THE EFFECTS OF NAPHTHENIC ACIDS AND OIL SANDS PROCESS WATER ON THE IMMUNE GENE EXPRESSION OF MICE

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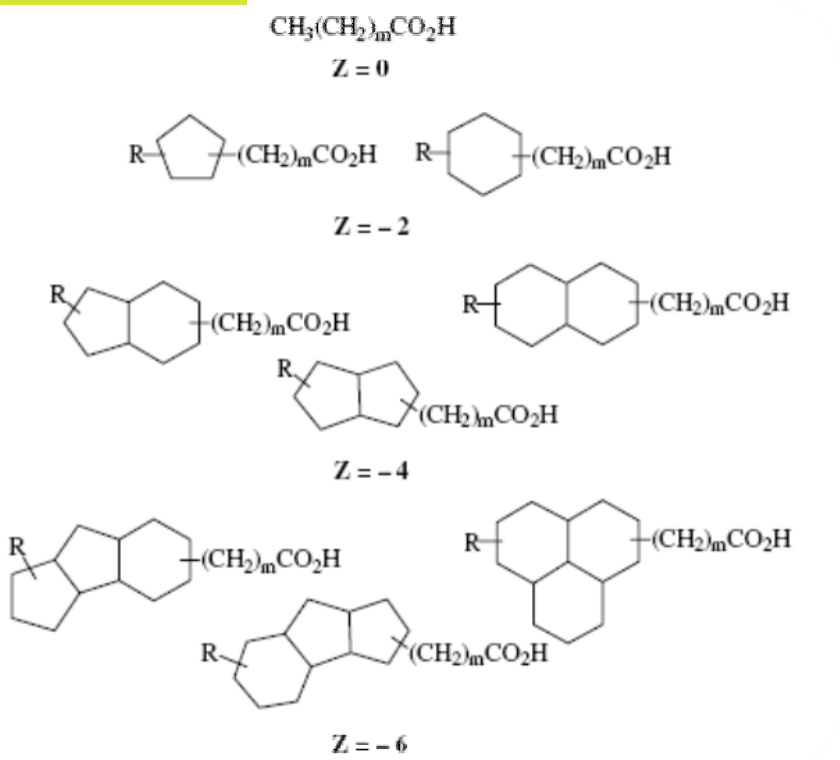
NAPHTHENIC ACIDS (NAS)



Clemente *et al.*, 2005.

- Naturally-occurring cyclic and acyclic alkyl-substituted aliphatic carboxylic acids found in petroleum
- Primary toxic component of Oil Sands Process Water (OSPW)
  - Concentration from 40-120 mg/L in tailing ponds.

OIL SANDS PROCESS WATER (OSPW)

NAVTEQ, 2008

- OSPW is accumulated on tailings ponds due to the zero discharge policy
- In order for this water to be released to the environment adequate remediation must be devised to reduce its toxicity
- Appropriate bio-markers must be developed, to allow for the evaluation of toxicity reduction

TOXICITY TESTS AND BIOMARKERS

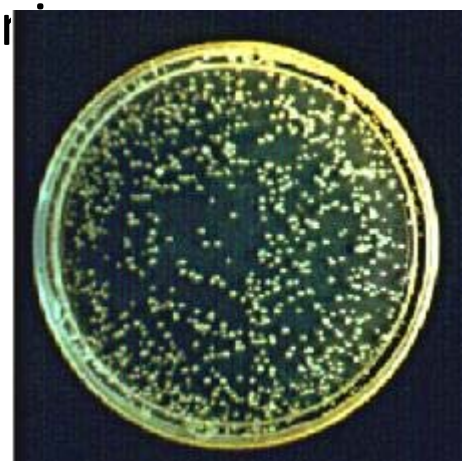
Microorganism-based test have become very popular for evaluating the toxicity of water contaminants.

E.g. the *Microtox* assay is based on the measurement of growth of a luminiscent bacteria (*Vibrio fischeri*) in presence of toxic substances.

http://1.bp.blogspot.com/_SZ24qCjKhRg/3ybqFw/s320/vibrio.jpg

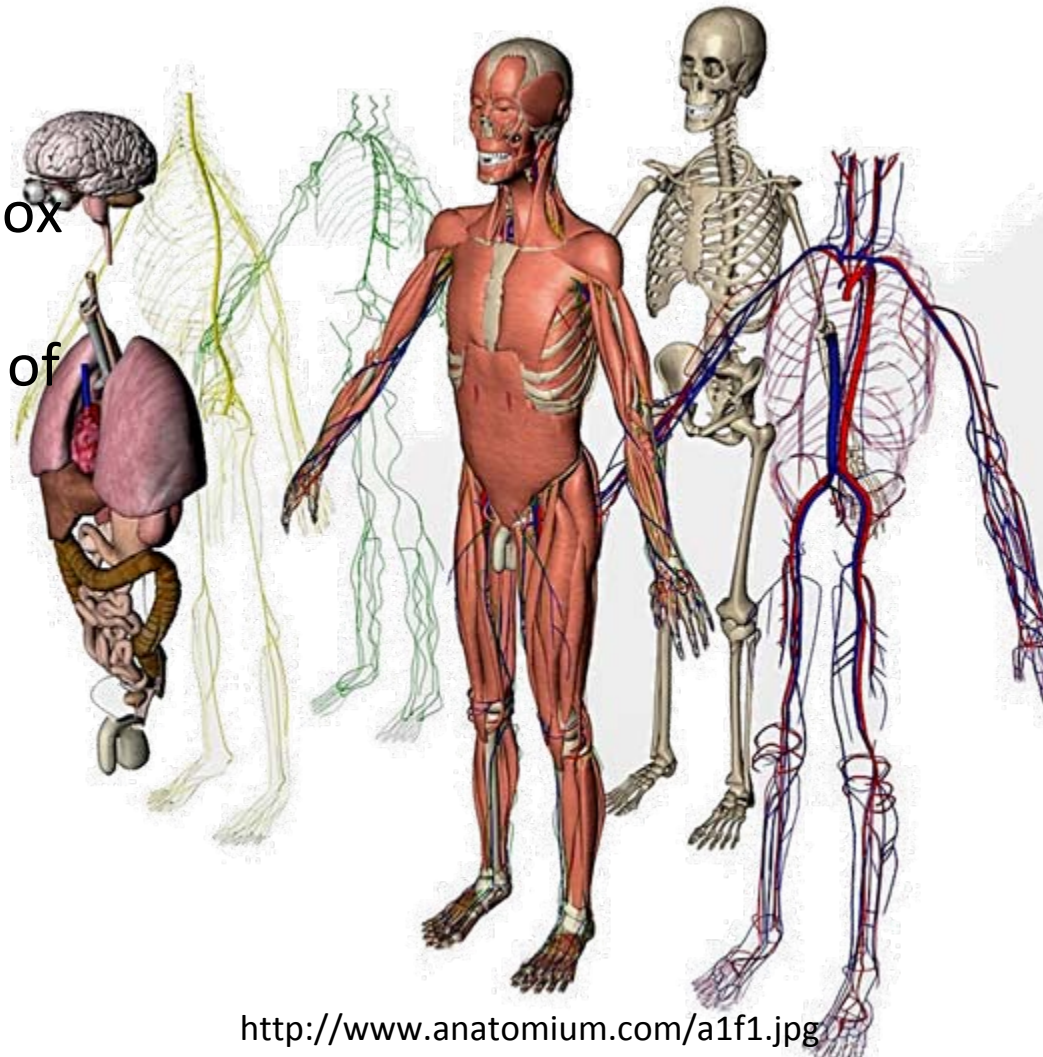
BACTERIA-BASED TOXICITY ASSAYS MAY NOT REFLECT THE EFFECTS OF CONTAMINANTS ON COMPLEX ORGANISMS

Although the Microtox assay is fast, and sensitive, “reduced toxicity” measured by the Microtox assay
may not necesarily reflect a lack of toxicity for more complex organi



www.ou.edu/.../faculty/pictures/vibrio.jpg

vs.



http://www.anatomium.com/a1f1.jpg

TOXICITY OF OSPW: FISH

- Mortality
  - Approx. 50%, in fish exposed to 25-75 mg/L NAs.
- Developmental abnormalities
- Gill and liver pathology
  - Excessive gill mucous
  - Necrosis of gill epithelial cells
  - Inflammatory cells in liver and gill
  - **Presence of virally induced tumors.**

http://www.learnnc.org/lp/media/uploads/2007/09/yellow_perch.jpg

TOXICITY OF OSPW: BIRDS

- Main focus on tree swallow (*Tachycineta bicolor),* as a sentinel species.
- Short-term exposure – few adverse effects
- Chronic exposure -
  - Blood biochemical changes
  - Inability to cope with harsh weather
  - **Increase in the incidence of parasitic infection by the blowfly *Protocalliphora* spp.**

