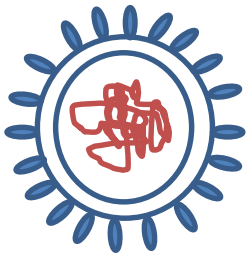
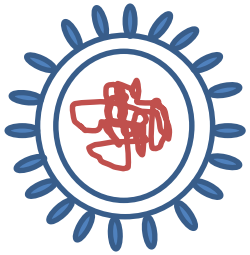
ADJUVANTS

Carolina Tafalla
Animal Health Research Center (CISA-INIA)

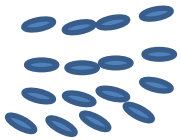
TYPES OF VACCINES



LIVE, ATTENUATED VACCINES

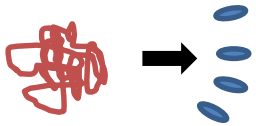


INACTIVATED VACCINES

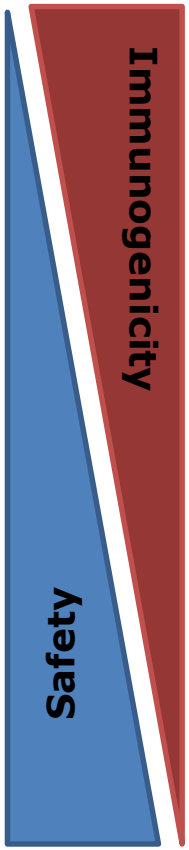


RECOMBINANT VACCINES

VIRUS LIKE PARTICLES (VLPs)



DNA VACCINES



WHY DO WE NEED ADJUVANTS?

-What is an adjuvant?

A substance that we add to a vaccine to increase its immunogenicity

-Not needed in live attenuated vaccines, as their replicative capacity already provides a delivery method to the antigen presenting cells. Components of the pathogen act as immunostimulants.

-Especially needed when we use subunit vaccines or when we want to immunize through mucosal routes

TYPES OF ADJUVANTS

Signal 1 facilitators

- They include all variants of delivery systems, including depot adjuvants

- They affect antigen residence time, the spatio–temporal behavior of the antigen (antigen geography) and the amount of the antigen that eventually reaches the adaptive immune cell receptors

- Examples: oil emulsions

Signal 2 facilitators

- Do not directly affect the concentration and distribution of antigen between injection site and draining lymph nodes over time

- They provide co-stimulatory signals during the antigen recognition phase, thus increasing the immune response or skewing it to provide the most suitable immune environment for the establishment of protection

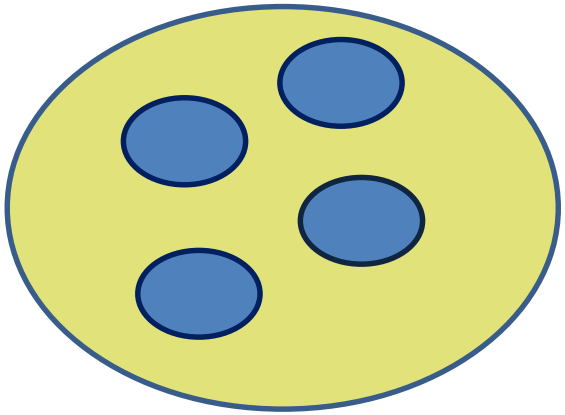
- Examples: ligands of innate receptors, cytokines

OIL-BASED EMULSIONS

Emulsion= dispered phase + continuos phase + surfactant
HLB= hydrophilic: lipohilic balance

W/O

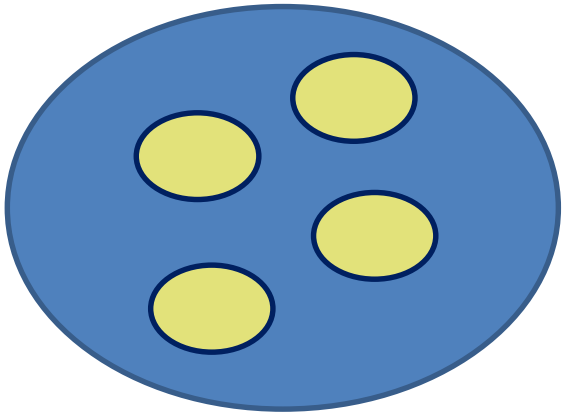
Low HLB value



long-term immune responses, but strong side effect

O/W

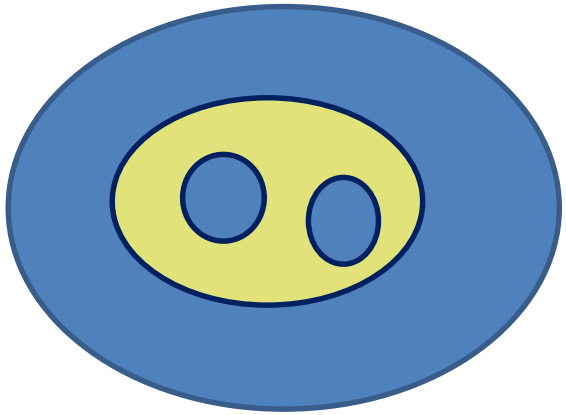
High HLB value



well tolerated but induce a shorter term immune response

W/O/W

Intermediate HLB value



generate long-term immune responses

SIGNAL 1 FACILITATORS

Freund´s complete adjuvant

-W/O. Heat-killed Mycobacteria and a mineral oil with surfactant
-Strong side effects
-Not always worked in fish

Freund´s incomplete adjuvant

-Lacks the mycobacterial components of the emulsion, being therefore just a W/O emulsion
-Less side effects, but still some like peritonitis
-Evidences of good effects in fish

Montanide

-Based on either mineral oil, non-mineral oil or a mixture of both
-May be used to manufacture different type of emulsions, W/O, O/W or W/O/W, for use in both mammals and fish
-Less side effects
-Evidences of good effects in fish

Other mineral oils

-AJ-oil (Alphaject 5200) used in some vaccines commercialised by Pharmaq

MONTANIDE™ for fish vaccines

FOR INJECTABLE VACCINES

Emulsion type	Mineral oil	Non-mineral Oil	Synthetic oil
 W in O	ISA 761 VG	ISA 763A VG	ISA 760 VG

ISA 763 A VG: Reference adjuvant

ISA 761 VG: Adjuvant for cellular immune response

FOR IMMERSION VACCINES

Micro-emulsions: IMS 1312 VG

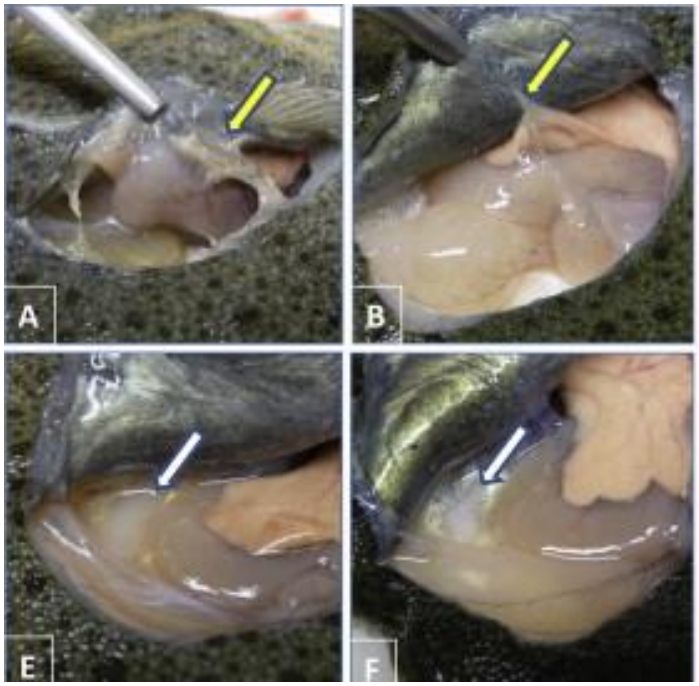
<u>MONTANIDE™ in the field</u>

Streptococcus
Aeronomas
Vibriosis
Yersinia
Pasteurellosis
Furonculosis

SIGNAL 1 FACILITATORS

- Work resonably well for antibacterial vaccines delivered intraperitoneally
- Not effective for viral or parasitic diseases
- Strong side effects

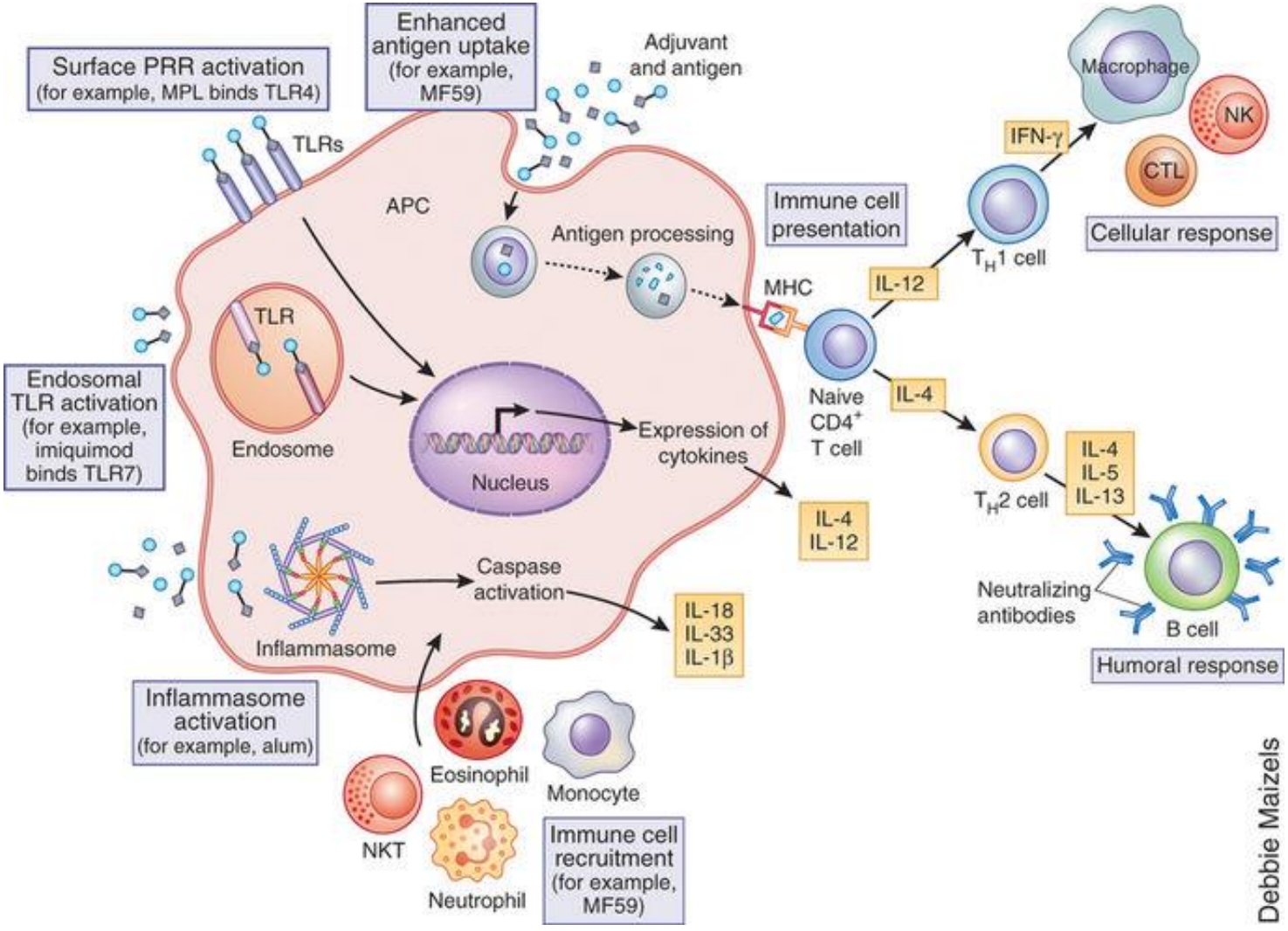
C. Secombes



Noia et al. FSI 2014; 38: 244-254.

SIGNAL 2 FACILITATORS

- provide co-stimulatory signals during the antigen recognition phase
- Skewing the immune response to provide the most suitable immune environment for the establishment of protection



SIGNAL 2 FACILITATORS

-Aluminium salts

Some of the few adjuvants that have been allowed and considered safe to use in human vaccines

