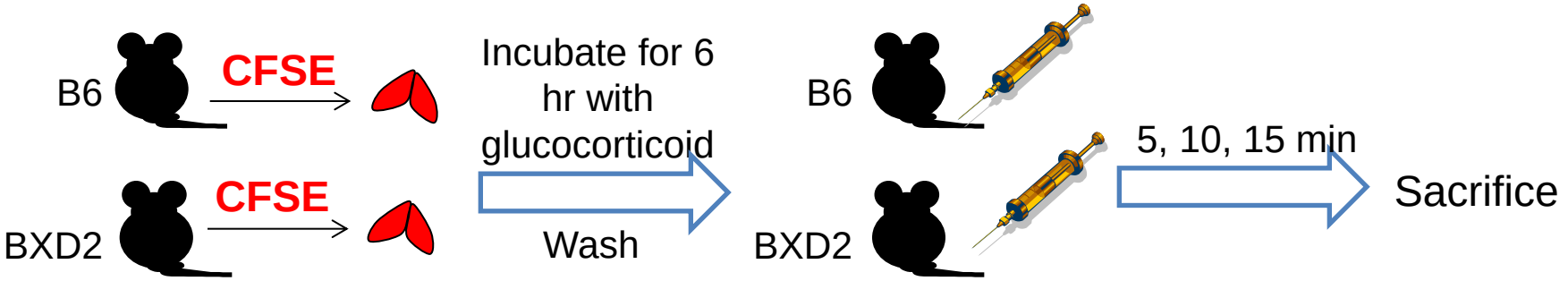
Age-related decrease in MZM and an increase in uncleared apoptotic cells (ACs) in BXD2 mice