http://www.animalpicturesarchive.com/ArchOLD-6/1188124544.jpg

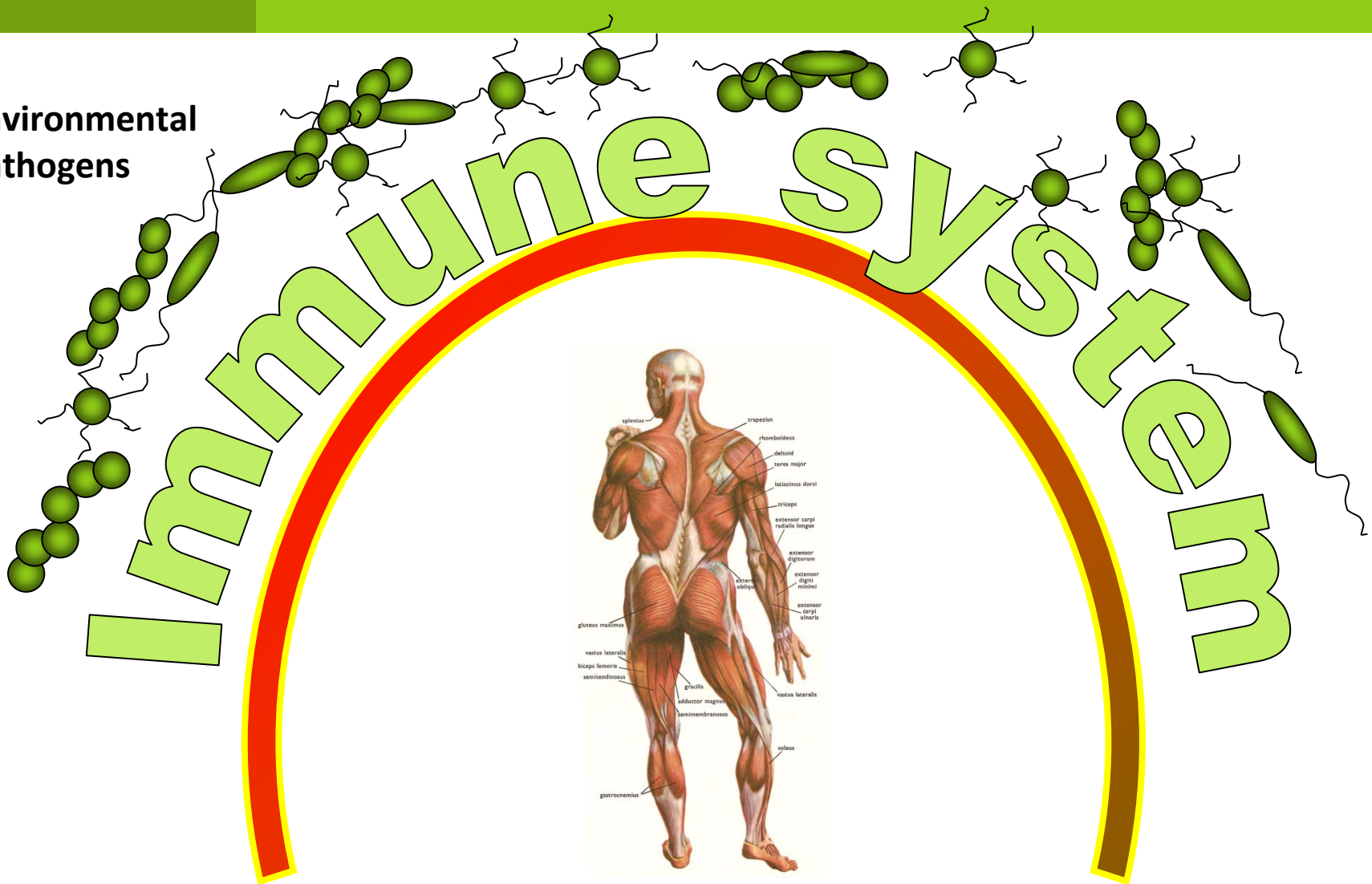
TOXICITY OF OSPW: MAMMALS

- Wistar rats tested
- Acute exposure
  - liver pathology
  - vascular damage
- Subchronic exposure
  - liver biochemistry is primarily affected
- **Effects of OSPW in mammalian immunity not evaluated**

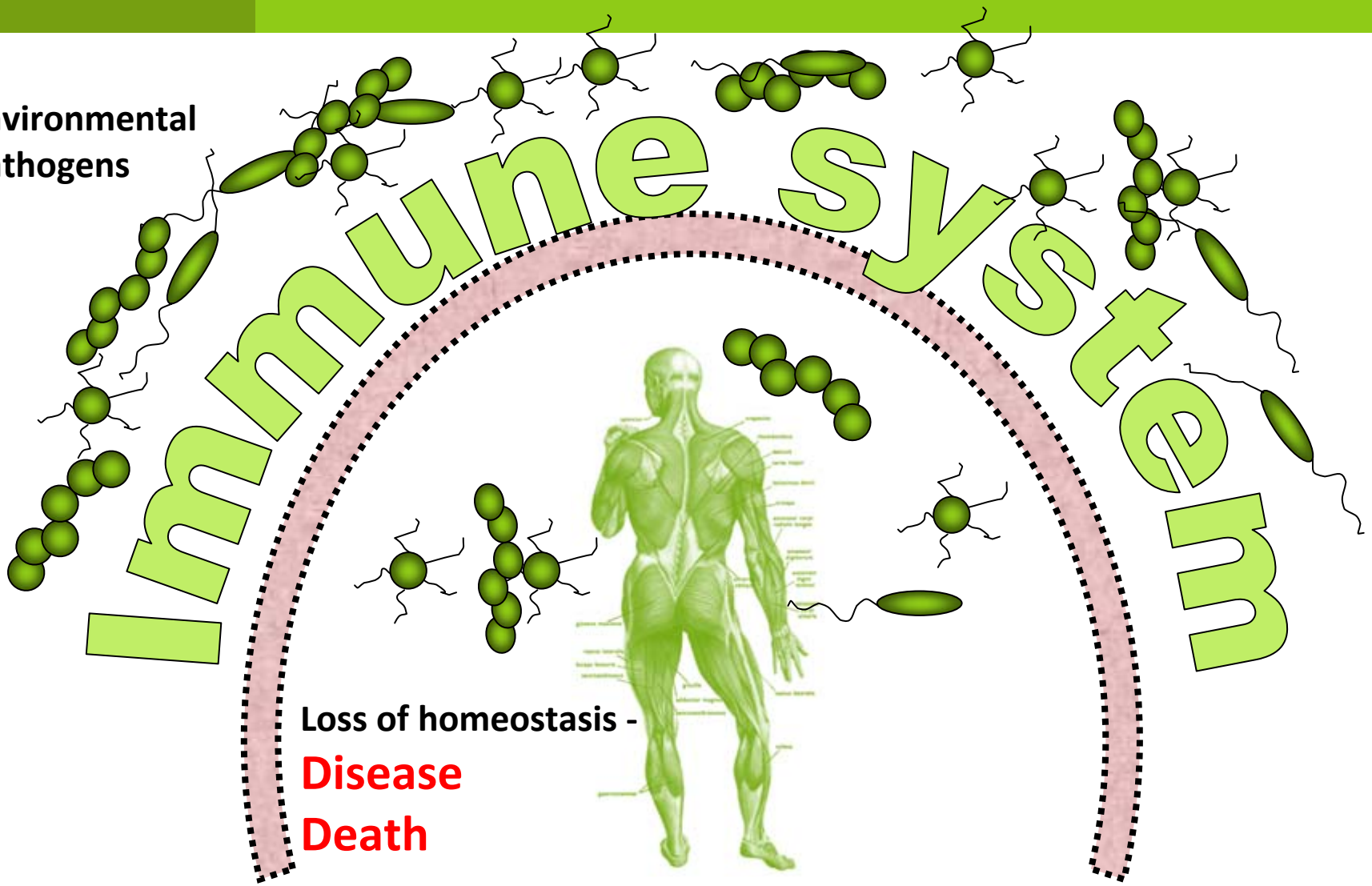
http://www.iar.or.jp/shodobutsu/wi_rat/images/wistar_image02.jpg

PROTECTIVE FUNCTION OF THE IMMUNE SYSTEM

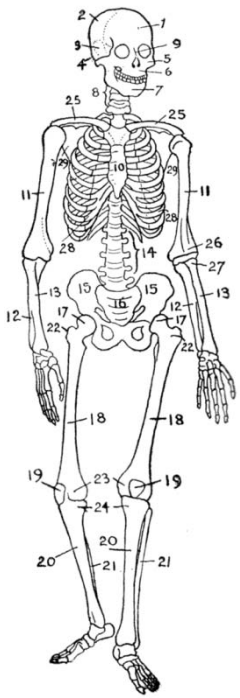
Environmental pathogens



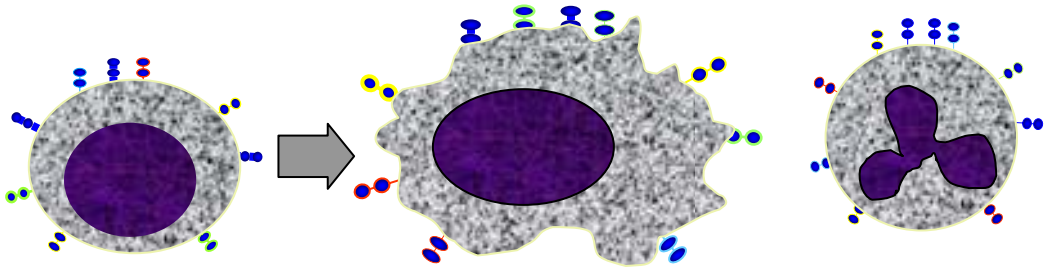
PROTECTIVE FUNCTION OF THE IMMUNE SYSTEM



