Avian immunology with an emphasis on vaccine-induced immunity and innate immune responses to avian influenza virus

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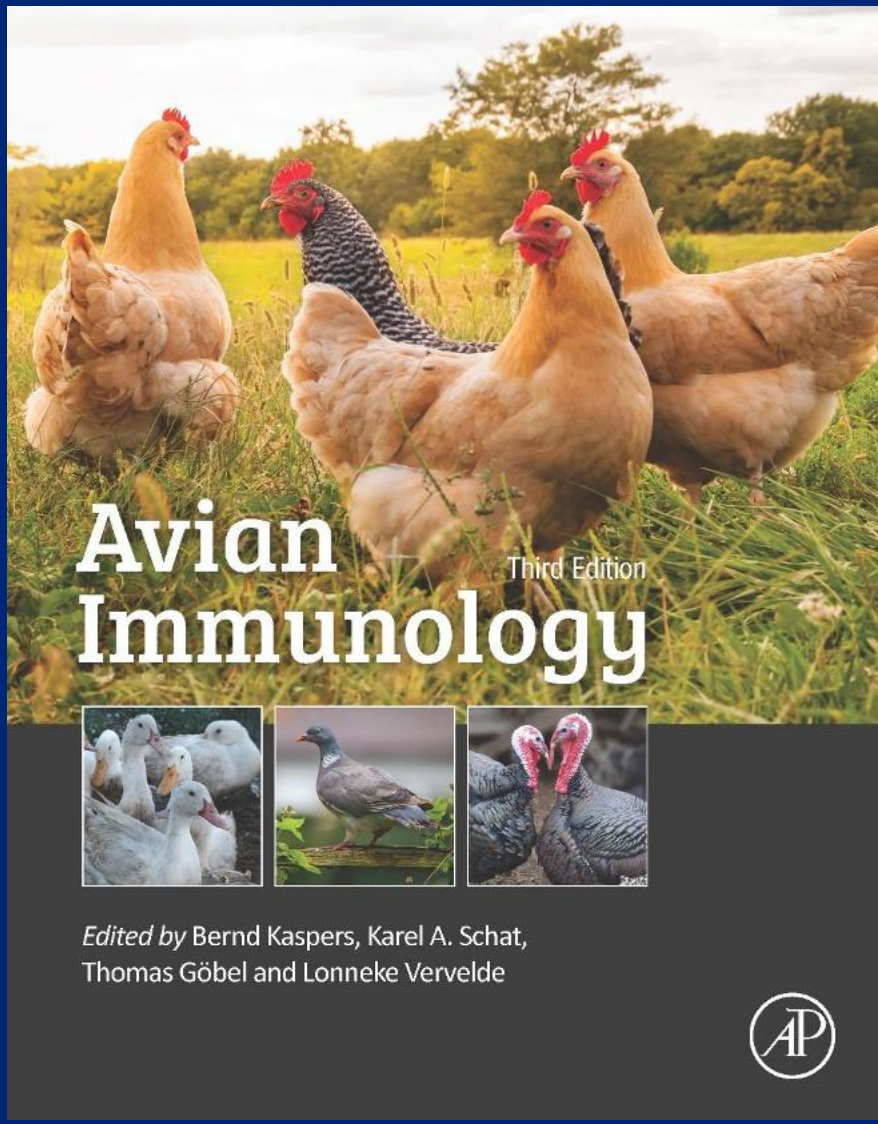
Avian Immunology in 50 Minutes

Impossible task!

The third edition of Avian Immunology (2022): 603 pages!

The alphabet soup of abbreviations is intimidating and confusing

Thus: only very selective aspects will be discussed with an emphasis on innate antiviral responses



The importance of different types of chickens for the immune responses: layers versus broilers

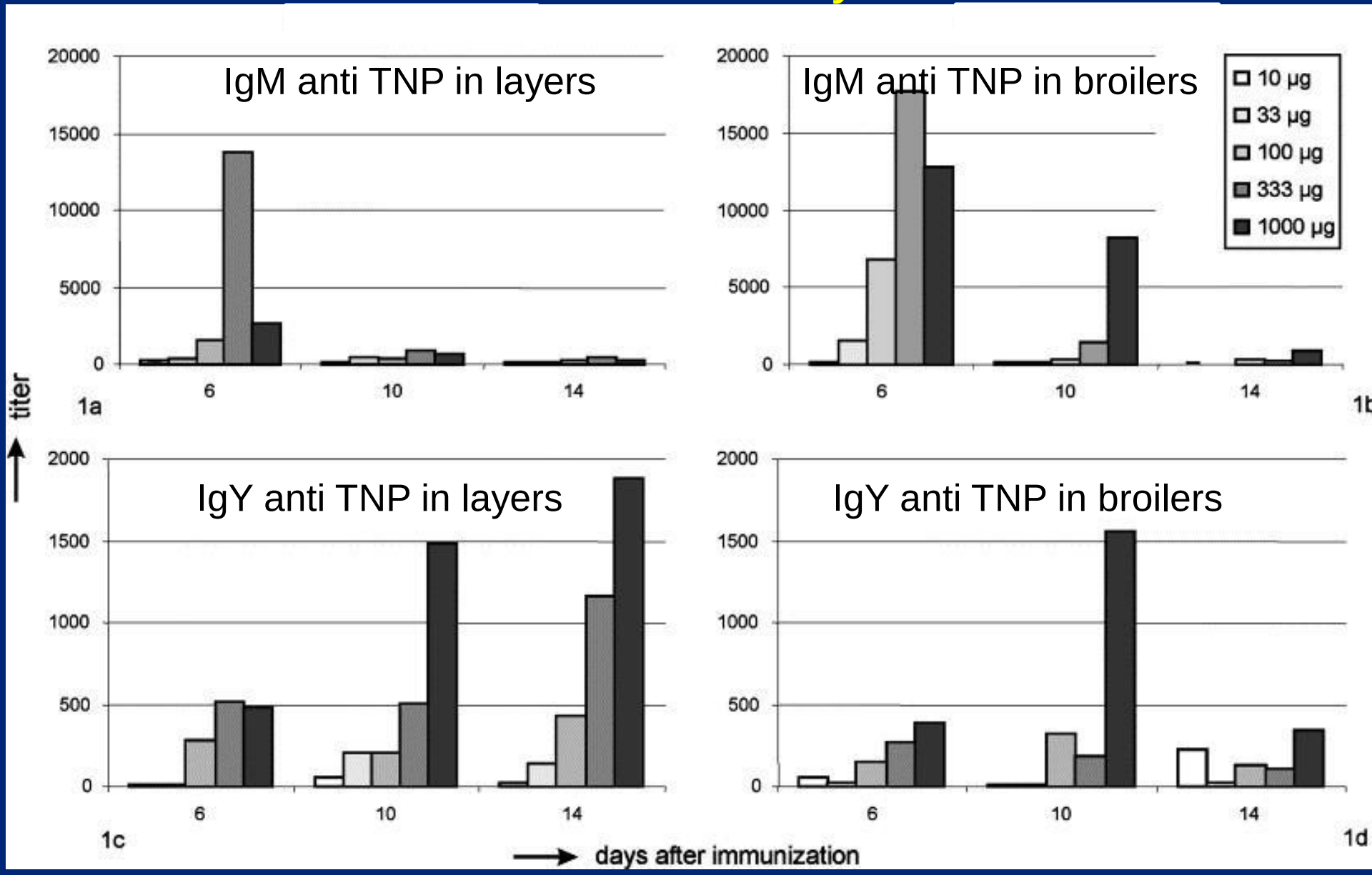
Requirements differ between these two types of chickens