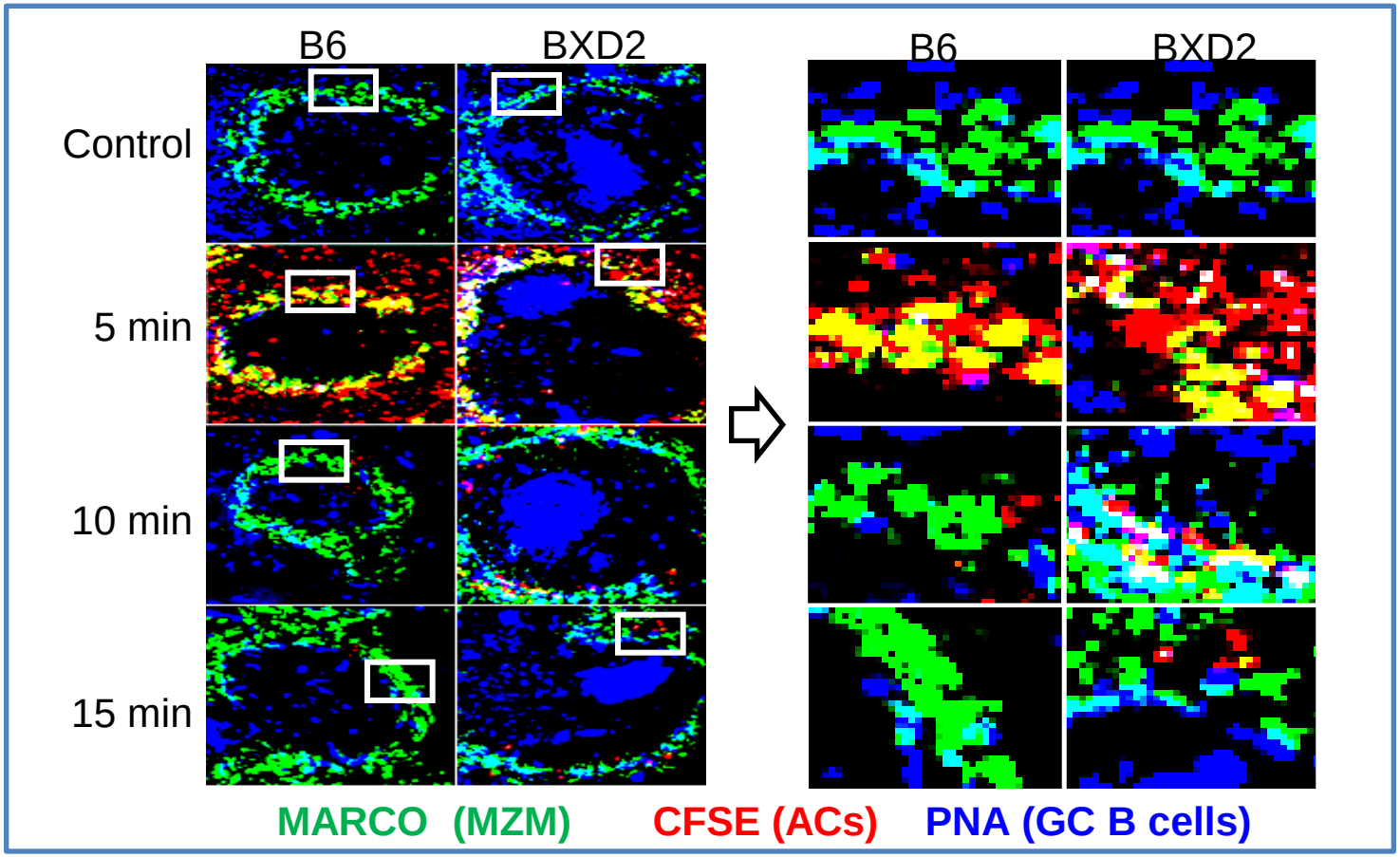




Li et al
Cutting Edge JI 2013

Decreased clearance of apoptotic cells (ACs) in BXD2 mice

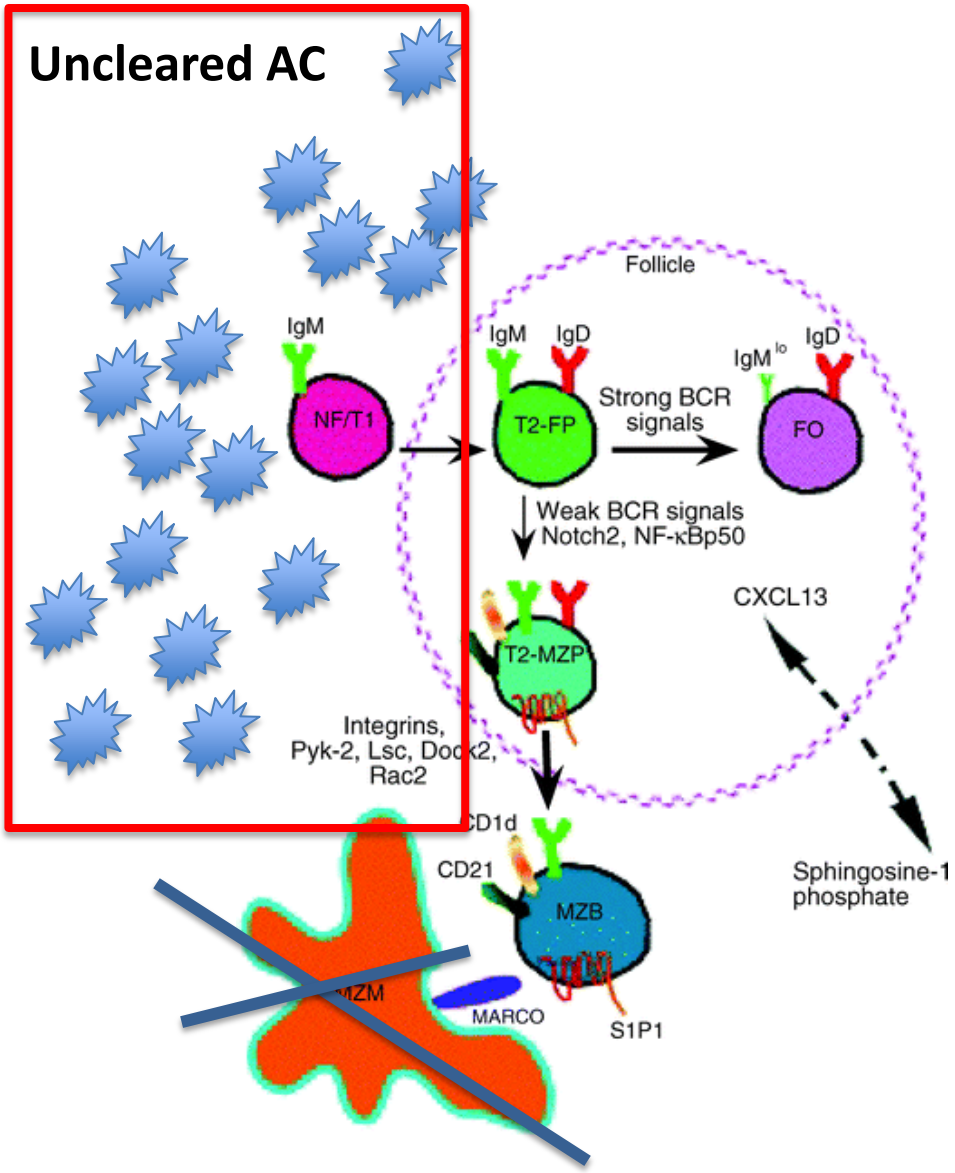




Li et al
Cutting Edge
JI 2013

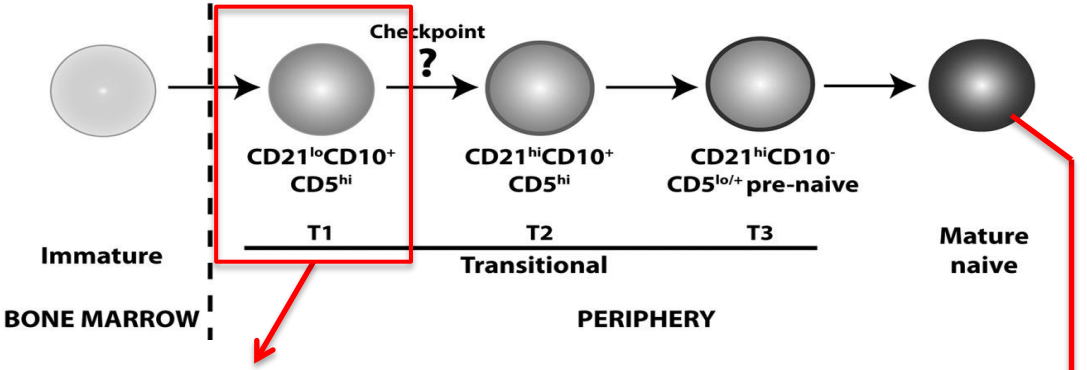
Summary

- BXD2 mice exhibit defective clearance of AC
- Inhibiting AC generation leads to decreased anti-DNA titers
- The uncleared AC is in the vicinity of T1 B cells

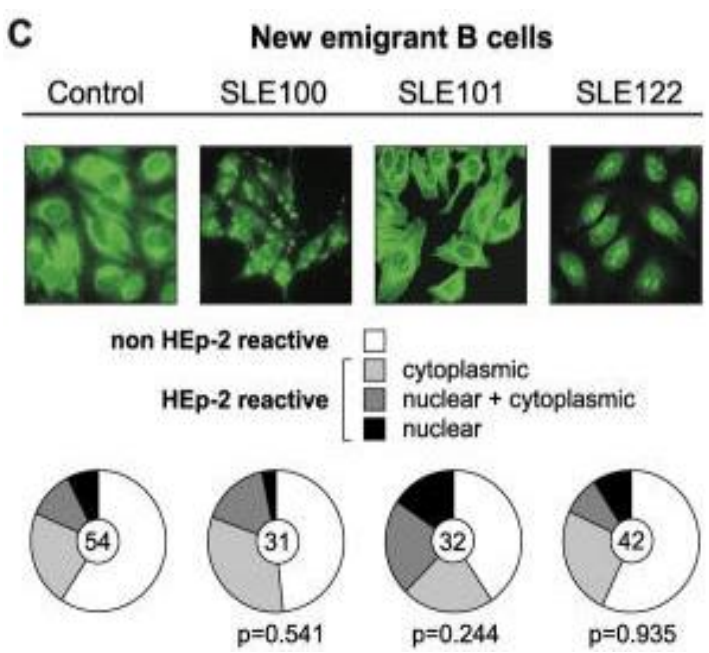


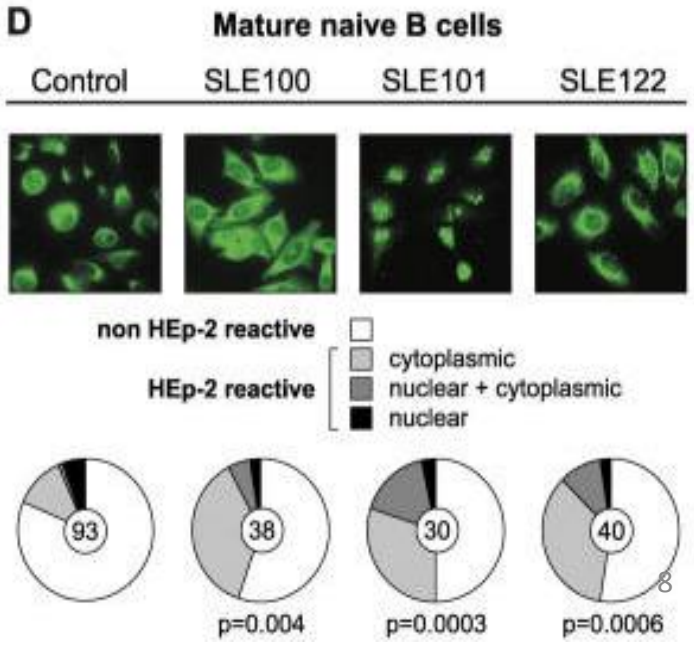
Modified from Pillai et al
Annu. Rev. Immunol. 2005. 23:161–96

