Natural killer receptor 1 dampens the development of allergic eosinophilic airway inflammation

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Innate immunity
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Allergic asthma: Type 1 hypersensitivity reaction

- Clinical manifestation of allergic hepersensitivity disease of the airways is characterized by:
  - Reversible airflow obstruction
  - Bronchial hyper-responsiveness
  - Airway inflammation

However, our understanding is still limited and central issues still being studied are the identification of fundamental molecules / pathways that govern the processes underlying inflammation in asthma.

  - Sub-mucosal mucus goblet cells hyperplasia/metaplasia
  - Smooth muscle cell hypertrophy
- All those lead to structural changes that in turn aggravate the hyper-responsiveness observed in this disease
- Research in the asthma field has provided rationale for the development of multiple therapeutic agents that interfere with specific inflammatory pathways

Allergic asthma: Type 1 hypersensitivity reaction

- In predisposed individuals, initial exposure(s) of professional antigen-presenting cells (APCs) to an allergen leads, mainly to:

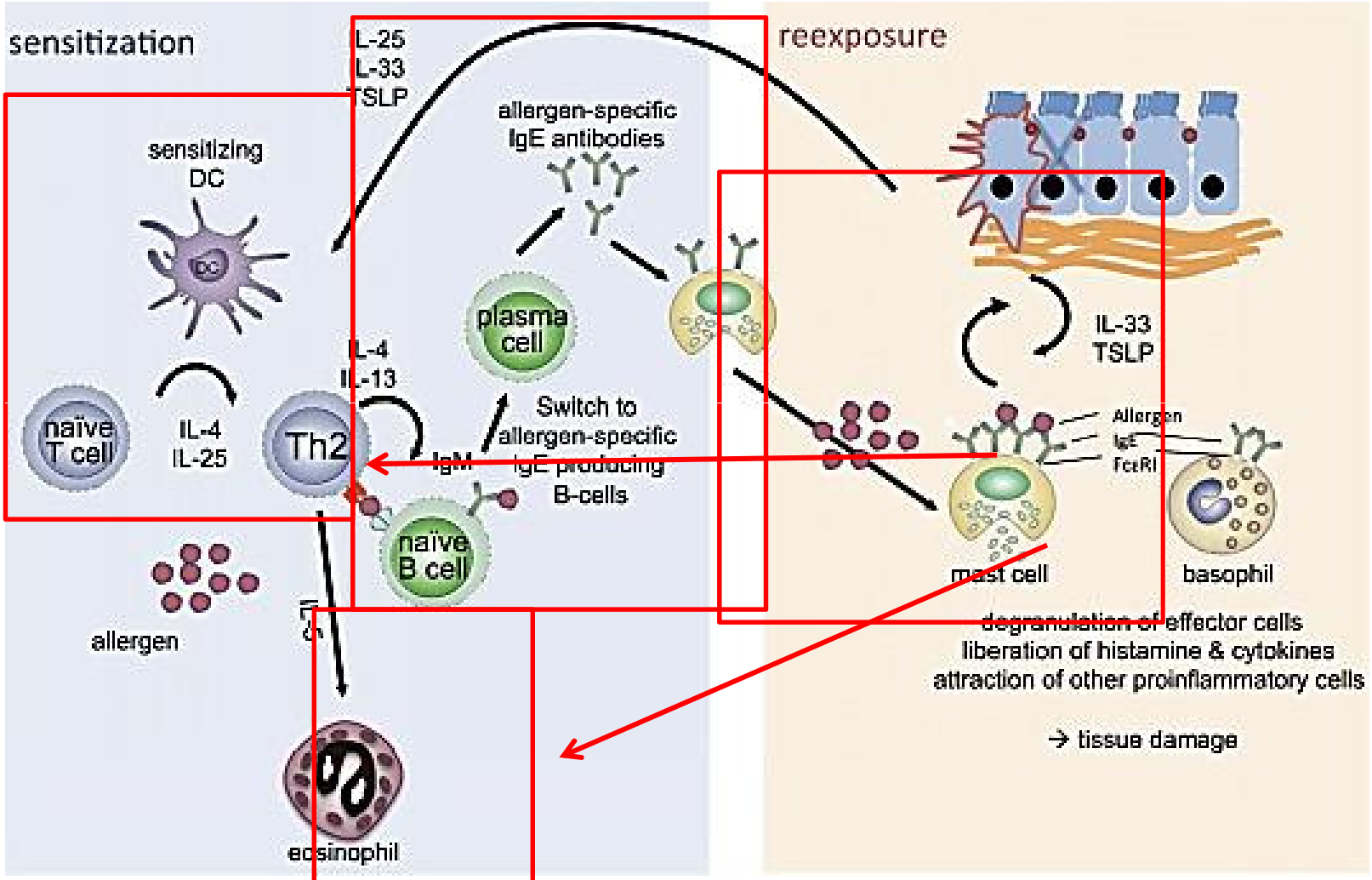
The eosinophils release pro-inflammatory mediators and toxic granules, which cause bronchoconstriction and damage to the epithelial cell layer

  - IgE-sensitized mast cells release both pre-formed and newly synthesized mediators, which:
    - promote vascular permeability