Most studies on immune responses are done in (SPF) layers

Koenen et al. 2002 demonstrated marked differences in antibody responses to TNP-KLH

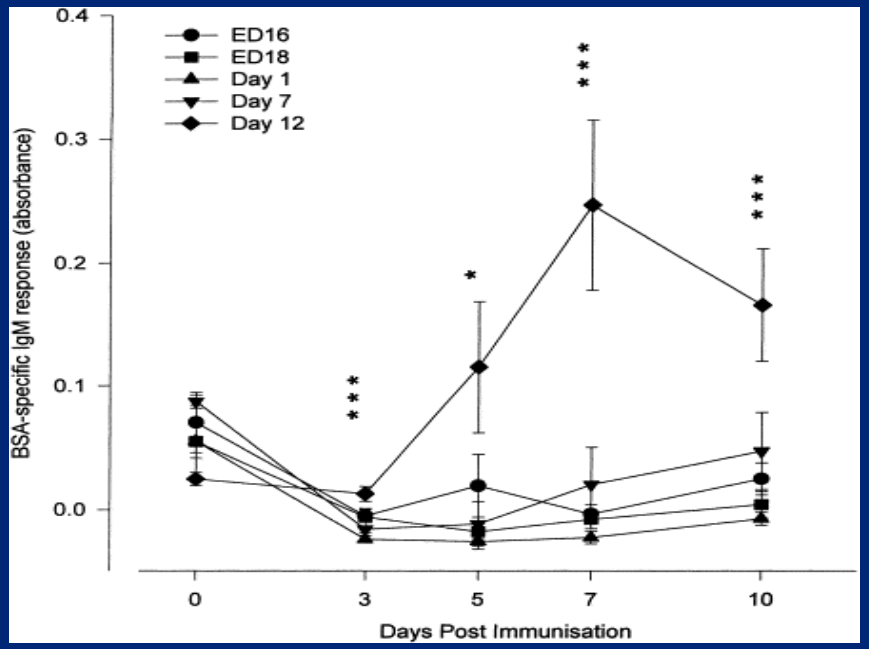
Mast and Goddeeris (1999) compared antibody responses in broilers after in ovo and after hatch immunizations with bovine serum albumen and noticed poor responses after vaccination in ovo and at 1 and 7 days of age

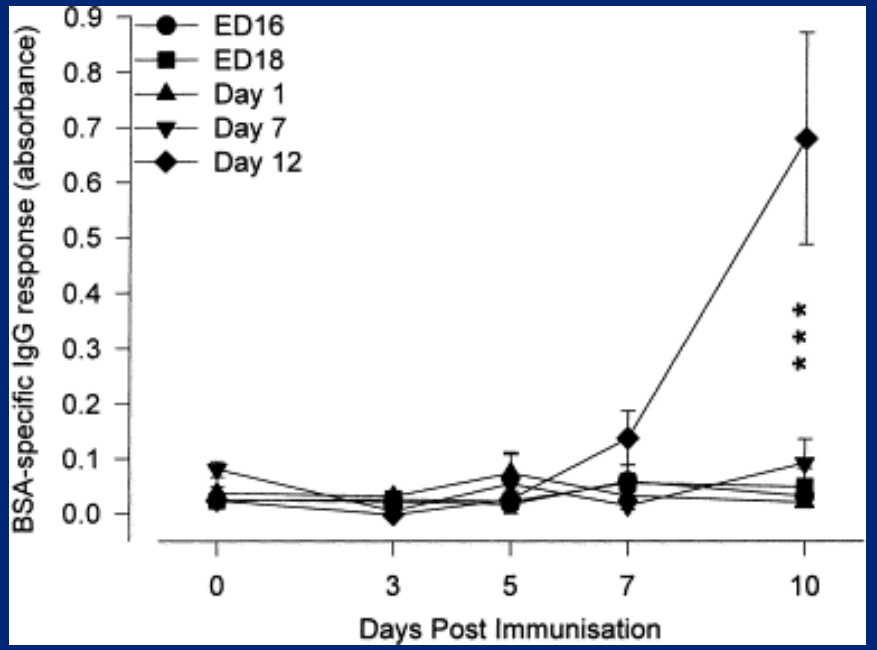
Immunization of 4-week-old layers and broilers