VERTEBRATE IMMUNE SYSTEM

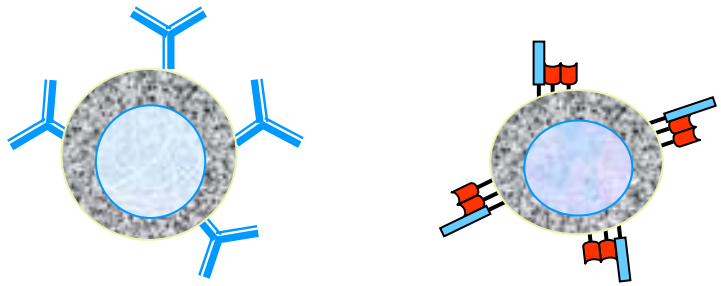


Innate Immunity



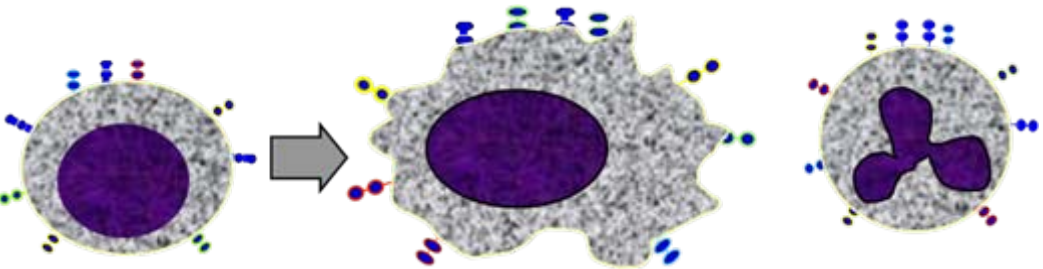
Monocytes **Macrophages** **Neutrophils**

Adaptive Immunity



B lymphocytes **T Lymphocytes**

INNATE IMMUNITY

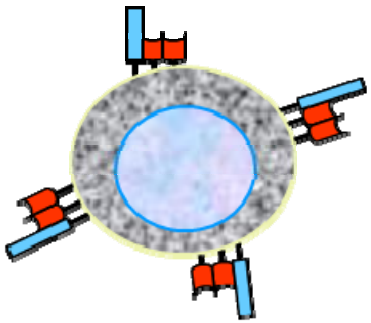


Monocytes Macrophages Neutrophils

Non-specific cell responses

- Phagocytosis
- Production of reactive oxygen species (ROI)
- Production of Nitric Oxide
- Release of antimicrobial proteins and peptides
- Release of pro-inflammatory molecules

ADAPTIVE IMMUNITY

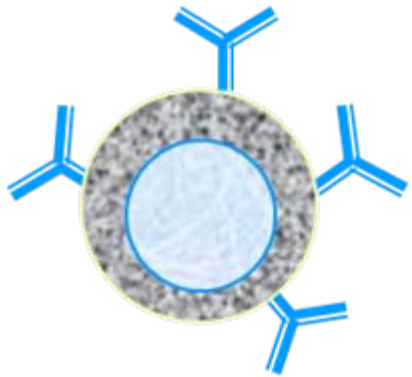


T Lymphocytes

Antigen-specific

$CD8^+$ Cytotoxic cells

$CD4^+$ Regulatory cells



B lymphocytes

Antigen-specific

Antibody production

MAIN OBJECTIVES

- To analyze the effect of OSPW contaminants on the immune system of mice
  - Commercial naphthenic acids
  - Organic fraction OSPW
- To assess the potential of advanced oxidation for reduction of OSPW toxicity

HYPOTHESES

a) Following exposure to NAs or OSPW the immune system of mice will be altered, possibly showing signs of enhanced inflammation and/or immune down-regulation.

b) Immuno-toxic effects of OSPW will be removed following advanced oxidation treatment.

AIMS OF RESEARCH

- **AIM 1:** Isolation of the organic fraction from OSPW.
- **AIM 2:** Elucidate the possible effects of acute and subchronic exposure to commercial NAs and the organic fraction of OSPW on immune gene expression and selected immune functions of mice.
- **AIM 3:** Examine the ability of animals exposed to OSPW to handle experimental infections (e.g. *Giardia muris).*
- **AIM 4:** Determine whether advanced oxidation decreases the observed toxic effects of OSPW.

AIM 1: ISOLATION OF ORGANIC FRACTION OF OSPW

Adjust pH of OSPW to 10.5 using 5M NaOH

↓

Clarify water by centrifugation

↓

Organic extraction: three times with 100 mL dichloromethane

→

Adjust pH to 2.0 using 5M HCL

↑

Organic extraction: three times with 100 mL dichloromethane

↑

Collect dichloromethane and evaporate solvent

→

Reconstitute organic fraction into a small volume of high pH aqueous solution

AIM 1

- Isolation of organic fraction from OSPW:
  - **40 Litres**
    - **Organic fraction weight: 2.48g**
    - **NA content: 1.07g (43% of total)**
- **40 Litres of Distilled Water were subjected to extraction protocol to be used as control**

AIM 2

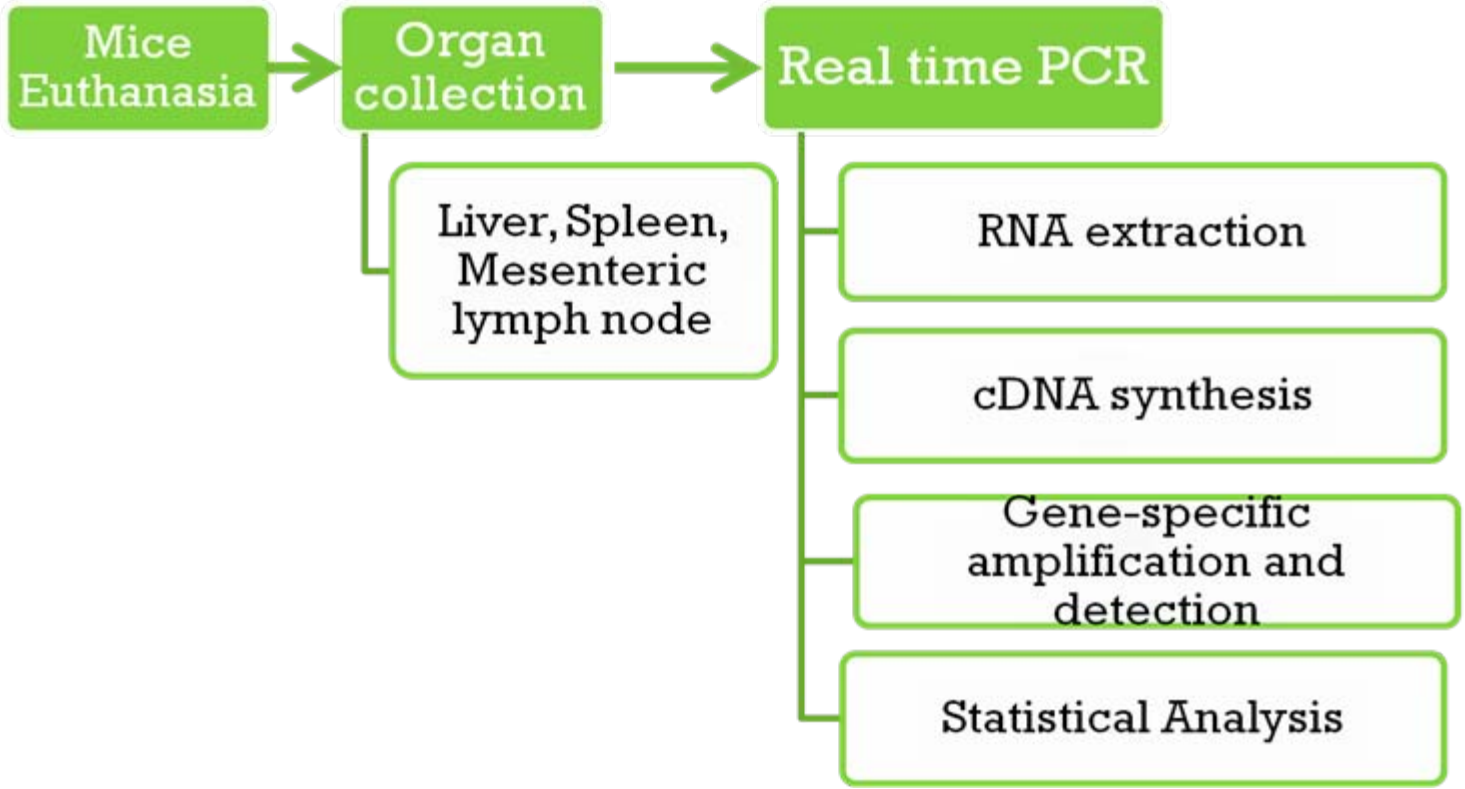
- Elucidate the possible effects of NAs and the organic fraction of OSPW on immune gene expression and selected immune functions of mice
  - **Analysis of expression of 20 immune genes, and 4 house keeping genes by real time PRC in livers and spleens**
  - Analysis of phagocytosis using peritoneal macrophages