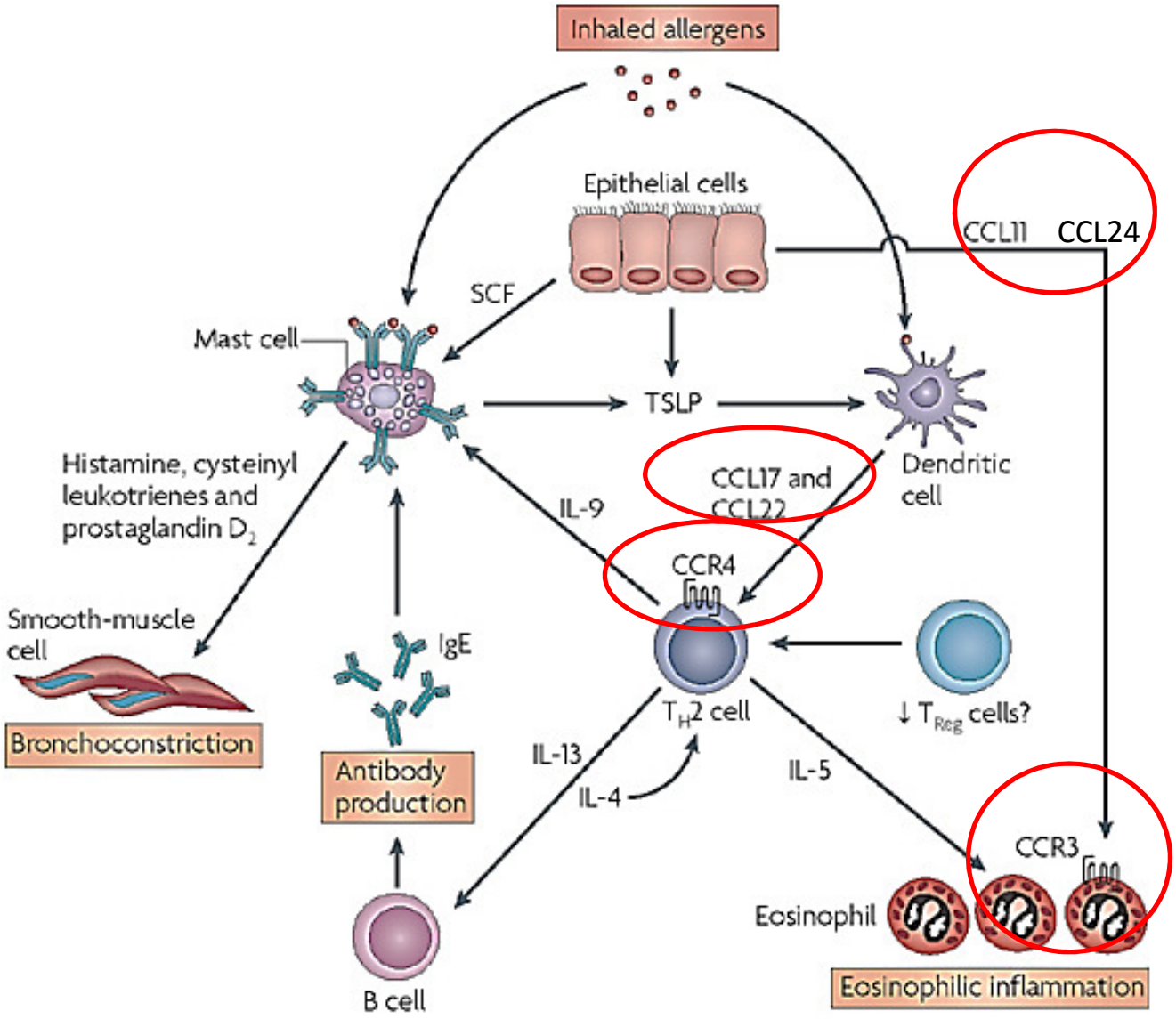
The polarized immunity toward Th2 phenotype in allergic respiratory disease involves the secretion of IL-4, IL-5, IL-9, and IL-13 that favors humoral antibody production (primarily IgE)

  - allergic response characterized by an influx of
    - eosinophils (via CCL24 and CCL11, which bind to CCR3)
    - Th2 cells (via CCL17 and CCL22, which bind to CCR4)

Sensitization and effector phase in allergic reactions



Michael B Soyka, Willem van de Veen, David Holzmann, Mübeccel Akdis, Cezmi A Akdis, Chest, Volume 146, Issue 5, 2014, 1347–1357


Inhaled allergens
Epithelial cells
CCL11
CCL24
SCF
Mast cell
TSLP
Dendritic cell
Histamine, cysteinyl leukotrienes and prostaglandin D2
CCL17 and CCL22
IL-9
CCR4
Smooth-muscle cell
IgE
TH2 cell
↓ TReg cells?
Bronchoconstriction
Antibody production
IL-13
IL-4
IL-5
CCR3
Eosinophil
B cell
Eosinophilic inflammation
Nature Reviews | Immunology

Natural killer cells

- The earliest contact between an antigen and the innate immune cells is thought to direct the subsequent antigen-specific T-cell response
- Thus, cells of the innate immune system, such as NK, NKT, and γδ T cells might regulate the development of allergic airway disease

Recent studies suggest contradictory roles for NK cells in immune modulation of allergy

Proinflammatory activity

Anti-inflammatory activity

- inflammation.
- This co-localization of DCs and NK cells results in a cross talk, which can influence the maturation and activation of both cells

Natural killer cells in asthma

NK cells accelerate allergic inflammation

- Increased NK cell number and high levels of IL-4^{+}CD56^{+} NK2 cells were found in PBMCs of allergic rhinitis patients
- In the peripheral blood of healthy individuals the CD56^{+}CD16$^{+/-}$ NK cells were able to respond to DCs by proliferation and production of IFNγ
- In patients with allergic rhinitis and intermittent asthma the capability of the NK cells to produce IFNγ in response to DCs were found to be significantly reduced

Natural killer cells and asthma

NK cells dampens allergic inflammatory response

Human NK cells were shown to induce neutrophil

Thus NK cells are a double edge sword and in contemplating NK cells as therapeutic targets, they should be approached cautiously and additional studies to understand the involvement of NK cells in allergic inflammation are needed.

probably controlled by the specialized pro-inflammation-resolving lipid mediators (SPMs) including Lipoxin A_4 (LXA_4) and Resolvin E1 (RvE1)

Natural killer cells activating receptors

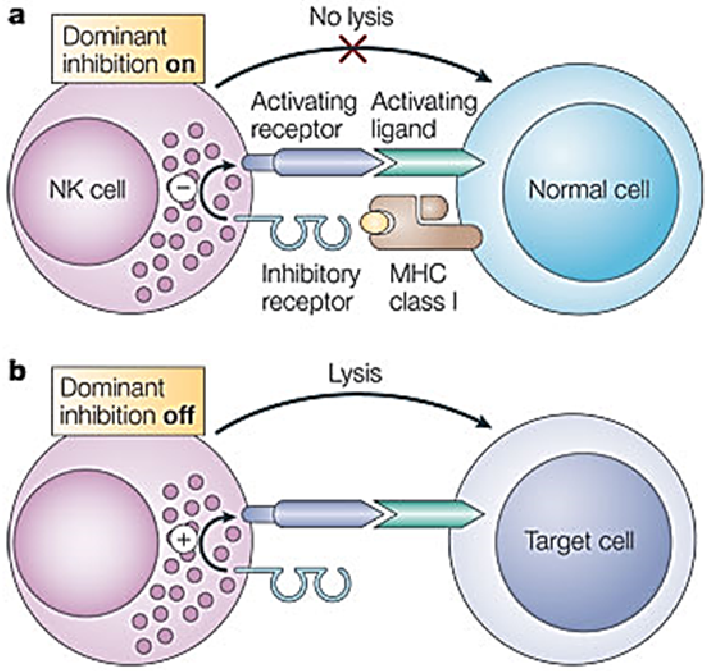
- Natural killer (NK) cells activation is controlled by the integration of signals from activating and inhibitory receptors interactions
- Upon ligation of the activating receptors with membrane-bound molecules on surrounding tissues, NK cells would undergo
  - blastogenesis
  - cytokine production

Knocking down of NCR1 in NK cells in mice reduces NCR1-mediated activity of NK cells, including NCR1-mediated lysis of target cells, but does not affect NK activity mediated by other NK receptors such as NKG2D

- All of the NCRs are transmembrane proteins, members of the Ig-Superfamily.
- Mice carry only the NKp46 activating receptor (NCR1) and NKG2D