Koenen et al. VII 89:47, 2002

Maturation of IgM and IG responses to BSA in broilers





Mast and Goddeeris, VII 70: 245, 1999

Unfortunately both groups did not directly compare responses in broiler versus layer-type chickens

Many factors influence immune responses in broilers

Short life span

Rapid growth

Relatively poor AB responses

Innate immunity is important

Stress

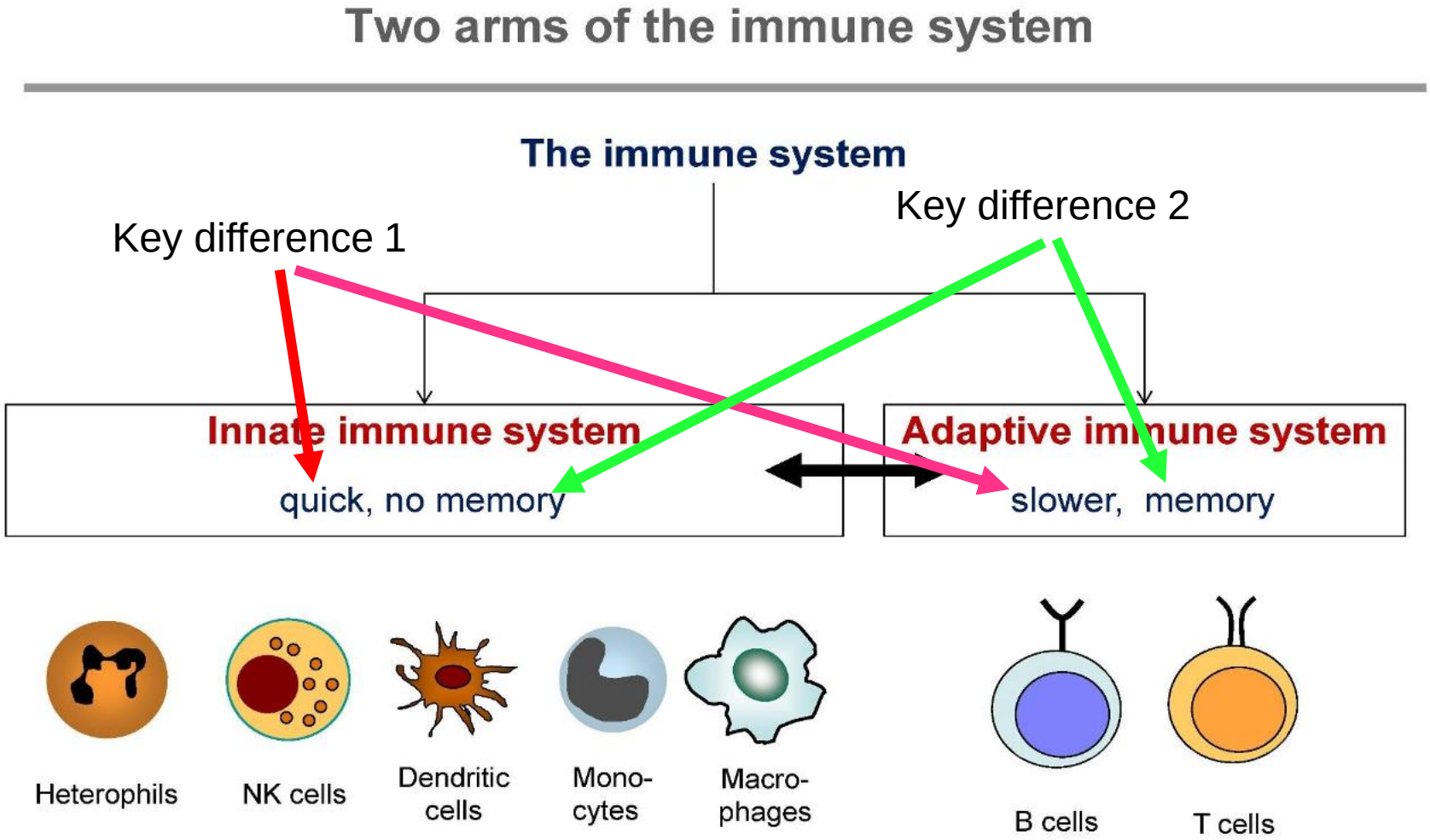
Microtoxins

Pathogens

Probiotics
Nutrition

Microbiome influences development of immune system

