Immunity
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A Beneficial Role for Immunoglobulin E in Host Defense against Honeybee Venom

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Bee Venom Phospholipase A2 Induces a Primary Type 2 Response that Is Dependent on the Receptor ST2 and Confers Protective Immunity

Noah W. Palm,[1,2] Rachel K. Rosenstein,[1,2] Shuang Yu,[1] Dominik D. Schenten,[1] Esther Florsheim,[1] and Ruslan Medzhitov[1,*]

Journal Club

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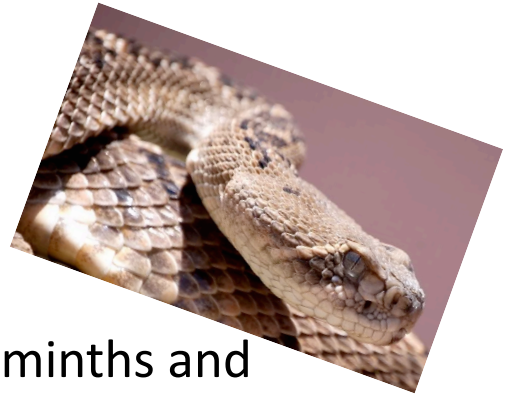
Background



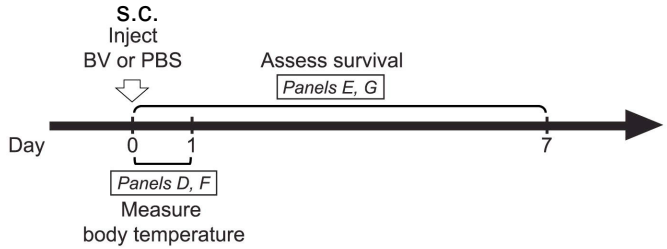
- IgE associated Th2 responses help the host to defend helminths and parasite infections.
- 20-30% of people worldwide suffer from allergies. *Pawankar, R. et al (2012), 12(1), 39–41.*
- Allergies are widely considered misguided Th2 cell responses
- „Allergy is the price we have to pay for the evolution of protection against multicellular parasites.“ *Artis, D., Maizels, R. M., & Finkelman, F. D. (2012). Forum: Immunology: Allergy challenged. Nature, 484(7395), 458–459.*
- The function of the acquired immunological response (allergic response) is the defense against toxins and venoms -> ‘‘toxin hypothesis’’ *(Profet, 1991)*
- Allergic responses are important for host defence against noxious environmental substances and evolved to promote avoidance of suboptimal environments *Palm, N. W. et al. (2012). Nature, 484(7395), 465–472*

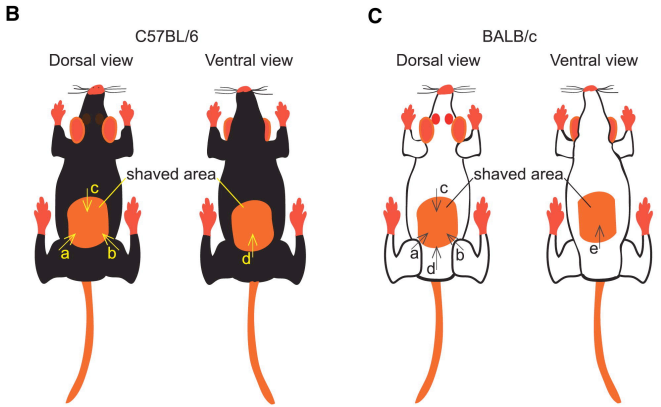
Background- venoms

- Venoms consist of complex mix of toxic components and represent a major class of noxious allergens.
- Bee venom major components:
  - Melittin (23-amino acid cationic cell lytic peptide)
  - Phospholipase A2 (PLA2)-> hydrolyzes membrane phospholipids to produce lysophospholipids and arachidonic acid
- PLA2 is an integral and conserved component of venoms from divergent species!
- Molecular mechanisms involved in innate sensing of allergens/ venoms and instruction of Th2 responses remain largely inkown.

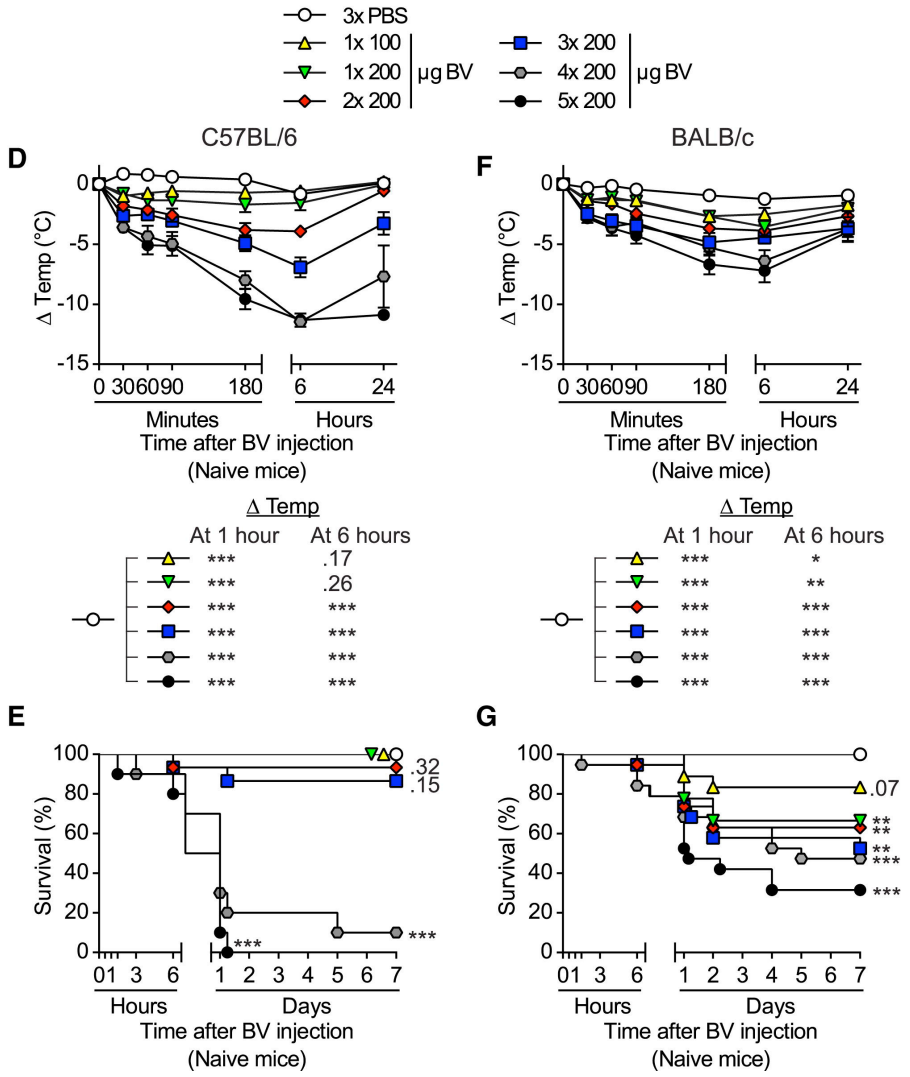
Acute systemic responses of naive mice to bee venom (BV)