Natural killer cells inhibitory receptors

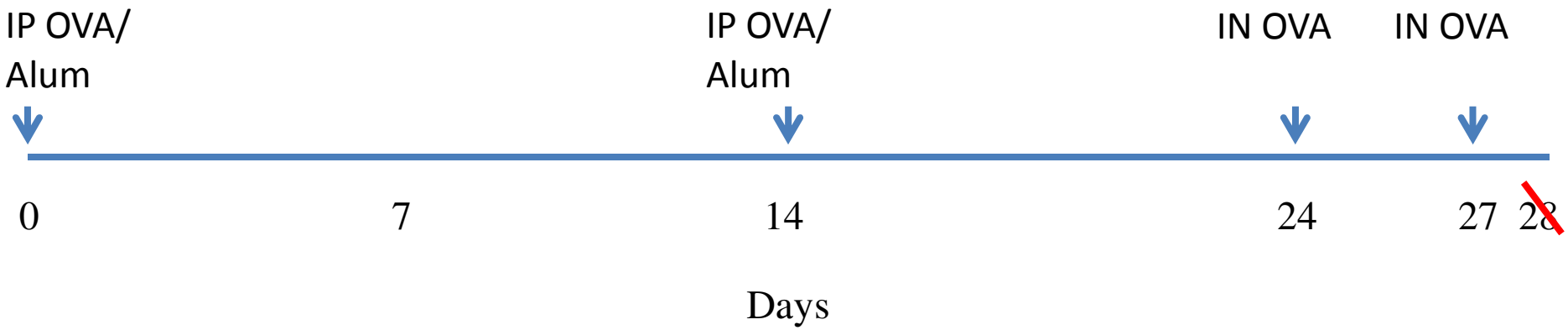
Colligation of activating receptors with inhibitory receptors results in a dominant inhibitory effect that down regulates signals initiated via the activating pathways



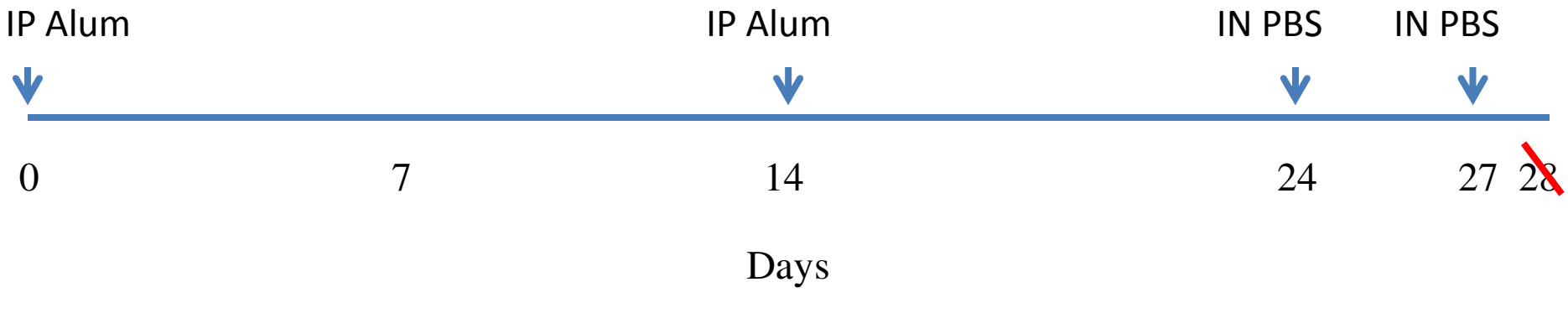
- Two types of inhibitory receptors
  - HLA class I killer cell Ig-like receptor (KIR)
  - Killer cell lectin-like receptors (KLR)

Allergic hypersensitivity model

OVA Allergic hypersensitivity



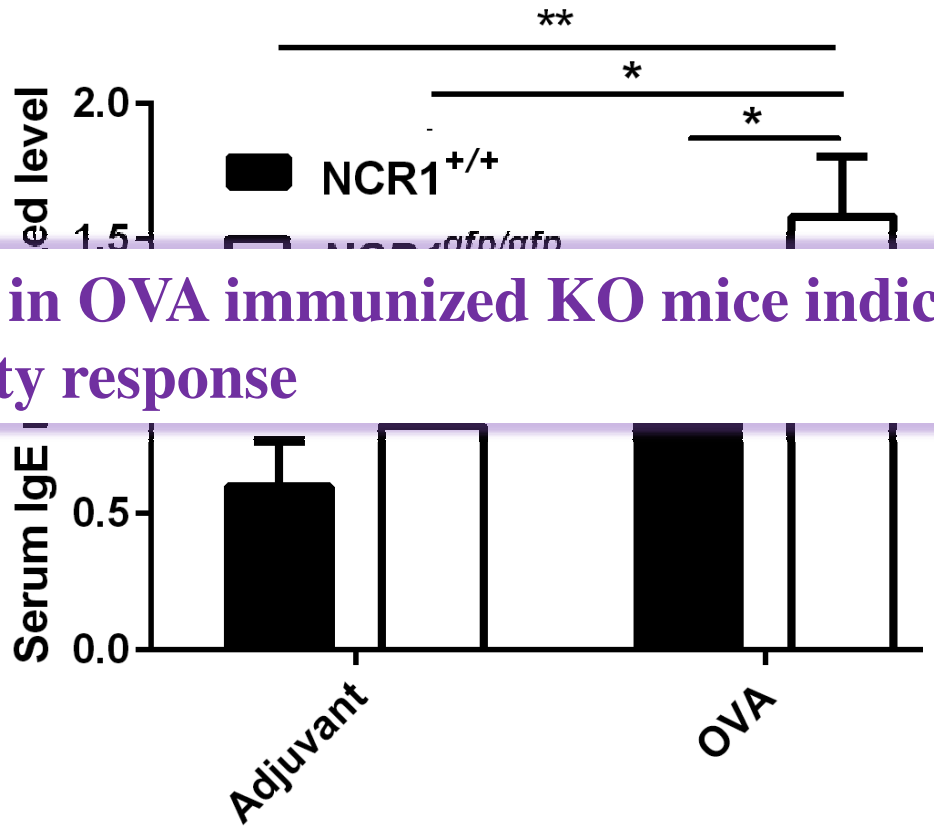
Adjuvant control



NCR1$^{gfp/gfp}$ and NCR1$^{+/+}$ Mice

- In C57BL/6 the gene encoding the NCR1 receptor (*NCR1*) was replaced with a green fluorescent protein (GFP) reporter cassette to generate *NCR1$^{gfp/gfp}$* mice
- *NCR1$^{gfp/gfp}$* mice and *NCR1$^{+/+}$* wild type mouse strains littermates were used