Induce Th2 responses

Activates NLRP3 inflammasome and DCs

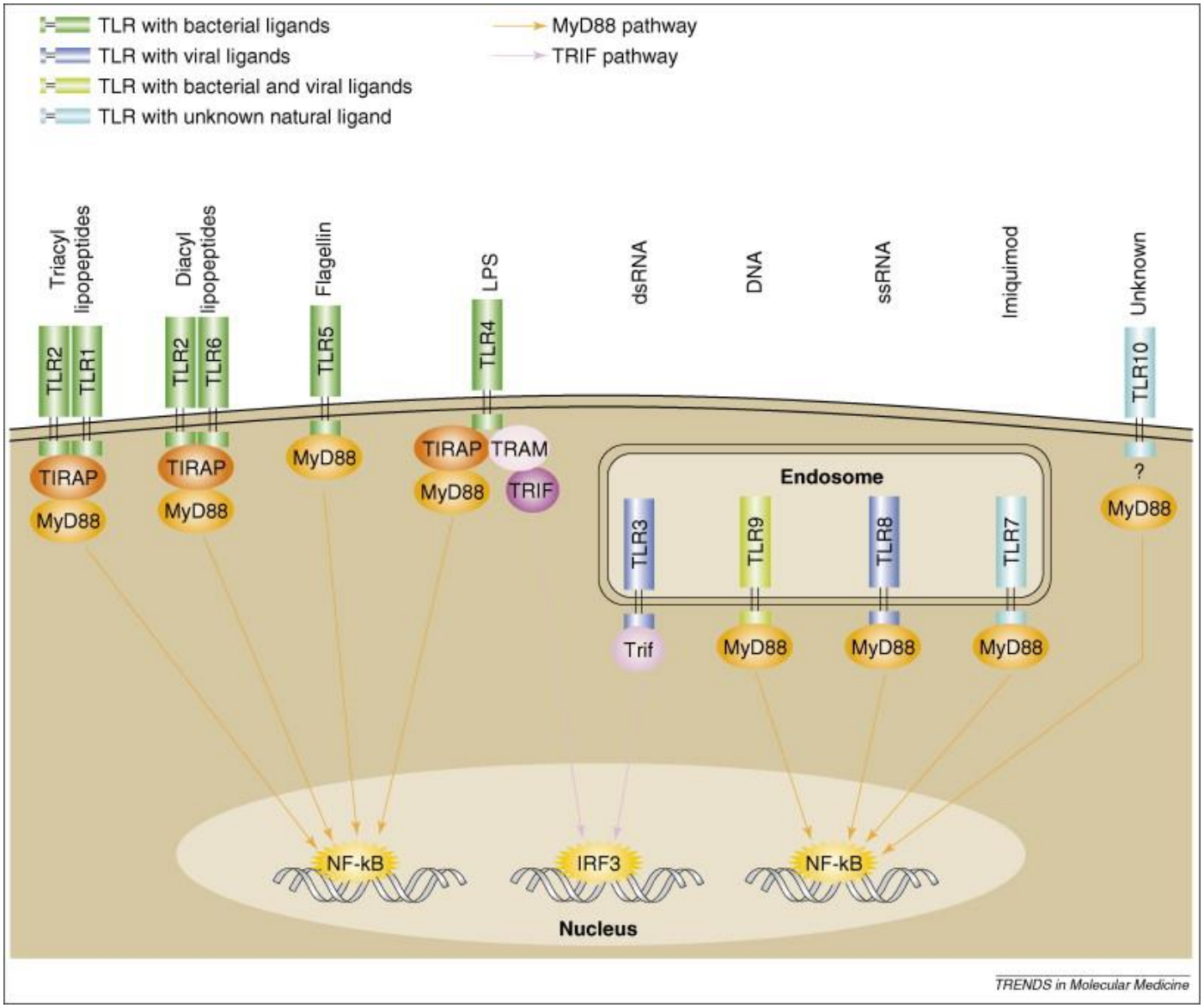
Only a few studies have used aluminium adjuvants in the optimization of fish vaccines

-TLR ligands

-Cytokines

Chemokines, pro-inflammatory cytokines, IFN-related cytokines

SIGNAL 2 FACILITATORS



Poly I:C

Lipopeptides

Flagellin

CpGs

LPS

β-glucans

Saponins

ISCOMs (cholesterol, phospholipid and saponin)

Enterotoxins

SIGNAL 2 FACILITATORS

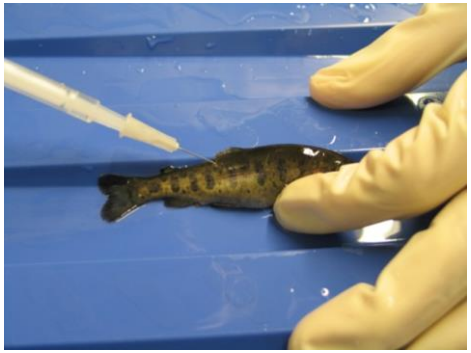
Table 1
Adjuvants, central components, receptors/process and principal immunological responses elicited by licensed and experimental adjuvants mainly for human medicine. Adapted from Coffman et al. [57].

Adjuvant	Central immunostimulatory component(s)	PPR/process	Principal immune response elicited
Alum	Aluminium salts	NLRP3 (?)	Ab, Th2 (+Th1 in humans)
MF59 and AS03	Squalene in water emulsions	Tissue inflammation	Ab, Th1 and Th2
AS04	MPL + Alum	TLR4 and NLRP3(?)	Ab and Th1
			Cash return
Adjuvants in experimental use or in late stage clinical development			
Poly I:C	Synthetic dsRNA		Ab, Th1, CTL
MPL, and in diff. formulations			Ab, Th1
Flagellin, flagellin-Ag fusion proteins	Recombinant flagellin from bacteria	TLR5	Ab, Th1 + Th2
Imiquimods	Imidazoquinoline derivatives	TLR7, TLR8 and both	Ab, Th1, CTL (when conjugated)
CpG, and in different formulations	Synthetic phosphorthioate-linked DNA oligonucleotides with optimised CpG motifs	TLR9	Ab, Th1, CTL (when conjugated)
ISCOMS	Saponins	Not defined	Ab, Th1 + Th2, CTL
IFA (and montanide formulations	Mineral or paraffin oil + surfactant	Not defined	Ab, TH1 + Th2
CFA	IFA + peptidoglycan, trehalose dimycolate	NLR, TLR?	Ab, Th1, Th17

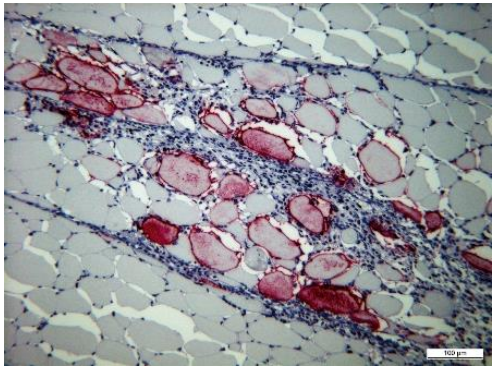
Abbreviations and descriptions: MF59 (Novartis proprietary adjuvant MF59 containing squalene, polyoxyethylene sorbitan monooleate and sorbitan trioleate), AS03 (GlaxoSmithKline) contains squalene, DL-a-tocopherol, polysorbate), AS04 (Aluminium hydroxide and monophosphoryl lipid A (MPL), ISCOMs (immune-stimulating complex; nanostructure of cholesterol, phospholipids and Quil-A saponins), IFA (incomplete Freund's adjuvants). Ab: antibodies, ?: not documented yet.

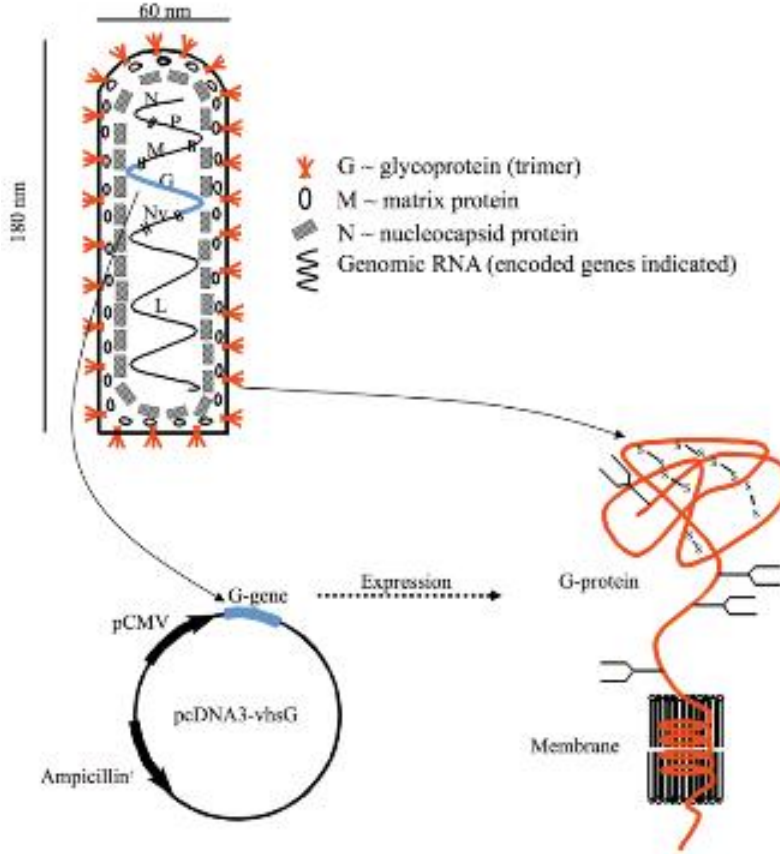
SEARCH FOR EFFECTIVE ADJUVANTS FOR DNA VACCINES IN FISH

DNA VACCINES AGAINST FISH RHABDOVIRUS



i.m. injection
0.01-10 μg

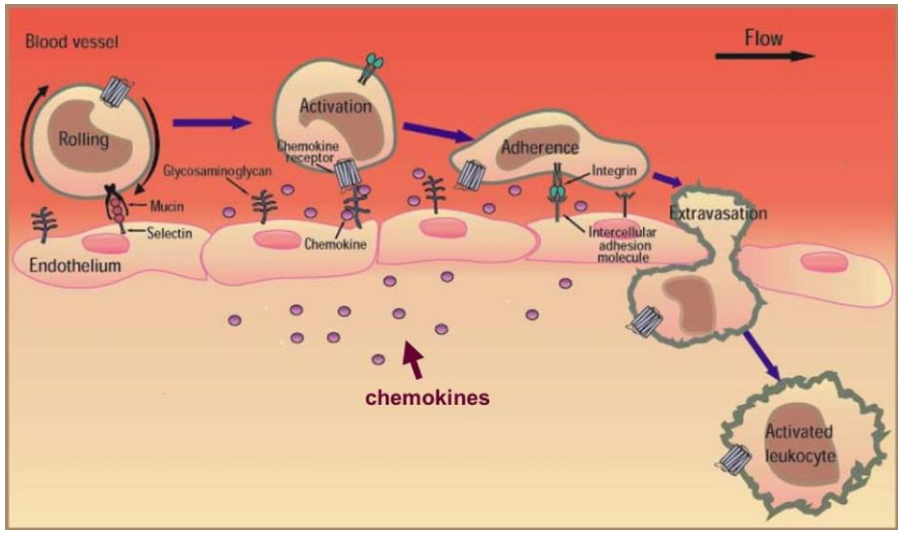




CHEMOKINES AS ADJUVANTS

CHEMOKINES

Attract immune cells to inflammation site
Regule immune function of recriuted cells
Conditionate the specific immune response



The Journal of Immunology

CCL19 and CCL28 Augment Mucosal and Systemic Immune Responses to HIV-1 gp140 by Mobilizing Responsive Immunocytes into Secondary Lymph Nodes and Mucosal Tissue

Kai Hu,[*,†] Sukun Luo,[*,†] Lina Tong,[*] Xin Huang,[*,†] Wei Jin,[*,†] Wenjie Huang,[*] Tao Du,[*,†] Yan Yan,[*,†] Siyi He,[*,†] George E. Griffin,[‡] Robin J. Shattock,[§] and Qinxue Hu[*,‡]

RESEARCH PAPER
Human Vaccines & Immunotherapeutics 8:11, 1607–1619; November 2012; © 2012 Landes Bioscience

Generation of antigen-specific immunity following systemic immunization with DNA vaccine encoding CCL25 chemokine immunoadjuvant

Noshin Kathuria,[1,†] Kimberly A. Kraynyak,[2,†] Diane Carnathan,[3] Michael Betts,[3] David B. Weiner[2] and Michele A. Kutzler[1,4,*]

Kuczkowska et al. Microb Cell Fact (2015) 14:169
DOI 10.1186/s12934-015-0360-z
MICROBIAL CELL FACTORIES
RESEARCH
Open Access

Lactobacillus plantarum displaying CCL3 chemokine in fusion with HIV-1 Gag derived antigen causes increased recruitment of T cells

Katarzyna Kuczkowska[1], Geir Mathiesen[1*], Vincent G. H. Eijsink[1] and Inger Øynebråten[2]

Experimental design

100 µl PBS
I.M.

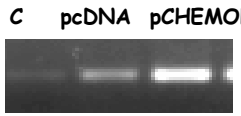
100 µl PBS
1 µg pcDNA
I.M.

100 µl PBS
1 µg pCK5B
I.M.

100 µl PBS
1 µg pCK6
I.M.

100 µl PBS
1 µg pCK7A
I.M.

VERIFICATION OF CHEMOKINE EXPRESSION IN THE MUSCLE

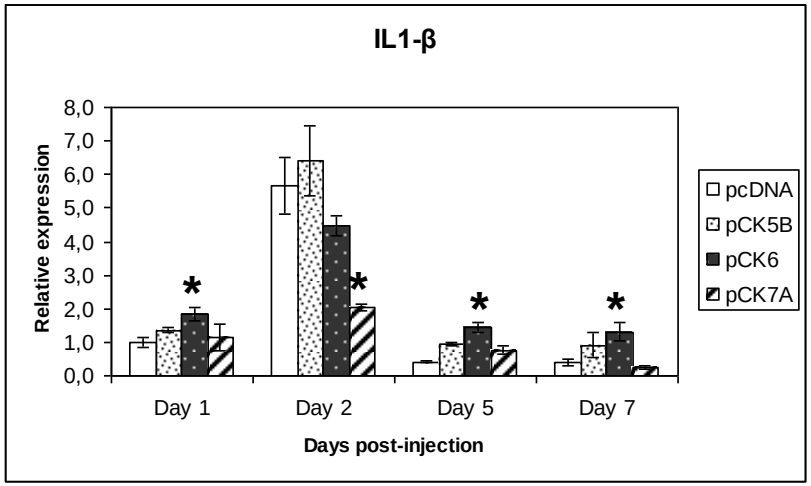


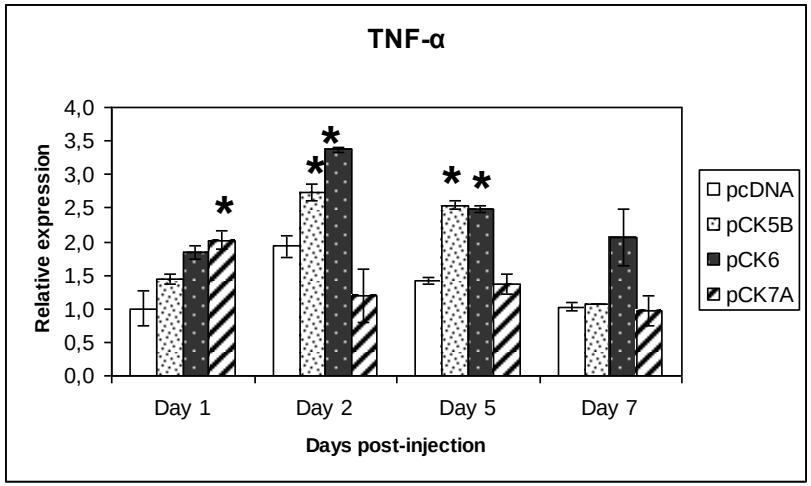
Head kidney and spleen removed for RNA extraction at days 1, 2, 5 and 7