Defect identified between the T1/T2 to mature naïve stage in SLE

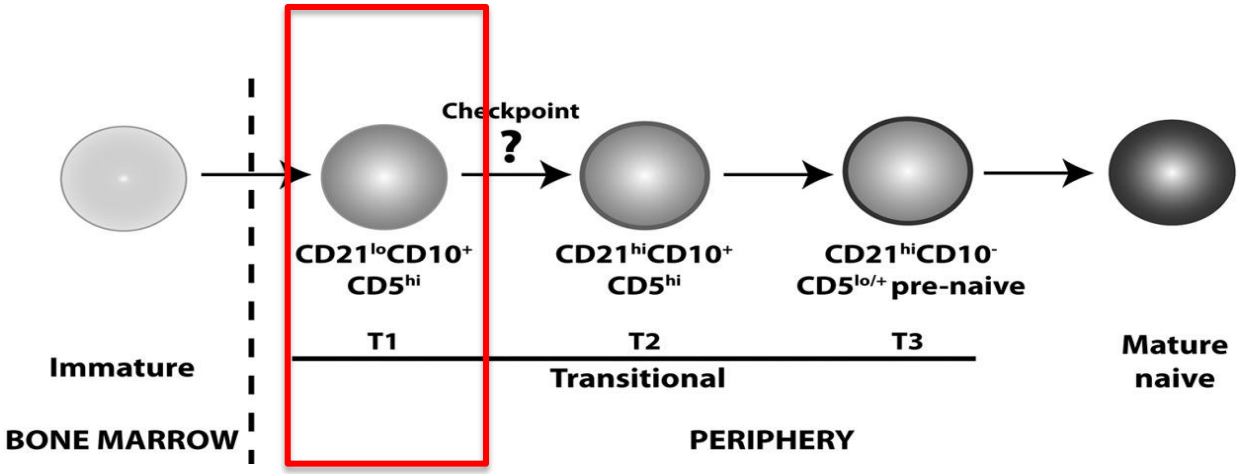


Suryani et al, Blood, 2010





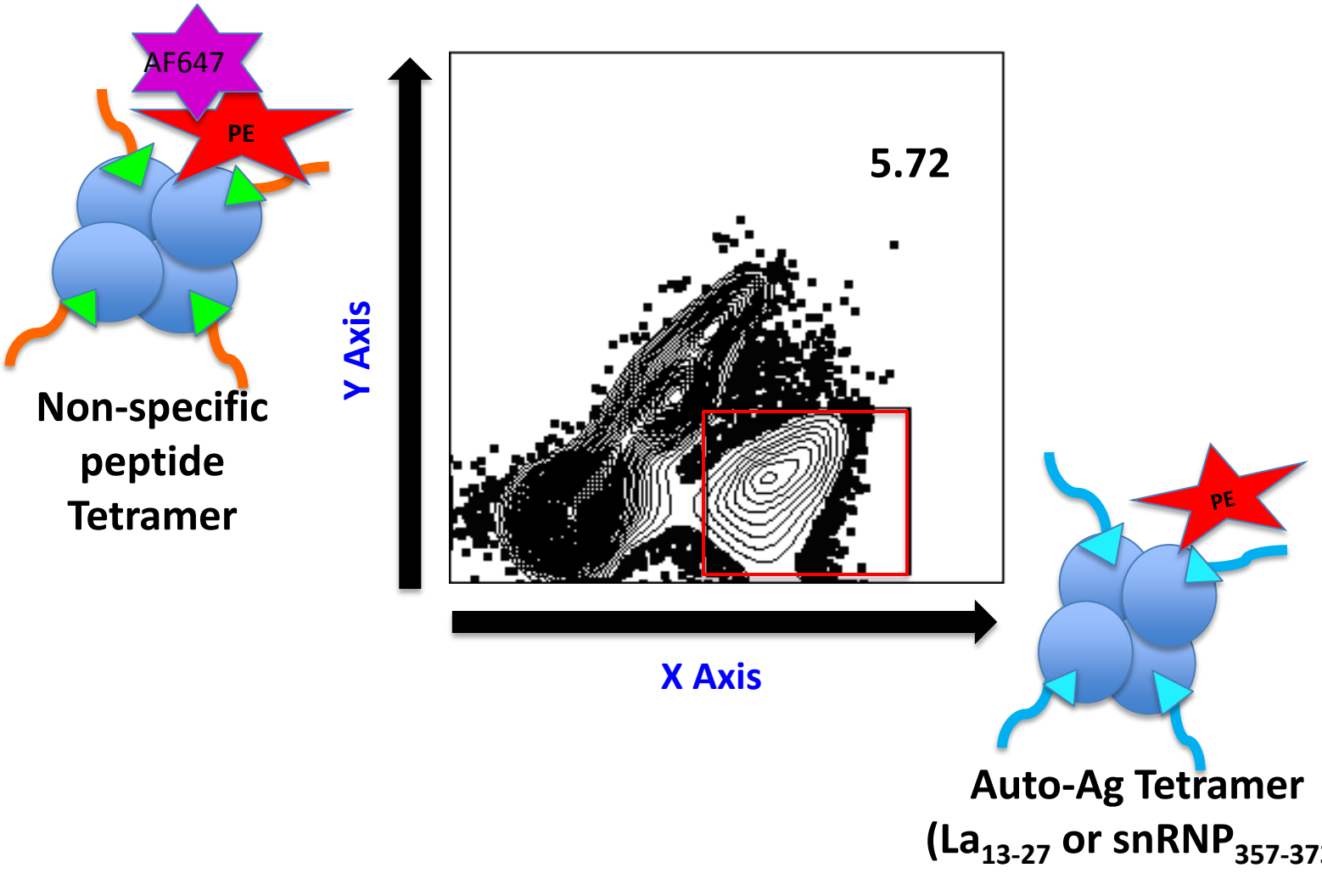
Yurasov *et al*. *JEM*. 2005.



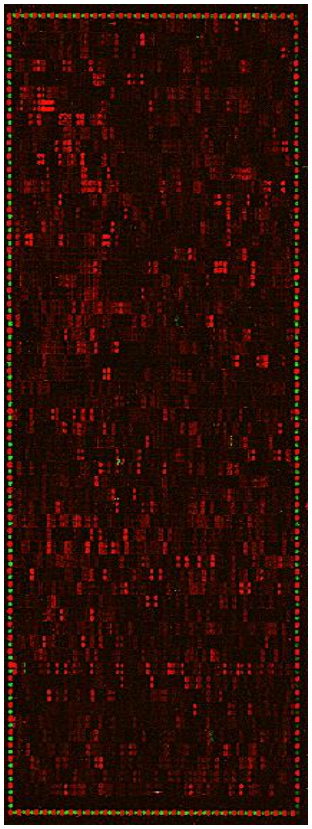
Suryani et al, Blood, 2010

How can we specifically study mechanisms leading to maturation of autoreactive T1 B cells in autoimmune individuals?