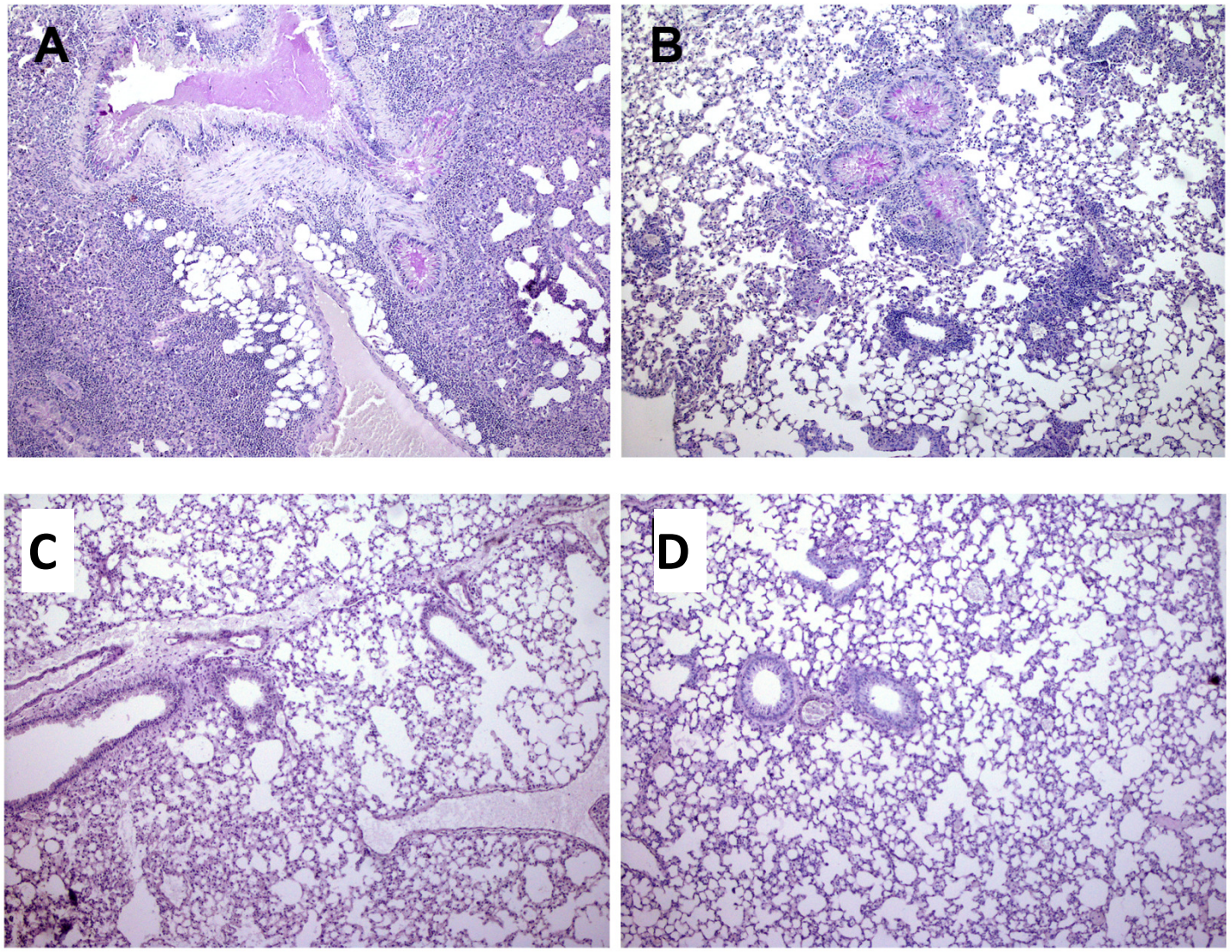
NCR1 involvement in IgE production in OVA/alum immunized mice: Increased IgE production in the absence of NCR1



Increased IgE in OVA immunized KO mice indicate increased in hypersensitivity response

* $p < 0.05$; **$p < 0.01$

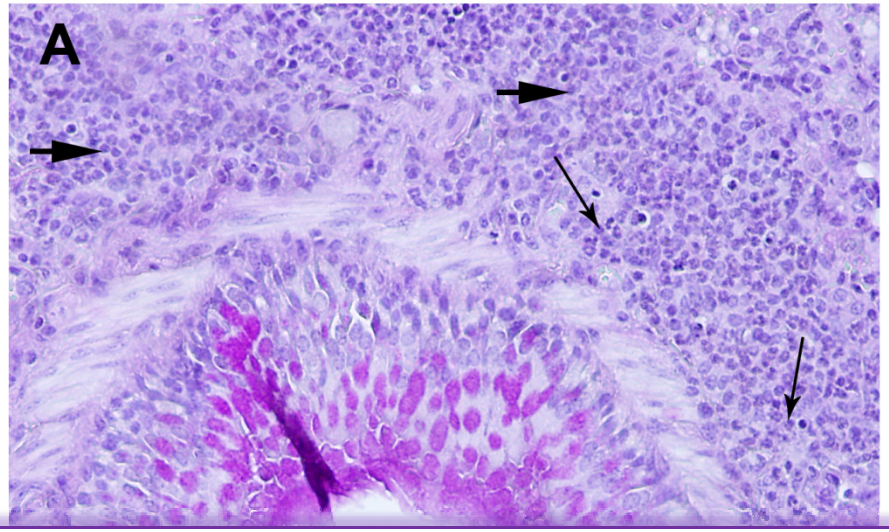
NCR1 involvement in inflammation in OVA/alum –immunized mice



H&E+PAS
x180

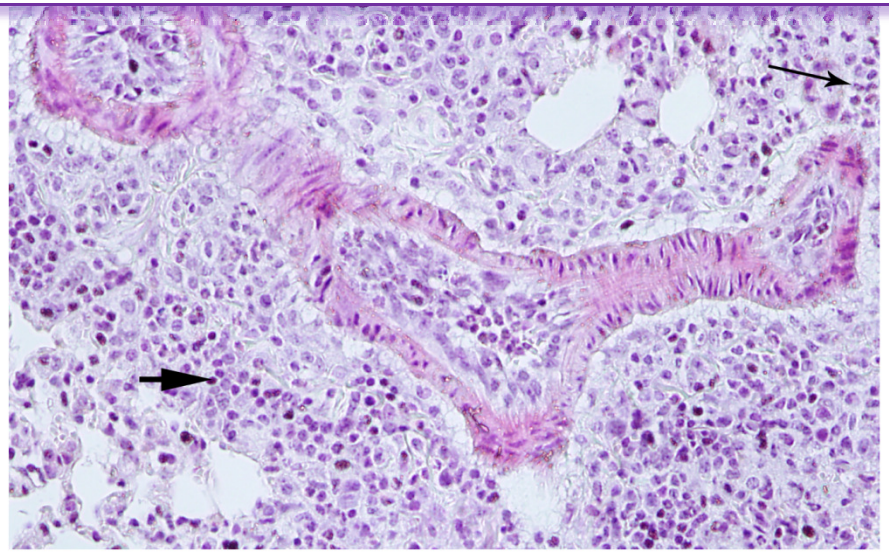
A. NCR1$^{gfp/gfp}$ OVA. B. NCR1$^{gfp/gfp}$ Alum
C. *NCR1*$^{+/+}$ OVA.
D. *NCR1*$^{+/+}$ Alum