EXPOSURE OF MICE TO NAS AND OSPW ORGANIC FRACTION

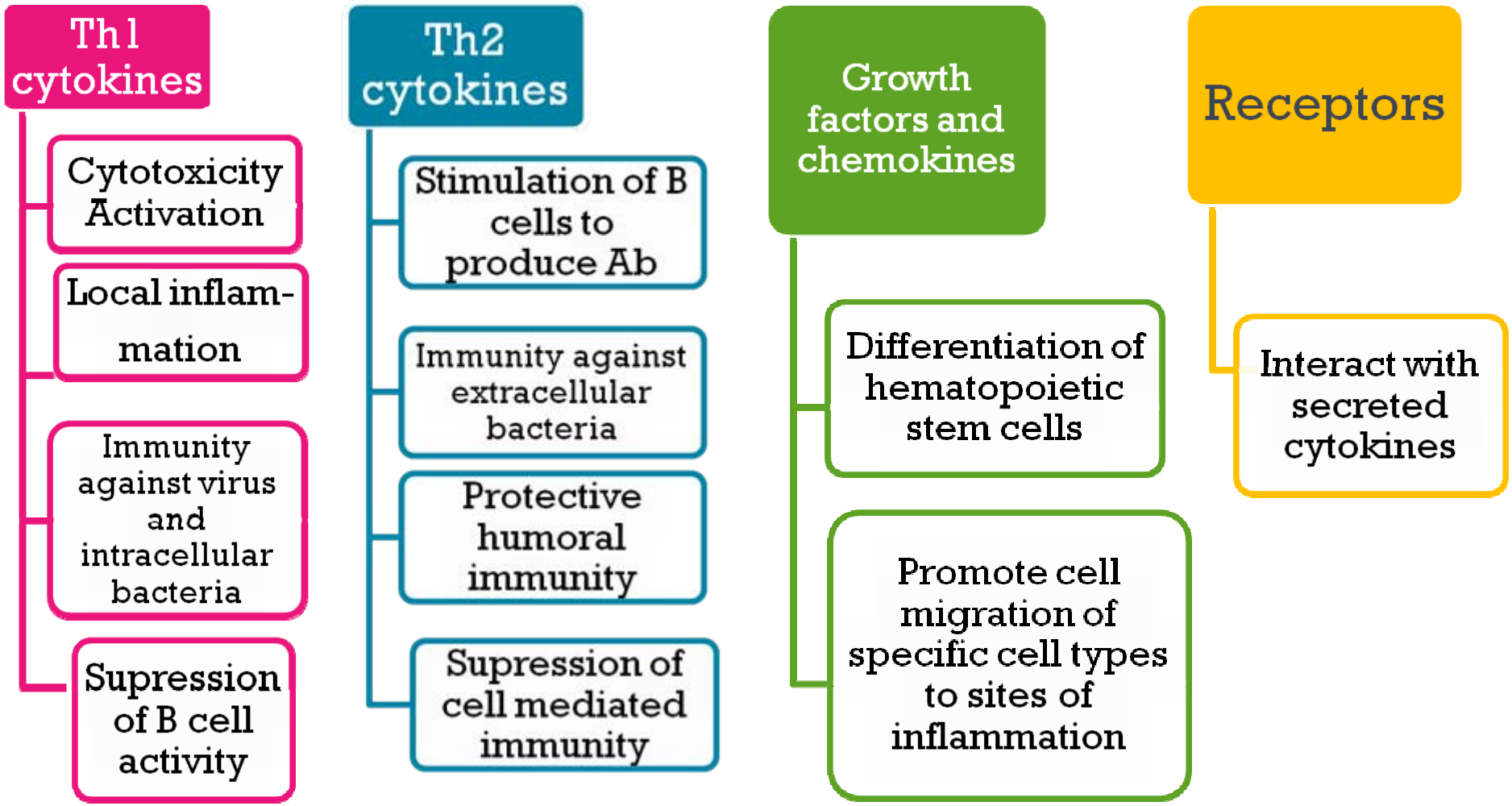
- **Exposure to commercial NAs**
  - **50 and 100 mg/Kg NAs. One dose weekly**
  - **Samples taken at:**
    - **Week 1, Week 2, Week 4, and Week 8**
- **Exposure to OSPW organic fraction (43.1% NAs – 56.9% other contaminants)**
  - **50 and 100 mg/Kg NAs. One dose weekly**
  - **Samples taken at:**
    - **Week 1,Week 2, Week 4, Week 8, Week 12**

http://www.criver.com/SiteCollectionImages/Images_255x164/rm_mice_black1_0015_lres.jpg

GENE EXPRESSION ANALYSIS

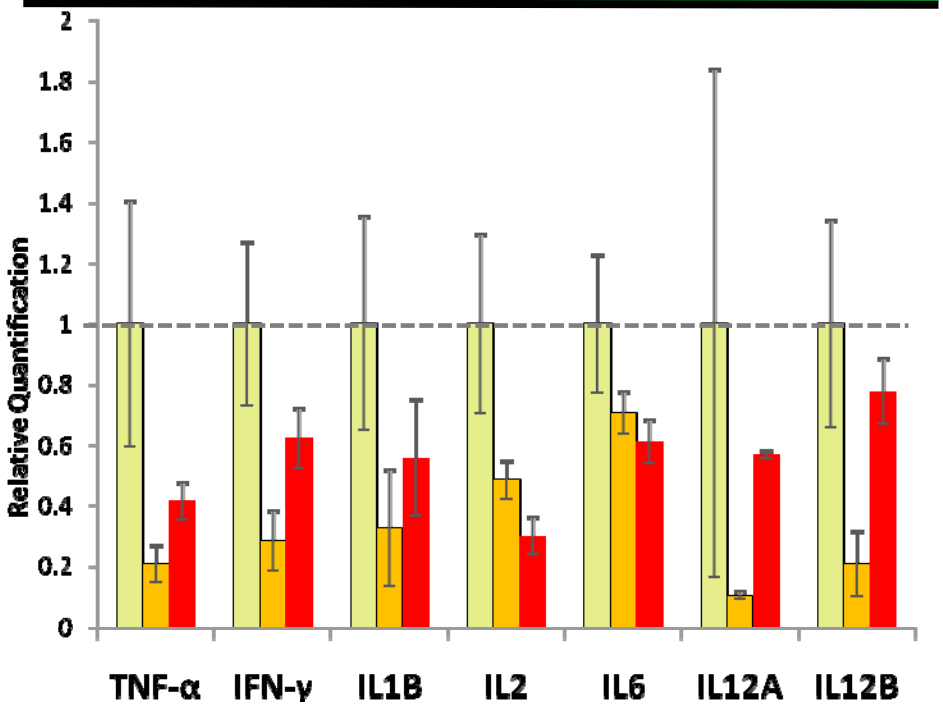


CATEGORIES OF IMMUNE GENES ANALYZED BY REAL-TIME PCR



LIVER – TH1 CYTOKINES WEEK 1

NAs



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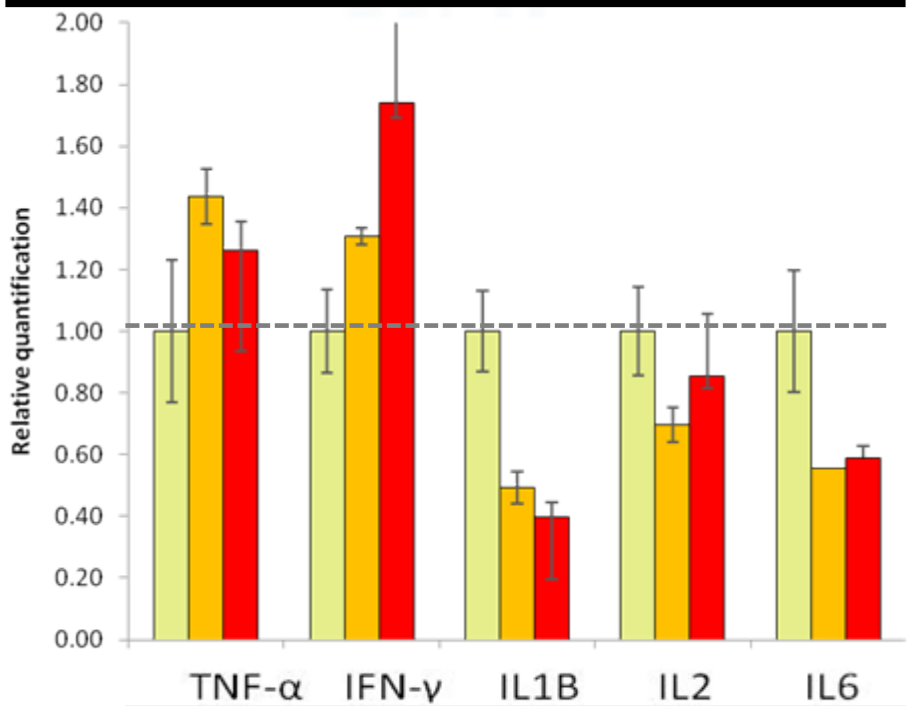
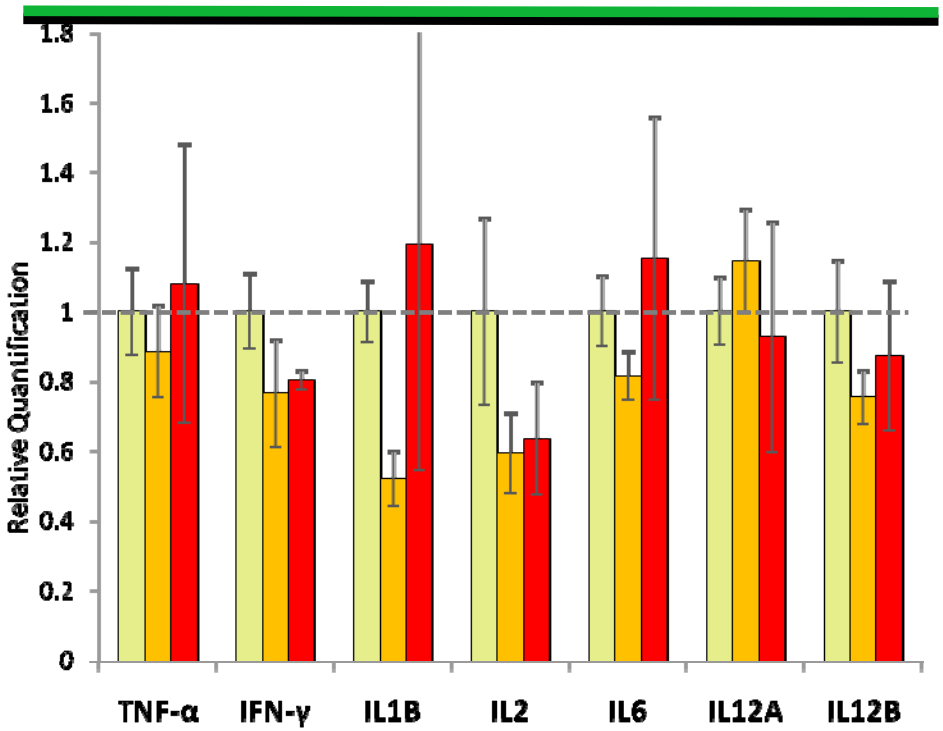


