The Role of Programmed Death Ligand 2 (PD-L2) in Regulation of Immunopathology in *Schistosoma mansoni* Infection

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I. Abstract

Schistosomiasis is a tropical infectious disease caused by parasitic helminths of the genus Schistosoma. Infection with schistosomiasis results in a CD4 T cell-mediated inflammatory response. Tissue-lodged parasite eggs cause granulomatous inflammation resulting in morbidity and mortality in both humans and in the murine model of the disease. Infection with the species Schistosoma mansoni results in uneven liver immunopathology among different mouse strains. CBA/J (CBA) mice develop severe immunopathology mediated by Th17 cells whileC57BL/6 (BL/6) mice exhibit mild immunopathology in a Th2-polarized cytokine environment. The PD-L2 - PD-1 inhibitory pathway is of interest in our study of schistosomiasis because of its known role in preventing overly aggressive immune responses. Previous lab observations have shown that when the PD-L2 gene is silenced in C57BL/6 mice, infected mice exhibit a marked increase in immunopathology compared with WT controls. I hypothesized that measurement of cytokine levels would show that PD-L2-deficient (PD-L2-/-) BL/6 mice had increased levels of proinflammatory cytokines associated with a Th17 response and reduced levels of anti-inflammatory Th2-associated cytokines compared with WT BL/6 controls. We now show that in the absence of PD-L2 there is an increase in pathology associated with elevated proinflammatory cytokines IL-23 and IL-17, and reduced anti-inflammatory cytokine IL-4. These results suggest that the PD-L2 - PD-1 system is involved in curtailing immunopathology in experimental schistosomiasis.

II. Schistosomiasis

-Second most prevalent tropical parasitic disease in the world
- •76 countries affected
- •207 million people infected, 85% in Africa
- •200,000 deaths per year

-Caused by trematode helminths of the genus schistosoma

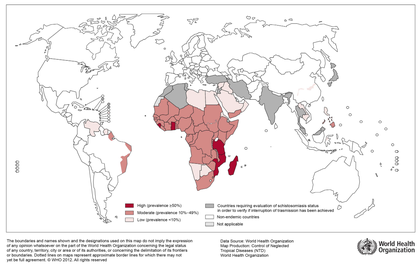
-3 main pathogenic species in humans
- •*Schistosoma mansoni, Schistosoma japonicum, Schistosoma haematobium*

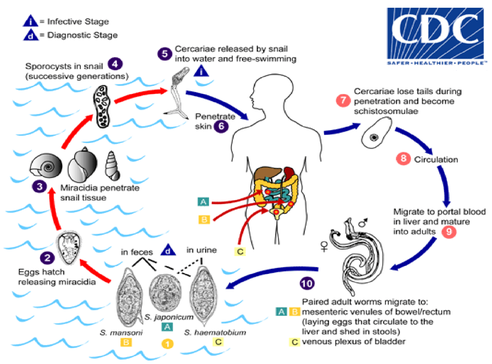
-Schistosomiasis is an immunologically mediated disease

-CD4+ T cells orchestrate inflammation

-Transmitted by contact with infected fresh water snails that serve as intermediate host

Distribution of schistosomiasis, worldwide, 2011





Source: http://www.cdc.gov/parasites/schistosomiasis/biology.html

III. Schistosomiasis Disease Pathology

Human pathology

SEVERE IMMUNOPATHOLOGY
- Hepatic fibrosis
- Portal hypertension
- Splenomegaly
- GI hemorrhage
- Death

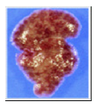
MILD IMMUNOPATHOLOGY
- Gastrointestinal symptoms

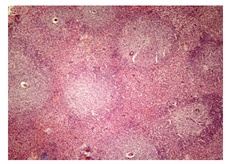
CLINICAL FORMS:

Murine mouse pathology

SEVERE IMMUNOPATHOLOGY

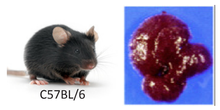
CBA/J

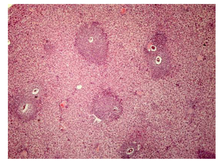




MILD IMMUNOPATHOLOGY

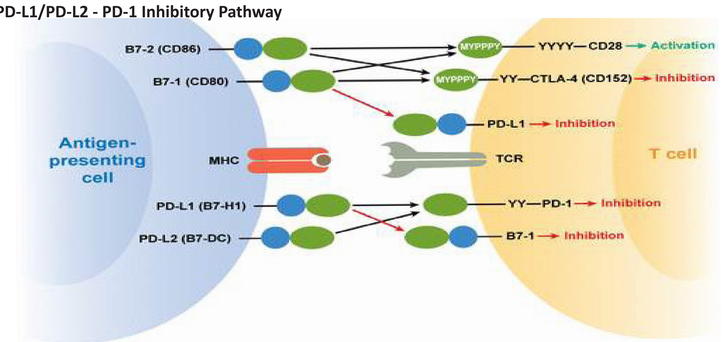
C57BL/6





IV. PD-L2

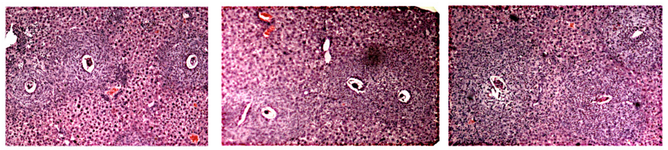
The PD-L1/PD-L2 - PD-1 Inhibitory Pathway



Keir ME, et al. 2008.
Annu. Rev. Immunol. 26:677–704

Liver Immunopathology in PD-L1-/-, PD-L2-/- and BL/6 WT Mice

A.



B.

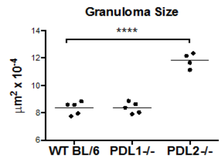
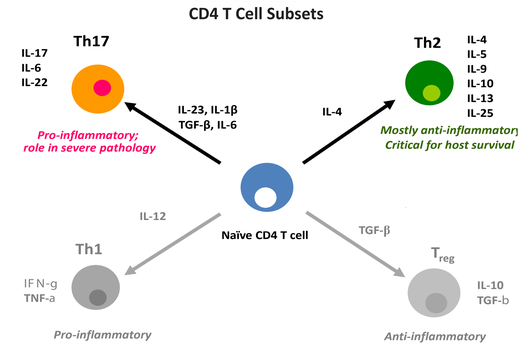


Figure 1. Granulomatous inflammation is more severe in PD-L2-/- mice. Focus shifted away from PD-L1 because of lack of change in PD-L1 deficient mice. A) Livers were removed 7 weeks post-infection, sectioned and stained. 10x magnification is shown. B) Morphometric analysis was used to determine granuloma size with individual dots representing the average granuloma size of individual mice. ****p<0.0001 (D. Brenda Salantes)

V. Objective

Measure levels of pro-inflammatory and anti-inflammatory cytokines in vivo in liver, spleen, and mesenteric lymph nodes (MLN) from schistosome-infected PD-L2-/- and control mice



VI. Methods

Acute Infection Model

WT CBA

WT BL/6

PD-L2-/-

i.p. injection with 85 *S. mansoni* cercariae

Sacrifice

0 1 2 3 4 5 6 7
Time (weeks)

Harvest:
Splenocytes
Mesenteric lymph node cells (MLNC)
Liver

Cytokine levels were measured by ELISA (protein) and qPCR (mRNA)