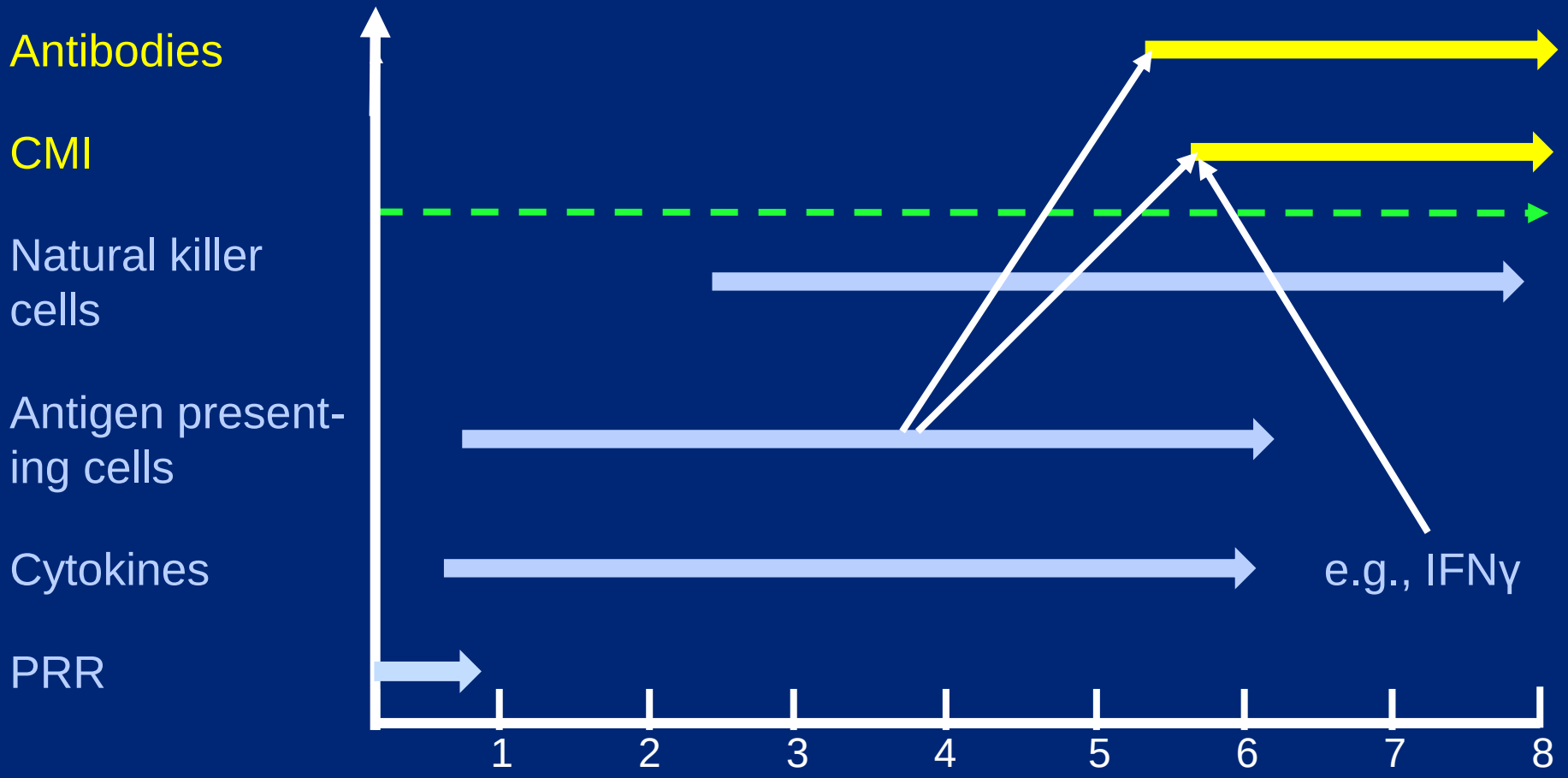
Key cells for innate and adaptive immune responses



Slide adapted from a lecture by Bernd Kaspers

Generation of immune responses in chickens

A virus or live virus vaccine enters the host by replicating in or passing through epithelial cell layers (skin, respiratory or intestinal tract)



Generation of immune responses in chickens: PRR

Immune responses start with the recognition of Pathogen-Associated Molecular Patterns (PAMPs) by Pattern Recognition Receptors (PRR).

Some examples of virus associated PAMS:

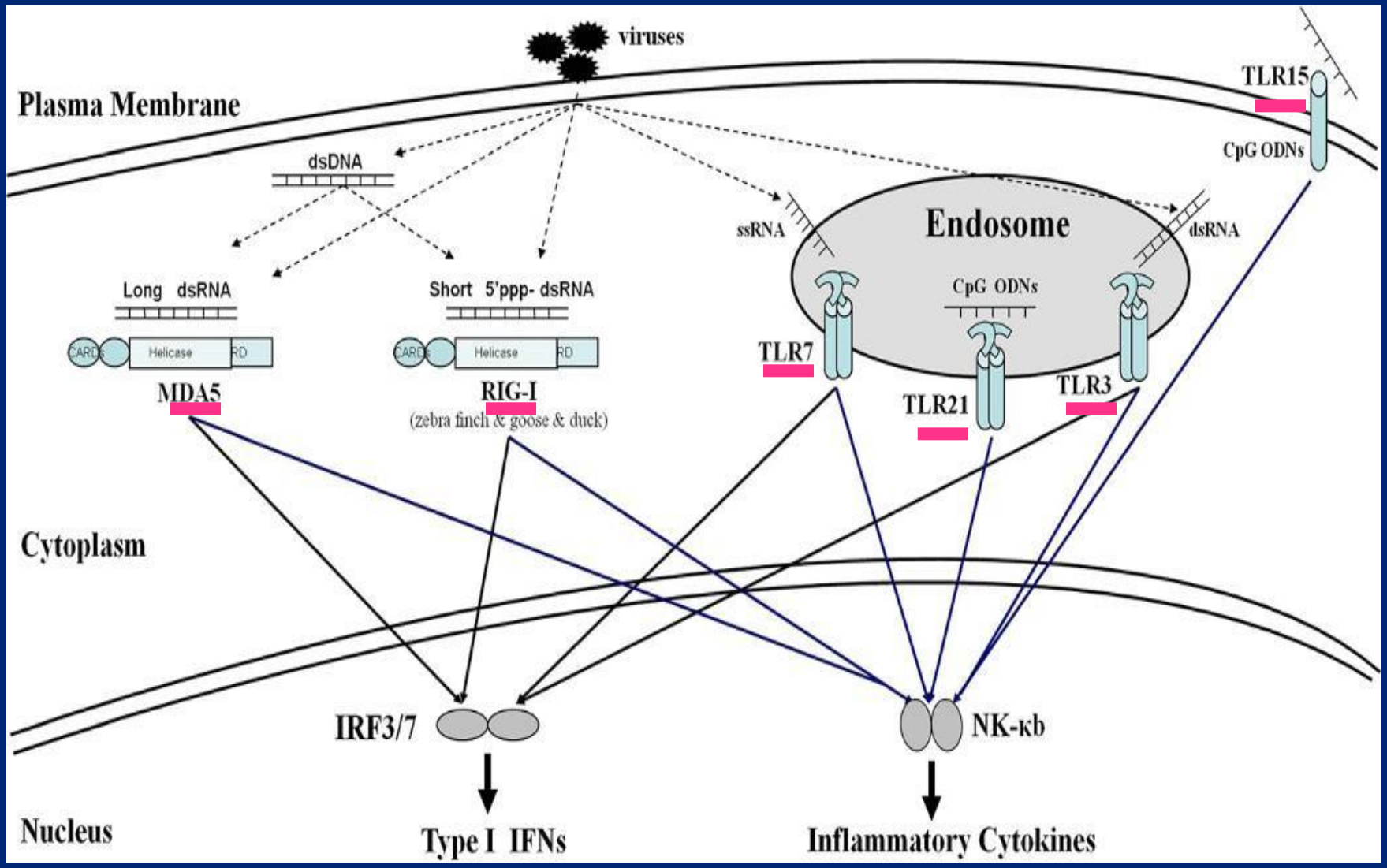
1. Glycoproteins

2. Unmethylated CpG DNA

3. Double-stranded and single-stranded RNA

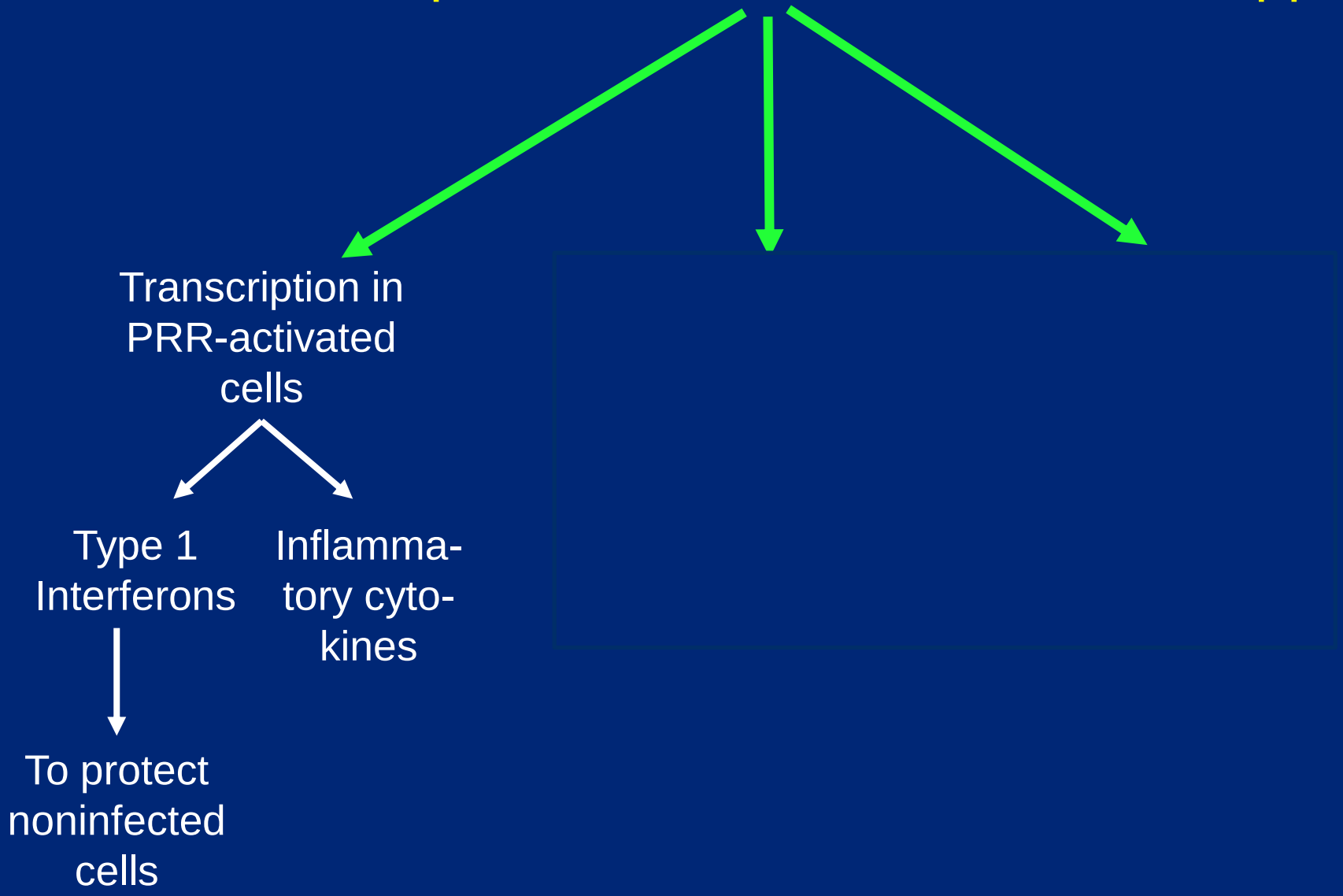
4. Double-stranded DNA fragments

Examples of PRR: Toll-like receptors (TLR), Melanoma differentiation-associated gene 5 (MDA5), Retinoic acid-inducible gene 1 (RIG-1)