NCR1 involvement in inflammation in OVA/alum –immunized mice: Increased inflammation in the lungs of NCR1$^{gfp/gfp}$ mice



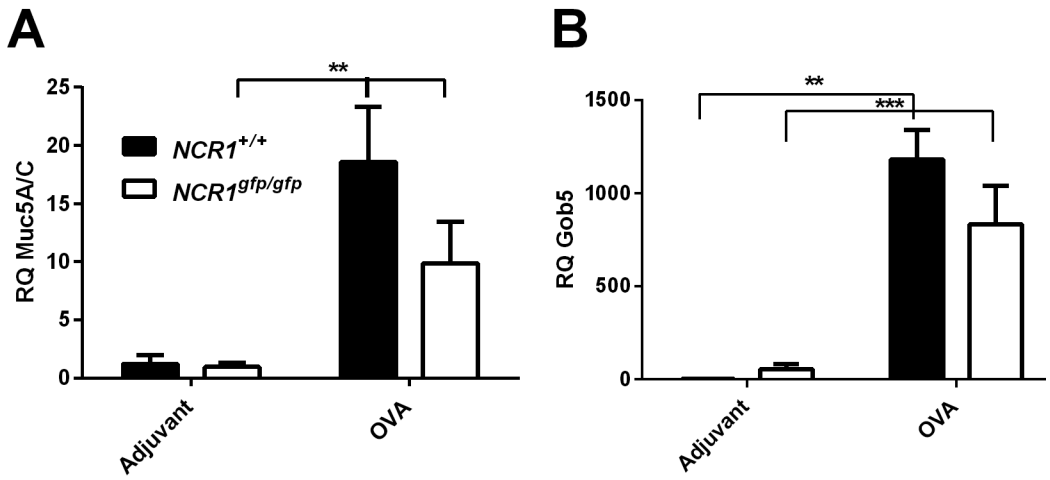
Loss of lung architecture and increase immune cell infiltration indicates increase hypersensitivity response in OVA immunized KO mice

H&E+PAS x340



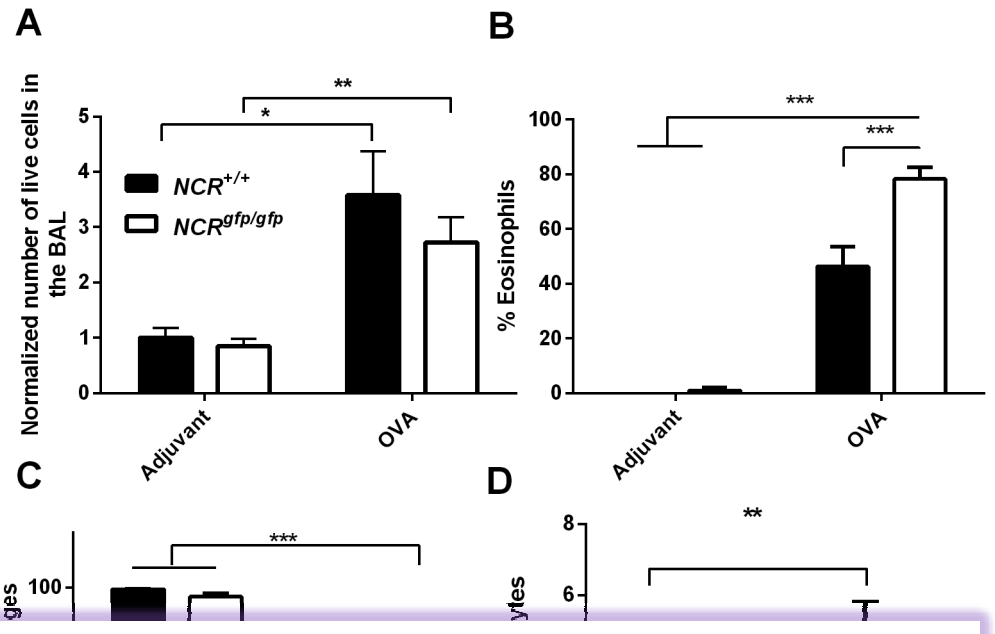
Thick arrow with a short tail identifies mononuclear cells. Thin arrow with a long tail identifies polymorphonuclear cells

NCR1 involvement in mucus genes expression

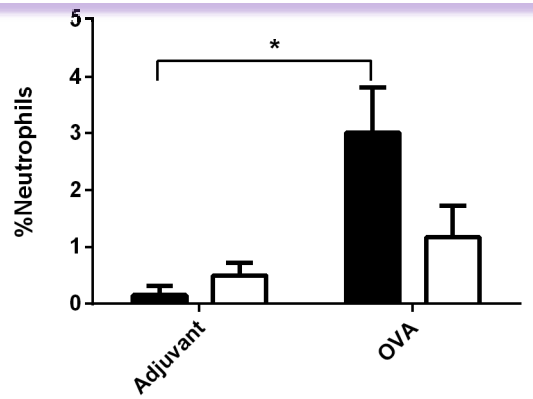


**p < 0.01; *** p < 0.001

Increased infiltration of eosinophil in *NCR1*$^{gfp/gfp}$ mice and increased infiltration of macrophages in *NCR1*$^{+/+}$ *mice* to the lung Bronchoalveolar l
s

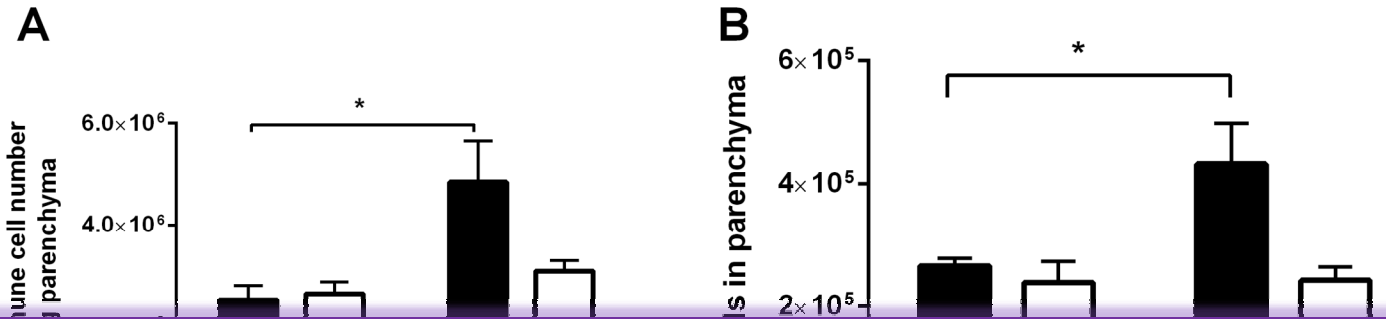


In the presence of NCR1, reduced percentage of eosinophils and increased percentage of macrophages were found in the BAL of OVA immunization in wild type mice indicating reduced hypersensitivity