Fig1 **A**

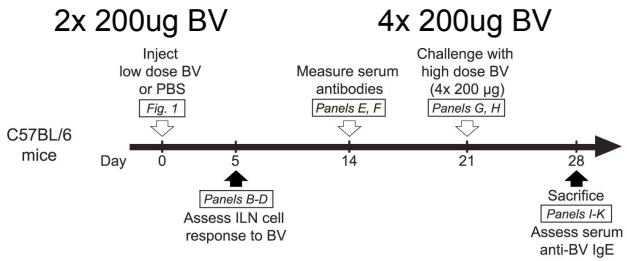


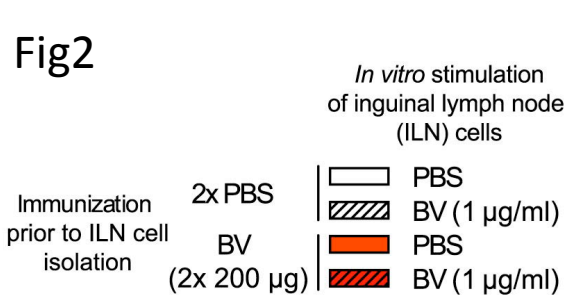


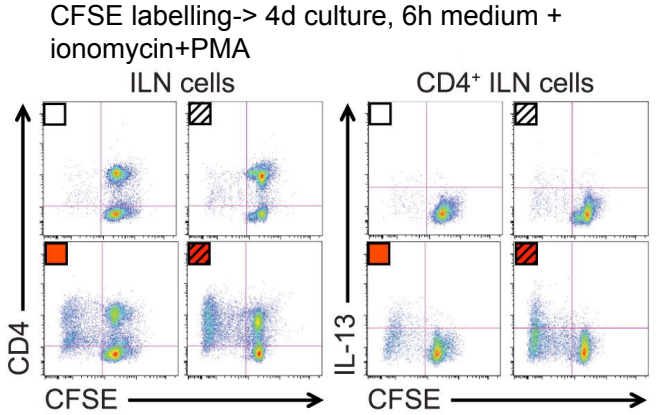
same results for Russel‘s viper venom



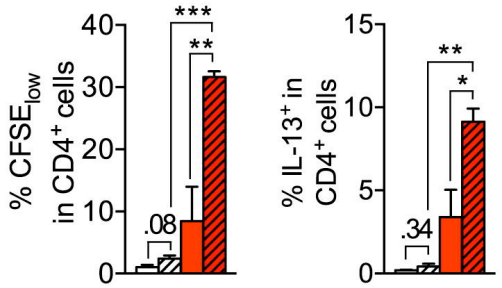
Sublethal BV dose induces Th2 response

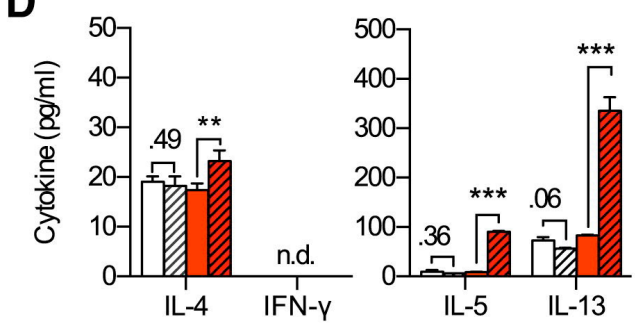




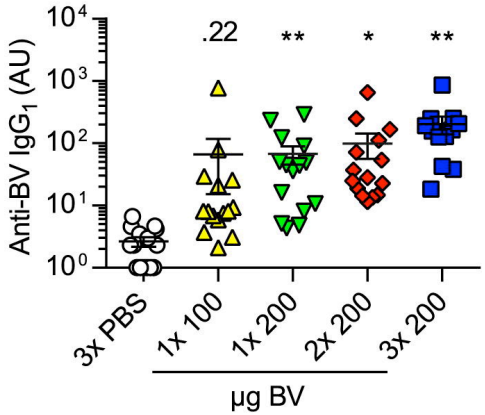


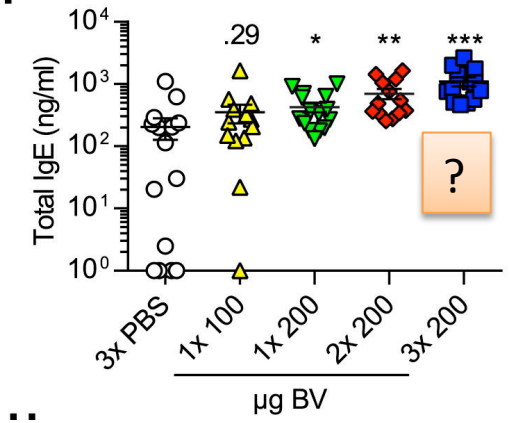
5d





14d



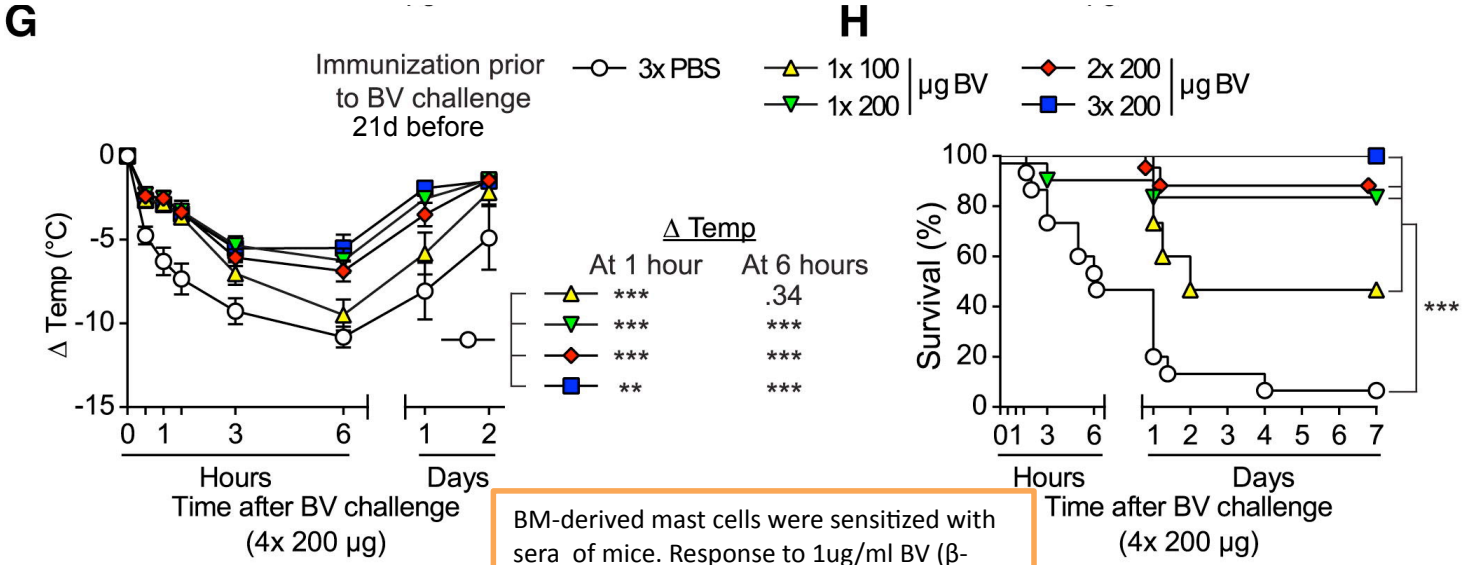


Mice can develop Th2 immune responses after exposure to BV at physiological doses.

Th2 immunity to BV increases resistence to high dose venom

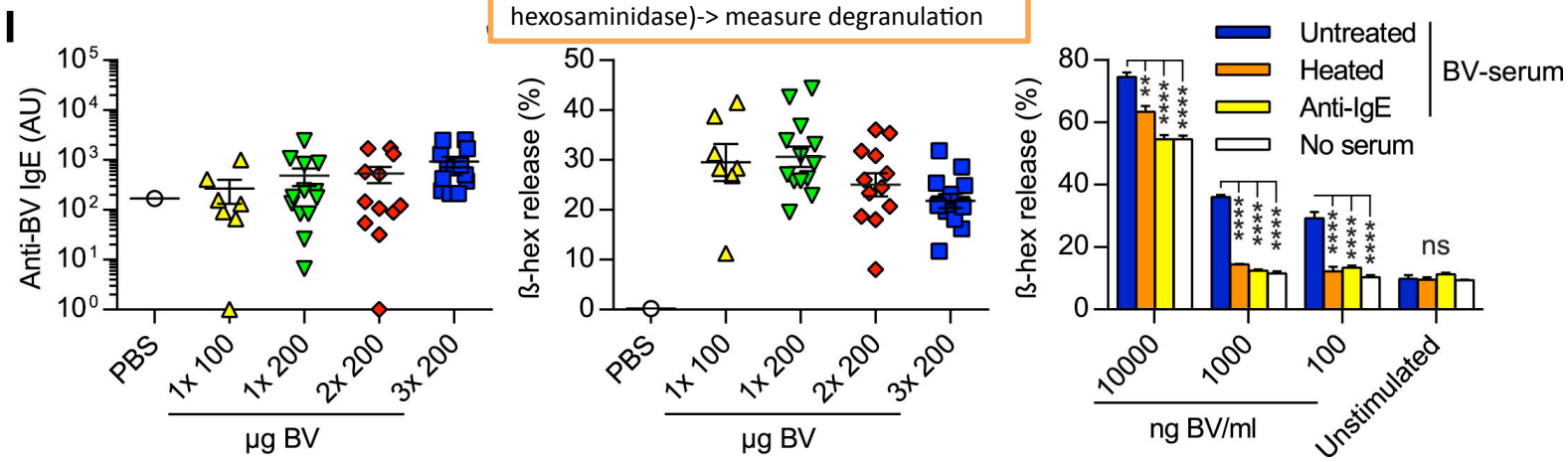
Fig2

d21 injection of lethal dose 4x200ug



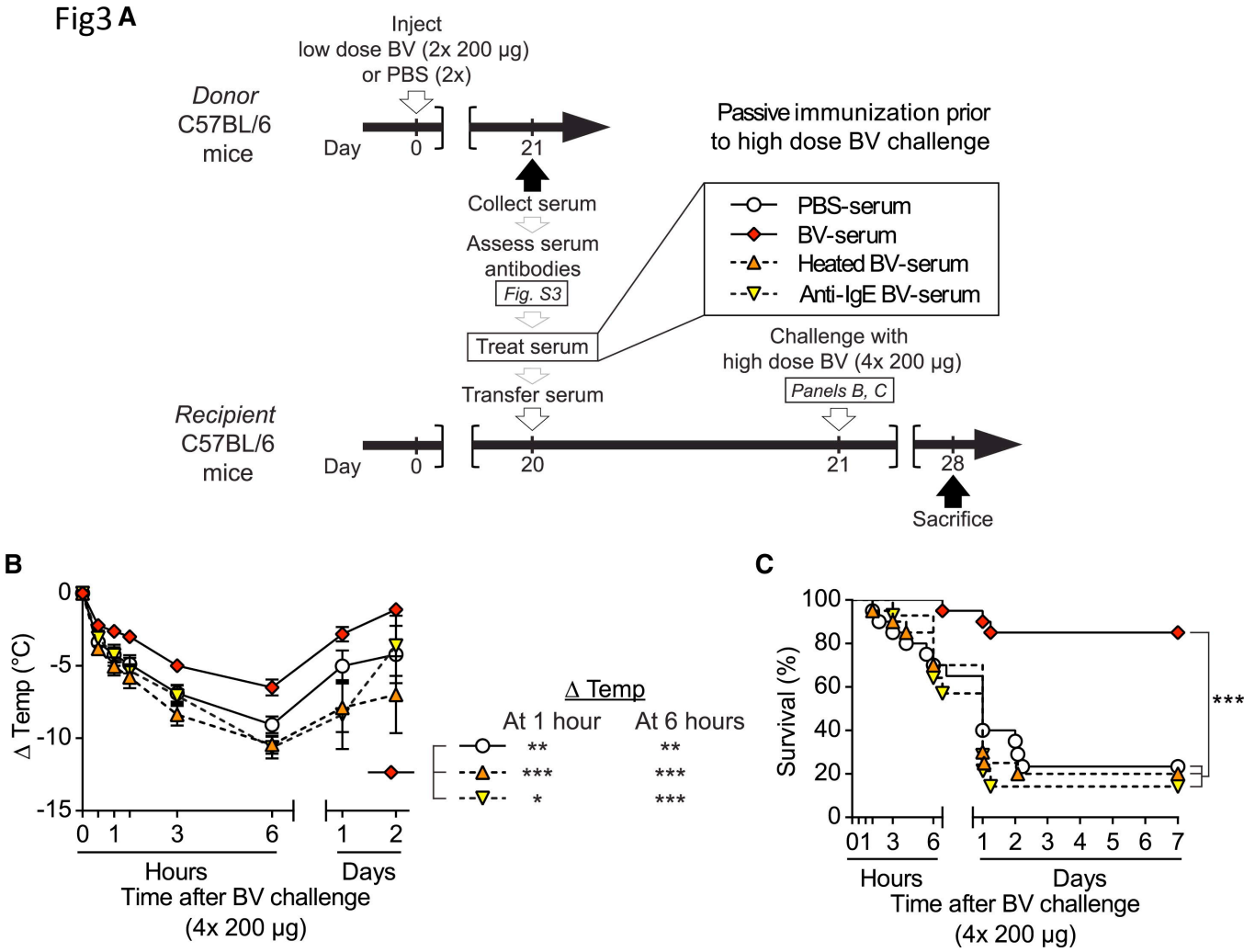
serum collected 7d post venom challenge (all surviving mice in H)