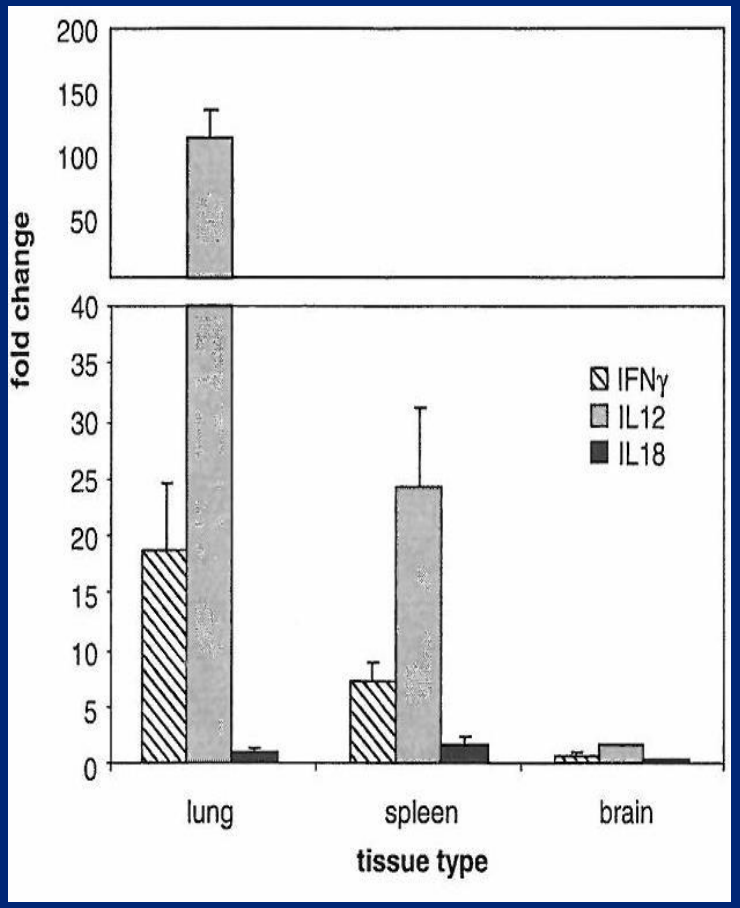


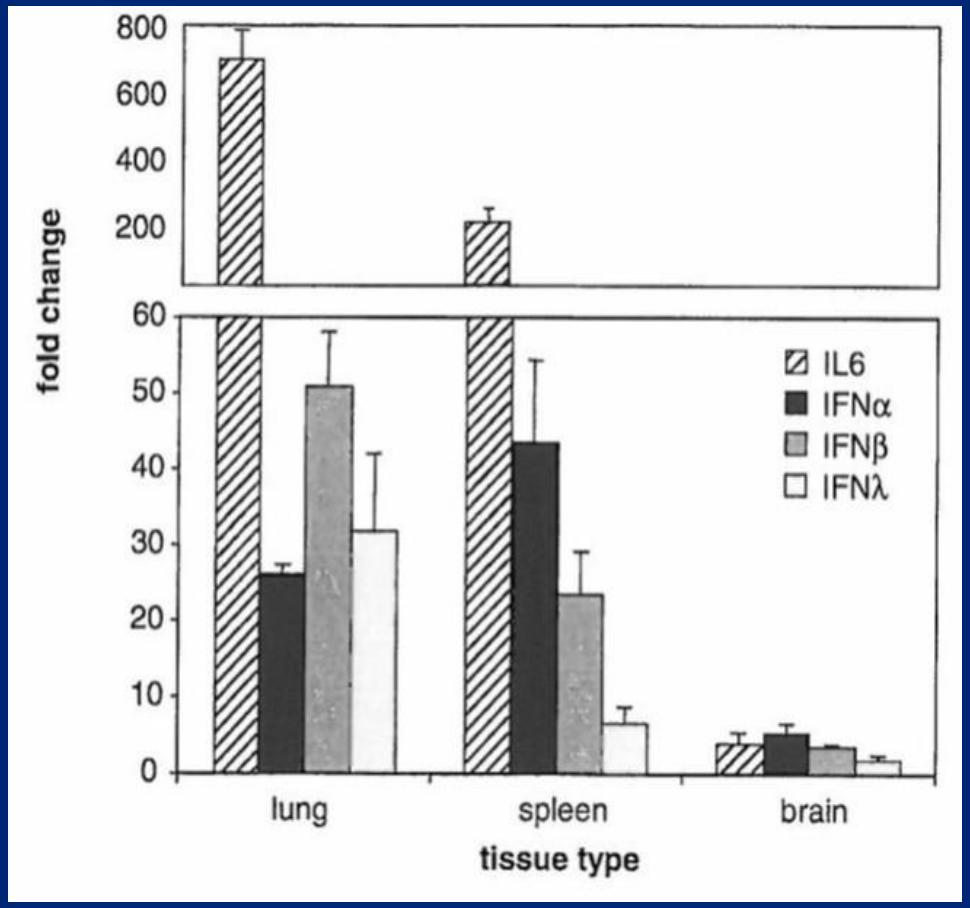
Chen *et al. Veterinary Research* 2013, 44:82

Once PRR are engaged many events start to occur in rapid succession and often overlapping



An example of rapid induction of interferons and proinflammatory cytokines. Karpala et al, 2012.

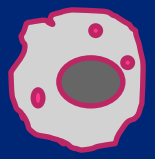




HP rgVN1203 causes a Th1 cytokine storm within 36 hrs pi.

The important cells. I. Antigen presenting and innate immune response cells

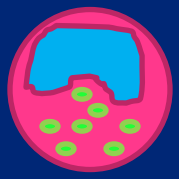
Dendritic cell (DC): presents antigen, produces IL-12 and other interleukins.