LIVER – TH1 CYTOKINES WEEK 2

NAs



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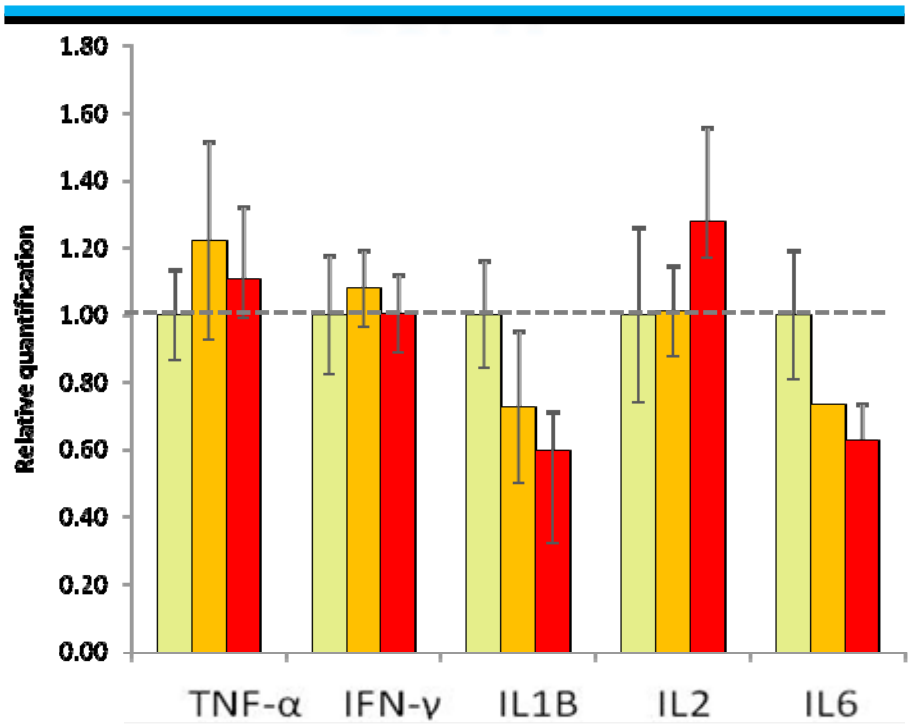
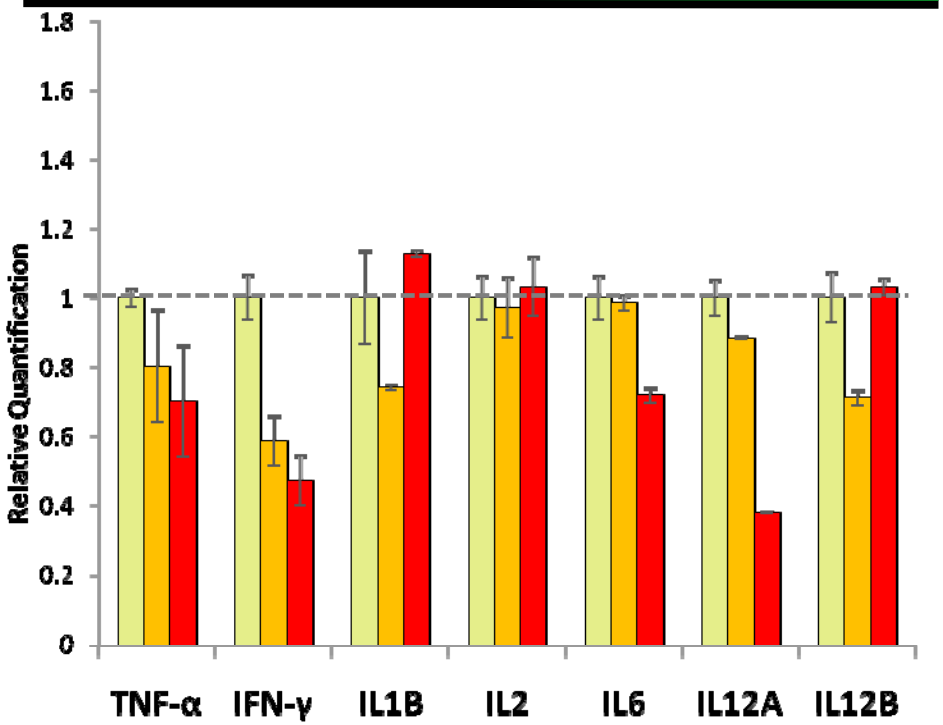


LIVER – TH1 CYTOKINES WEEK 4

NAs



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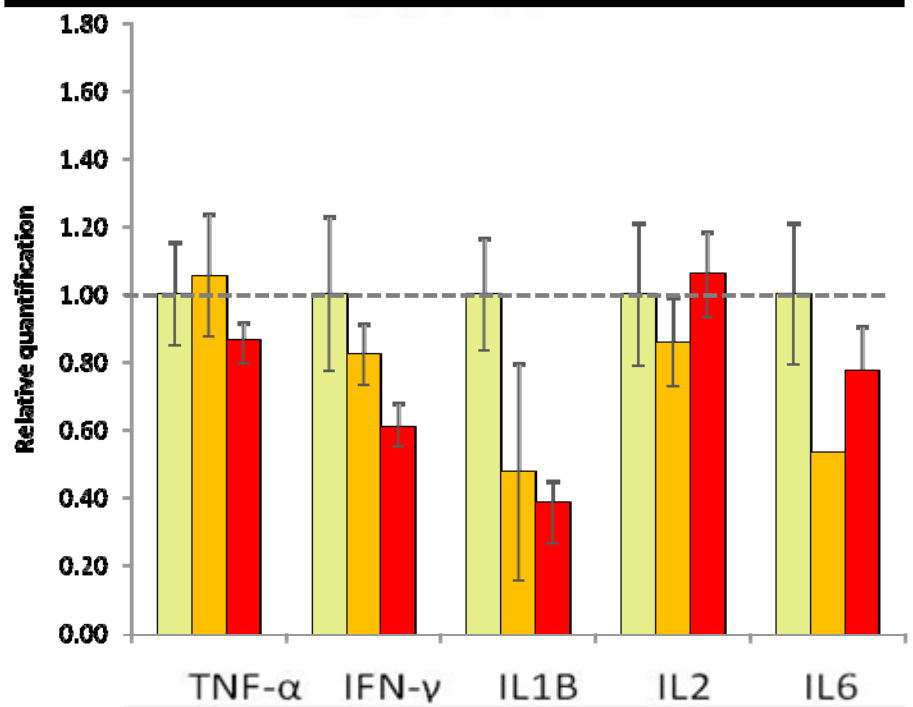
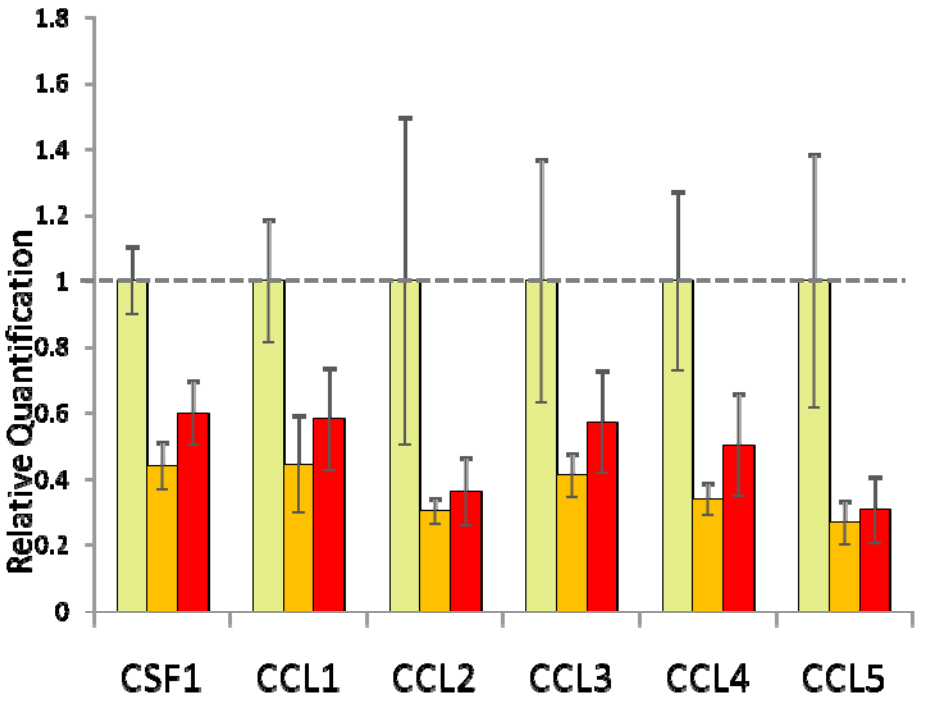