Evaluation through real-time PCR of the levels of expression of different immune genes

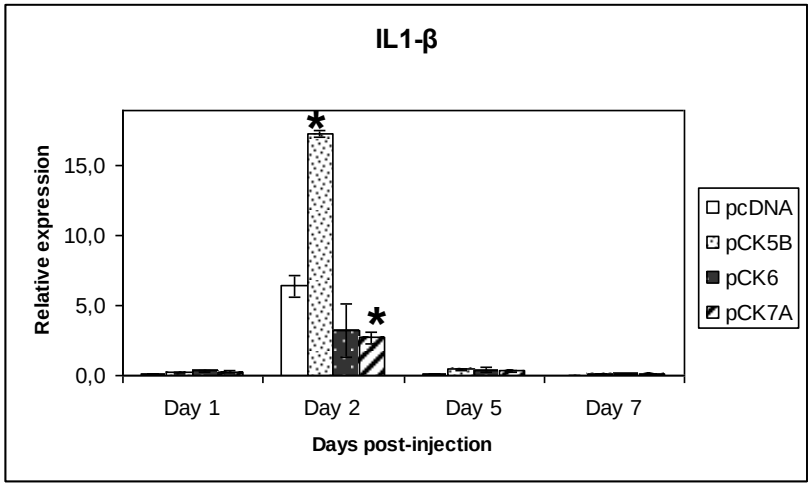
Levels of expression of pro-inflammatory genes

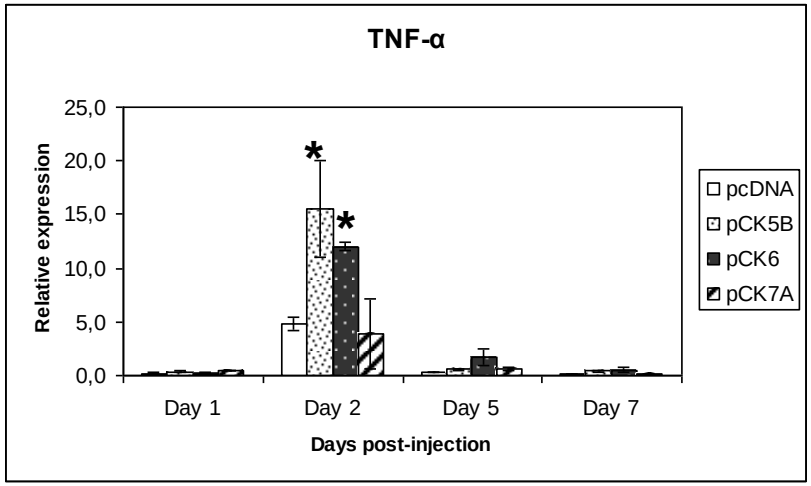
Head Kidney





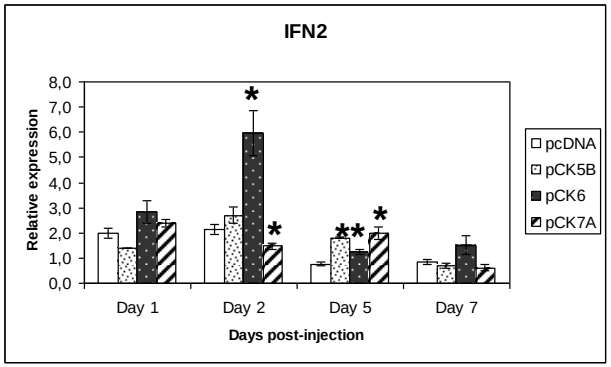
Spleen

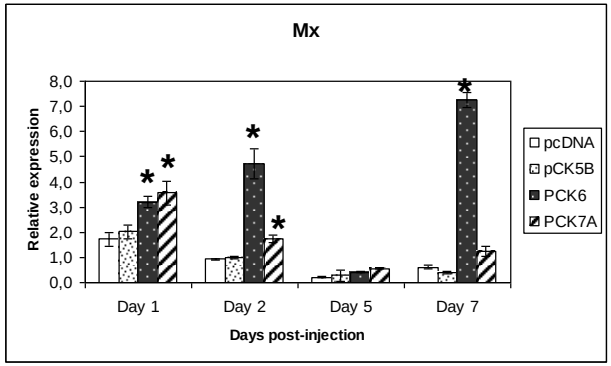


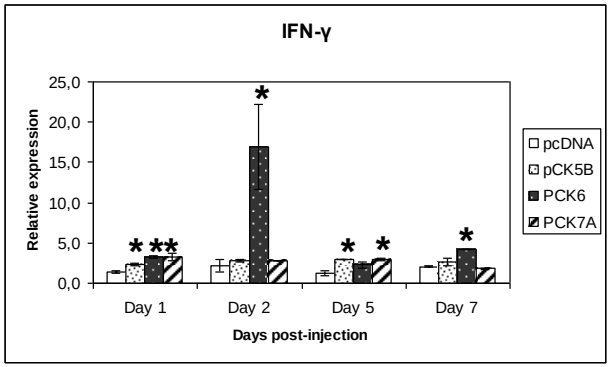


Levels of expression of interferon-related genes

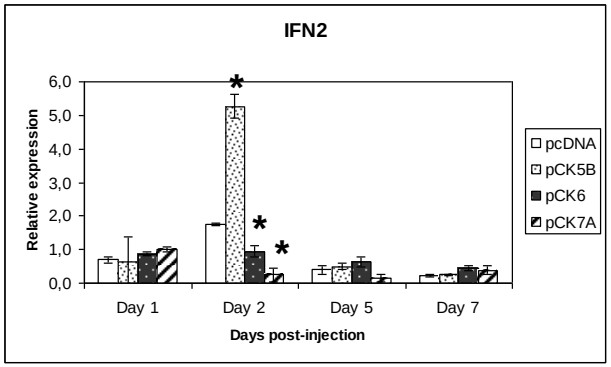
Head Kidney

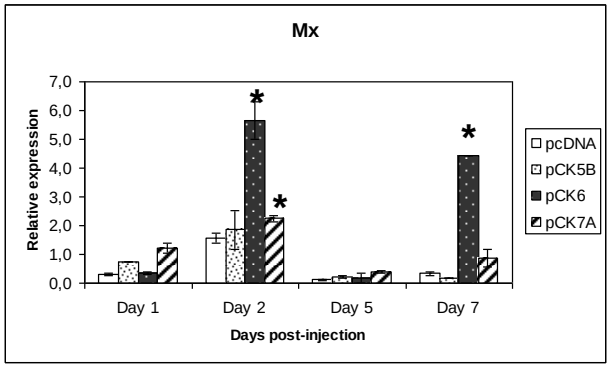


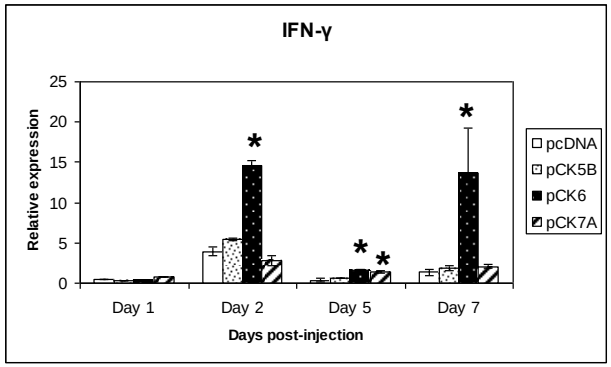




Spleen

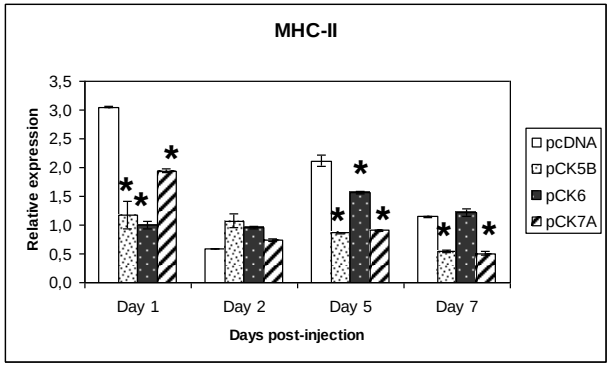


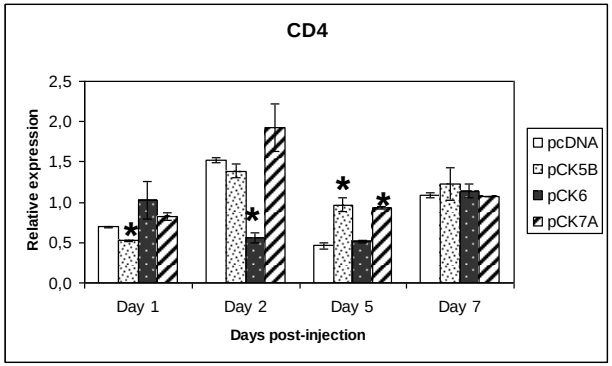


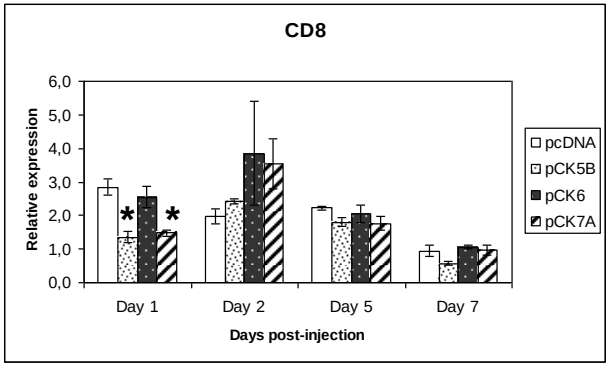


Levels of expression of "marker" genes

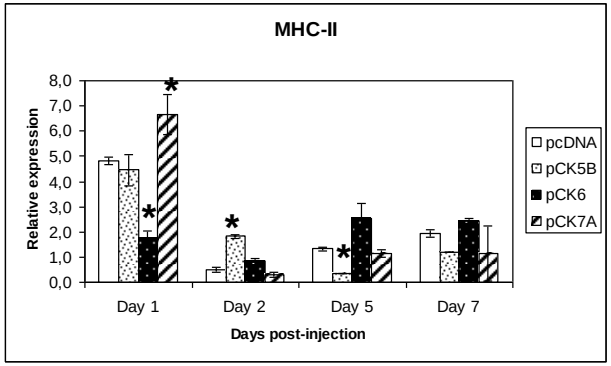
Head Kidney

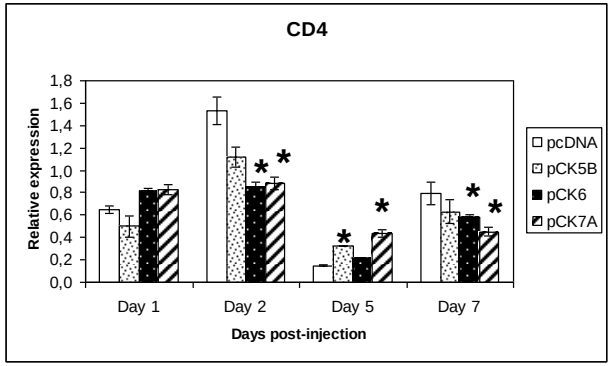


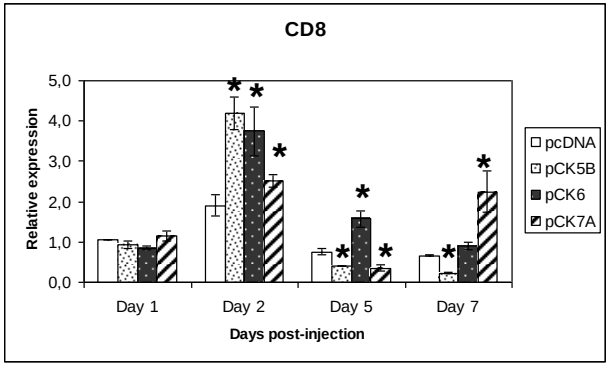




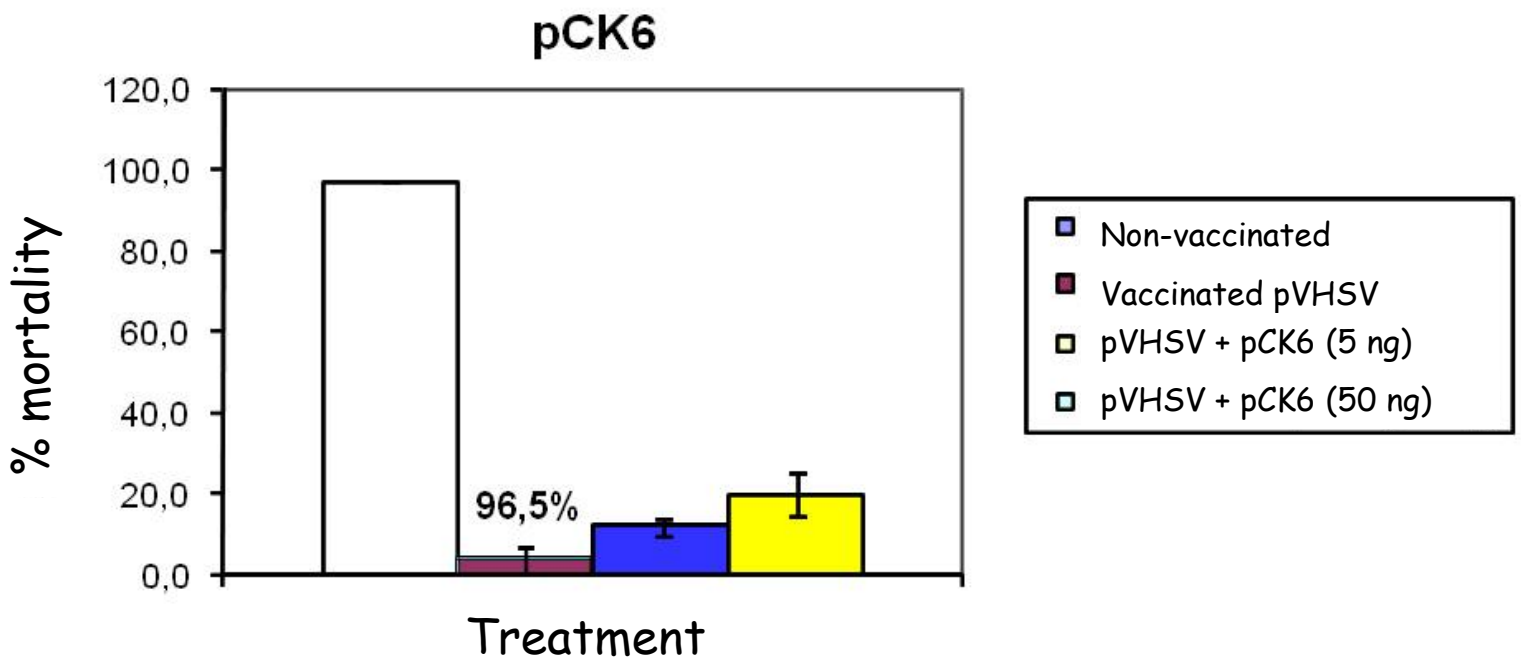
Spleen







Adjuvant effect of pCK6 in DNA vaccination



Despite these immunostimulatory effects co-injection of pCK6 with a VHSV DNA vaccine did not increase the protection conferred.