Macrophage presents antigen, phagocytosis, iNOS

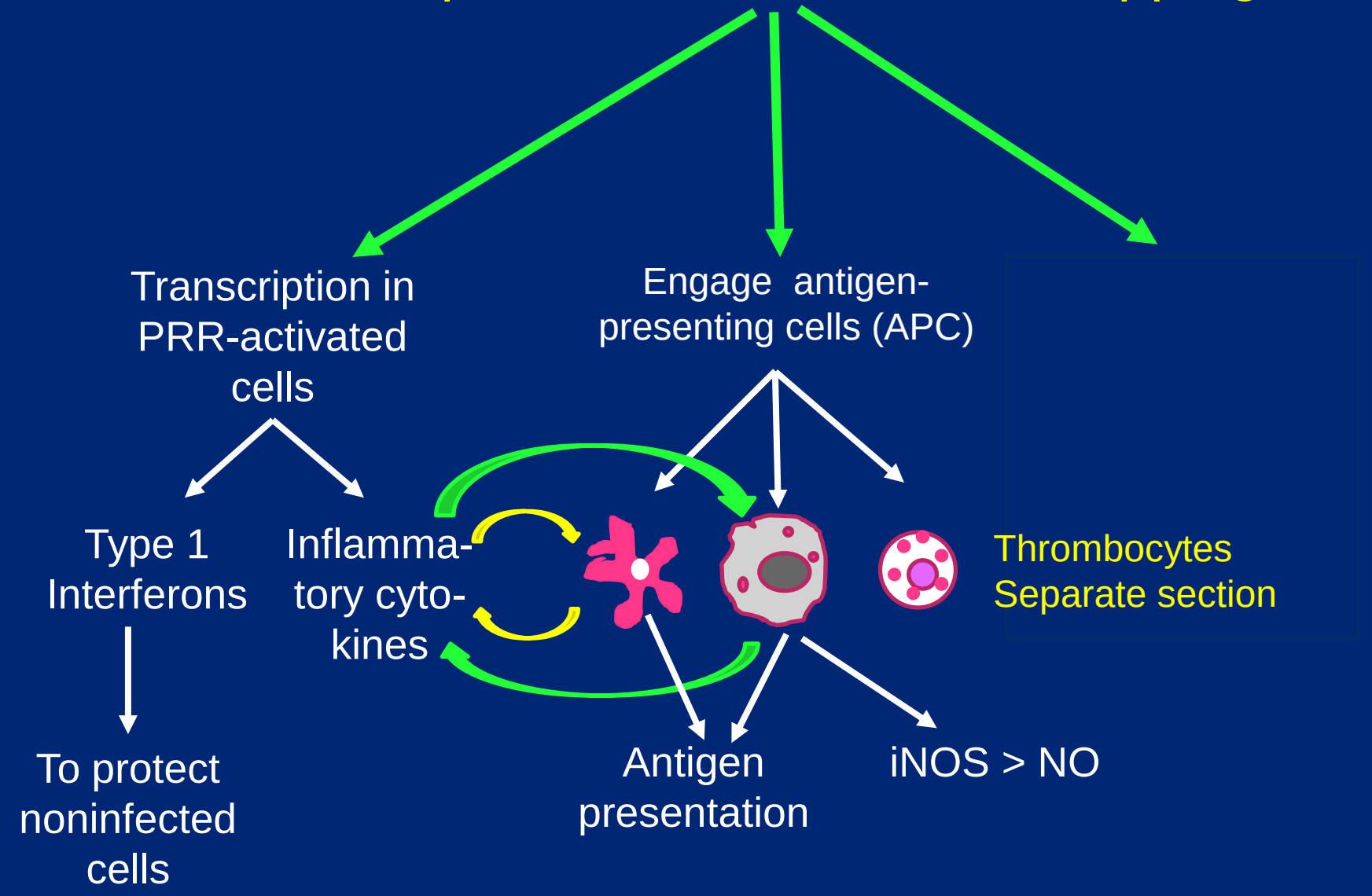
Heterophil: phagocytosis, produces chemokines.

Thrombocyte: phagocytosis, produces interleukins, etc, blood clotting.