BM-derived mast cells were sensitized with sera of mice. Response to 1ug/ml BV (β-hexosaminidase)-> measure degranulation



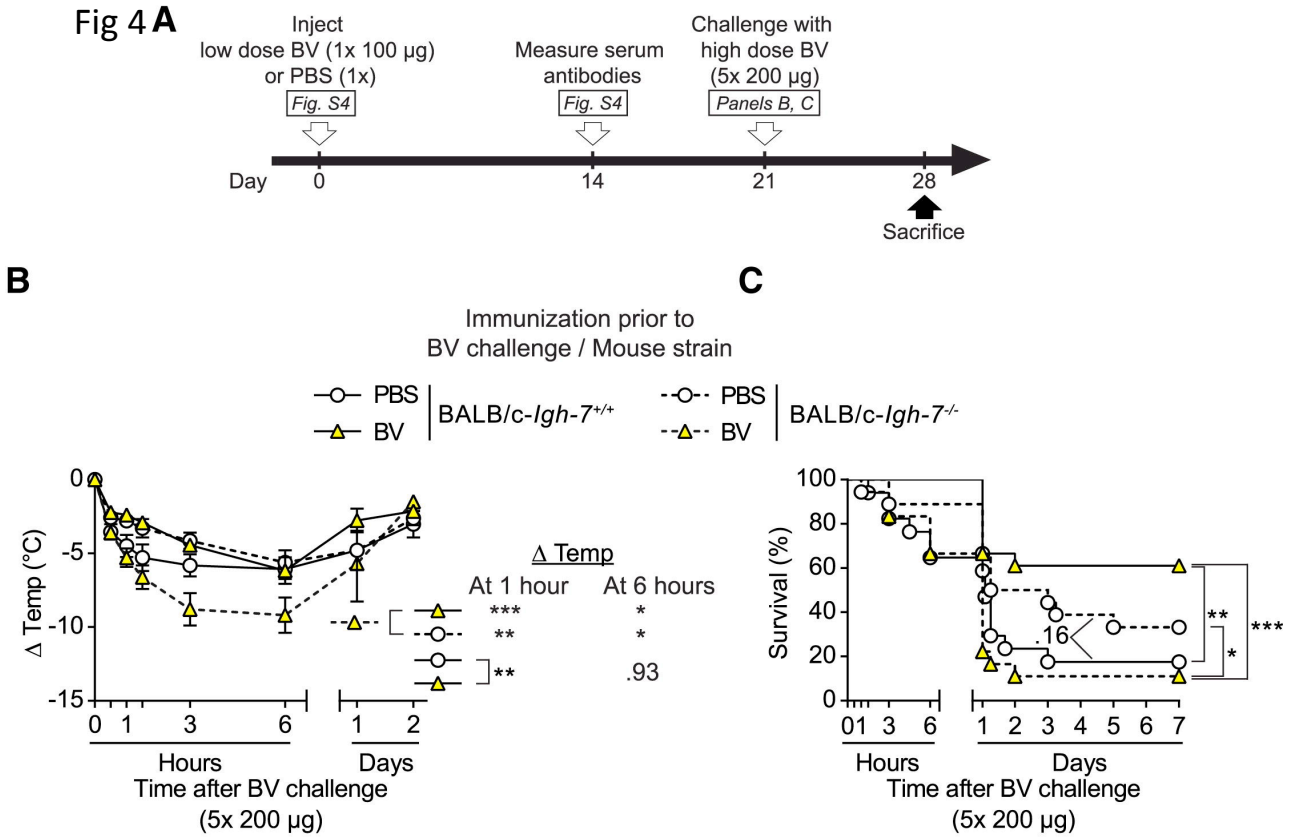
Th2 response after priming with sublethal amount of bee venom is protective against a subsequent lethal BV dose-> classically it was thought to exacerbate the outcome!

Acquired resistance to high-dose venom challange is dependent on functional IgE



IgE contributes or may be fully responsible for the immune serum‘s protective effect!

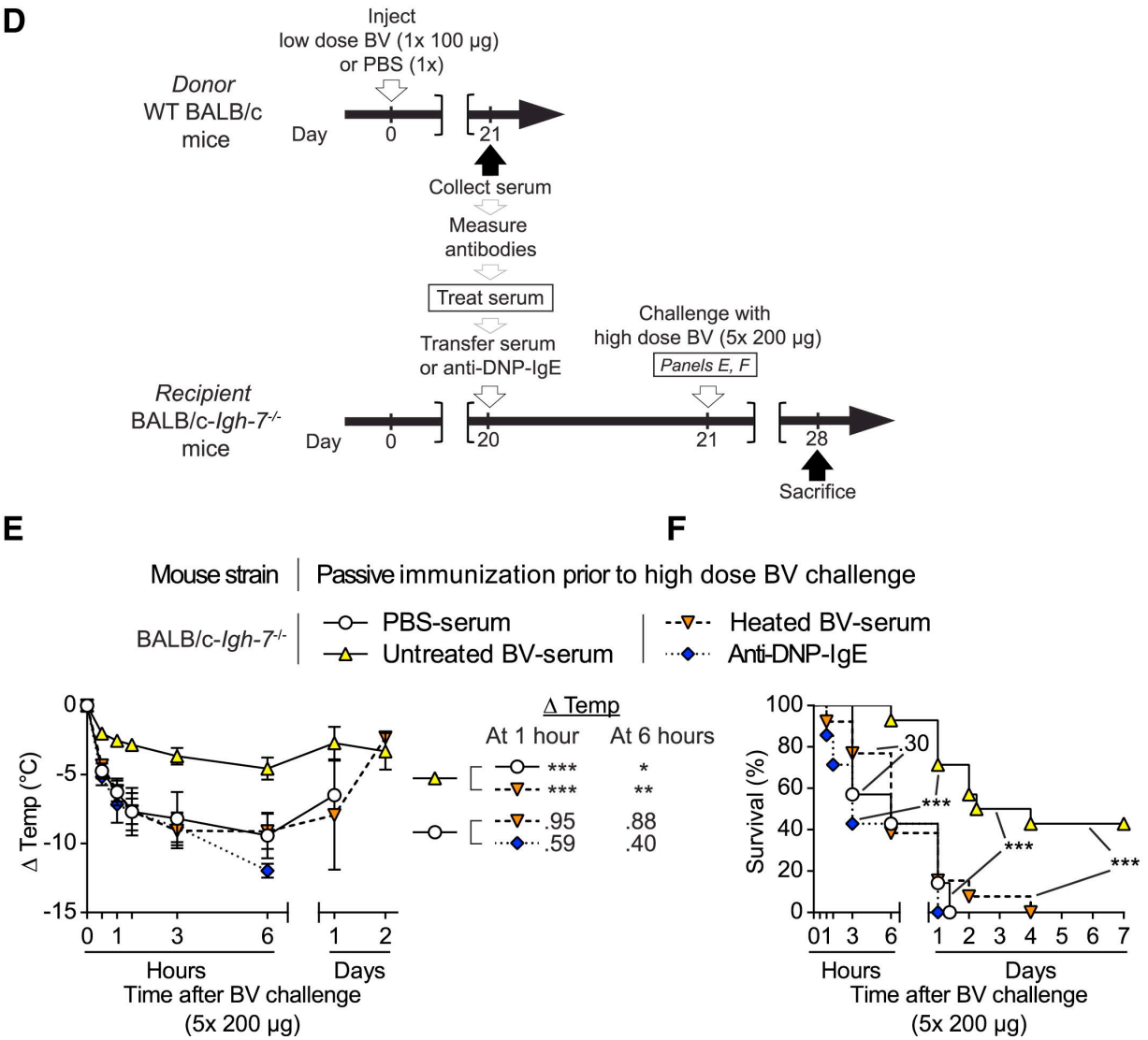
BV immunized IgE deficient mice do not develop enhanced resistance to the venom



IgE deficient mice are less reistant to high-dose BV challenge.