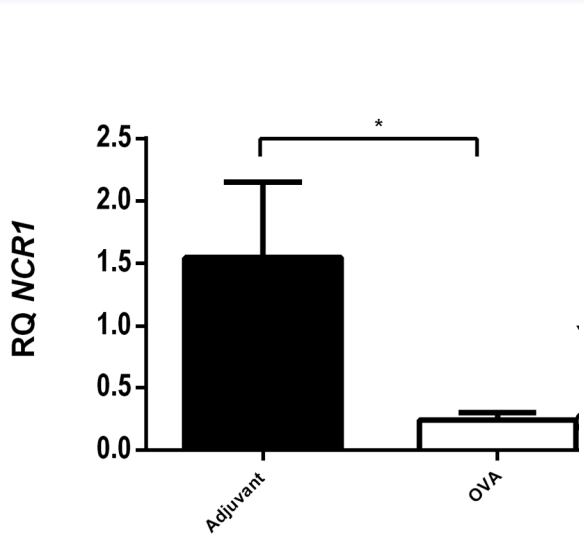


*p < 0.05; **p < 0.01; *** p < 0.001

NCR1 expression during allergic airway inflammation (Lung parenchyma)



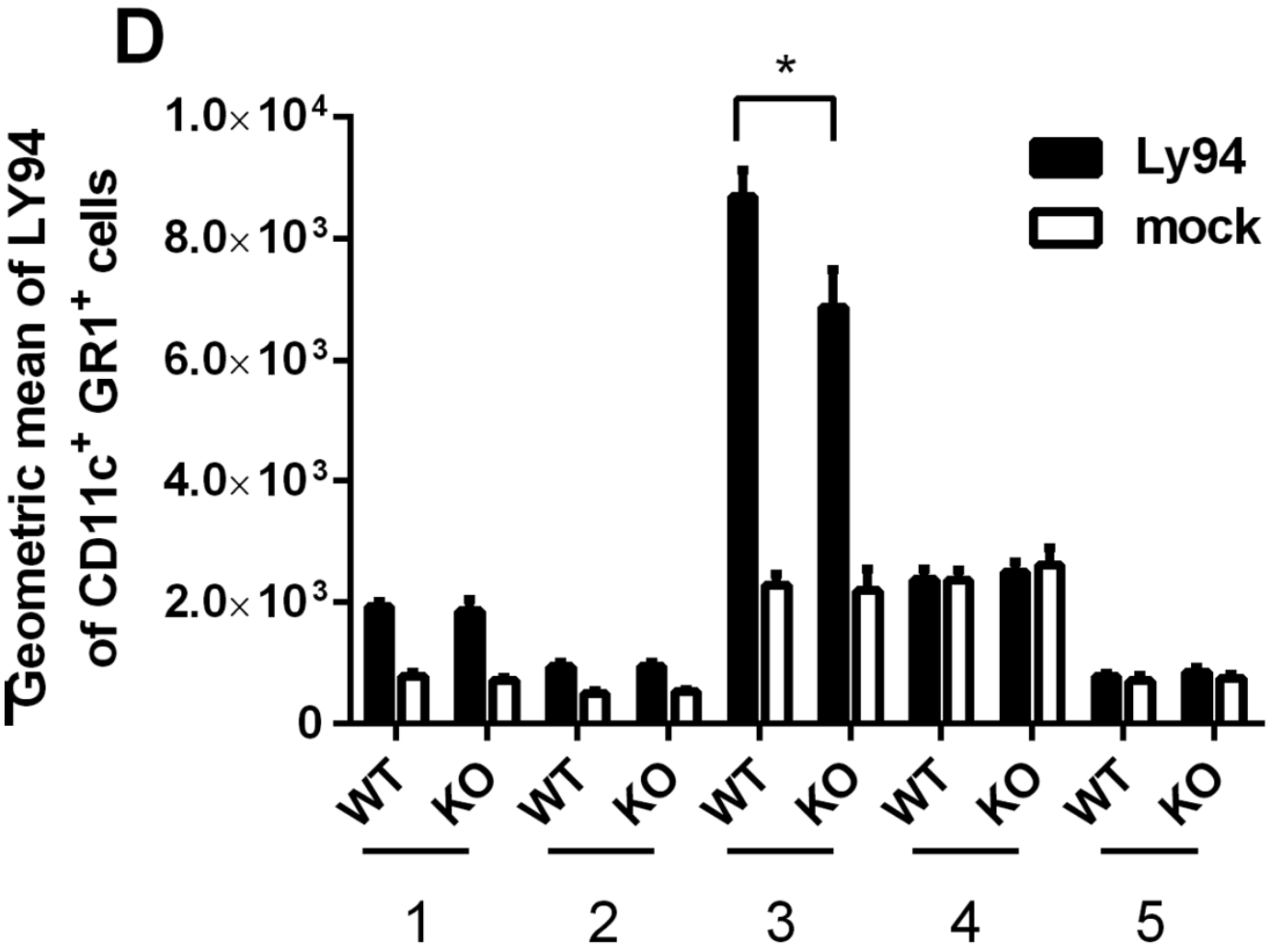
An increase in NK cells could be found in the lung parenchyma in OVA immunized WT mice suggesting that NK cells dampens the allergic response



NCR1 and its ligand expression during allergic airway inflammation

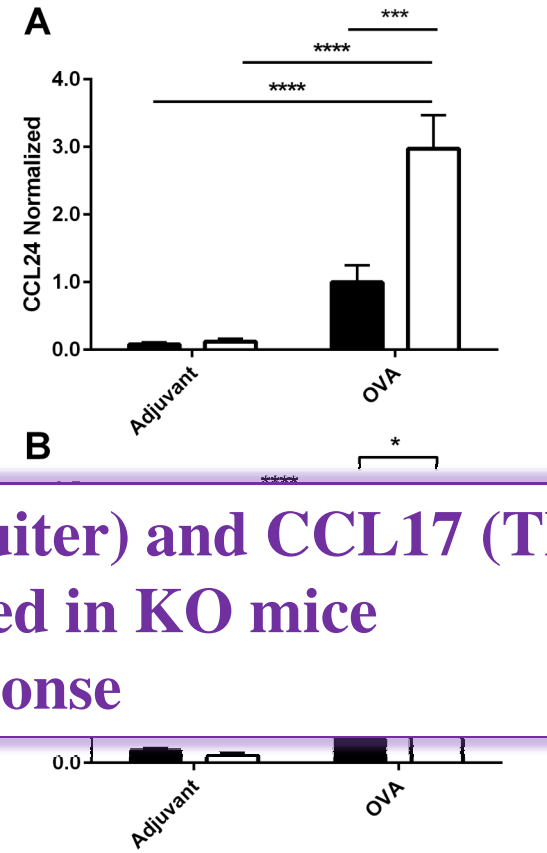
1. $CD11c^{+}GR1^{-}$ cells (myeloid DCs or interstitial macrophages)
2. $CD11c^{++}GR1^{-}$ cells (Th1 prone lung DCs and alveolar macrophages)
3. $CD11c^{+}GR1^{+}$ cells (plasmocytoid DCs)
4. $CD11c^{-}GR1^{+}$ cells (Siglec F^{-} SSC^{high} =Neutrophils)
5. $CD11c^{-}$ $GR1^{int}$ cells (Siglec F^{+} SSC^{high} = eosinophils)