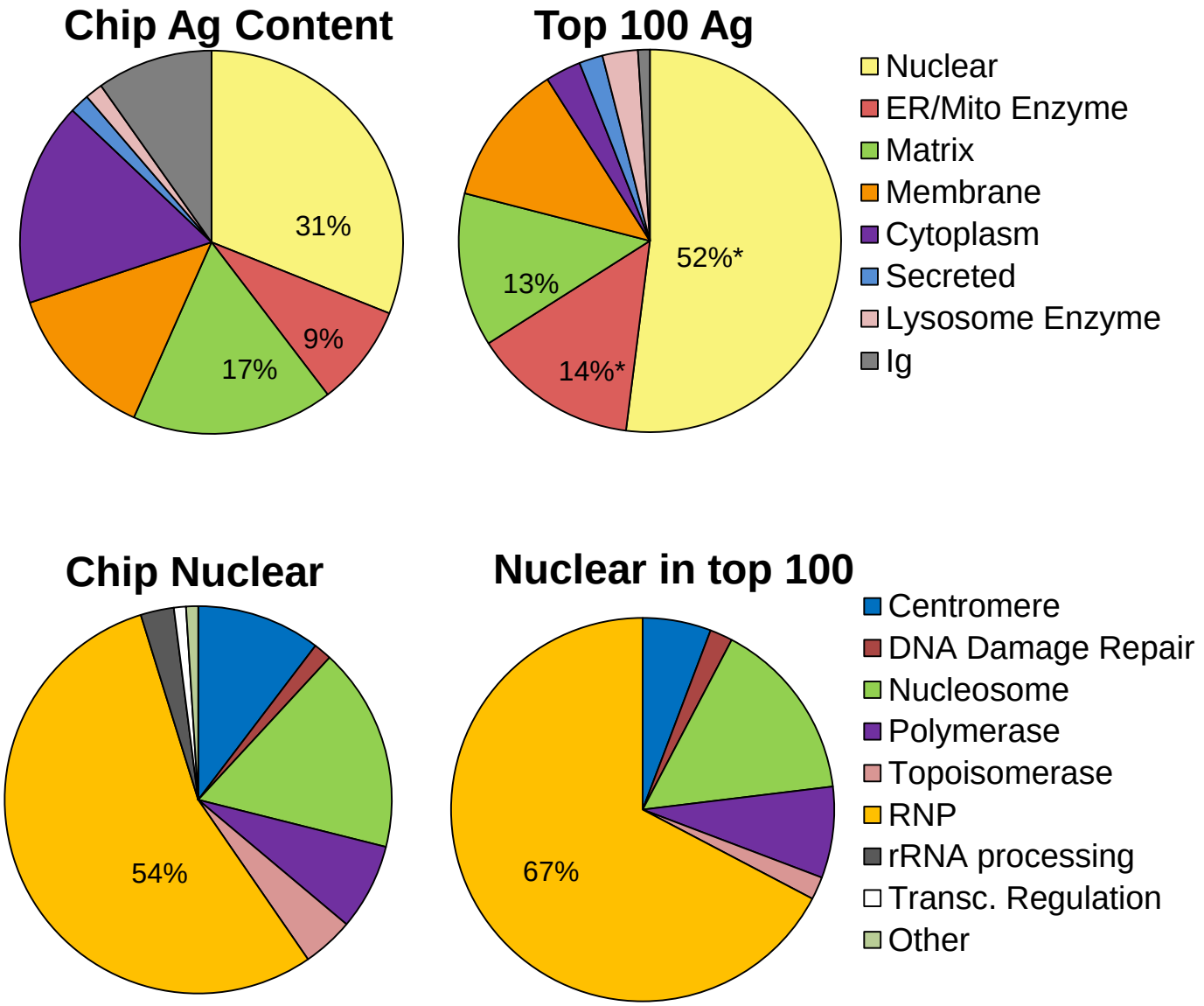
Use of a PE-Avidin – biotin-peptide tetramer strategy to identify autoreactive B cells in BXD2



Pepperprint identification of peptides for tetramer generation

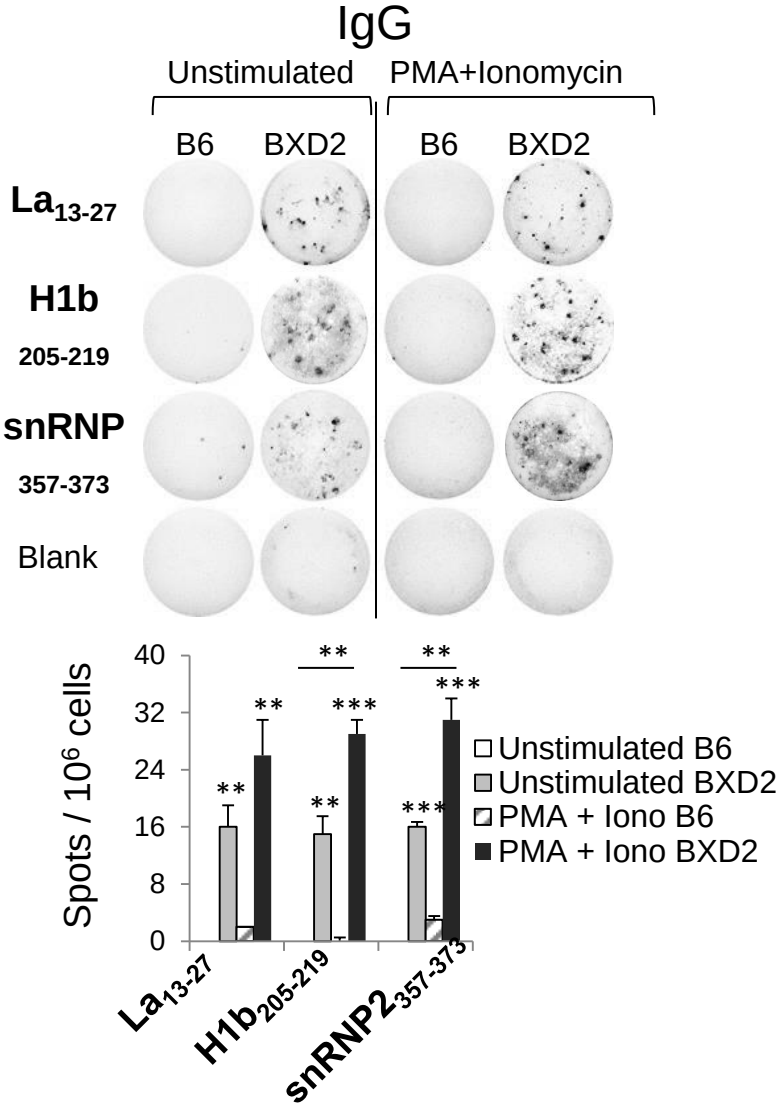


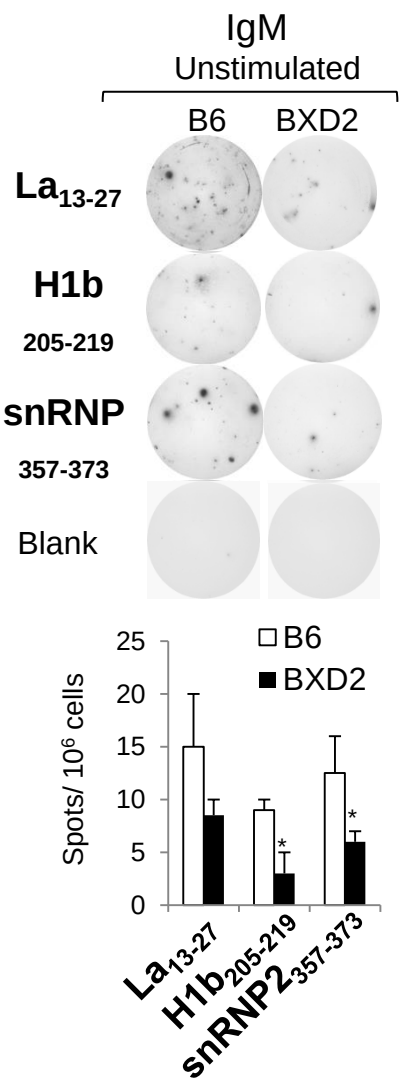
PEPperPRINT Peptide Autoimmunity Microarray probed with BXD2 serum