LIVER – GROWTH FACTORS AND CHEMOKINES WEEK 1

NAs



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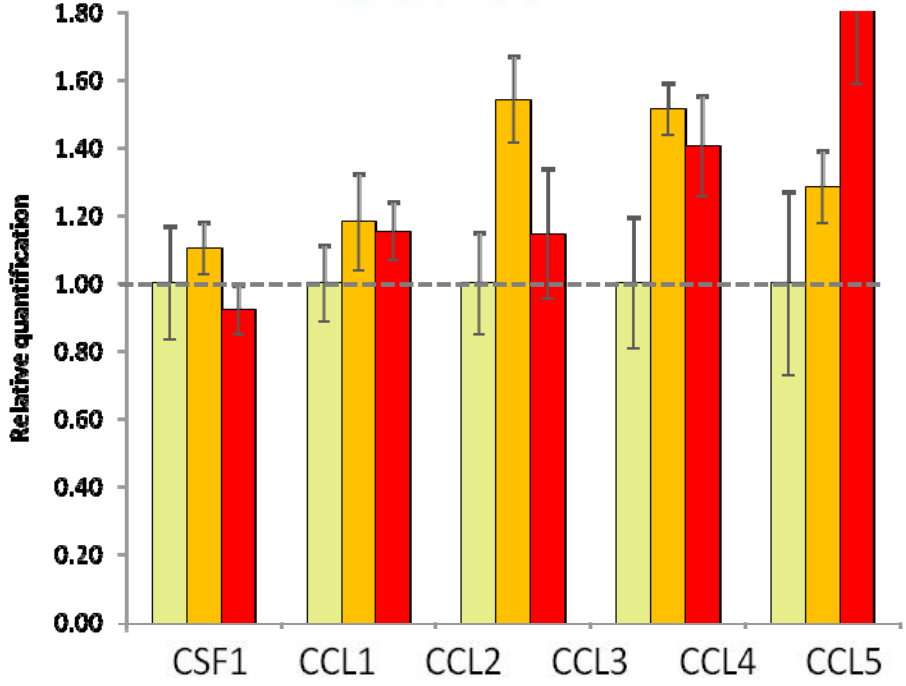


SUMMARY - EFFECT OF NAS ON IMMUNE GENE EXPRESSION IN THE LIVER

		TNF-α		IFN-γ		IL-1β		IL-2		IL-6		IL-12A		IL-12B	
Th1 cytokines	NAs (mg/kg)	50	100	50	100	50	100	50	100	50	100	50	100	50	100
	Week1	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
	Week2	NC	NC	NC	NC	NC	NC	↓	↓	NC	NC	NC	NC	NC	NC
	Week4	NC	↓	↓	↓	NC	NC	NC	NC	NC	↓	NC	↓	NC	NC

		TGF-β1		IL-4		IL-5		IL-10	
Th2 cytokines	NAs (mg/kg)	50	100	50	100	50	100	50	100
	Week1	↓	↓	NC	NC	↑	NC	↓	↓
	Week2	↑	↑	NC	NC	NC	NC	↑	↑
	Week4	NC	↑	NC	NC	NC	NC	NC	NC

		CSF1		CCL1		CCL2		CCL3		CCL4		CCL5	
Growth factors and chemokines	NAs (mg/kg)	50	100	50	100	50	100	50	100	50	100	50	100
	Week1	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
	Week2	↑	↑	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
	Week4	NC	↓	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC

		CSF1R		TNFRSF1A		TNFRSF1B	
Cytokine Receptors	NAs (mg/kg)	50	100	50	100	50	100
	Week1	↓	↓	↓	↓	NC	NC
	Week2	↑	↑	↑	↑	↑	↑
	Week4	NC	↓	NC	↓	NC	↓

SUMMARY - EFFECT OF OSPW ON IMMUNE GENE EXPRESSION IN THE LIVER

		TNF-α		IFN-γ		IL-1β		IL-2		IL-6	
Th1 cytokines	NAs (mg/kg)	50	100	50	100	50	100	50	100	50	100
	Week1	NC	NC	↑	↑	↓	↓	NC	NC	↓	↓
	Week2	NC	NC	NC	NC	↓	↓	NC	NC	↓	↓
	Week4	NC	NC	↓	↓	↓	NC	NC	NC	↓	↓
	Week8	NC	NC	↑	NC	↓	↓	NC	NC	↓	↓
	Week12	NC	NC	NC	NC	↓	↓	↑	NC	NC	NC

		TGF-β1		IL-4		IL-10	
Th2 cytokines	NAs (mg/kg)	50	100			50	100
	Week1	↑	↑	NC	↑	↑	↑
	Week2	NC	NC	↓	↓	↑	↑
	Week4	NC	NC	NC	NC	↑	↑
	Week8	↓	↓	NC	NC	↑	↑
	Week12	NC	NC	NC	NC	NC	NC

		CSF1		CCL2		CCL3		CCL4		CCL5	
Gowth factors and chemokines	NAs (mg/kg)	50	100	50	100	50	100	50	100	50	100
	Week1	NC	NC	NC	NC	NC	NC	↑	↑	↑	↑
	Week2	NC	NC	NC	NC	↑	NC	↑	NC	↑	NC
	Week4	NC	NC	NC	NC	↑	NC	↑	↓	↑	↓
	Week8	NC	↓	NC	NC	NC	NC	NC	NC	NC	NC
	Week12	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC

		CSF1R		TNFRSF1A	
Cytokine receptors	NAs (mg/kg)	50	100	50	100
	Week1	NC	NC	NC	↑
	Week2	↑	↑	NC	NC
	Week4	NC	NC	NC	NC
	Week8	↓	↓	↓	↓
	Week12	NC	NC	NC	NC

SUMMARY -EFFECT OF NAS ON IMMUNE GENE EXPRESSION IN THE SPLEEN

		TNF-α		IFN-γ		IL-1β		IL-2		IL-6		IL-12A		IL-12B	
Th1 cytokines	NAs (mg/kg)	50	100	50	100	50	100	50	100	50	100	50	100	50	100
	Week1	NC	NC	↓	↓	NC	↓	NC	NC	NC	NC	↑	↑	NC	↓
	Week2	↑	↑	↑	↑	NC	NC	NC	↑	NC	↑	↑	↑	↑	↑
	Week4	NC	NC	NC	NC	↓	↓	NC	NC	↓	↓	NC	NC	NC	NC

		TGF-β1		IL-4		IL-5		IL-10	
Th2 cytokines	NAs (mg/kg)	50	100	50	100	50	100	50	100
	Week1	NC	NC	↑	↑	NC	NC	↓	↓
	Week2	↑	↑	NC	NC	↑	↑	↑	↑
	Week4	↓	↓	NC	NC	NC	NC	NC	NC

		CSF1		CCL1		CCL2		CCL3		CCL4		CCL5	
Growth factors and chemokines	NAs (mg/kg)	50	100	50	100	50	100	50	100	50	100	50	100
	Week1	↑	↑	↑	NC	↓	↓	↓	↓	↓	↓	↓	↓
	Week2	↑	↑	↑	↑	NC	NC	↑	↑	NC	↑	↑	↑
	Week4	↓	↓	↓	NC	NC	NC	NC	NC	NC	NC	↓	NC

		CSF1R		TNFRSF1A		TNFRSF1B	
Cytokine Receptors	NAs (mg/kg)	50	100	50	100	50	100
	Week1	NC	NC	↓	↓	↓	↓
	Week2	NC	NC	NC	↓	NC	NC
	Week4	NC	NC	NC	NC	NC	NC

SUMMARY - EFFECT OF OSPW ON IMMUNE GENE EXPRESSION IN THE SPLEEN

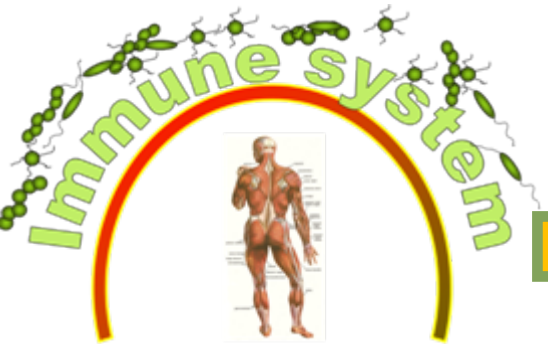
		TNF-α		IFN-γ		IL-1β		IL-2		IL-6	
Th1 cytokines	NAs (mg/kg)	50	100	50	100	50	100	50	100	50	100
	Week1	NC	↓	↓	↓	↓	↓	↓	↓	↓	↓
	Week2	↑	↑	NC	NC	NC	NC	NC	NC	NC	NC
	Week4	↓	NC	↓	↓	↓	NC	↓	NC	↓	NC
	Week8	NC	↓	↑	↓	↓	↓	↓	↓	↓	↓
	Week12	↓	↓	↓	↓	↓	↓	↓	↓	↓	NC