IgE but not IgG AB are necessary for the protective effect in the adaptive response to BV

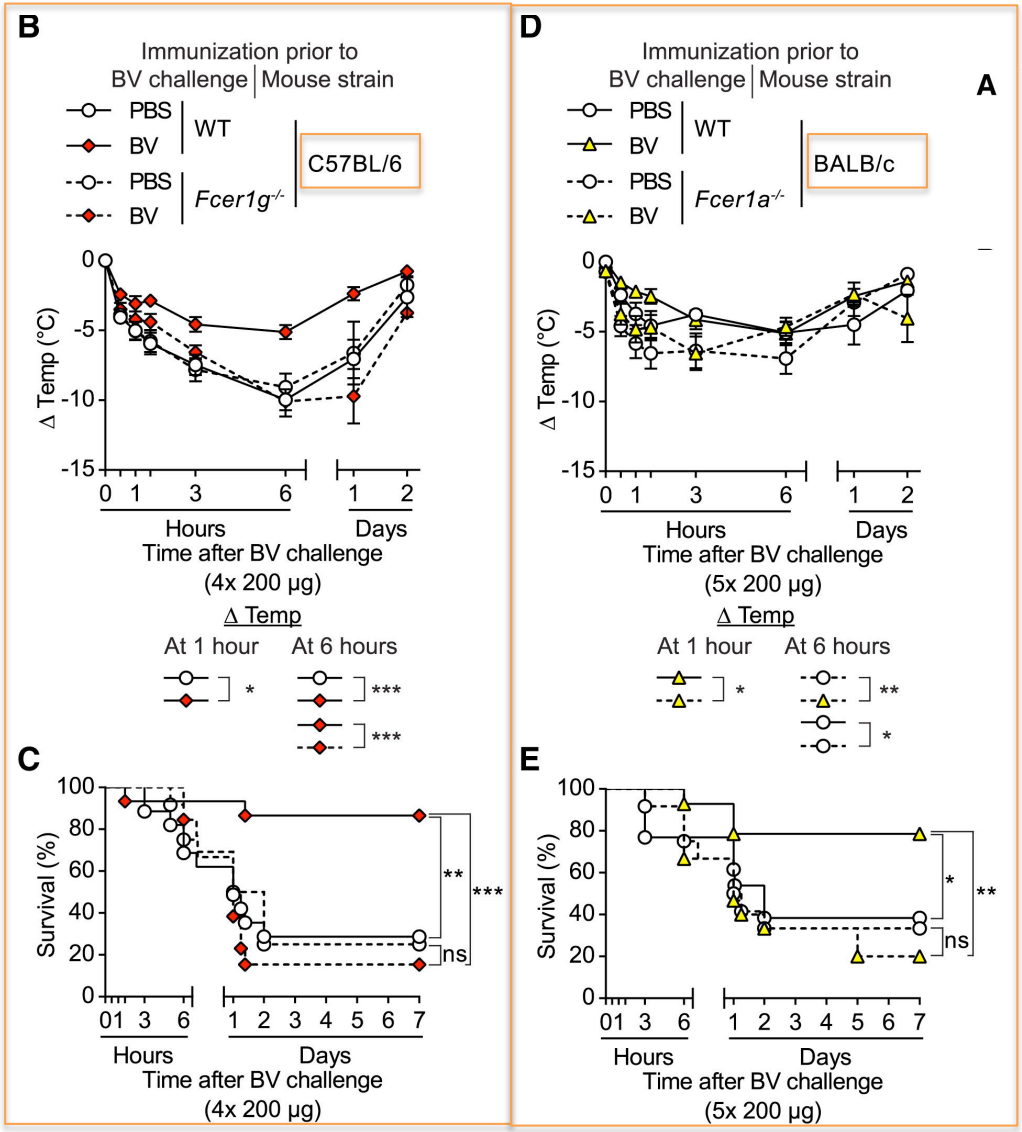
Fig 4

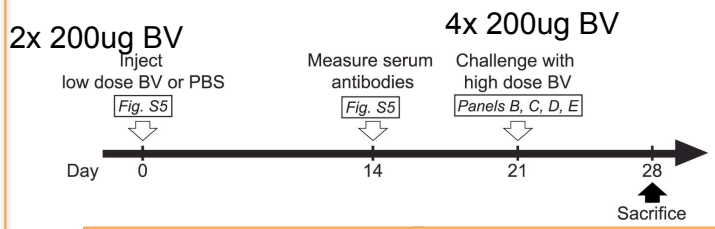


IgE deficient mice could be rescued by passive immunization with untreated BALB/c BV serum.

Importance of FcεRI - BV immunization is not protective in FcεRI deficient mice

Fig5

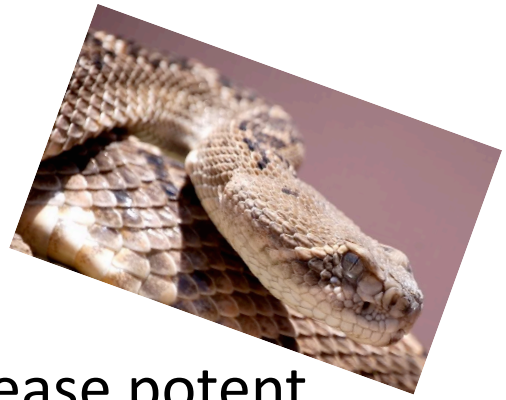




Acute response and AB production is similar in wt and FcεRIγ or α deficient mice.

FcεRIα and FcεRIγ signaling is required BV immunized mice to exhibit increased resistance to lethal dose of BV. (no rescue by passive immunization)

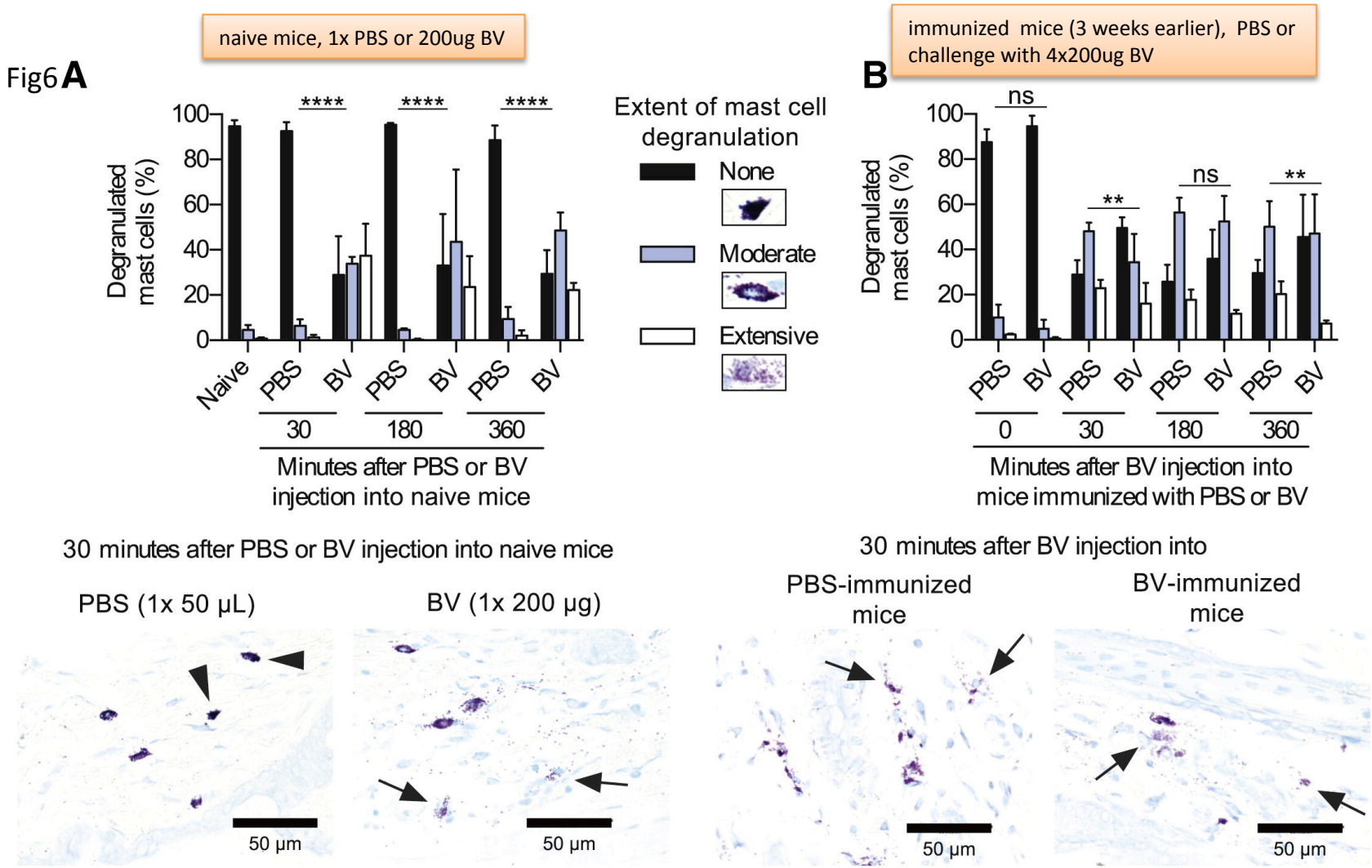
Background – Mast cells



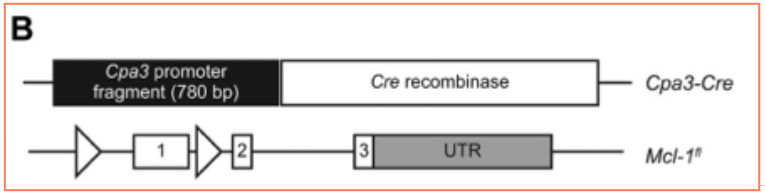
- In response to snake venom, mast cells (MCs) release potent biologically active mediators which can promote an increase in vascular permeability, local inflammation, abnormalities of the clotting and fibrinolysis systems, and shock . *Metz, M. (2006) . Science, 313(5786), 526–530*

- MCs are protective against the snake venom sarafotoxin (that is a homologue of Endtothelin 1): They cleave off the C-terminal Tryphophan -> exactly the structure required for toxicity *Schneider, L. A. et al. (2007). Journal of Experimental Medicine, 204(11), 2629–263*