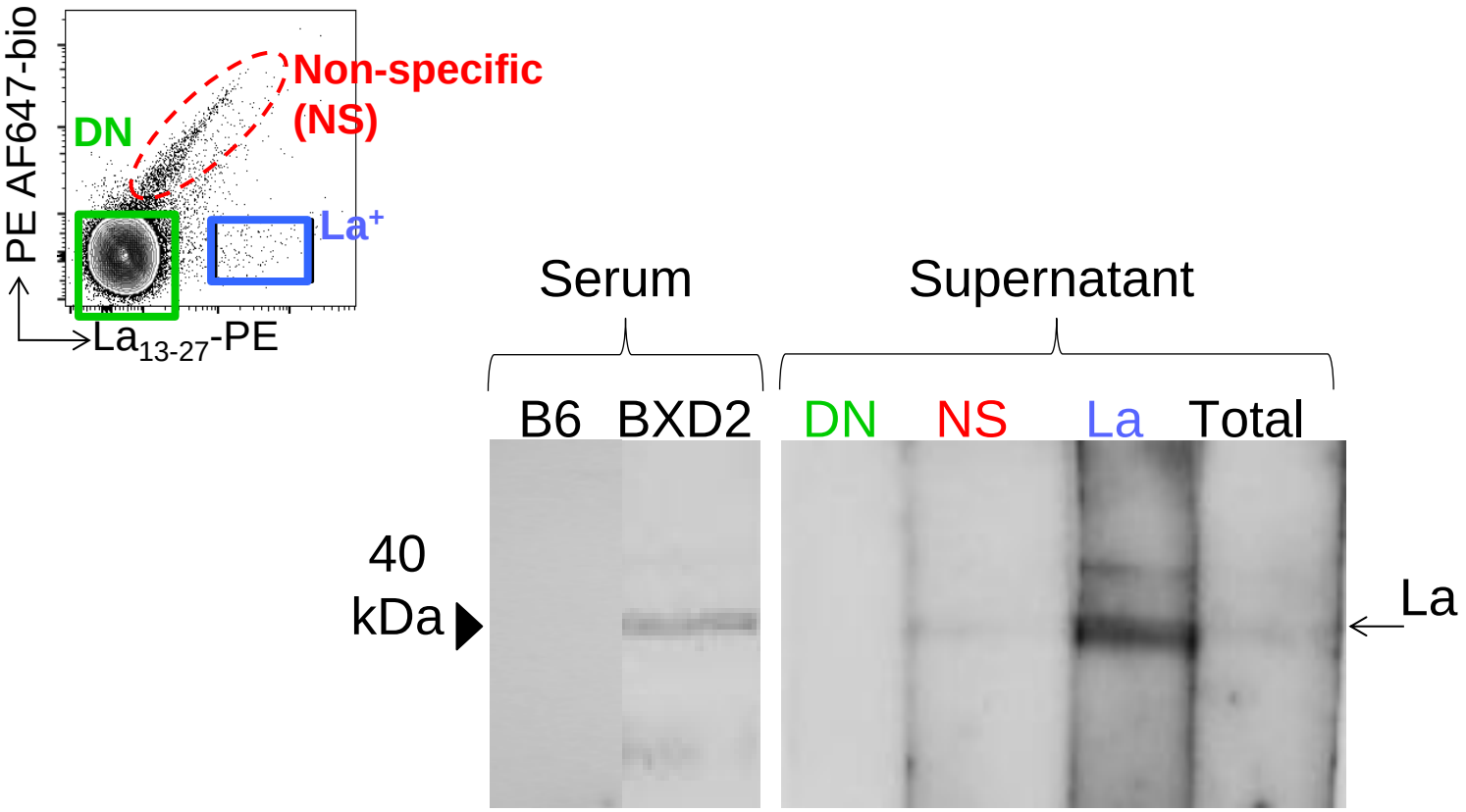
***Predominance of nuclear and RNP autoAg in BXD2 mice**

Selecting peptides for tetramer generation:
Anti-La, histone and RNP peptide autoAbs in BXD2 mice





Antibody maturation of La tetramer^{+} B cells

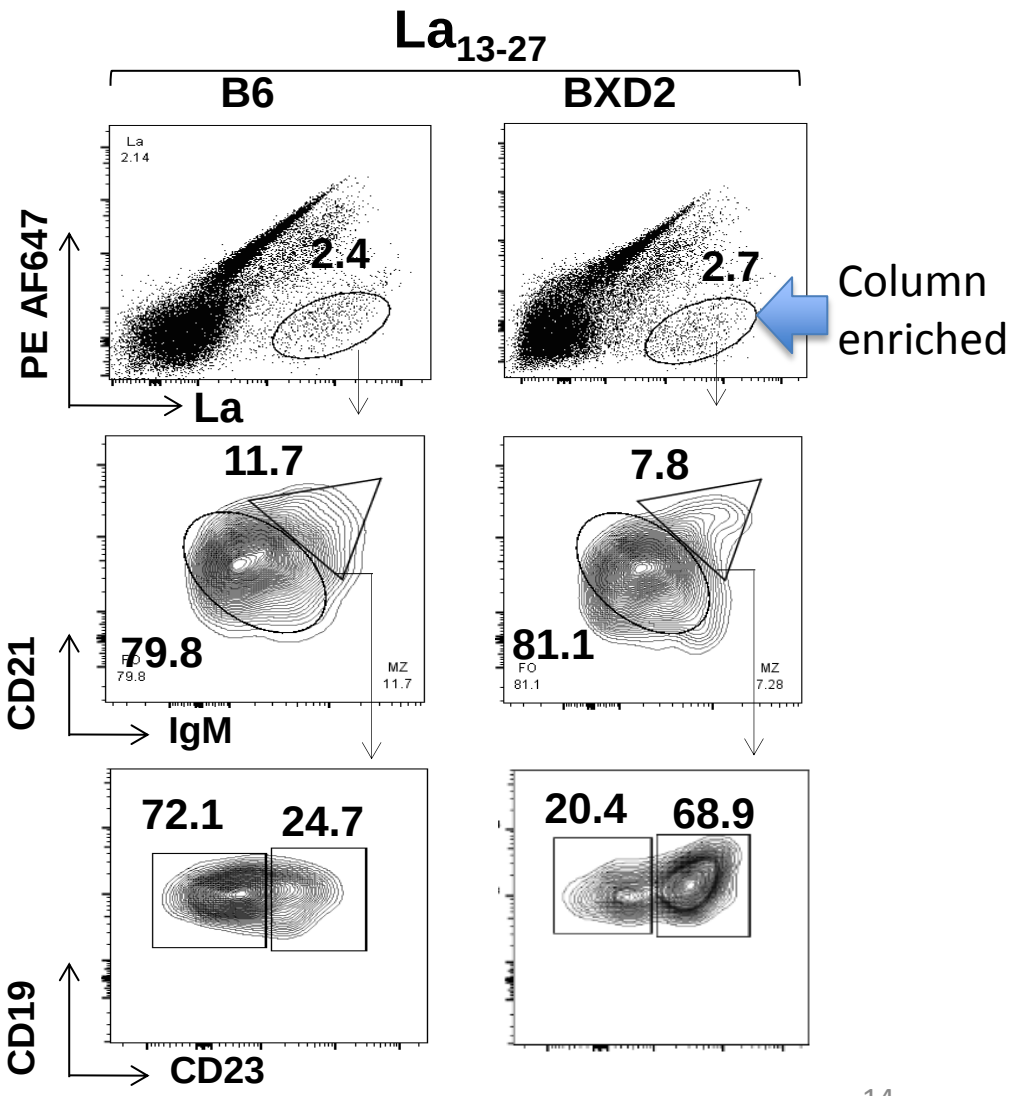


Probed with serum or supernatant from tetramer sorted cells and detected with anti-mouse IgG