		TGF-β1		IL-4		IL-10	
Th2 cytokines	NAs (mg/kg)	50	100	50	100	50	100
	Week1	NC	↑	NC	↓	NC	NC
	Week2	NC	NC	NC	NC	↑	↑
	Week4	↓	NC	NC	NC	NC	NC
	Week8	↓	↓	↓	↓	↓	↓
	Week12	↓	↓	↓	↓	NC	NC

		CSF1		CCL2		CCL3		CCL4		CCL5	
Gowth factors and chemokines	NAs (mg/kg)	50	100	50	100	50	100	50	100	50	100
	Week1	NC	NC	NC	NC	NC	NC	NC	NC	NC	↑
	Week2	↑	↑	NC	NC	NC	NC	NC	NC	NC	NC
	Week4	↓	NC	NC	NC	NC	NC	↓	↓	↓	↓
	Week8	↓	↓	NC	↓	↓	↓	↓	↓	↓	↓
	Week12	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓

		CSF1R		TNFRSF1A	
Cytokine receptors	NAs (mg/kg)	50	100	50	100
	Week1	NC	NC	NC	NC
	Week2	↑	↑	NC	↑
	Week4	↓	NC	↓	NC
	Week8	↓	↓	↓	↓
	Week12	↓	↓	↓	↓

IMMUNE DOWN-REGULATION CAN LEAD TO DISEASE AND POSSIBLY DEATH



Homeostasis

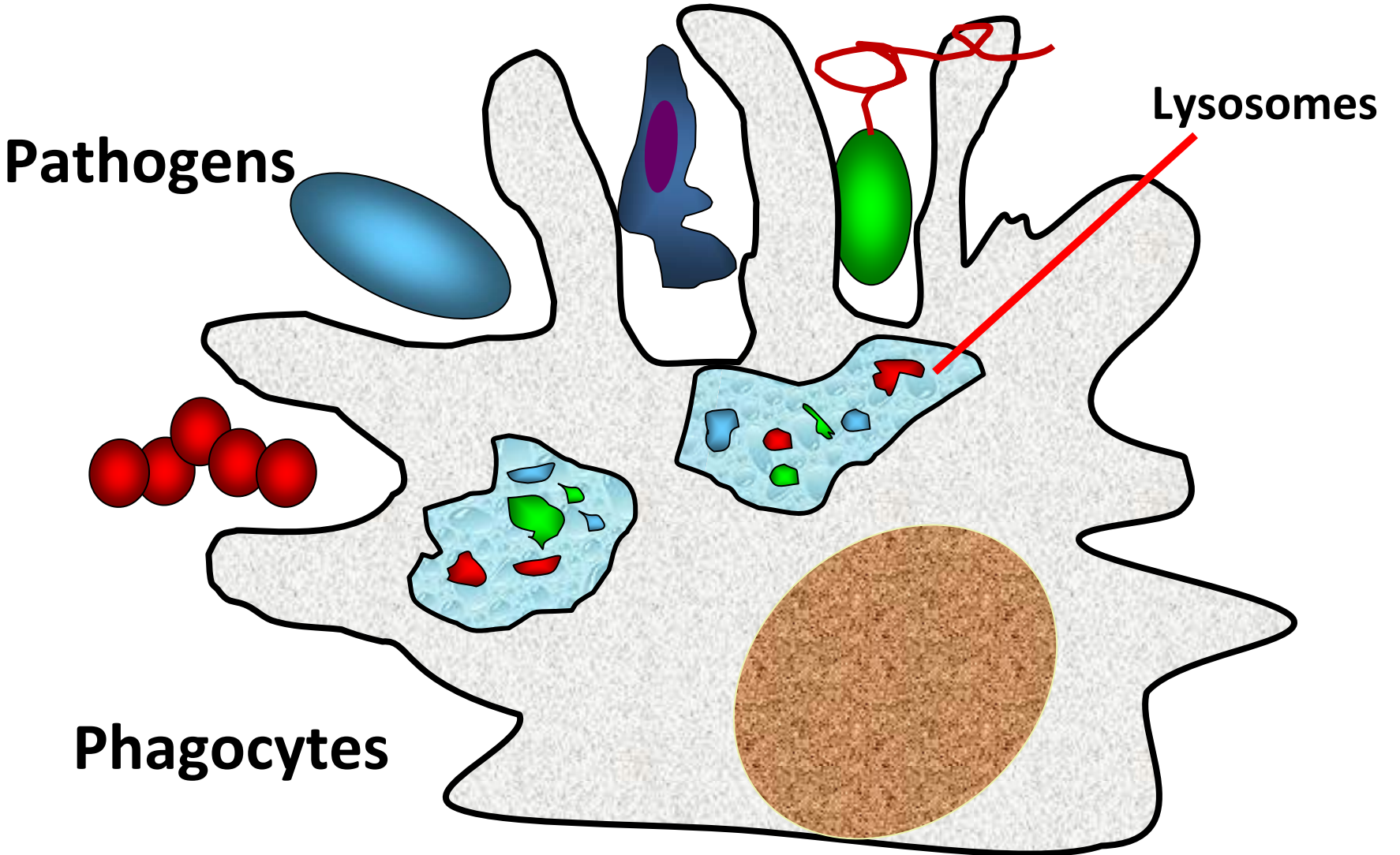
Down-regulation

- Disease
- Death

AIM 2:

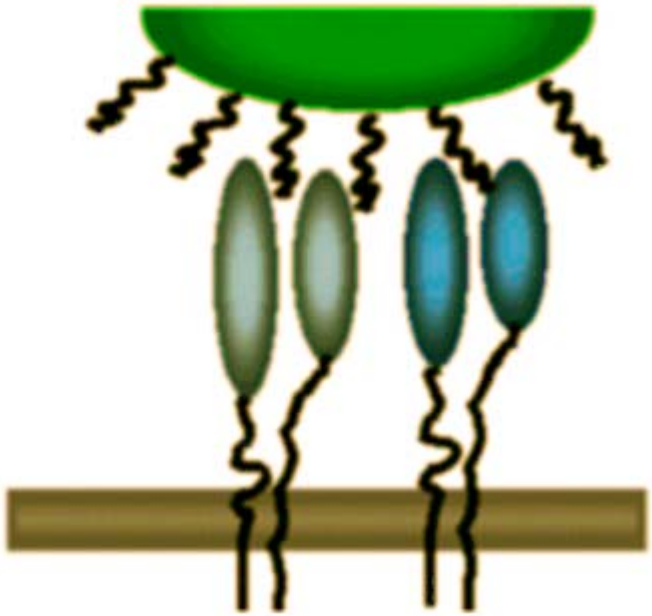
- Elucidate the possible effects of NAs and the organic fraction of OSPW on immune gene expression and selected immune functions of mice
  - Analysis of expression of 20 immune genes, and 4 house keeping genes by real time PRC (liver, spleen, and mesenteric lymph node)
  - **Analysis of phagocytosis using peritoneal macrophages**

PHAGOCYTOSIS IS AN ESSENTIAL CELL MECHANISM OF INNATE IMMUNITY



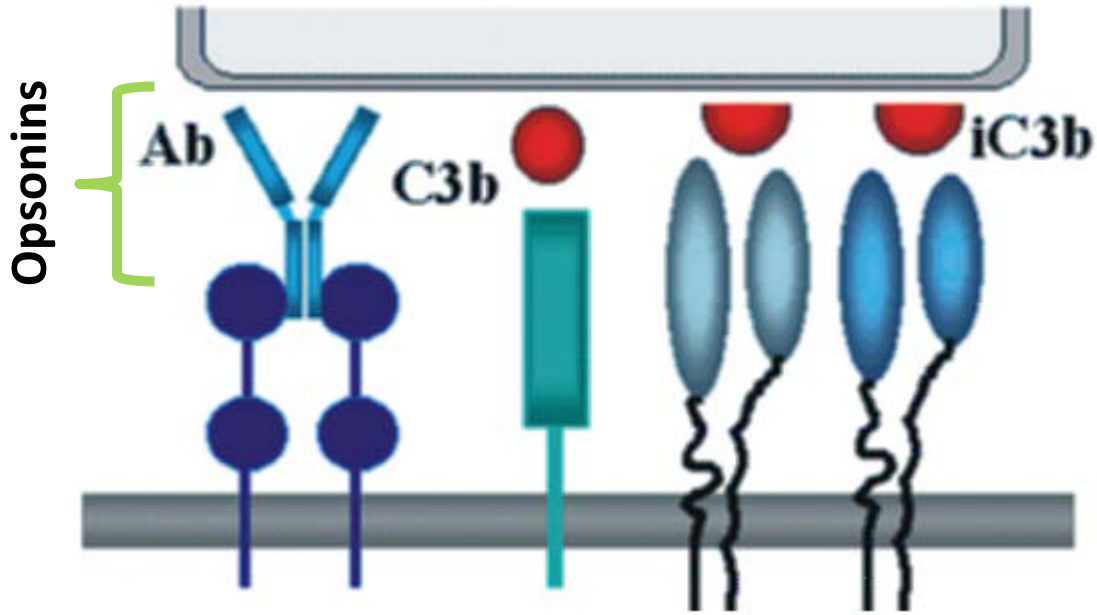
OPSONIN-DEPENDENT AND -INDEPENDENT PHAGOCYTOSIS