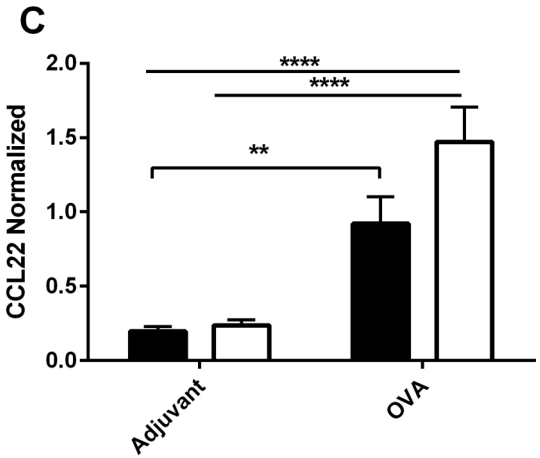
NCR1 ligand expression during allergic airway inflammation (Lung parenchyma)



Increased allergy related chemokine production in the lungs of *NCR1*$^{gfp/gfp}$

The chemokines CCL24 (eosinophil recruiter) and CCL17 (Th2 cell recruiter) proteins expression increased in KO mice suggesting increased hypersensitivity response

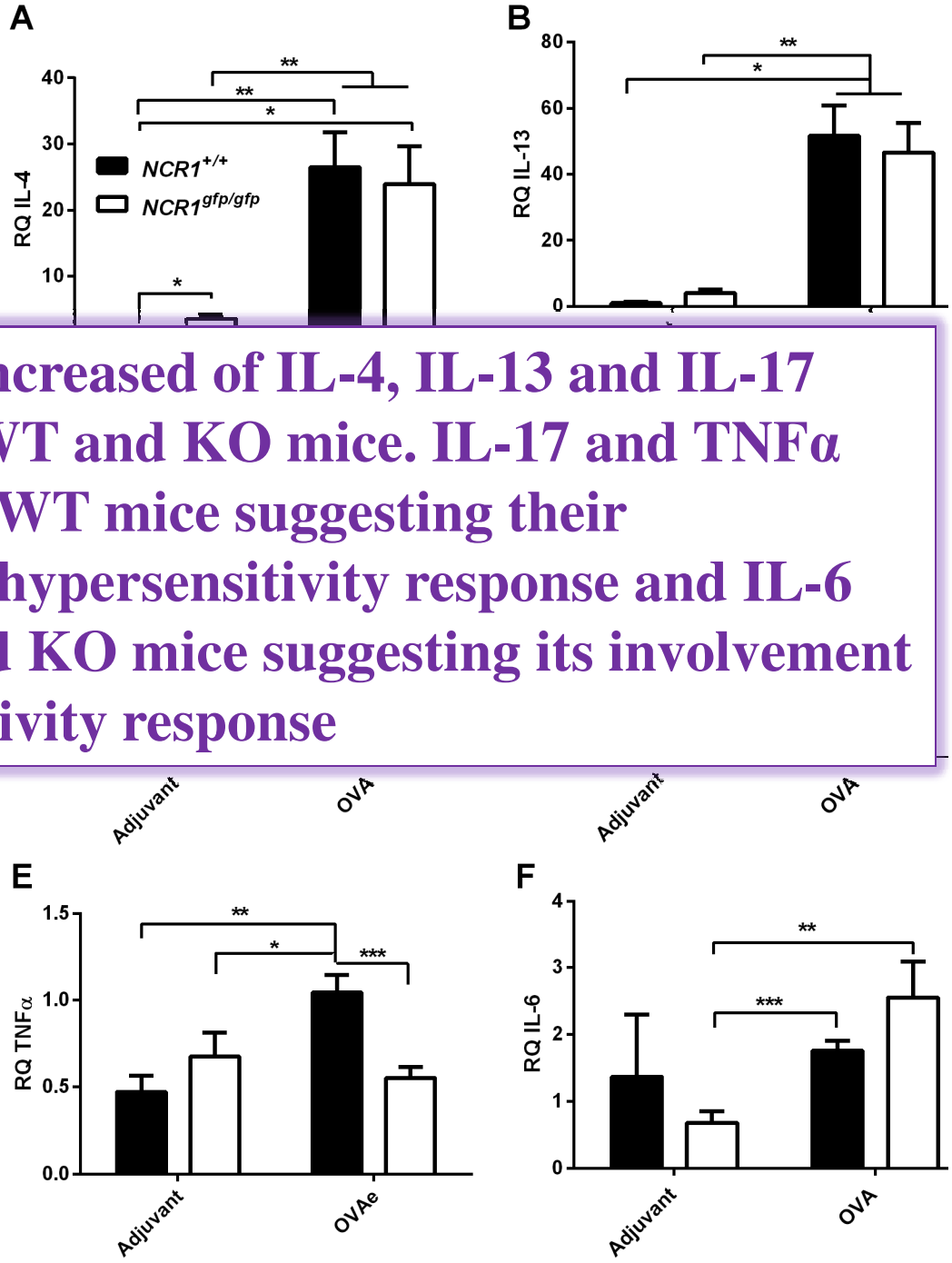




* p < 0.05; **P < 0.01; ***p < 0.001;
****p < 0.0001

Increased Th2 inflammation

OVA immunization lead to increased of IL-4, IL-13 and IL-17 mRNA in OVA immunized WT and KO mice. IL-17 and TNFα increase in OVA immunized WT mice suggesting their involvement in reducing the hypersensitivity response and IL-6 increased in OVA immunized KO mice suggesting its involvement in increasing the hypersensitivity response



* p < 0.05; **P < 0.01;
***p < 0.001

Natural killer receptor 1 involvement in the development of allergic eosinophilic airway inflammation