Summary of autoreactive B cell analyses using Ag tetramer

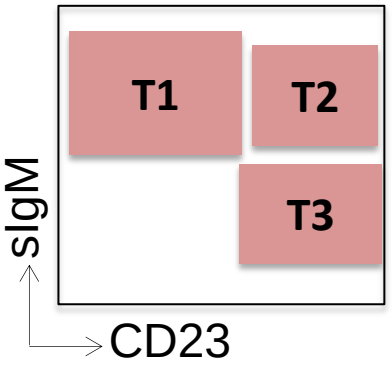
- Overall % of La and snRNP tetramer reactive B cells not significantly different B6 vs BXD2
- Most autoreactive cells are FO, with a higher % and number in BXD2 mice.
- There is a skew in the IgM^{hi}CD21^{hi} population to the MZ-P (IgM^{hi}CD21^{hi}CD23^{+}) in BXD2 mice.
- These autoreactive MZ-Ps also express activation markers CD69 and CD86

- **Is MZ-P dysregulation a result of earlier B cell defect?**

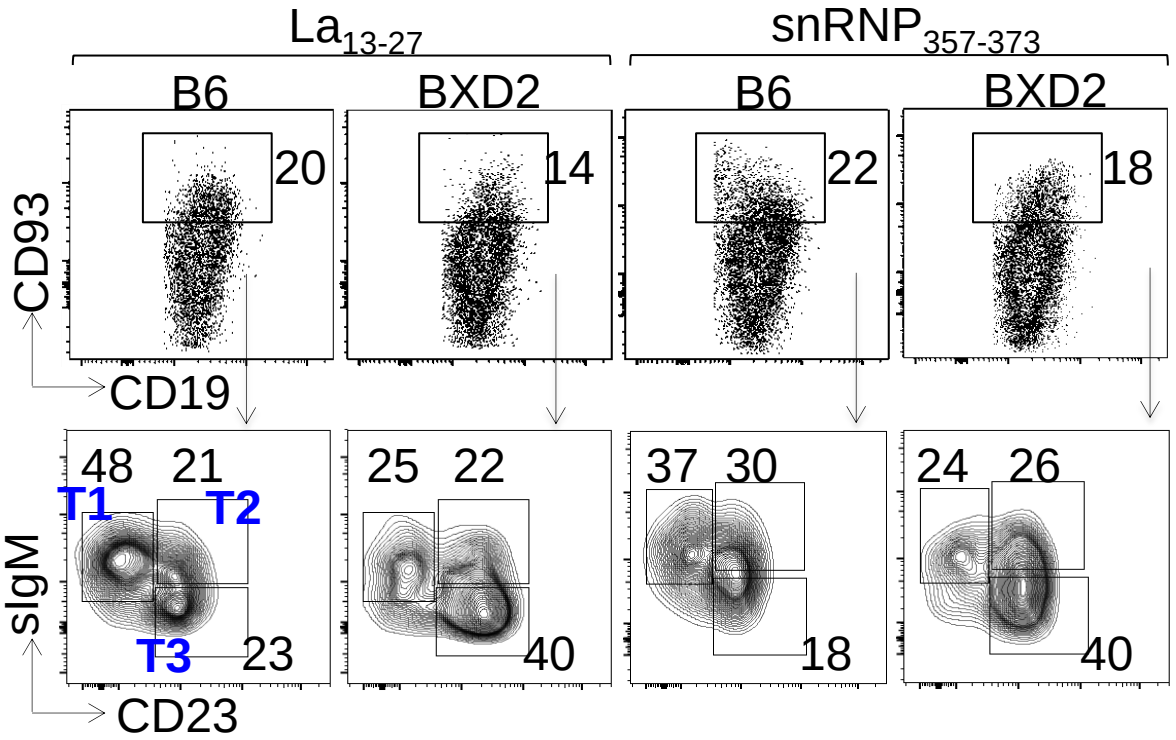


Dysregulation of autoreactive cells at the T1 stage in BXD2 mice

	T1	T2	T3	FO B
sIgM	++	++	+	+/++
IgD	-	++	++	++
CD93	++	+	+	-
CD23	-	+	+	+

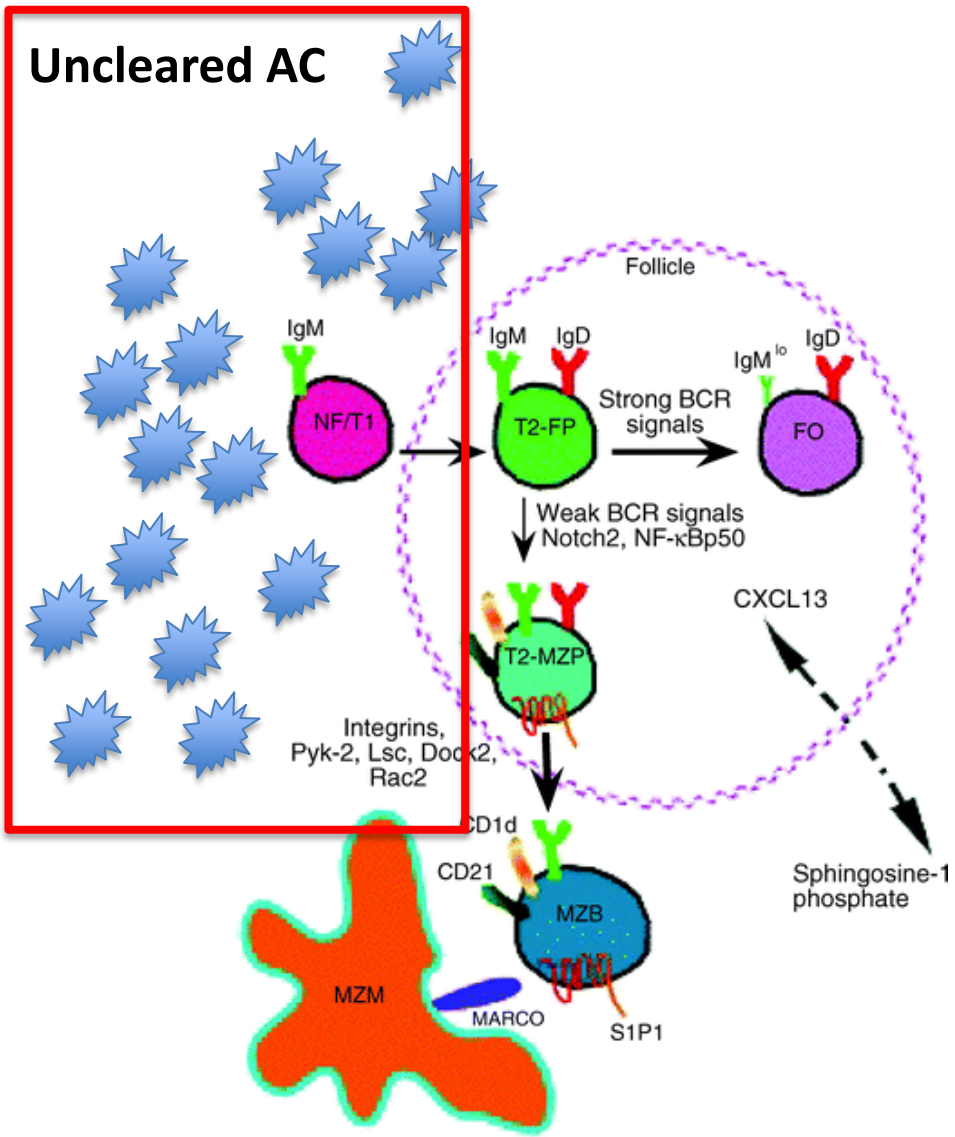


*CD93 = C1q receptor



Summary

- BXD2 mice exhibit IgG response to peptides derived from common apoptotic cell antigens
- Established a general method to identify autoreactive B cells
- Identified skewing in the development of early transitional autoreactive B cells including T1 and MZ-P subsets
- Abnormal maturation in autoreactive T1 cells is found in SLE patients and BXD2 mice. What is the underlying mechanism?