Experimental design

www.impactjournals.com/oncotarget/ Oncotarget, Vol. 7, No. 14

Rainbow trout CK9, a CCL25-like ancient chemokine that attracts and regulates B cells and macrophages, the main antigen presenting cells in fish

Carolina Aquilino[1], Aitor G. Granja[1], Rosario Castro[1], Tiehui Wang[2], Beatriz Abos[1], David Parra[3], Christopher J. Secombes[2] and Carolina Tafalla[1]

[1] Animal Health Research Center (CISA-INIA), Valdeolmos (Madrid), Spain

[2] Scottish Fish Immunology Research Centre, School of Biological Sciences, University of Aberdeen, Aberdeen, UK

[3] Animal Physiology Unit, Department of Cell Biology, Physiology and Immunology, School of Biosciences, Universitat Autonoma de Barcelona, Cerdanyola del Valles, Spain

Molecular Immunology 48 (2011) 1102–1113

Contents lists available at ScienceDirect

Molecular Immunology

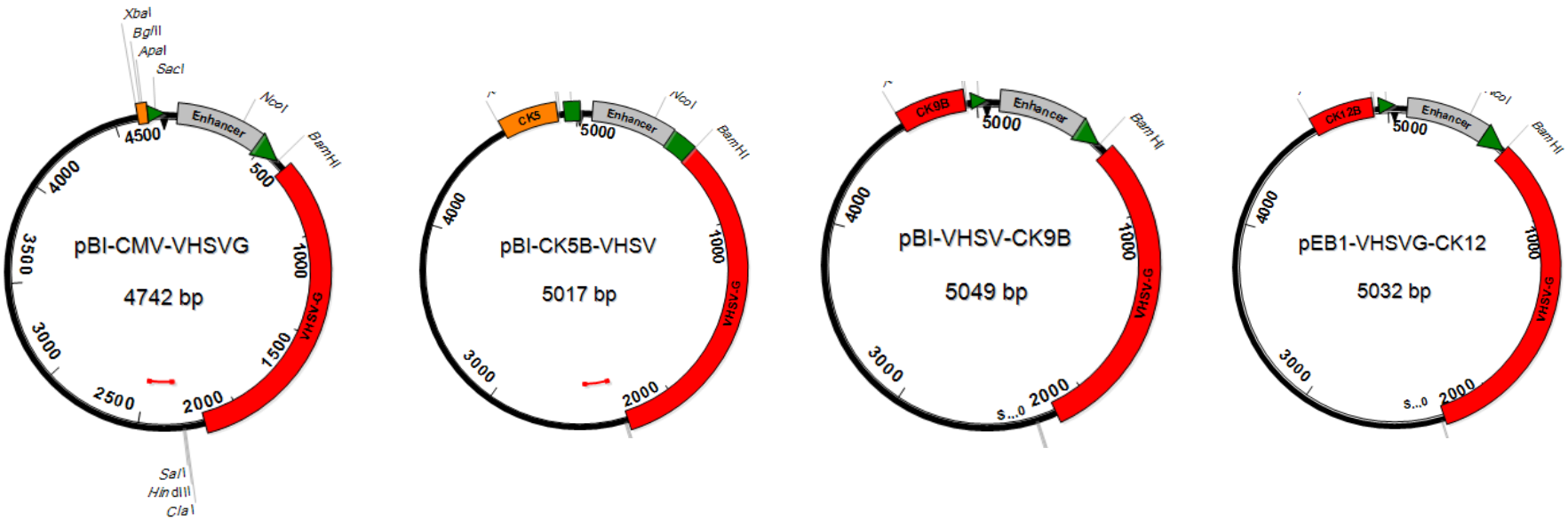
journal homepage: www.elsevier.com/locate/molimm

CK12, a rainbow trout chemokine with lymphocyte chemo-attractant capacity associated to mucosal tissues

Jana Montero, M. Camino Ordas, Alí Alejo, Lucia Gonzalez-Torres, Noemi Sevilla, Carolina Tafalla*

Centro de Investigación en Sanidad Animal (CISA-INIA), Carretera de Algete a El Casar km. 8.1, Valdeolmos 28130, Madrid, Spain

-Plasmids coding for VHSV G protein and CK5B, CK9 and CK12 chemokines

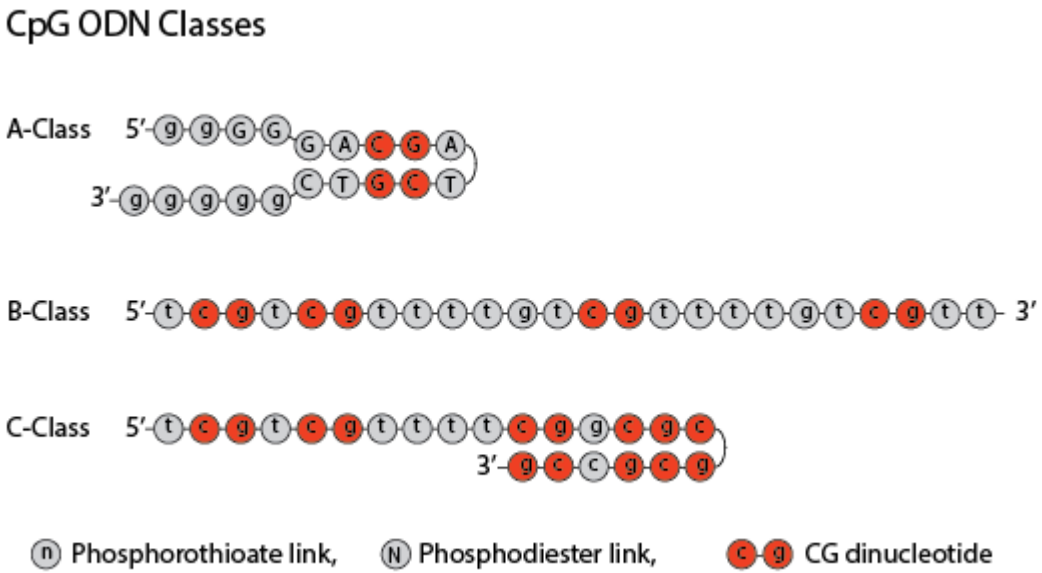


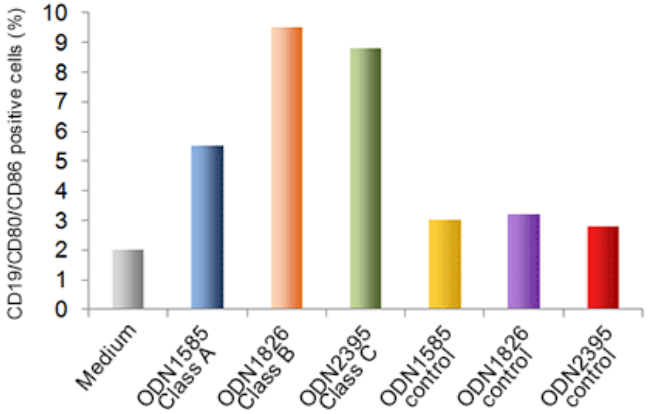
- 12 g rainbow trout
- Plasmids were encapsulated in alginate
- Feed to the fish 1 week, 1 week out, 1 week
- Challenge with VHSV after 6 weeks

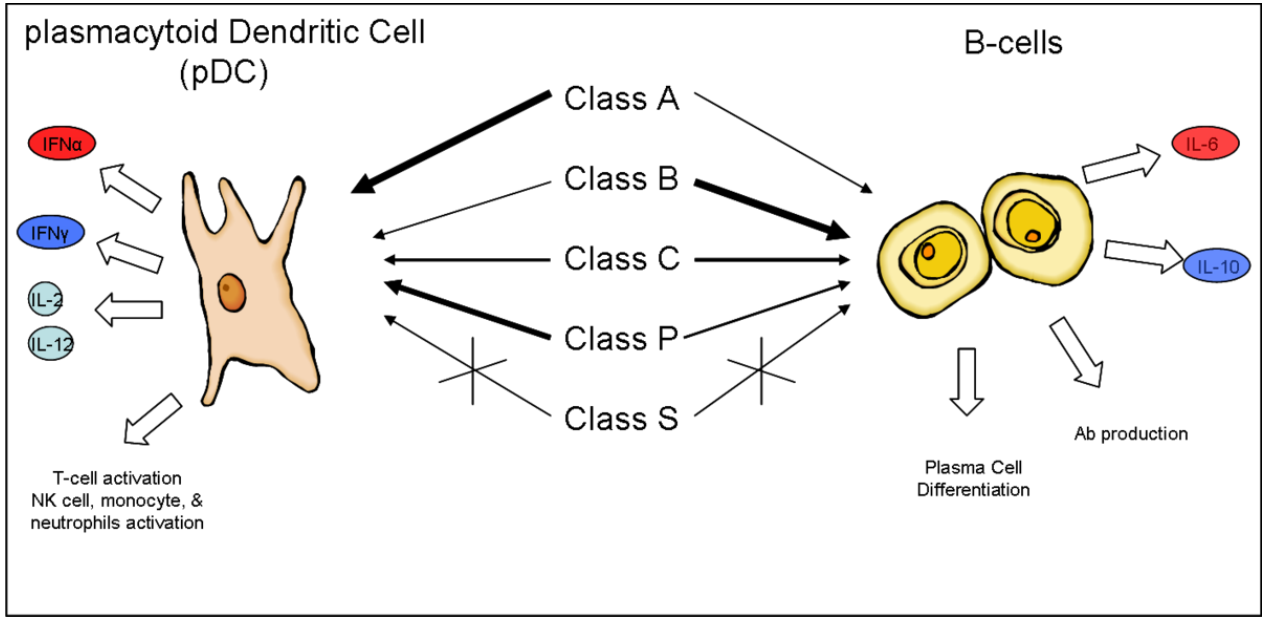
Results

Antigen	Delivery	% Mean accumulated mortality
Alginate/pBI	Feed	91
Plasmid pBI	Intramuscular injection	91
Plasmid pcDNA3-vhsG	Intramuscular injection	0
Plasmid pBI-vhsG	Intramuscular injection	9
Alginate/pBI-vhsG	Feed	78
Alginate/pBI-vhsG-CK5/9/12	Feed	81
Alginate/pBI-vhsG	Intubation	80
Alginate/pBI-vhsG-CK5/9/12	Intubation	92