- Our major finding is that NCR1 dampens the allergic reaction following OVA/alum immunization and challenge with OVA
  - The highest concentration of total IgE in the serum was demonstrated in *NCR1*$^{gfp/gfp}$ mice following OVA immunization in comparison to OVA immunized *NCR1*$^{+/+}$ mice
  - In OVA immunized *NCR1*$^{gfp/gfp}$ mice the lung architecture was not preserved in contrast to *NCR1*$^{+/+}$ mice, the adjuvant immunized *NCR1*$^{gfp/gfp}$ and *NCR1*$^{+/+}$ mice

Natural killer receptor 1 dampens the development of allergic eosinophilic airway inflammation

Infiltration of immune cells to the lungs was higher in the mice receiving OVA/Alum immunization compared with Alum immunization alone, demonstrating the ability of OVA to induce airway inflammation as previously described

- However, in the *NCR1* KO mice the leukocytes were mainly eosinophils
- In *NCR1* wild type mice there was an increase in eosinophils accompanies with a substantial increase in macrophages

- In wild type mice following OVA immunization:
  - An increase in total NK cells number in the lungs was observed,
  - A decrease in NCR1 mRNA expression was observed following sensitization
  - The NK ligand expressing cells were found to be plasmocytoida DCs
  - The number of NCR1 ligand carrying cells was significantly higher in *NCR1*$^{+/+}$ in comparison to *NCR1* $^{gfp/gfp}$ mice

Natural killer receptor 1 dampens the development of allergic eosinophilic airway inflammation

- CCL24, the eosinophil chemoattractant , protein expression increased significantly more in NCR1 lacking mice

Natural killer receptor 1 dampens the development of allergic eosinophilic airway inflammation

- IL-4 and IL-13 cytokines mRNA increased in OVA immunized mice without significant differences observed in the presence or absence of NCR1

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- The Israel Ministry of Health

Allergen sensitization and challenge

Experimental

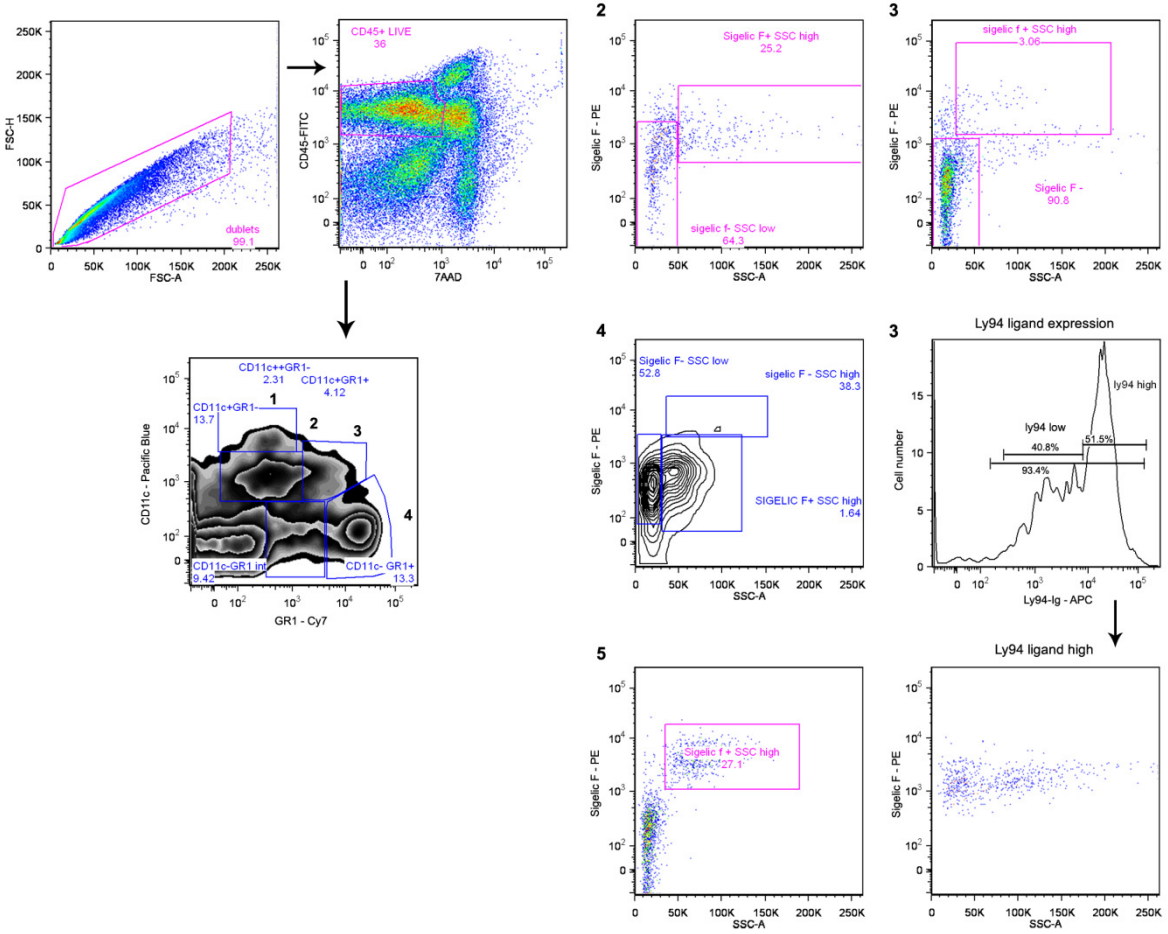
Day 0: OVA and aluminum Oxide (Intraperitoneal)
Day 14: OVA and aluminum Oxide (Intraperitoneal)
Day 24: OVA (Intranasal)
Day 27: OVA (Intranasal)
Day 28: Mice euthanatized

Controls

Day 0: aluminum Oxide (Intraperitoneal)
Day 14: aluminum Oxide (Intraperitoneal)
Day 24: PBS (Intranasal)
Day 27: PBS OVA (Intranasal)
Day 28: Mice euthanatized

NCR1 and its ligand expression during allergic airway inflammation.

1. $CD11c^{+}GR1^{-}$ cells (myeloid DCs or interstitial macrophages),
2. $CD11c^{++}GR1^{-}$ cells (Th1 prone lung DCs and alveolar macrophages)
3. $CD11c^{+}GR1^{+}$ cells (plasmocytoid DCs).
4. $CD11c^{-}GR1^{+}$ cells (Siglec F^{-} SSC^{high} =Neutrophils
5. $CD11c^{-}GR1^{int}$ cells (Siglec F^{+} SSC^{high} = eosinophils



Natural killer receptor 1 dampens the development of allergic eosinophilic airway inflammation

- The importance of NK cells in the development of allergic inflammation is starting to emerge
- However, two contradictory roles for NK cells in allergic inflammation have been suggested:
  - Enhancement of the proinflammatory response
  - Inhibition of the proinflammatory response
- To further understand the role of NK cells in allergic inflammation we studied the role of NCR1 in a model of experimental asthma, classified as type 1 hypersensitivity reaction, in mice.