Role for Mast cells in IgE mediated resistance to high dose BV challenge

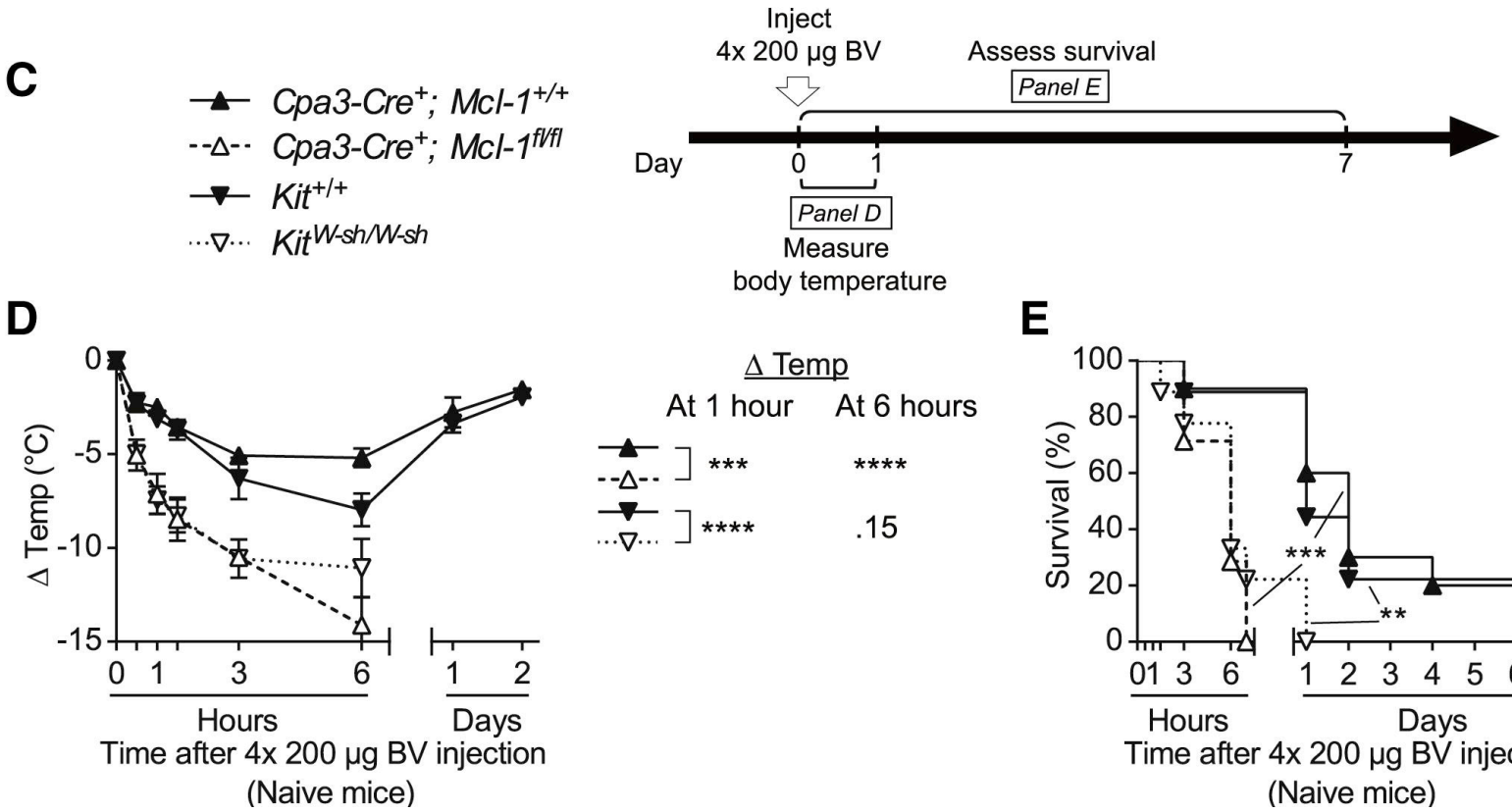


Role for Mast cells in IgE mediated resistance to high dose BV challenge



Cpa3: Carboxypeptidase A3
Mcl-1 which is survival factor for mast cells and basophils becomes deleted -> ca 95% reduced MC and 65% reduced basophils.

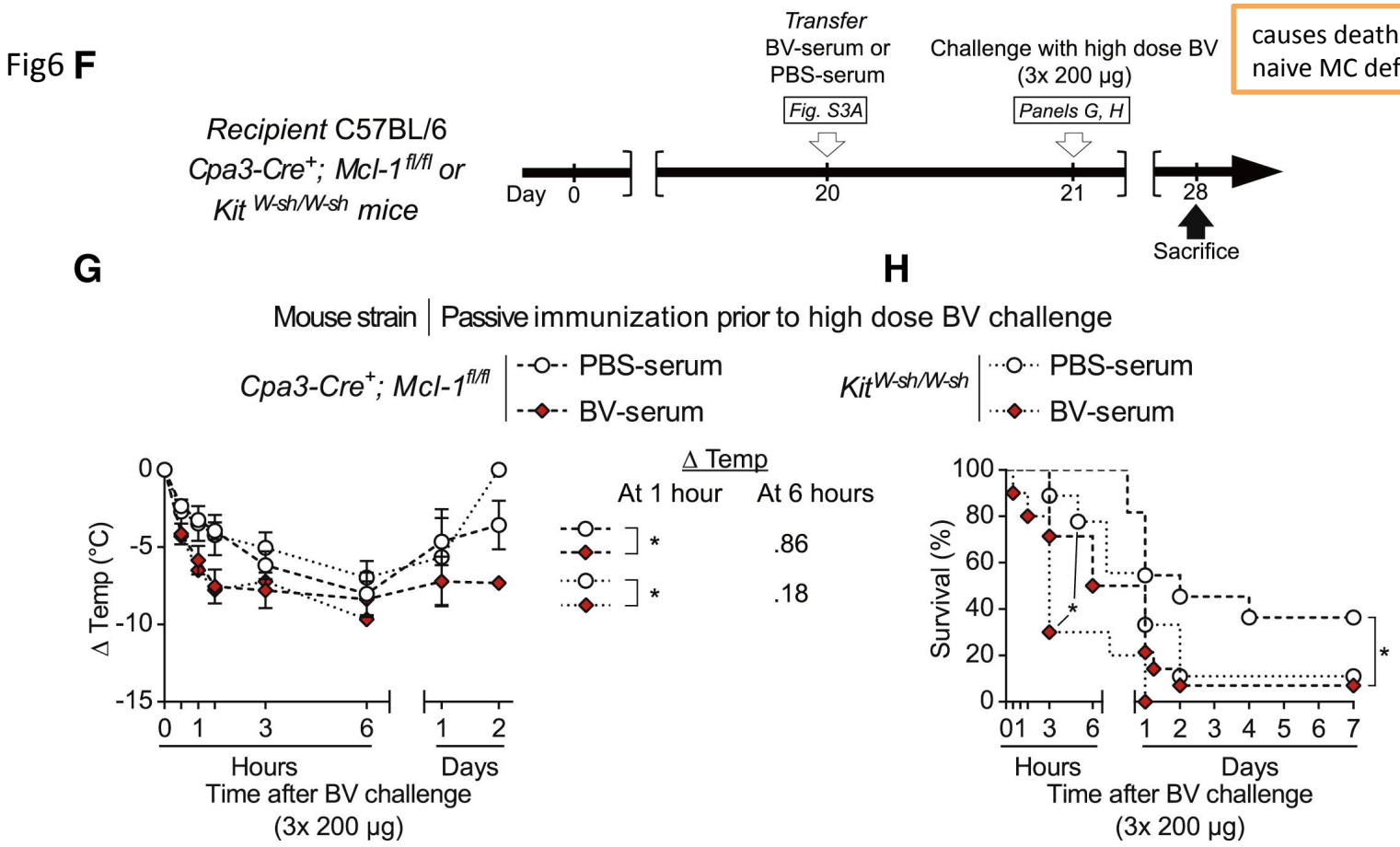
Fig6



C57BL/6-Kit $^{W\text{-}sh/W\text{-}sh}$, receptor for MC survival and maturation factor stem cell factor (c-kit) is mutated
C57BL/6-Cpa3-Cre^{+};Mcl-1 $^{fl/fl}$: MC deficient and basophils are reduced

No rescue in MC deficient mice by passive immunization to challenge with potenial lethal dose of BV

causes death in 60-90% of naive MC defcient mice

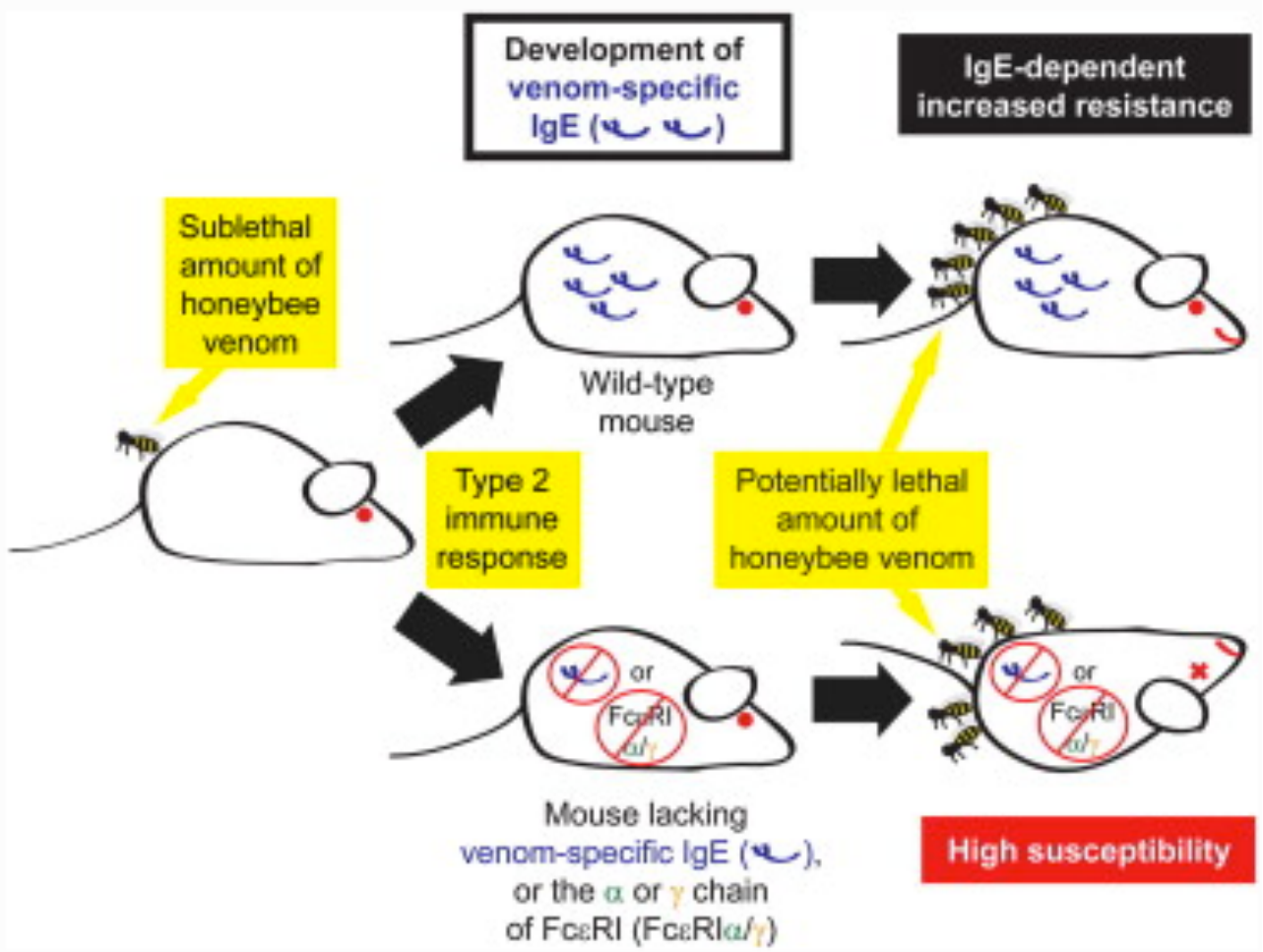


Worse survival of MC deficient animals that received BV serum in response to high dose BV compared to mice that received PBS-> MC may contribute to IgE mediated resistance againse BV.