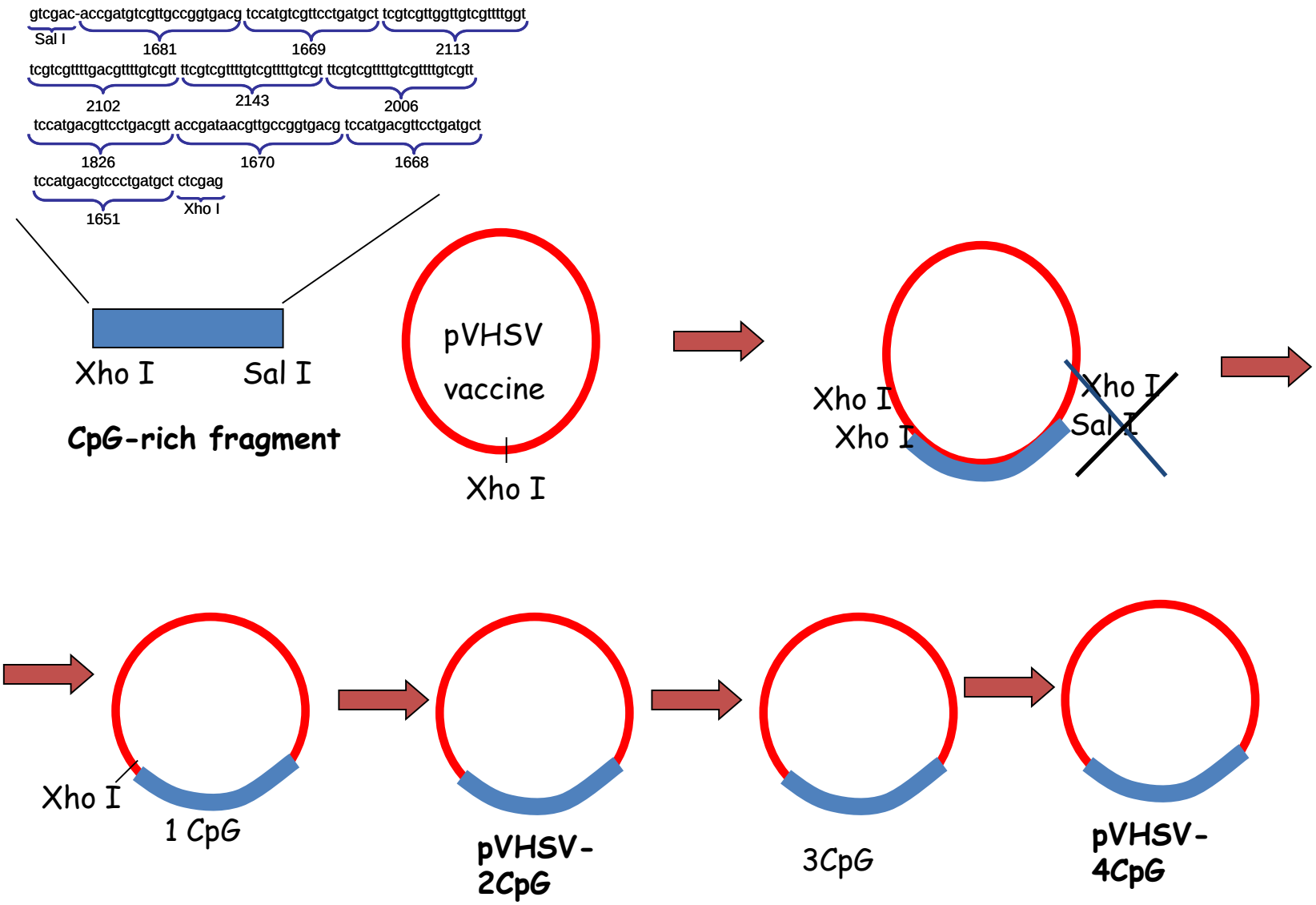
CpGs AS ADJUVANTS



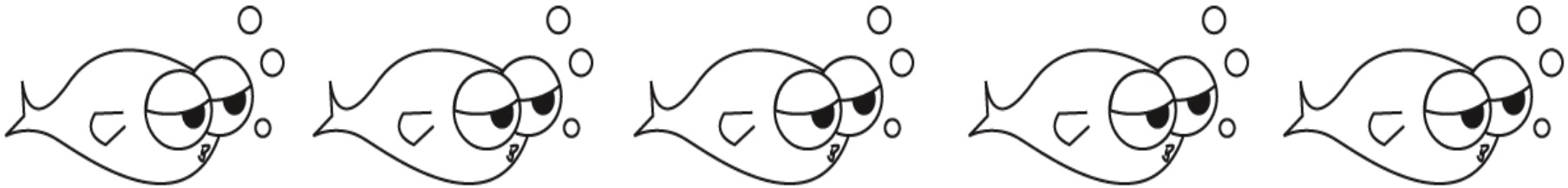




Introduction of a multi-copy CpG fragment in a VHSV DNA vaccine



Experimental design of the vaccination experiment



GROUP 1
100 ul PBS
intramuscular

GROUP 2
100 ul PBS
+ 1 ug pcDNA
intramuscular

GROUP 3
100 ul PBS
+ 1 ug pVHSV
intramuscular

GROUP 4
100 ul PBS
+ 1 ug pVHSV-2CpG
intramuscular

GROUP 5
100 ul PBS
+ 1 ug pVHSV-4CpG
intramuscular

Days 2, 7 and 14 p.v.

Spleen and muscle

RNA extraction to study gene transcription

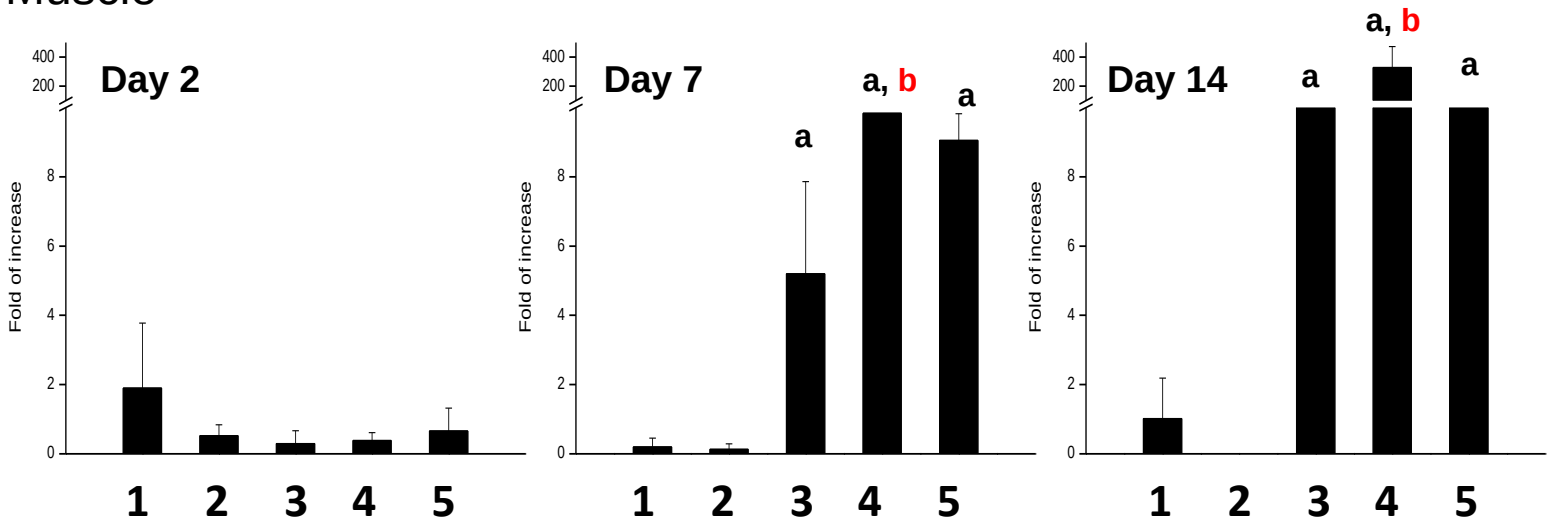
Day 30 p.v.

Serum

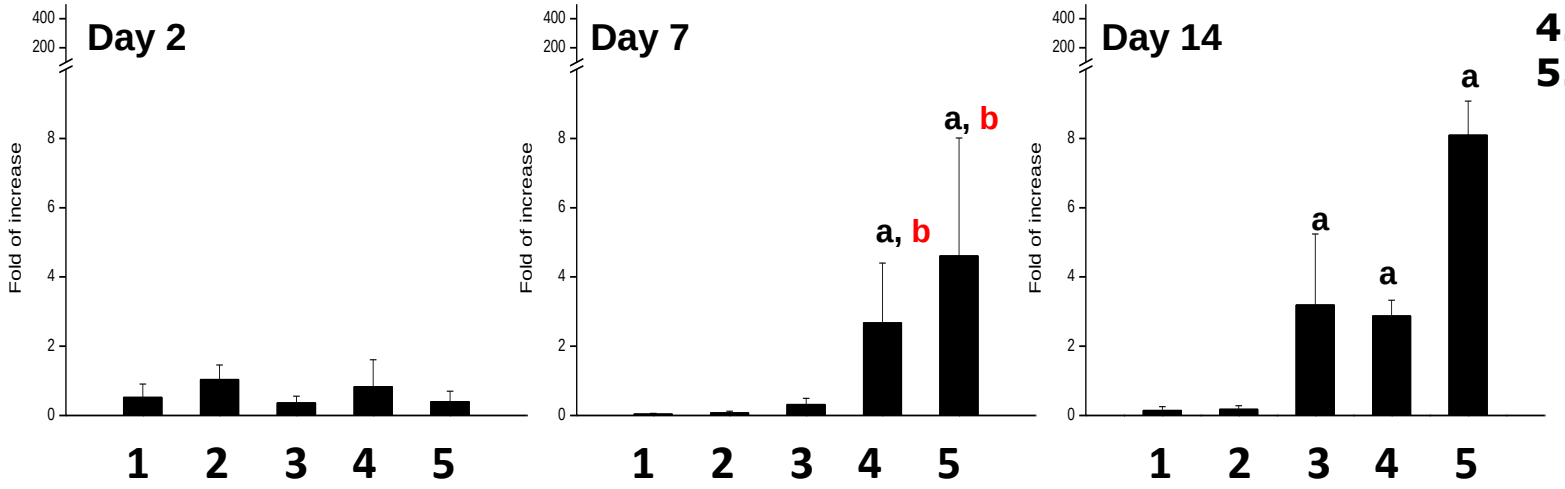
Neutralizing capacity

Mx transcription levels after vaccination

Muscle



Spleen

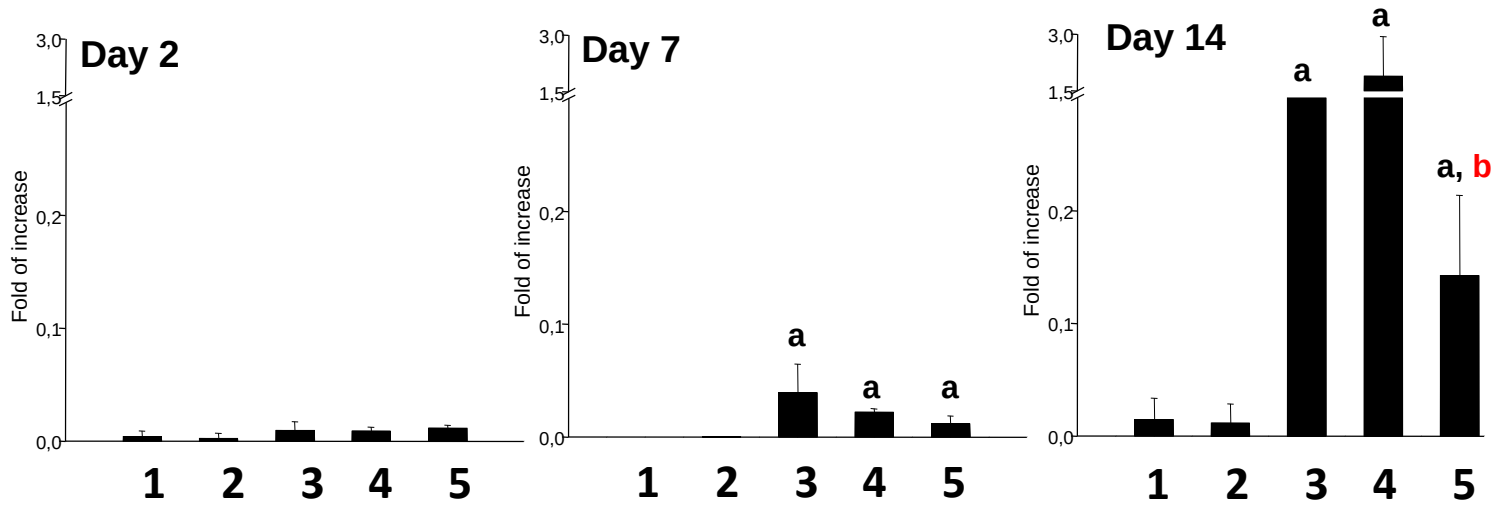


1. Control
2. pcDNA
3. pVHSV
4. pVHSV-2CpG
5. pVHSV-4Cpg

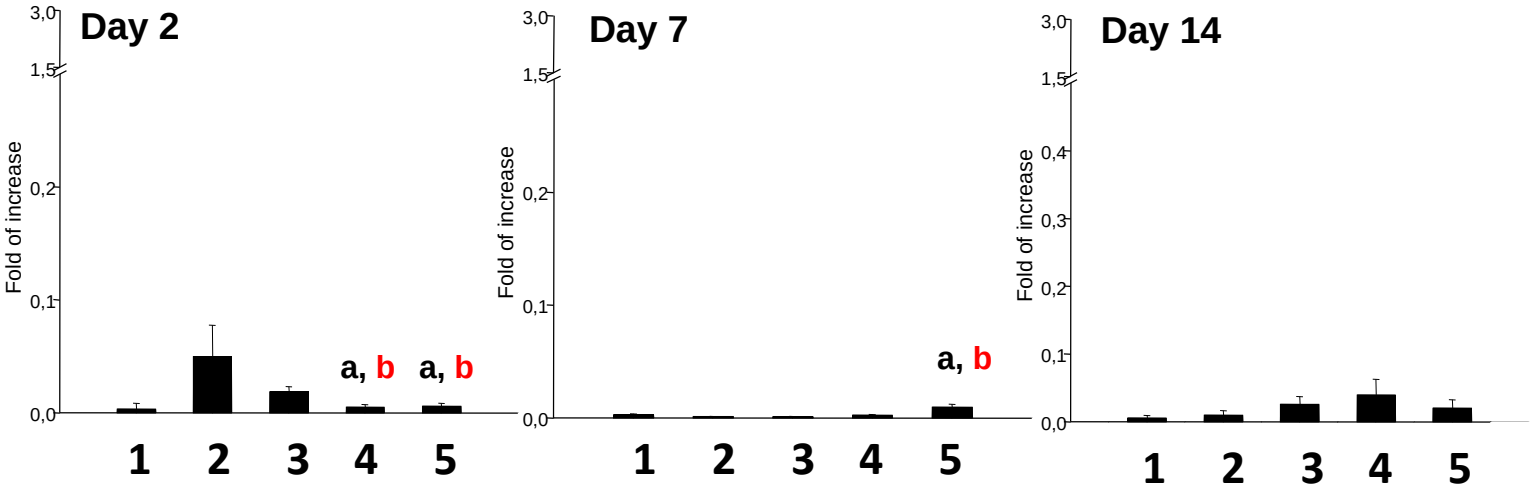
Martinez-Alonso et al. Vaccine 2011; 29: 1289-1296.