VII. Cytokine Levels in 7 week-infected PD-L2-/- and WT Control Mice

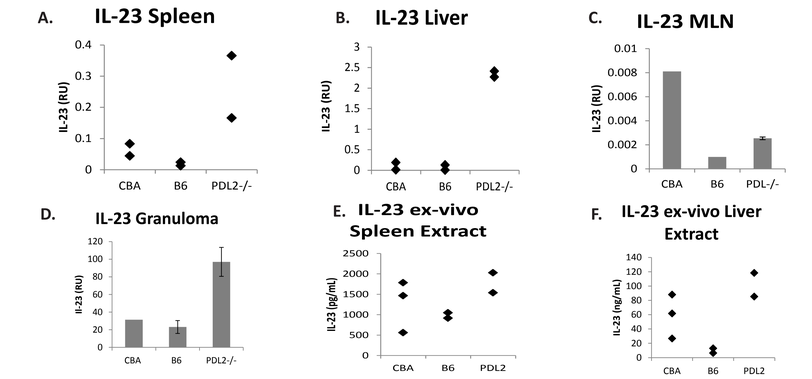


Figure 1. Proinflammatory Th17-associated cytokine IL-23 is increased in PD-L2-/- mice. MLN and granuloma cells were pooled. A, B, C, and D depict IL-23 levels measured by qPCR. E and F depict IL-23 levels measured by ELISA.

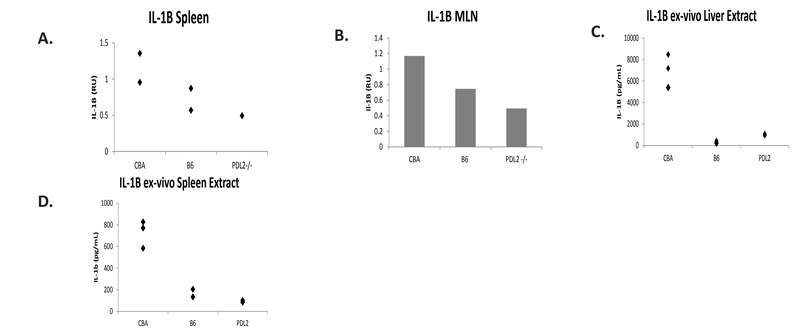


Figure 2. There is no correlation between proinflammatory Th17-associated cytokine IL-1β and high pathology in PD-L2-/- mice. MLN were pooled. A, and B depict IL-1β levelsmeasured by qPCR. C and D depict IL-1β levels measured by ELISA.

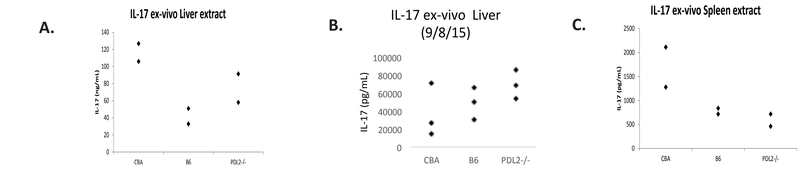


Figure 3. Proinflammatory Th17-associated cytokine IL-17 is increased in PD-L2-/- mice in liver, but not in spleen. A, B, C, and D depict IL-17 levels measured by ELISA. C and D are repeatd experiments.

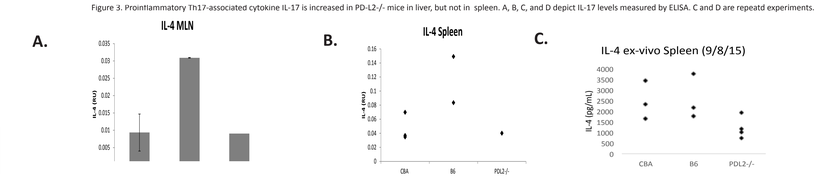


Figure 4. Anti-Inflammatory Th2-associated cytokine IL-4 is decreased in PD-L2-/- mice. A and B depict IL-4 levels measured by qPCR, C depicts IL-4 levels measured by ELISA.

VIII. Summary and Conclusions

CBA are high-pathology mice that express high levels of pro-inflammatory cytokines IL-1β, IL-23, IL-17

BL/6 are low-pathology mice that express high levels of anti-inflammatory cytokine IL-4

In the absence of PD-L2, BL/6 mice exhibit a dramatic increase in pathology associated with elevated pro-inflammatory cytokines IL-23 and IL-17, and reduced anti-inflammatory cytokine IL-4. IL-1β levels do not differ significantly

The PD-L2 - PD-1 system is involved in curtailing immunopathology in experimental murine schistosomiasis

IX. Acknowledgements

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