Direct pathogen recognition



Phagocytosis

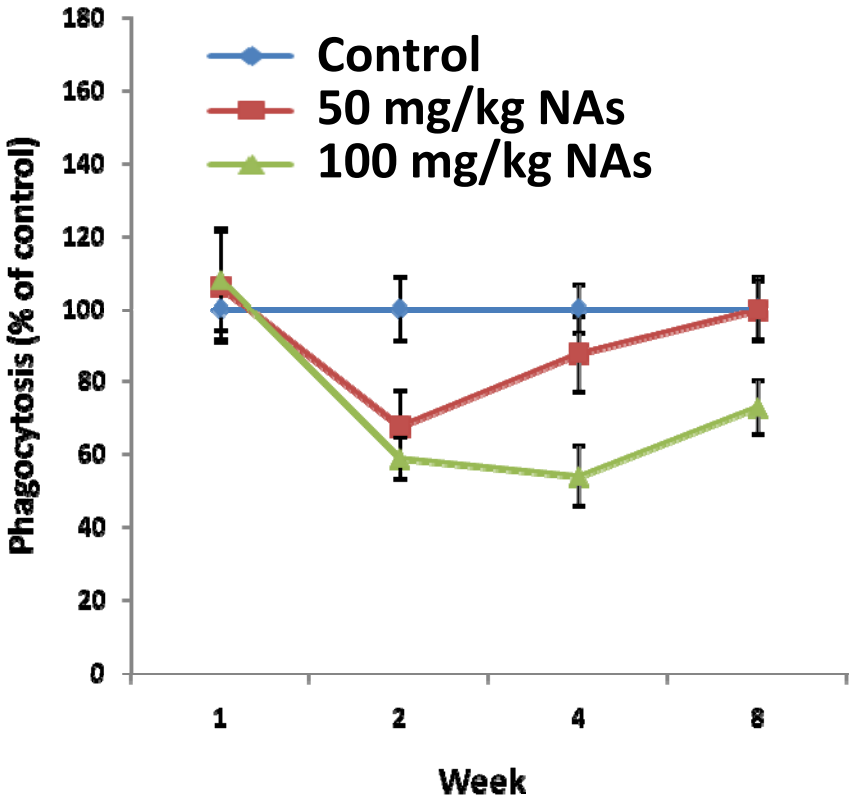
Indirect pathogen recognition



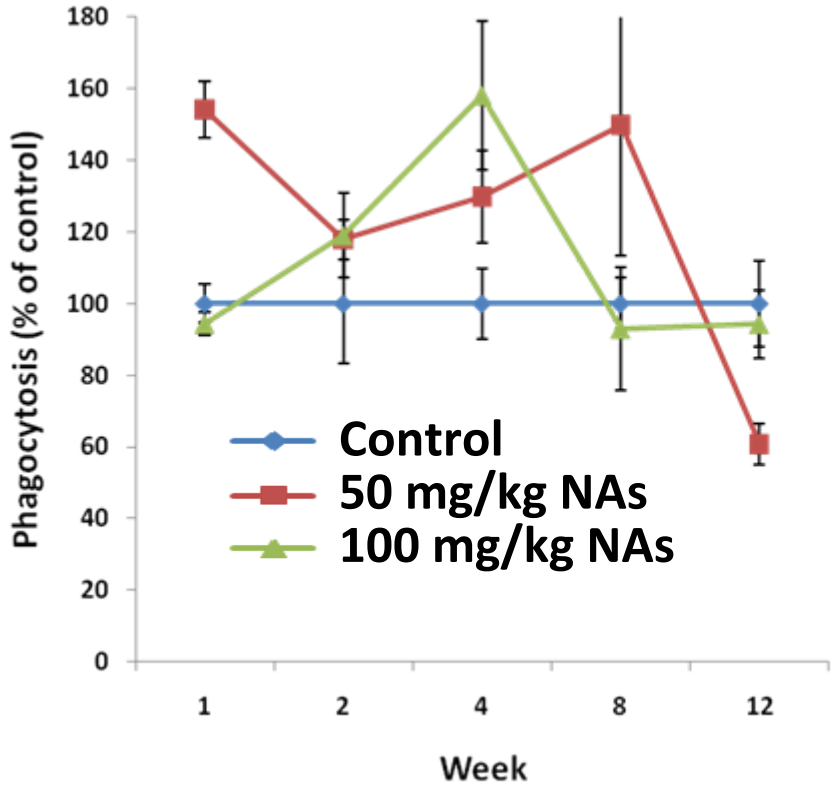
Phagocytosis

EFFECT OF NAS OR OSPW ON OPSONIN-INDEPENDENT PHAGOCYTOSIS

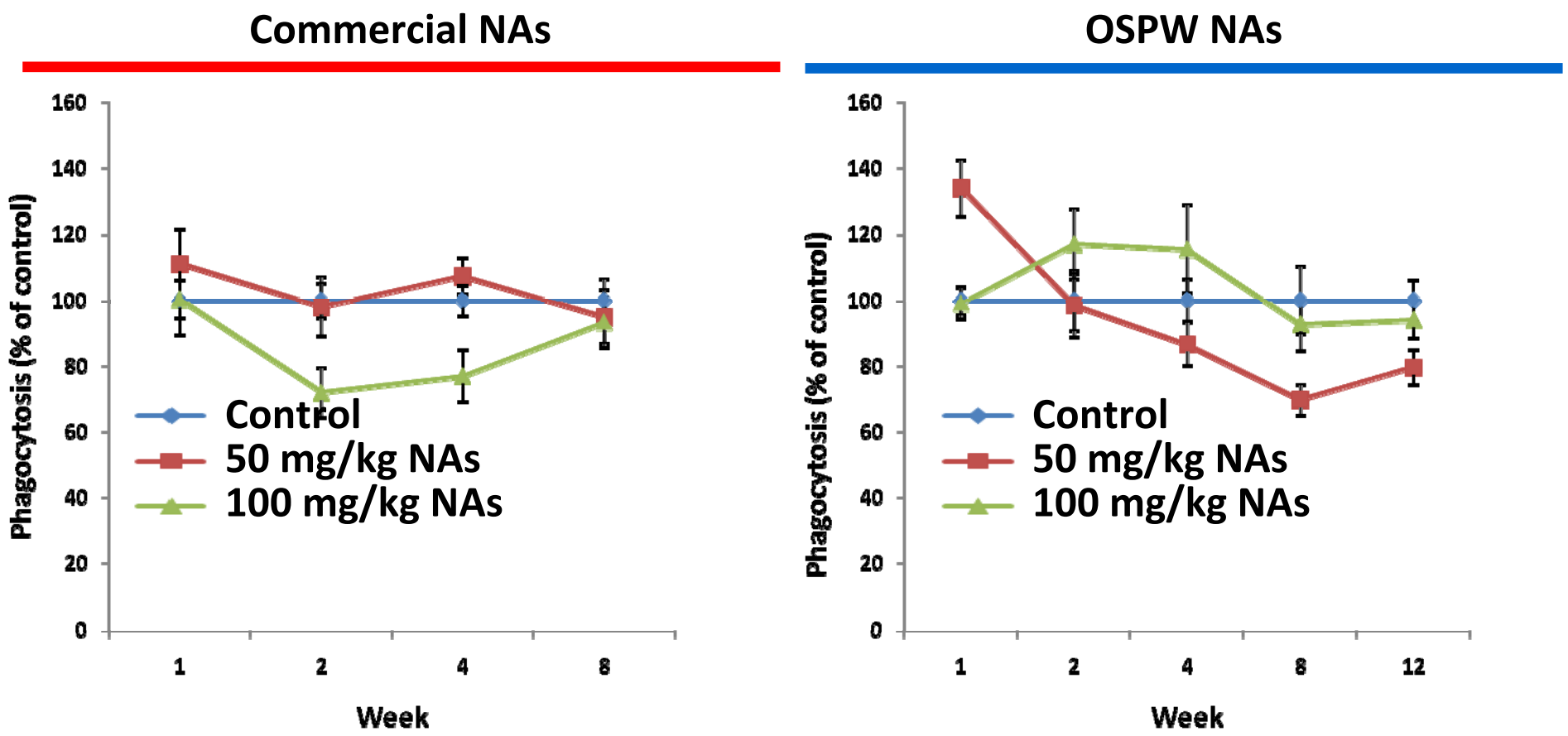
Commercial NAs



OSPW NAs



EFFECT OF NAS OR OSPW ON OPSONIN-DEPENDENT PHAGOCYTOSIS



CONCLUSIONS

•Different expression patterns of immune genes were observed in the livers and spleens of mice, after exposure to either commercial NAs, or OSPW

•Opsonin-independent and –dependent phagocytosis by peritoneal macrophages was reduced in animals treated with commercial NAs, but not in animals treated with OSPW

•These differences may be due to either composition of the NAs in the two preparations, or to the additional organic components present in OSPW

•The observed down-regulation of immune functions at different time points could make animals more susceptible to pathogens during these periods.

THANK YOU!