IFNγ transcription levels after vaccination

Muscle



Spleen

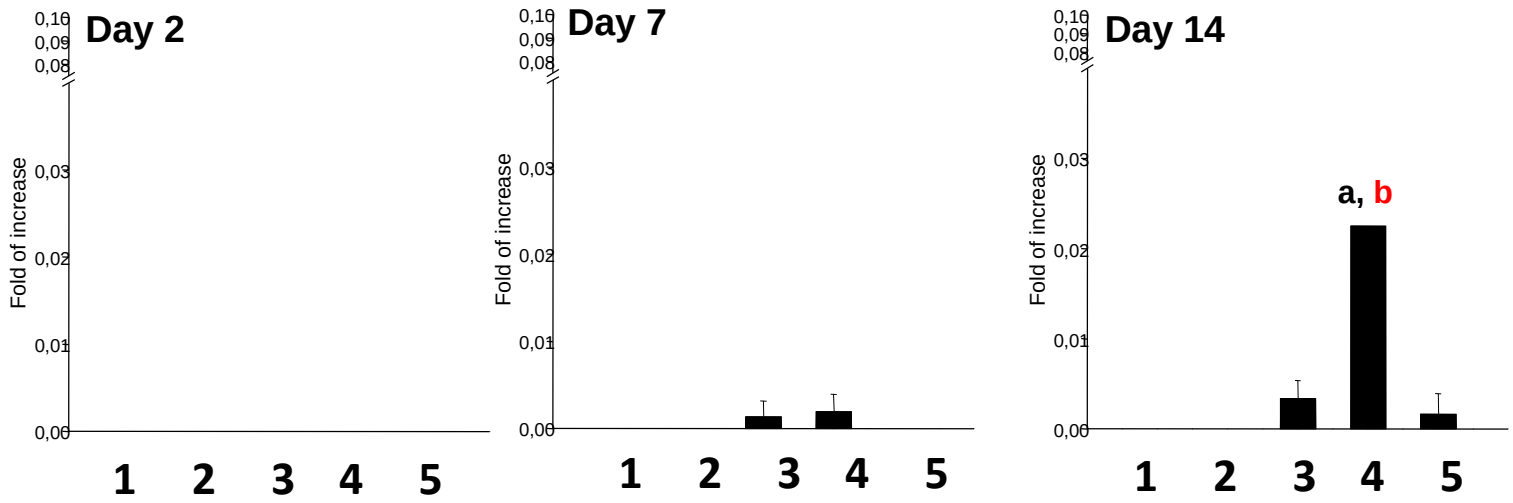


1. Control
2. pcDNA
3. pVHSV
4. pVHSV-2CpG
5. pVHSV-4CpG

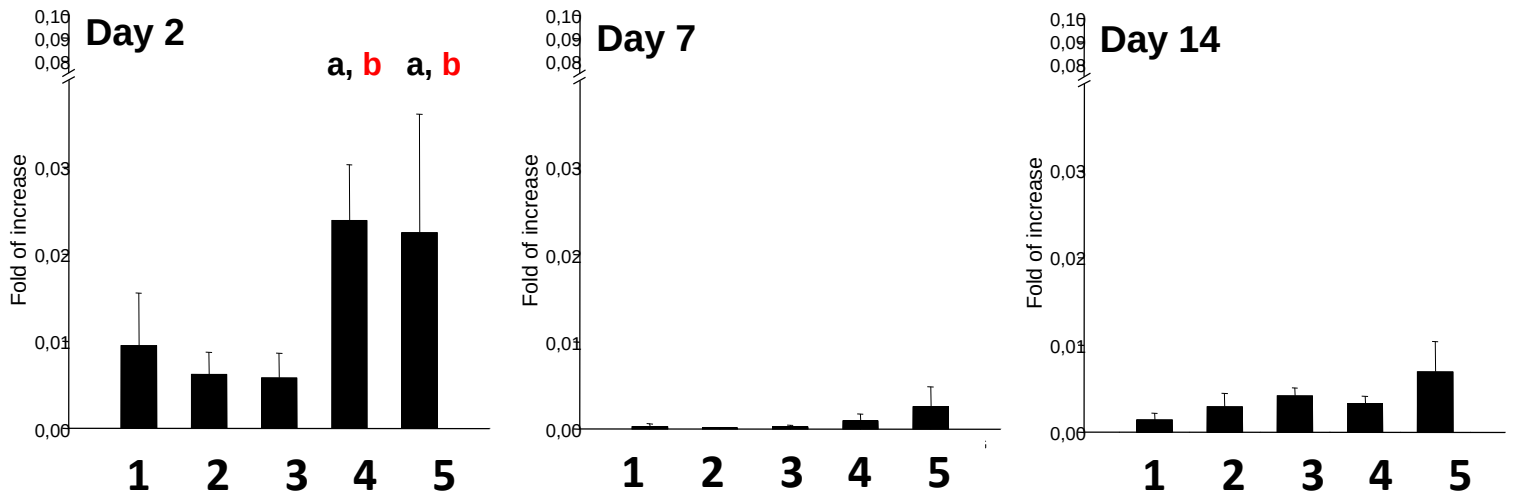
Martinez-Alonso et al. Vaccine 2011; 29: 1289-1296.

MHC I transcription levels after vaccination

Muscle



Spleen

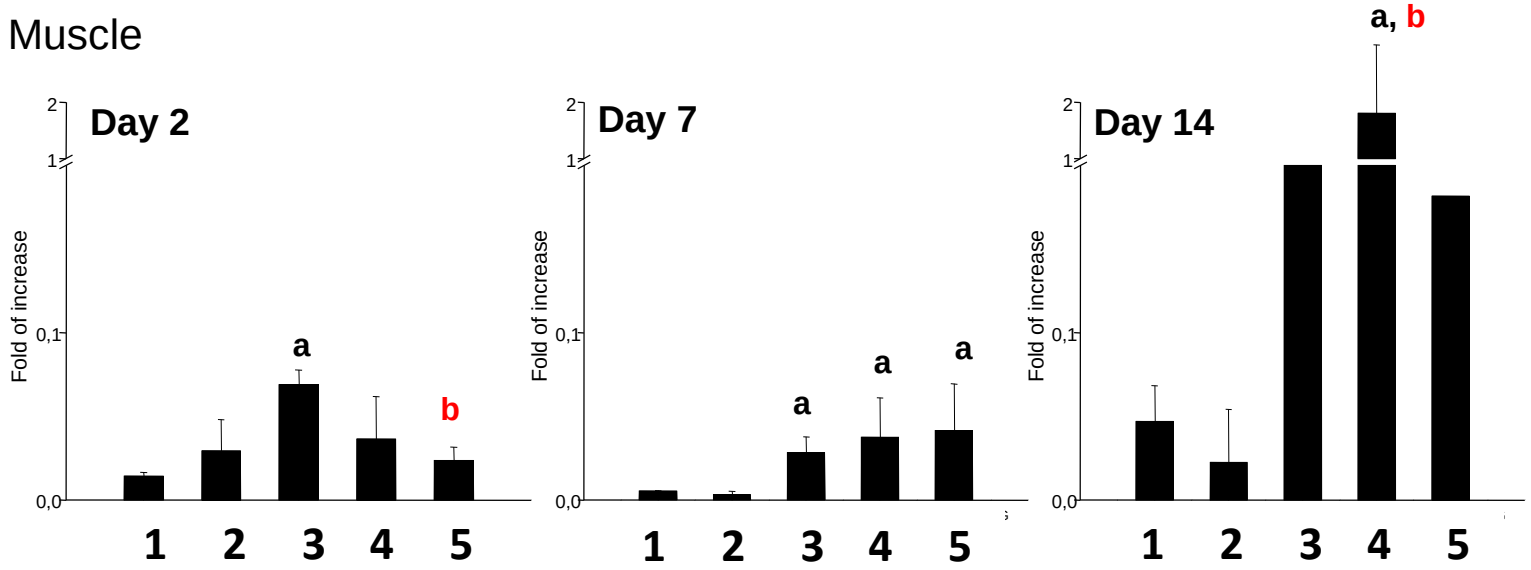


1. Control
2. pcDNA
3. pVHSV
4. pVHSV-2CpG
5. pVHSV-4CpG

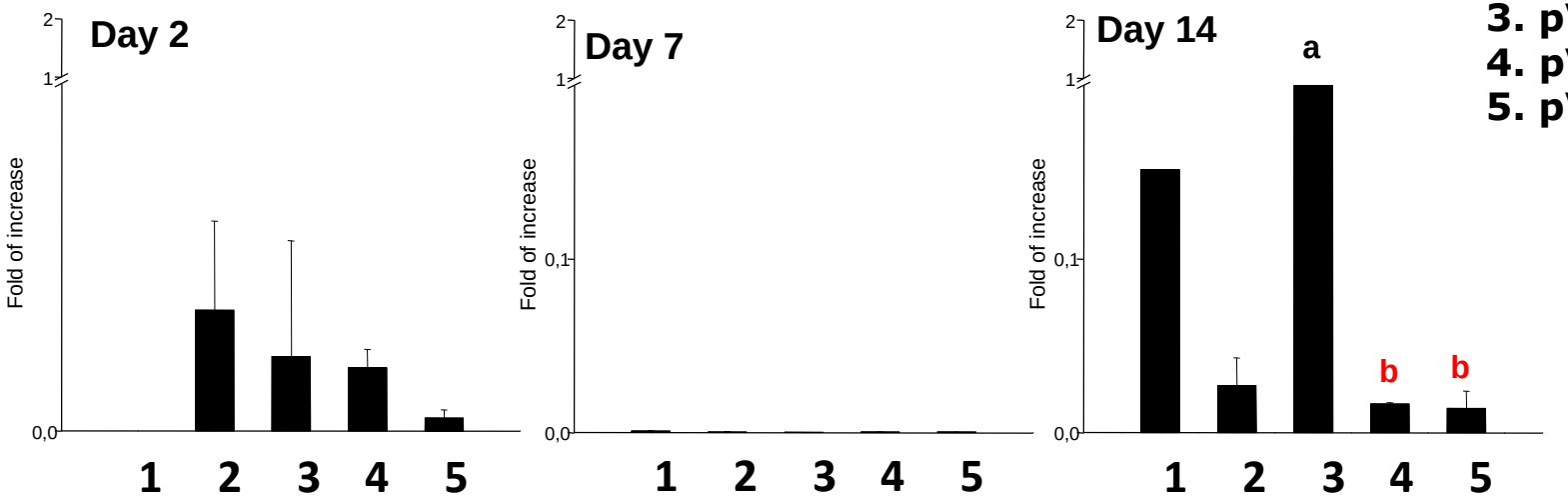
Martinez-Alonso et al. Vaccine 2011; 29: 1289-1296.

IL-1β transcription levels after vaccination

Muscle



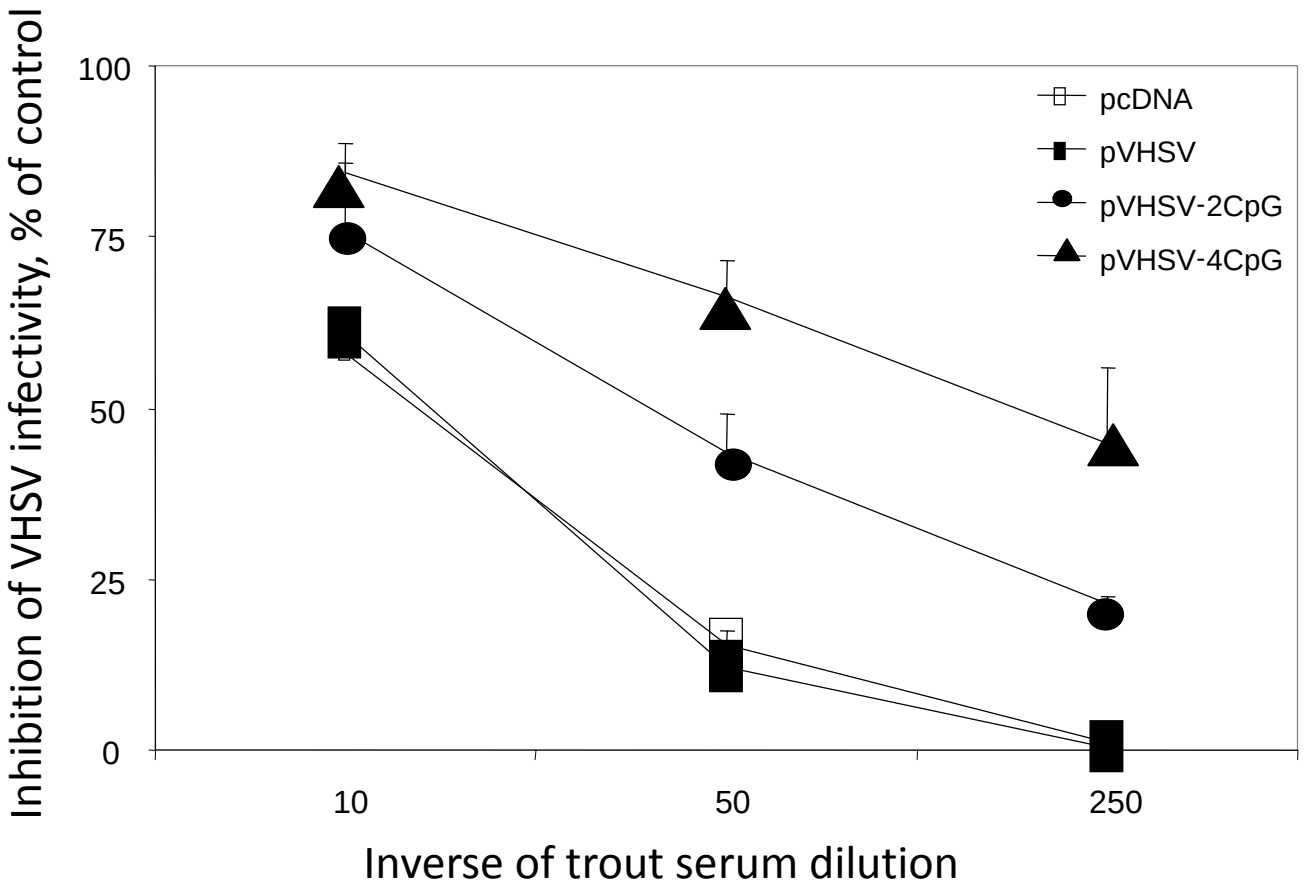
Spleen



1. Control
2. pcDNA
3. pVHSV
4. pVHSV-2CpG
5. pVHSV-4CpG

Martinez-Alonso et al. Vaccine 2011; 29: 1289-1296.

Serum neutralizing activity after vaccination



Martinez-Alonso et al. Vaccine 2011; 29: 1289-1296.

USE OF RECOMBINANT IL6 AS AN ADJUVANT FOR AN IPNV INACTIVATED VACCINE

Experimental design of the vaccination experiment

GROUP 1
100 ul PBS
intraperitoneal

GROUP 2
100 ul PBS
+ 100 ng IL6
intraperitoneal

GROUP 3
100 ul PBS
+ $2x10^9$ $TCID_{50}$ IPNV
intraperitoneal

GROUP 4
100 ul PBS
+100 ng IL6
+$2x10^9$ $TCID_{50}$ IPNV
intraperitoneal

Days 2, 6 and 15 p.v.