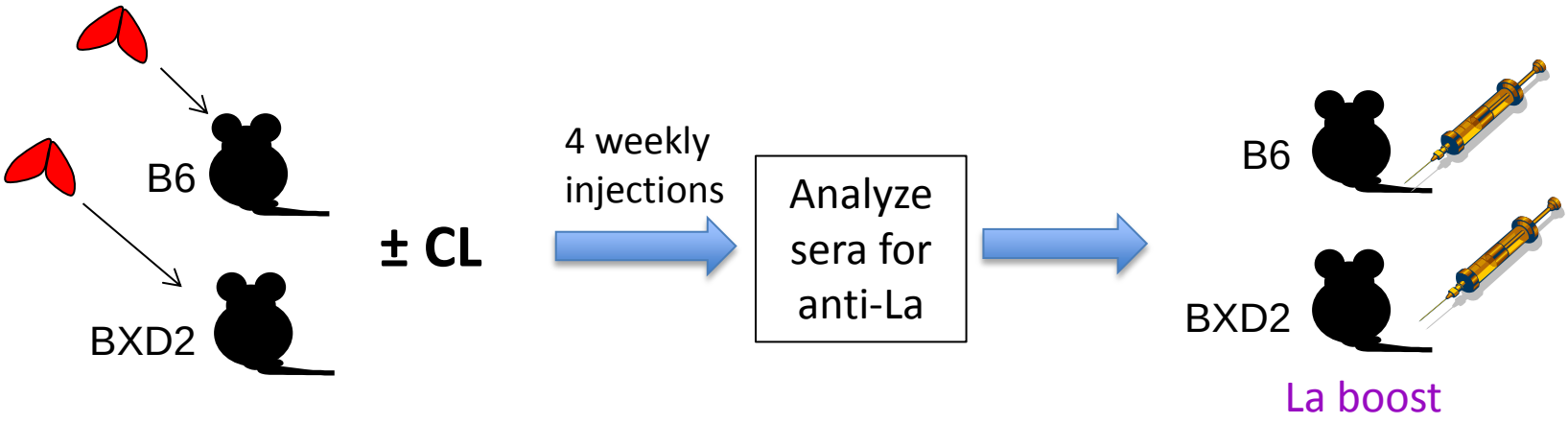


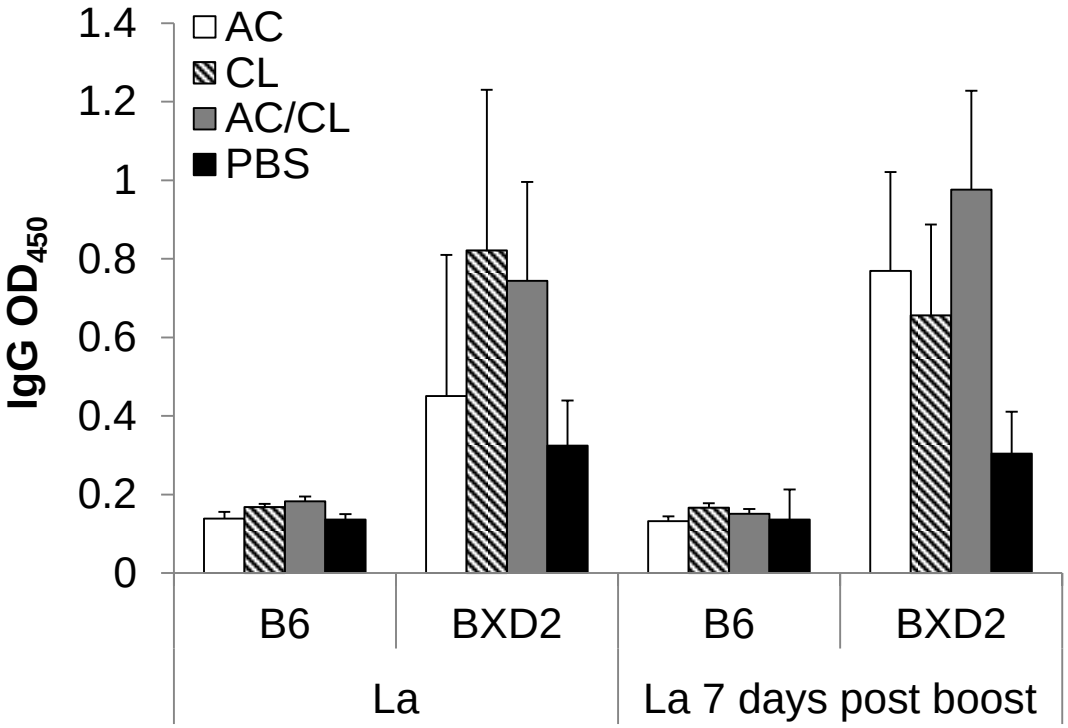
Modified from Pillai et al
Annu. Rev. Immunol. 2005. 23:161–96

Research in Progress

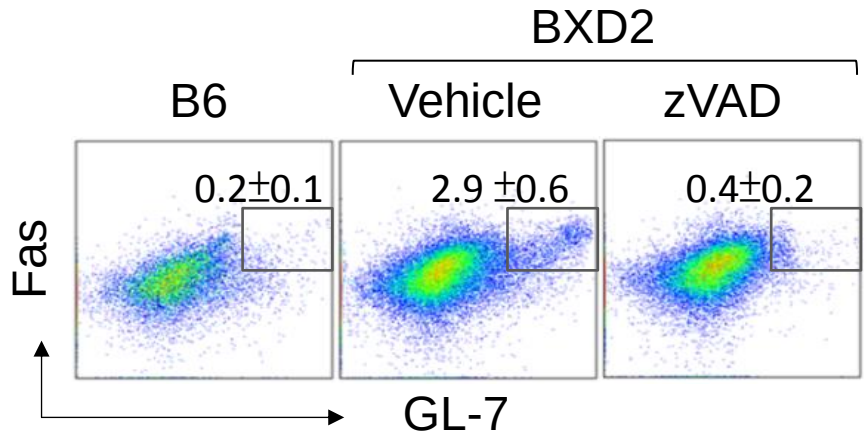
- Do transitional B cells from BXD2 mice exhibit immunoreactivities to apoptotic cell self antigens?
- And if so, is this related to defects in MZMs which then promoted the maturation of autoreactive B cells in BXD2 mice?