Summary

- Th2 cell immunity can enhance mouse resistance to honeybee or Russell's viper venoms.
- IgE and FcεRI contribute to such acquired increased resistance to honeybee venom
- IgE-associated immune responses can protect the host against noxious substances.-> beneficial effect of IgE and first experimental support for „toxin hypothesis".

Summary


Development of venom-specific IgE
IgE-dependent increased resistance
Sublethal amount of honeybee venom
Wild-type mouse
Type 2 immune response
Potentially lethal amount of honeybee venom
or
FcεRI α/γ
Mouse lacking venom-specific IgE, or the α or γ chain of FcεRI (FcεRIα/γ)
High susceptibility

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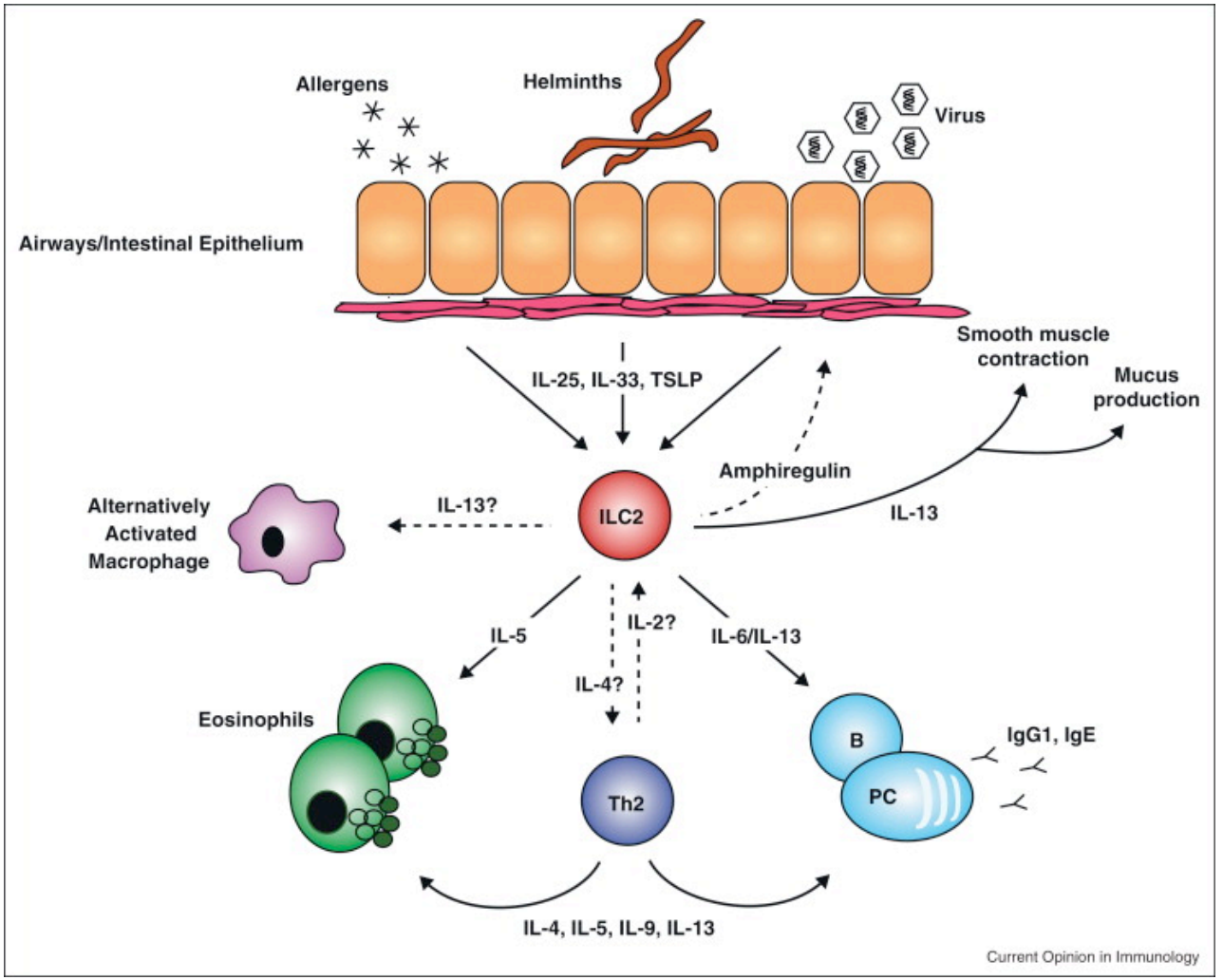
Bee Venom Phospholipase A2 Induces a Primary Type 2 Response that Is Dependent on the Receptor ST2 and Confers Protective Immunity

Noah W. Palm,[1,2] Rachel K. Rosenstein,[1,2] Shuang Yu,[1] Dominik D. Schenten,[1] Esther Florsheim,[1] and Ruslan Medzhitov[1,*]

Background

- Mechanisms by which innate immune system recognizes helminths and allergens (Th2 + IgE response) remain largely unknown.
  - detected by their enzymatic activities (e.g. proteases)
  - detected by sensing of tissue damage (response is aimed at repairing the damage) *Palm, N. W., (2012) Nature, 484(7395), 465–472*
  - Epithelial derived cytokines (IL-25, TSLP, IL-33) induce Th2 response to helminths and allergens. *Pulendran, B., & Artis, D. (2012). Science, 337(6093), 431–435.*

Group 2 innate lymphoid cells



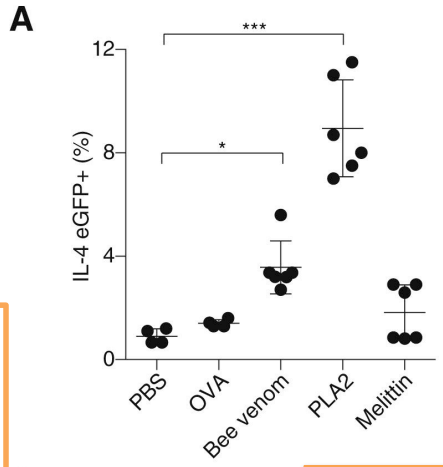
Walker, J. A., & McKenzie, A. N. (2013). Current Opinion in Immunology, 25(2), 148–155. 0

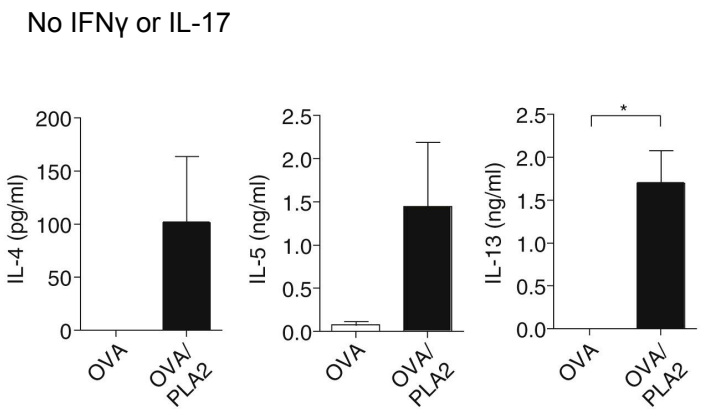
Bee venom PLA2 induces a Type 2 immune response

IL-4 IRES-eGFP reporter mice-:
4 get mice
(IL-4/GFP-enhanced transcript)

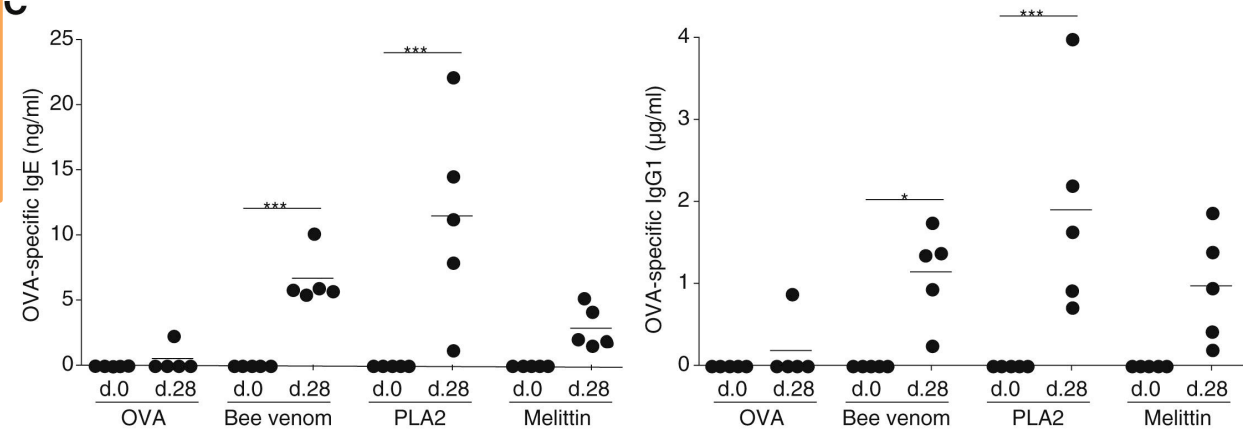
Cell culture: CD4⁺ MACS sorted cells, $1.5x10^5$ cells incubated with irratdiated splenocytes with OVA (900ug/ml-100ug/ml) for 5-6d,

Antibody production:
Immunization d0 and d21.