Spleen and kidney cells

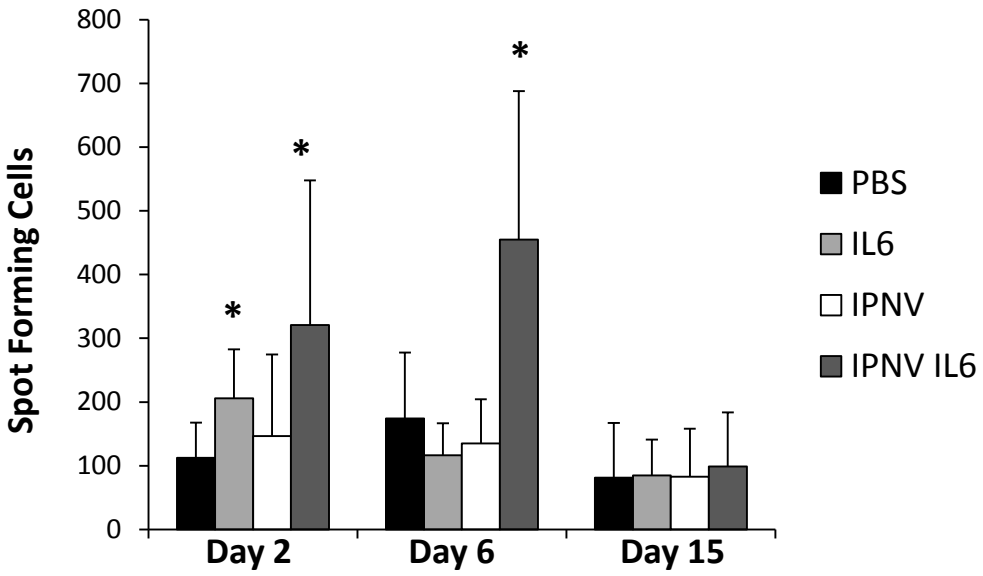
ELISPOT and levels of MHC II

Days 2, 6, 15 and 30 p.v.

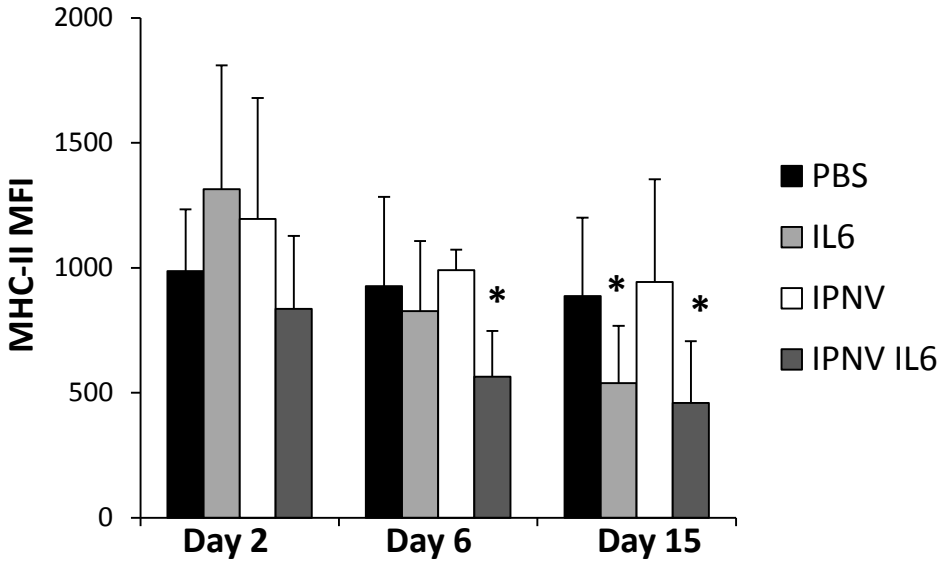
Serum

Antibody titers

Effects of IL6 on spleen IgM+ B cells after vaccination

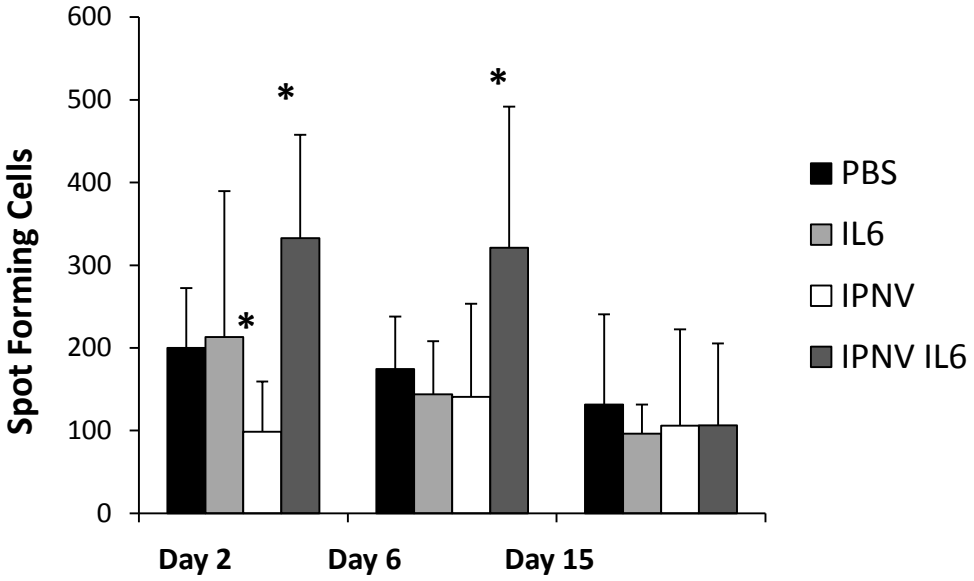


The addition of IL6 to the vaccine significantly increases the number of IgM-secreting cells in the spleen

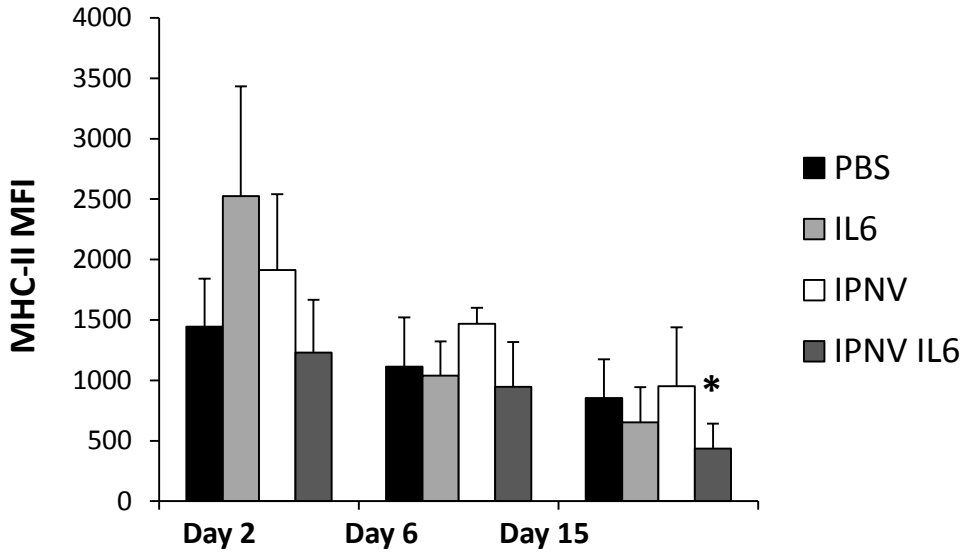


The addition of IL6 to the vaccine significantly decreases the amount of surface MHC II on splenic IgM B cells

Effects of IL6 on kidney IgM+ B cells after vaccination

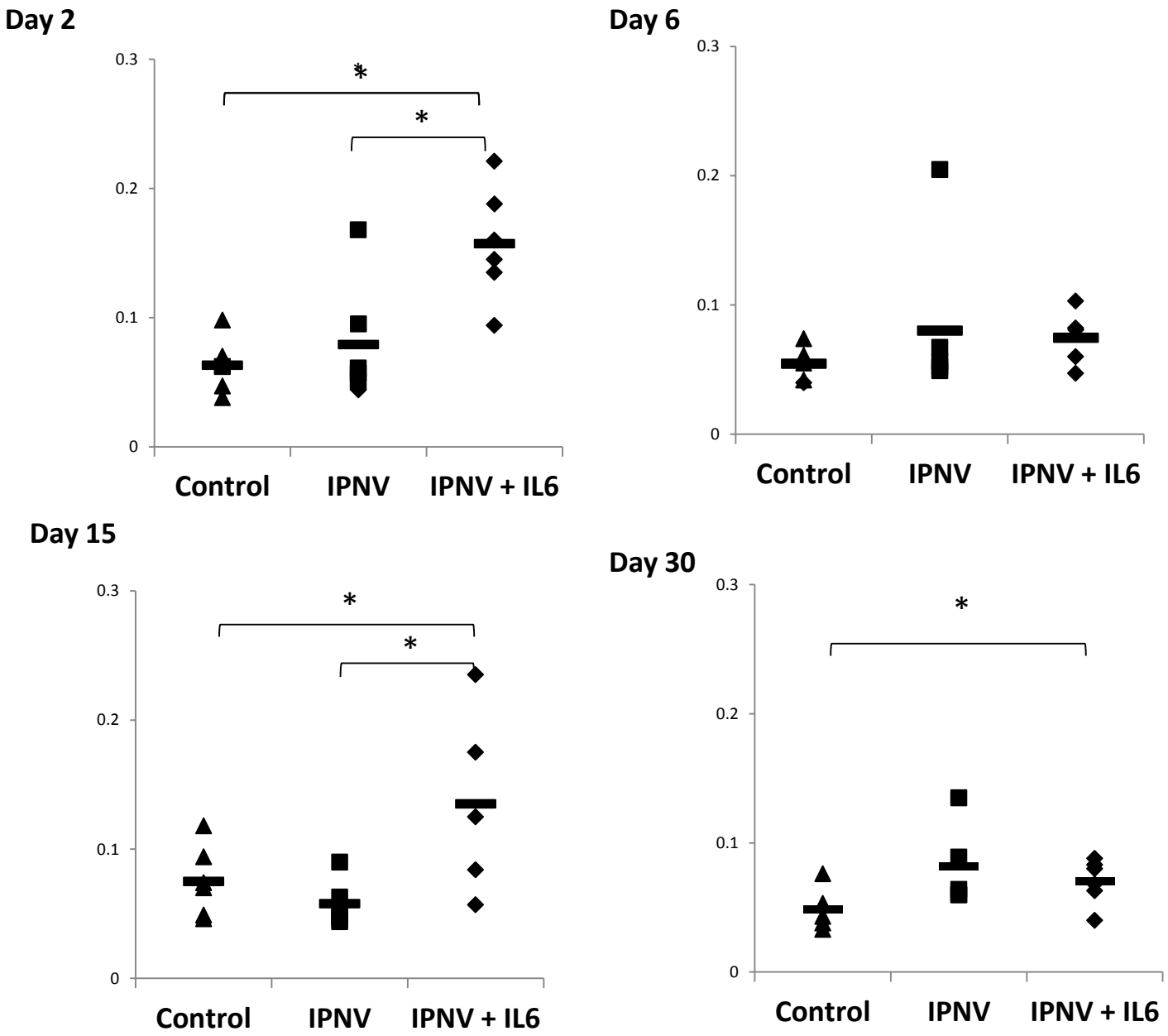


The addition of IL6 to the vaccine significantly increases the number of IgM-secreting cells in the kidney



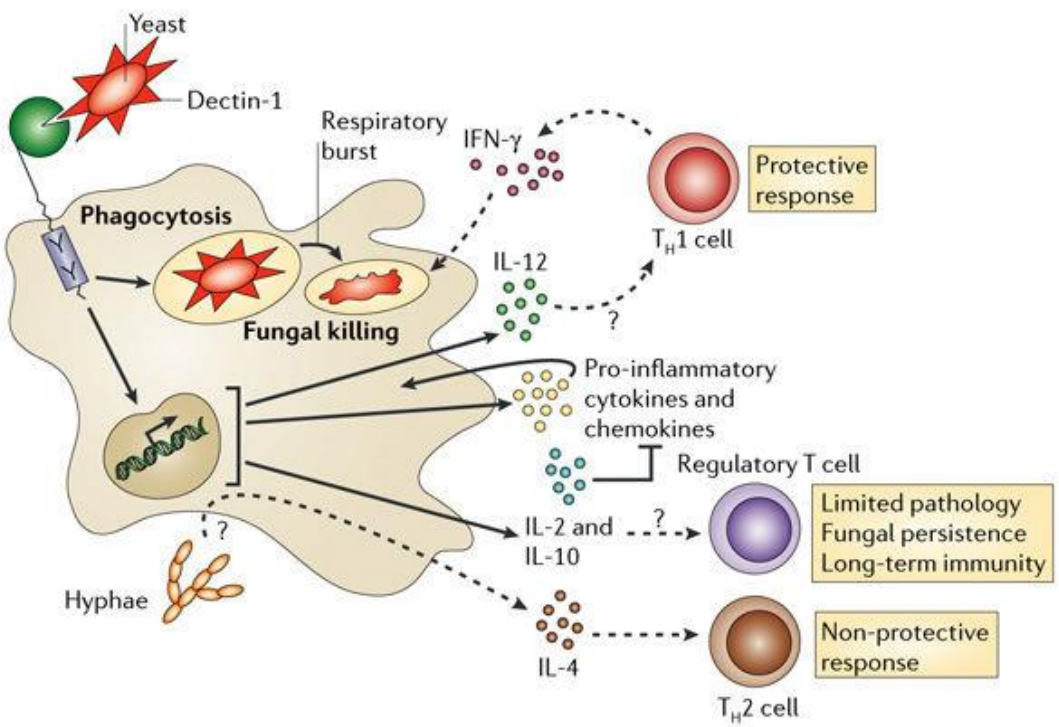
The addition of IL6 to the vaccine significantly decreases the amount of surface MHC II on kidney IgM B cells