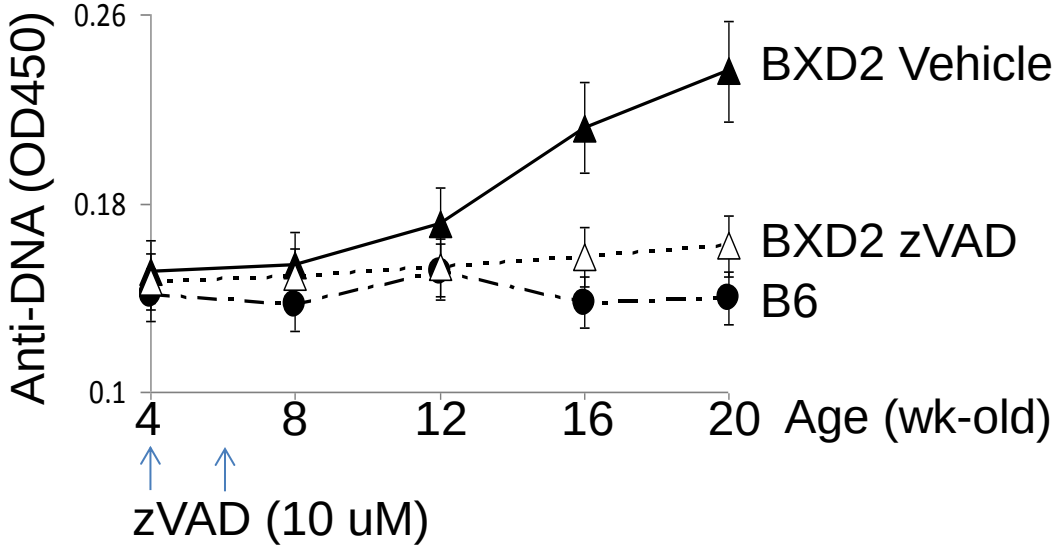
Anti-La IgG and chronic AC stimulation





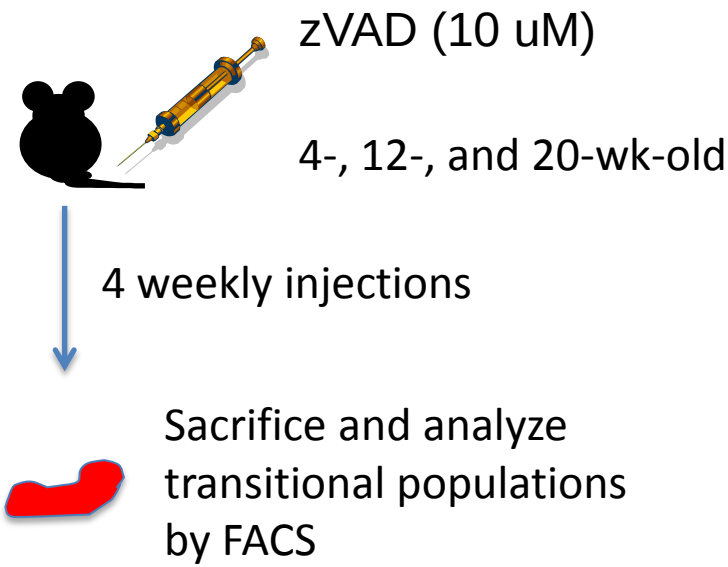
Inhibition of apoptosis suppressed anti-DNA antibody





Question-Does prevention of the generation of AC before the onset of autoimmunity blocks the skew toward the more mature T3 phenotype

- This will be done in mice in which ACs have been blocked with Z-VAD caspase inhibitor.

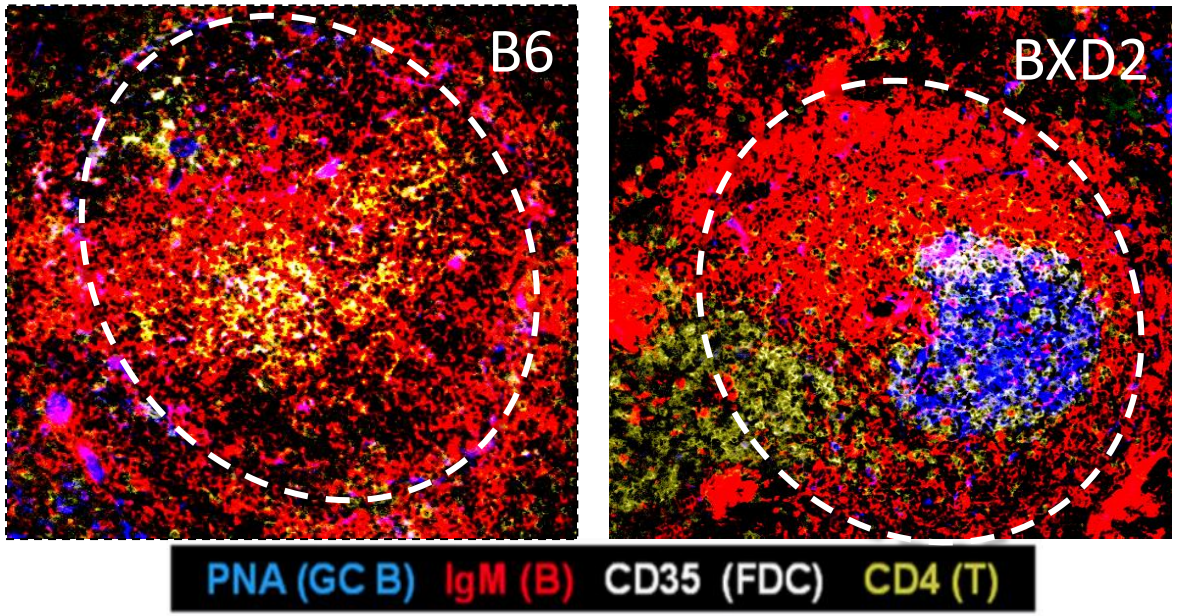


- **EXPECTED RESULTS**: Z-VAD treatment will shift the T1-T3 skewing to a more normalized ratio in BXD2 mice at young age. However, blocking of apoptotic responses in older BXD2 mice after disease onset may be an issue since this also prevent clonal deletion of autoreactive B cells.

Question – Does excessive ACs or elimination of MZM induce follicular localization of transitional B cells in autoimmune BXD2 mice

- **EXPERIMENTAL DESIGN**: The anatomical location of CD93^{+} transitional B cells will be determined by confocal imaging in normal B6 and lupus BXD2 mice at various ages.

- **EXPECTED RESULTS**: There will be increased CD93^{+} B cells within the follicle of BXD2 mice.



More follicular entry of IgM^{hi} CD93+ B cells in BXD2?

Hsu *et al. Nature Immunology*. 2008

Future Experimental Plans

- **Increased follicular entry** of transitional B cells in BXD2 mice compared to B6.
- Features of **anergy reversal** in transitional cells from BXD2 mice, including lower basal levels of pTyr, increased pTyr response after anti-BCR stimulation, increased baseline intracellular Ca2+ levels, and increased Ca2+ levels after BCR stimulation. This will be associated with B-cell proliferative responses following anti-IgM stimulation.
- **Elevated pSyk** in the expanded T3 B cells of MZM depleted BXD2 mice.
- Abnormal phenotypes of transitional B cells in BXD2 mice will be further augmented by disruption of MZMs or exogenous ACs. This will suggest integrity of MZMs or normal clearance of ACs can prevent maturation of autoreactive T1 B-cells to maintain B-cell tolerance.

Thank you