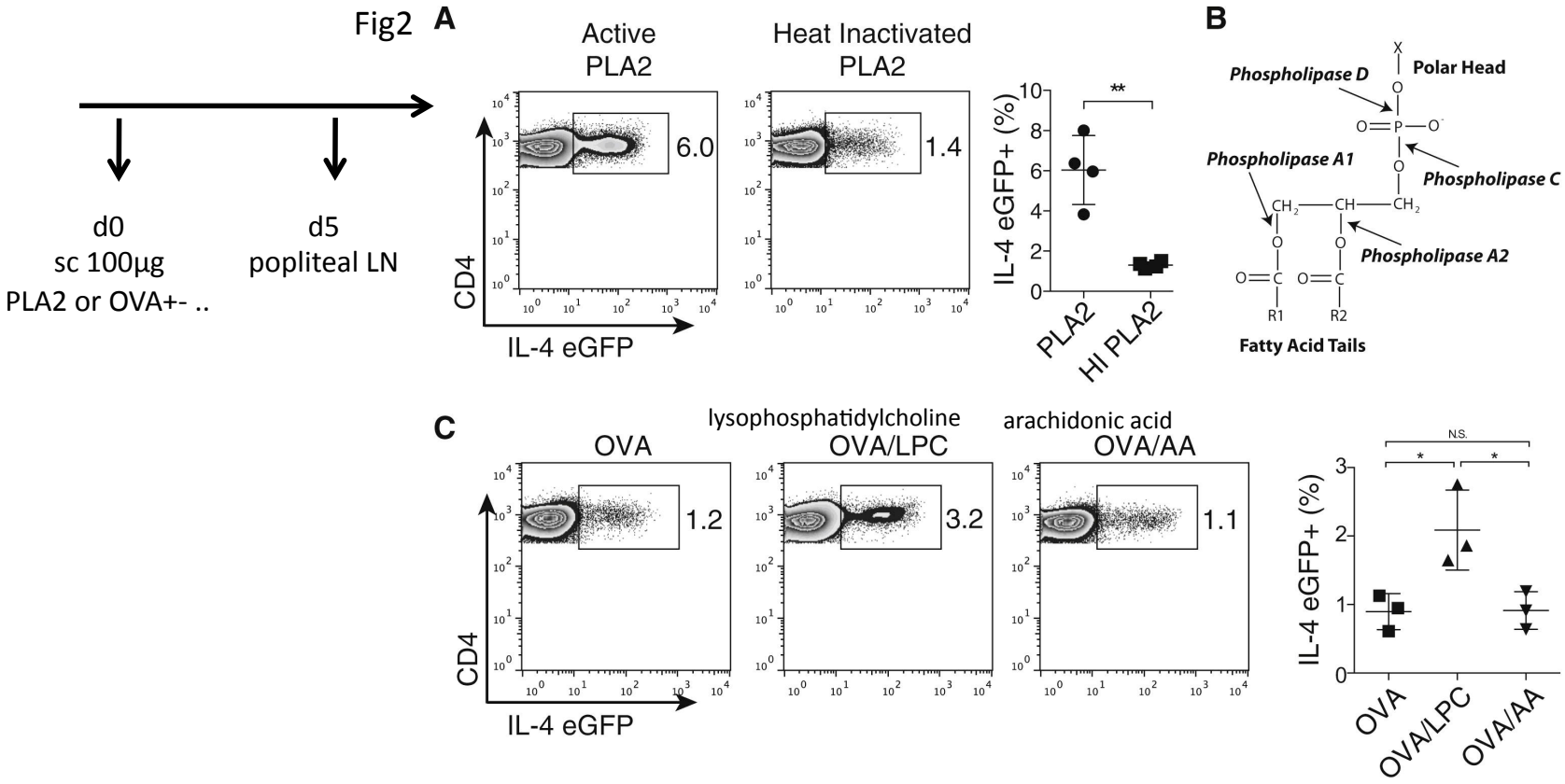


PLA2 is potent inducer of Th2 response



⇧total IgE after BV or PLA2 immunization. (IgE response to bvPLA2remained intact in TLR2 or $TLR4^{-/-}$ -> not effect of contaminated PAMPs.)

PLA2 induces Th2 response via cleavage of membrane phospholipids (to produce lysophospholipids)



PLA2 induces Th2 response by hydrolyzing membrane phospholipids to produce lysophospholipids (such as LPC)

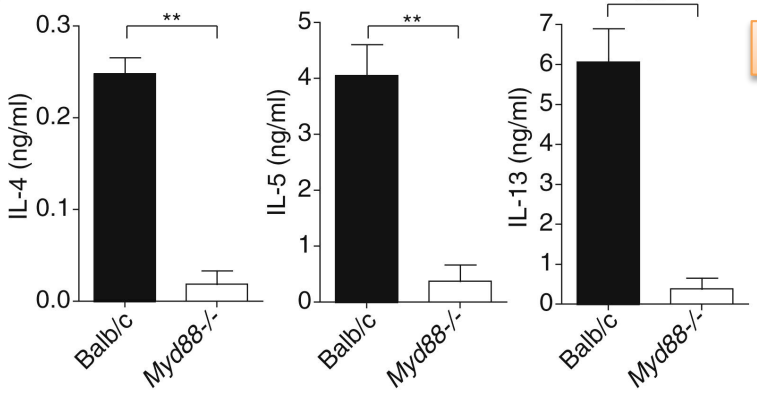
Mechanism-> not clear!! knock out receptor of LPC (Gpr132)-> intact Th2 cell differentiation in response to PlA2-> different receptor or receptor independent effect? (induction of cell lysis?)

Th2 responses induced by bvPLA2 are dependent on MyD88 and ST2

Mice immunizes s.c. with 100ug bvPLA2+OVA-> 4-5d->poplieatal LN-> 1.5x10^5 CD4+ T cells, cocultured with irradiated (850rads) splenocytes (3x10^5) plus titrating doses of OVA (900ug/ml- 100ug/ml)
A-B Cytokine production after in vitro restimulation of LN CD4+
D restimulation of LN CD4+ cells 5d after OVA +/- LPC

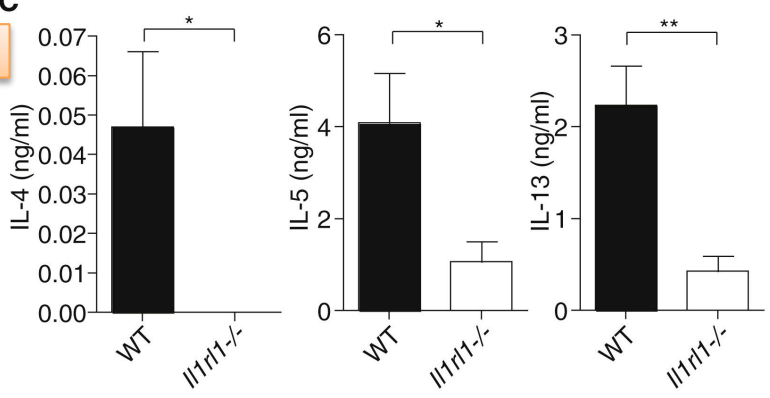
Fig3 A

Balb/c



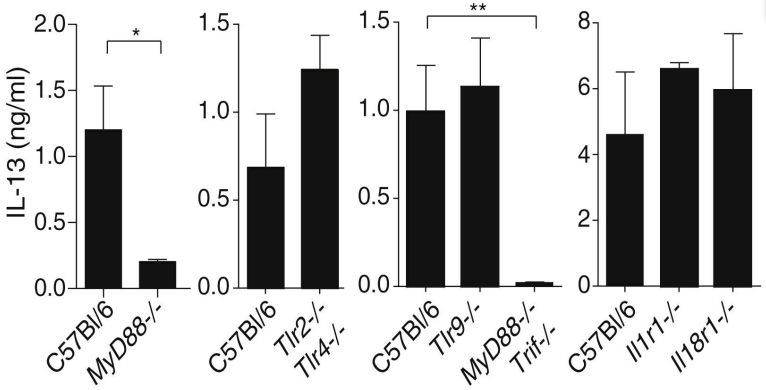
C

C57BL/6



B

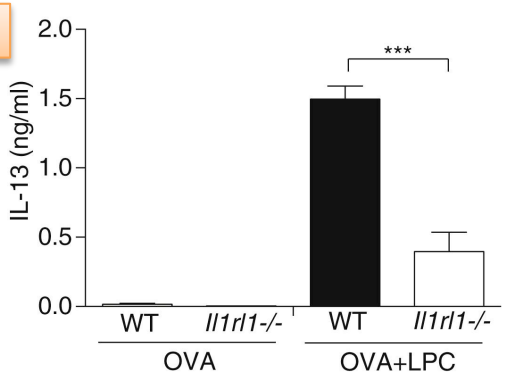
C57BL/6



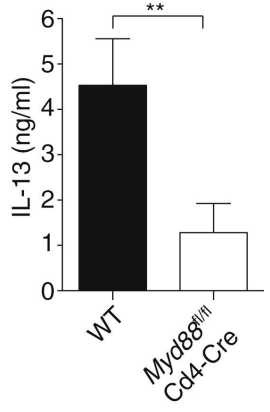
D

Il1rl1=ST2

C57BL/6



E



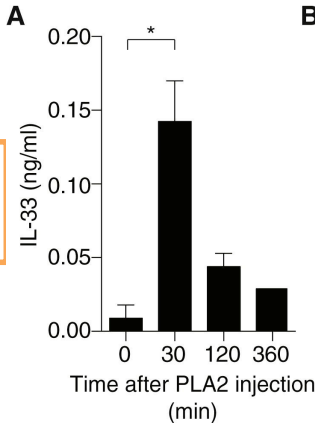
C

lysophosphatidylcholine activation of Th2 is dependent on ST2. IL-33 acts directly on ST2 on CD4 T cells and not on DC (data not shown).