Effects of IL6 on serum IPNV-binding IgM titers



OTHER PROMISING SIGNAL 2 ADJUVANTS FOR USE IN FISH

β-GLUCANS AS ADJUVANTS



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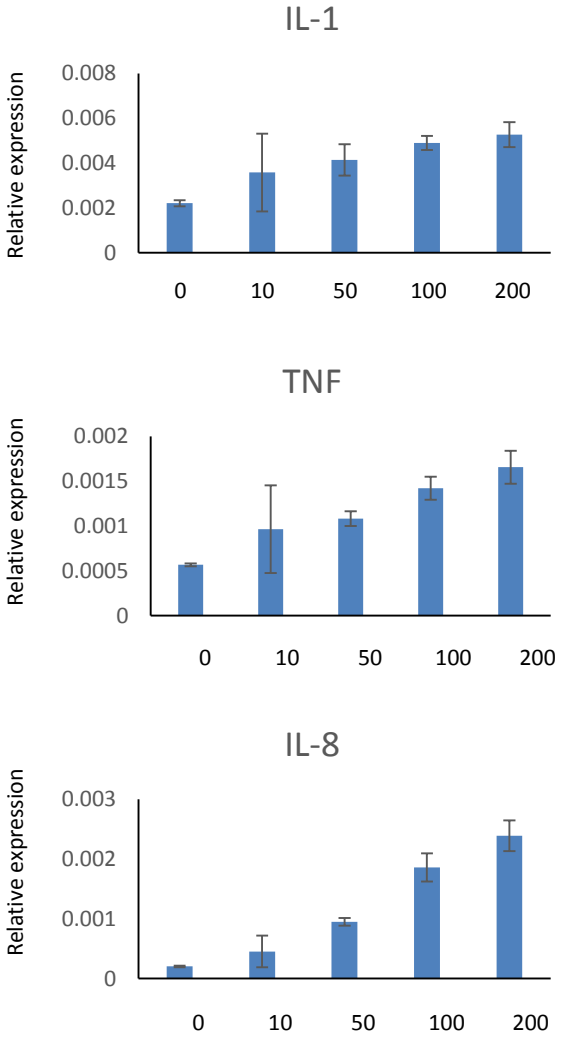
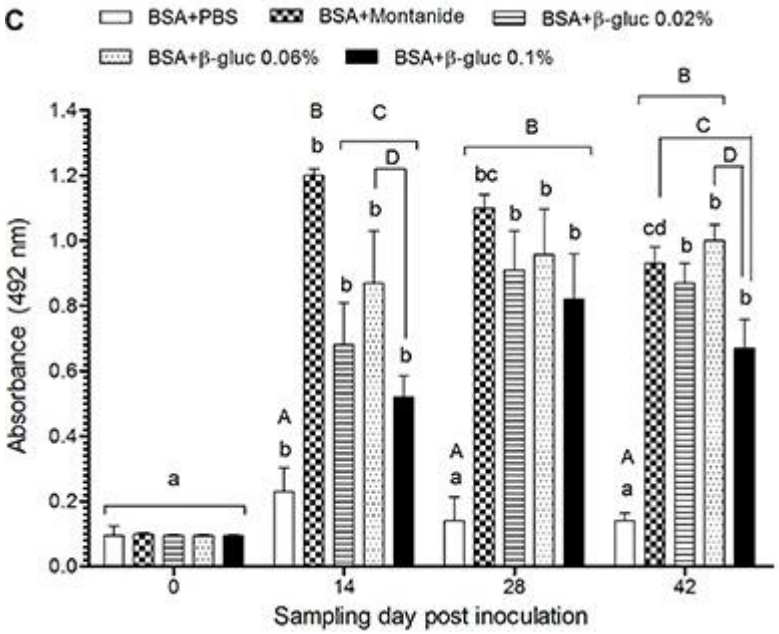
RTS11 stimulated with different glucan doses



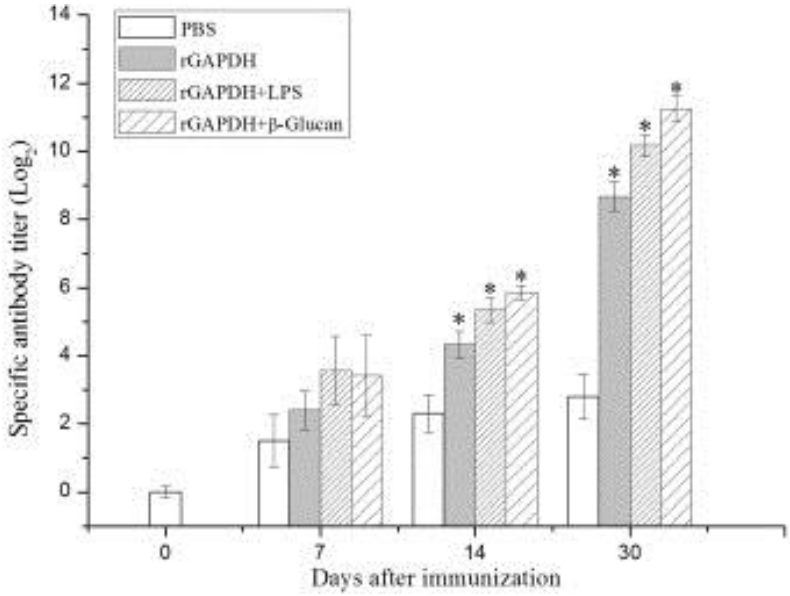
β-GLUCANS AS ADJUVANTS IN FISH

Silver catfish; BSA as model antigen; ip vaccination



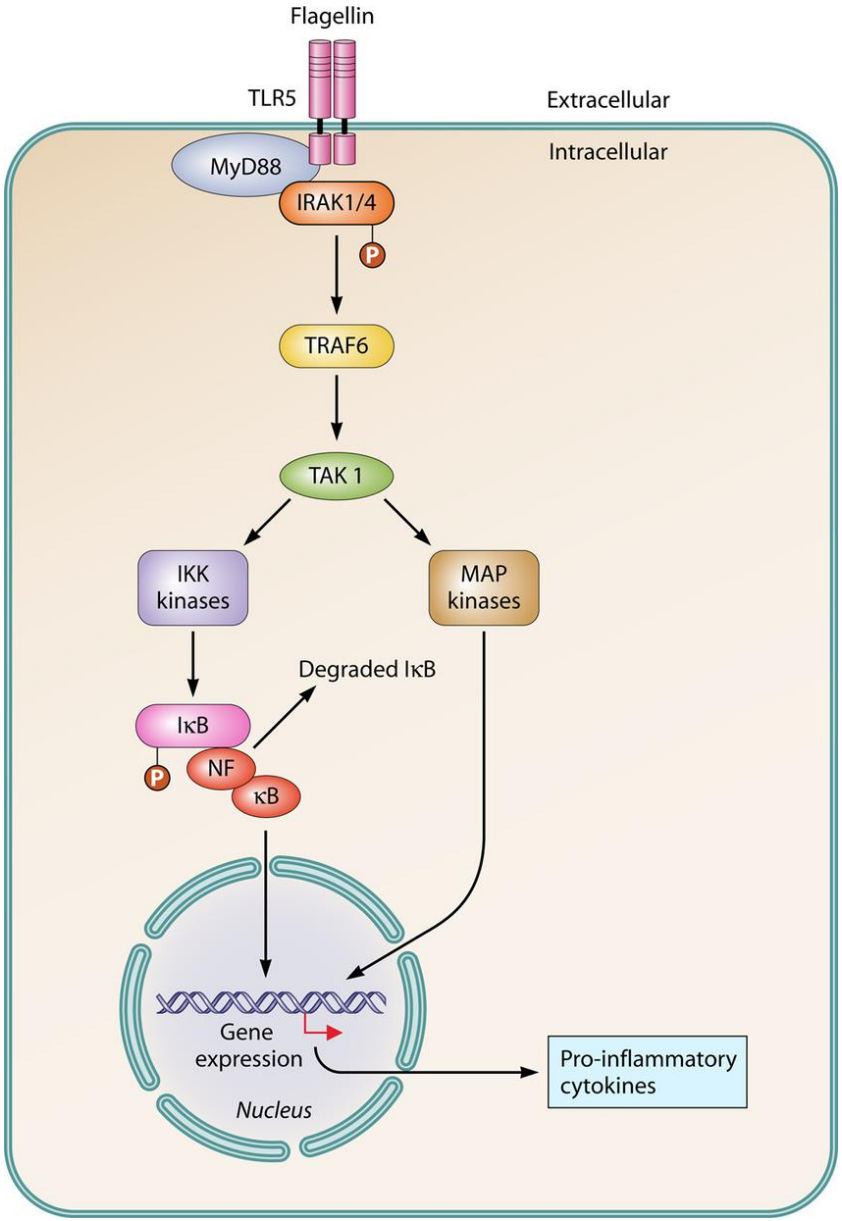
Pvana et al. 2016. Braz J Med Biol Res 25; 49(8)

Flounder; E. tarda recombinant; im vaccination



Diao et al. 2013. Vet Immunol Immunopathol 156: 167-175

FLAGELLIN AS ADJUVANT



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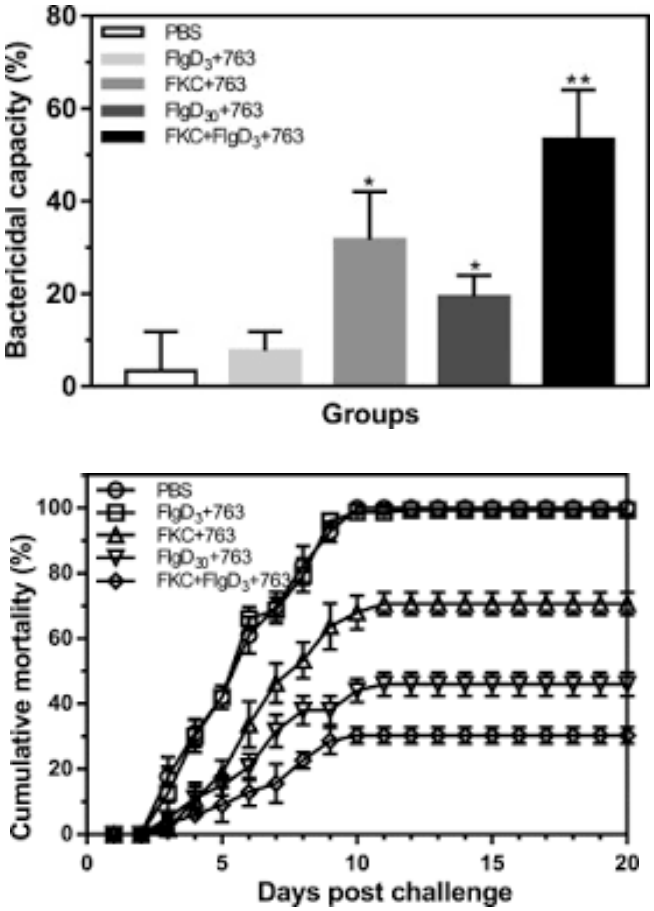
Re-examination of the rainbow trout (*Oncorhynchus mykiss*) immune response to flagellin: *Yersinia ruckeri* flagellin is a potent activator of acute phase proteins, anti-microbial peptides and pro-inflammatory cytokines *in vitro*

Eakapol Wangkahart [a, b], Callum Scott [a], Christopher J. Secombes [a, **], Tiehui Wang [a, *]

[a] Scottish Fish Immunology Research Centre, School of Biological Sciences, University of Aberdeen, Aberdeen, AB24 2TZ, UK
[b] Division of Fisheries, Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Khamriang Sub-District, Kantarawichai, Mahasarakham, 44150, Thailand

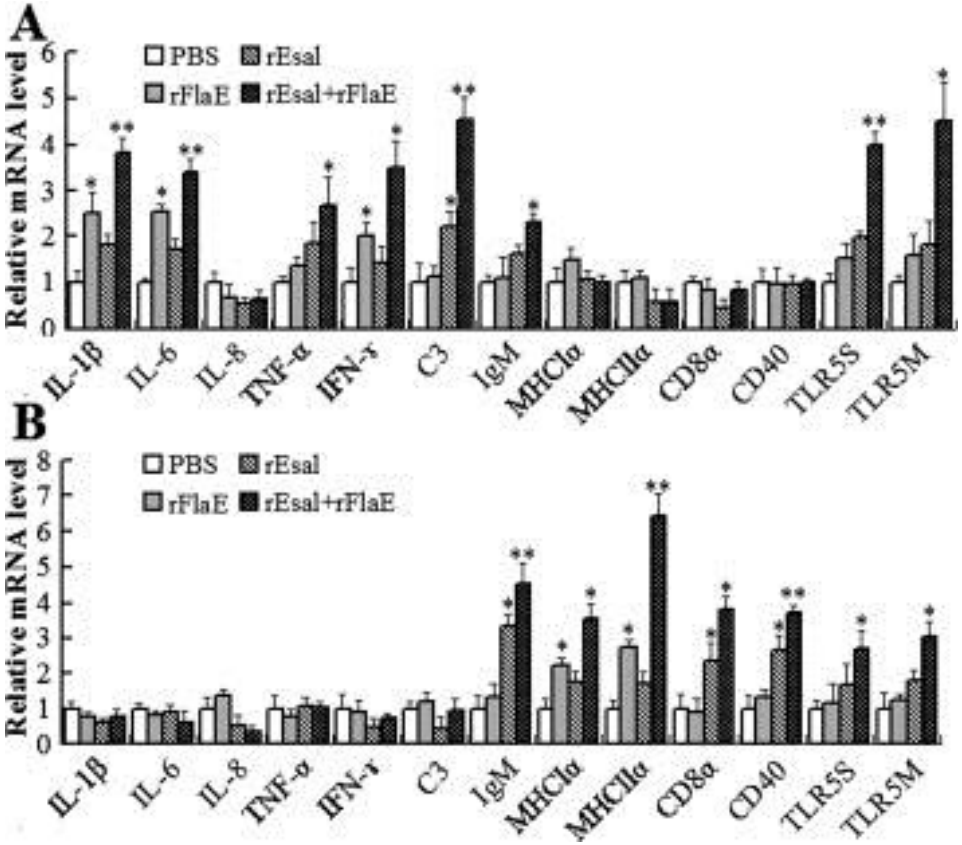
FLAGELLIN AS ADJUVANT IN FISH

Turbot; Formalin-killed cells (FKC); ip injection.



Liu et al. 2017. Vaccine 35(2):369-374.

Flounder; subunit vaccine V. anguillarum; ip injection.



Jia et al. 2013; Fish Shellfish Immunol 34: 514-520

CONCLUSIONS

- In fish, signal 1 adjuvants work efficiently for intraperitoneally delivered antibacterial vaccines although strong local side effects are elicited

- New adjuvants should be developed for antiviral and antiparasitic vaccines, as well as for vaccines delivered through alternative administration routes

- Chemokines do not seem as adequate adjuvants for fish DNA vaccines against rhabdovirus

- The introduction of instrinsic CpG motifs in DNA vaccines appears as promising strategy to increase their immunogenicity

- Recombinant IL6 increases the immunogenicity of an inactivated virus

– β-glucans and flagellin also seems as promising adjuvants for use in aquaculture.

THANK YOU FOR YOUR ATTENTION