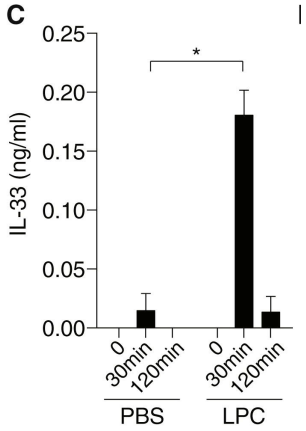
bvPLA2 induces IL-33 release and ST2 dependent activation of ILC2s

Fig4

ip injection of 100ug bvPLA2-
ELISA of peritoneal fluid



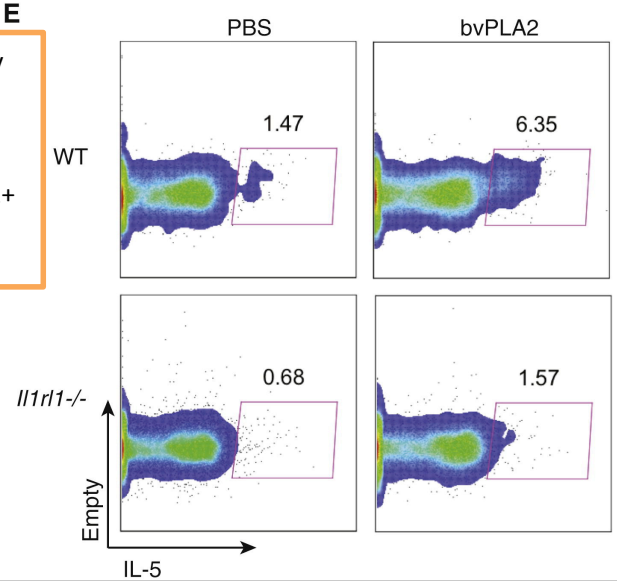
ip injection 100ug LPC
-> ELISA of peritoneal fluid



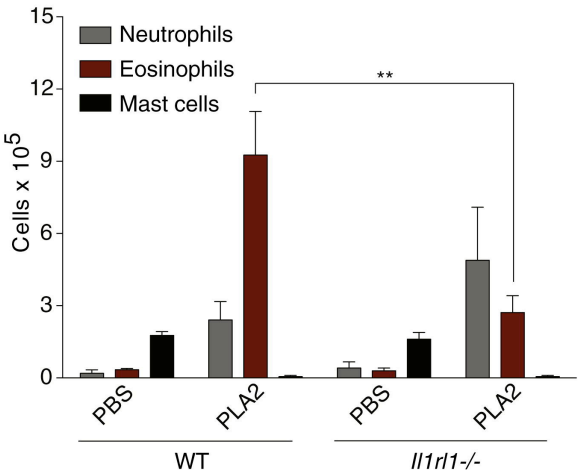
bvPLA2 might act by producing LPC (membrane damage!) which then leads to IL-33 release.

Peritoneal wash after 3d daily ip injection with PLA2, d4 analysis

ILC2 gating: CD45+Lin-CD90.2+ ex vivo stimulation with PMA and ionomycin



cells identified by morphology-> PLA2 induces eosinophilia!



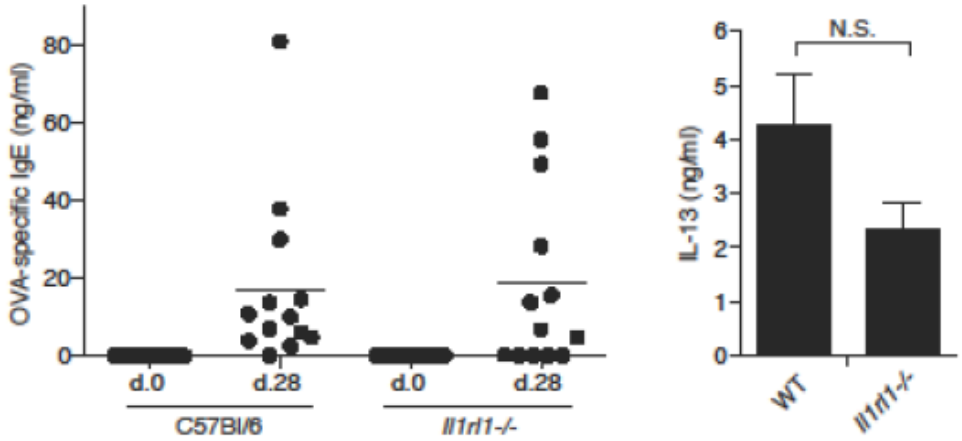
bvPLA2 induces ILC2 activation by triggering the release of IL-33.

The bvPLA2-induced IgE response is independent of ST2

IgE response after secondary immunization

Th2 response after secondary immunization

Suppl 4 **A**

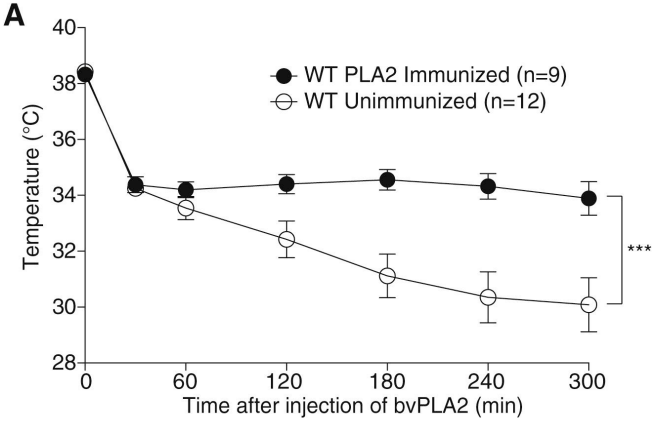


Anti-OVA IgE production and T cell IL-13 production after immunization with bvPLA2 and OVA on day 0 and day 21 in wild type and ST2 deficient mice on day 28.

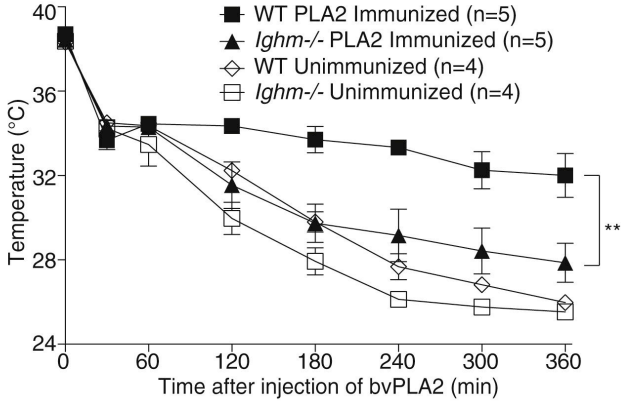
Th2 response is dependent on ST2 but IgE response is not? ->Th2 response is measured after primary immunization whereas IgE response can only be measured after secondary immunization. (Th2 after secondary immunization is also ST2 independent!)

FcεR1α and B cells dependent immune response to bvPLA2 helps to protect against bvPLA2 mediated toxicity

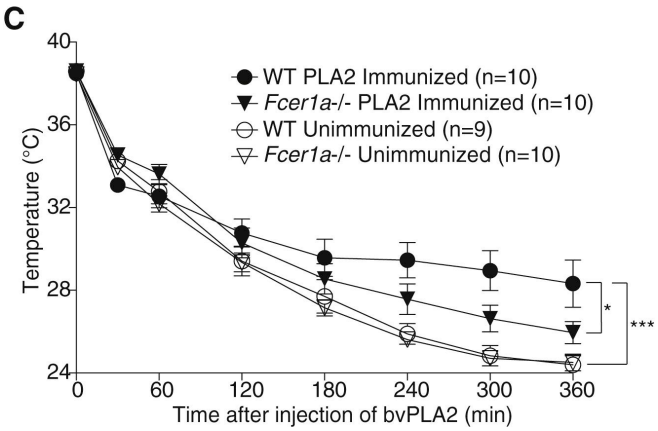
Fig 5 **A**



B

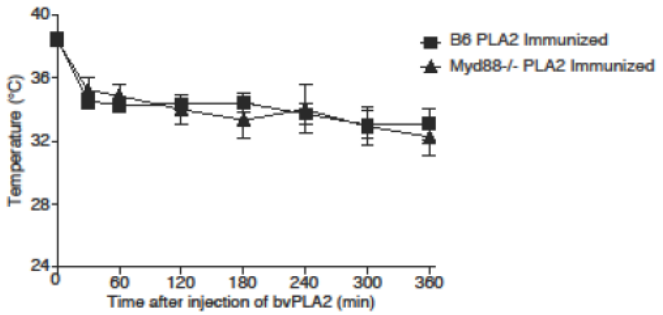


C



A: 6 weeks weekly ip low dose(50ug) bvPLA2.
Challenge with 150ug PLA2/250ul PBS/25g mouse
B: No B cells
C: no FcεRα (normal function of MC)
Suppl 4C: MyD88 deficient

Suppl 4C **C**



Authors are not able to passivlely immunize mice by serum transfer!

Link between Type 2 immunity and sensing tissue damage

- bvPLA2 hydrolyzes membrane phosphplipids-> lysophospholipids-> disrupt cellular mebranes-> cell death-> IL-33- release -> supports Th2 differentiation by binding to ST2 on T cells
- ST2-deficient (and Myd88 deficient) mice exhibit diminished Th2 cell and ILC2 in primary responses to bvPLA2.
- IgE response to PLA2 could protect mice from future challenge with a near-lethal dose of PLA2
- IgE responses appeared to be largely unaffected by ST2 deficiency. (ST2 is required for primary but not secondary T cell responses-> IgE undetectable after primary immunization!)
- FcεR1α contributes to protection from bvPLA2 toxicity.
- *When is IgE protective and when does it lead to allergy/anaphylaxis?? not clear..*