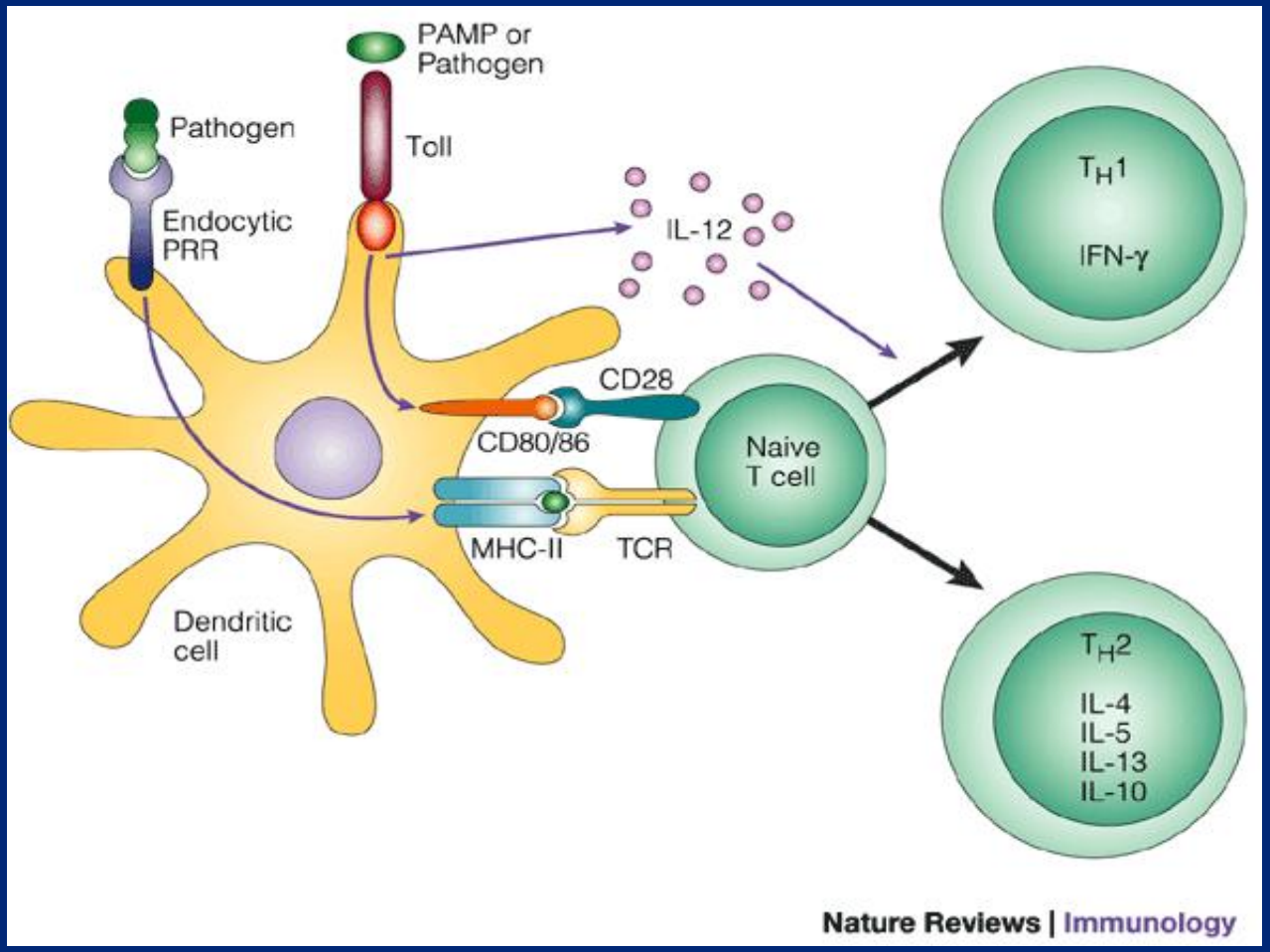


Natural killer (NK) cell: kills infected and tumor cells, produces IFN-γ

Once PRR are engaged many events start to occur in rapid succession and overlapping



A simplistic model of the role of a dendritic cell

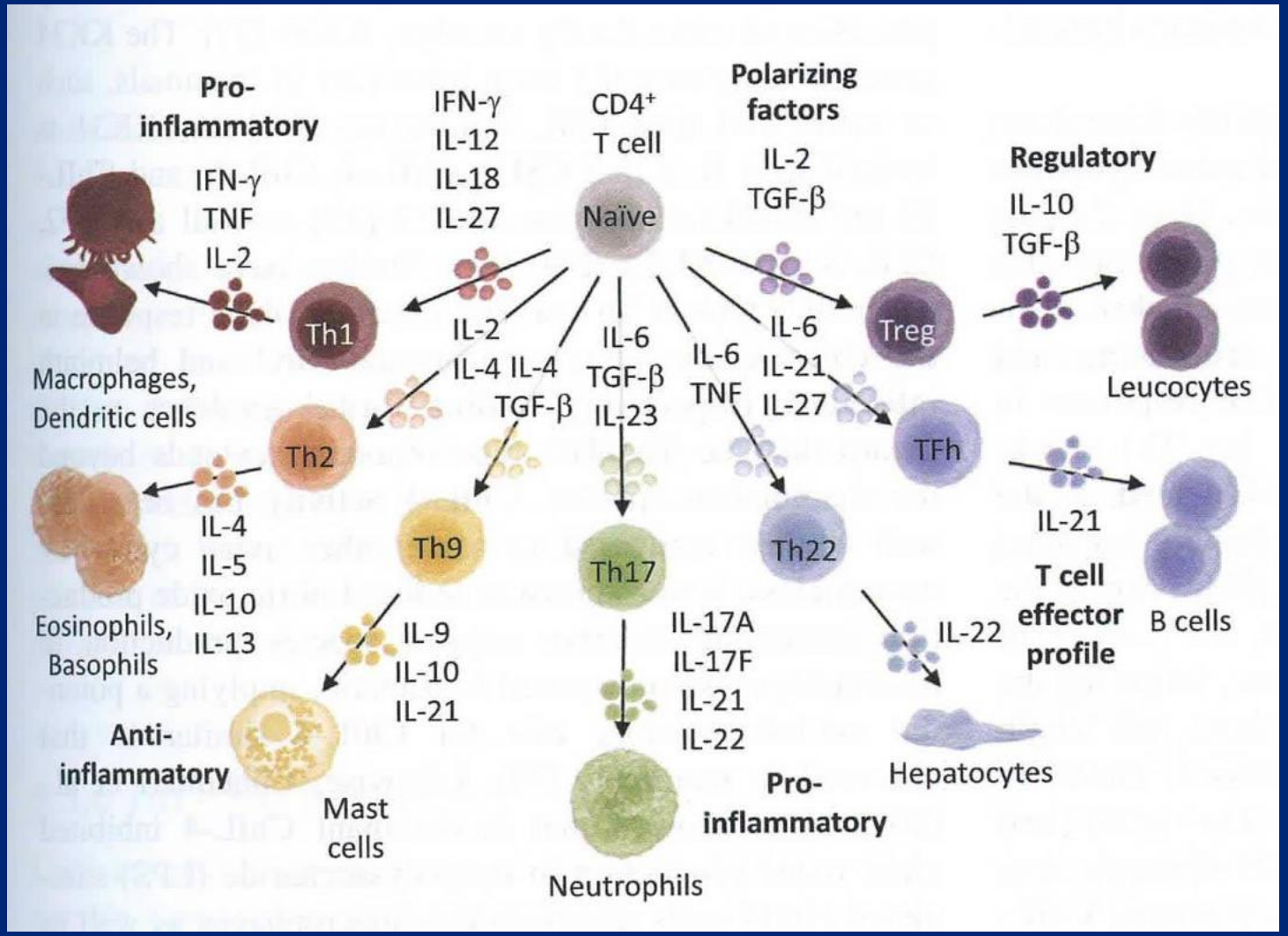


T_H1 response against intracellular pathogens

T_H2 response against extracellular pathogens

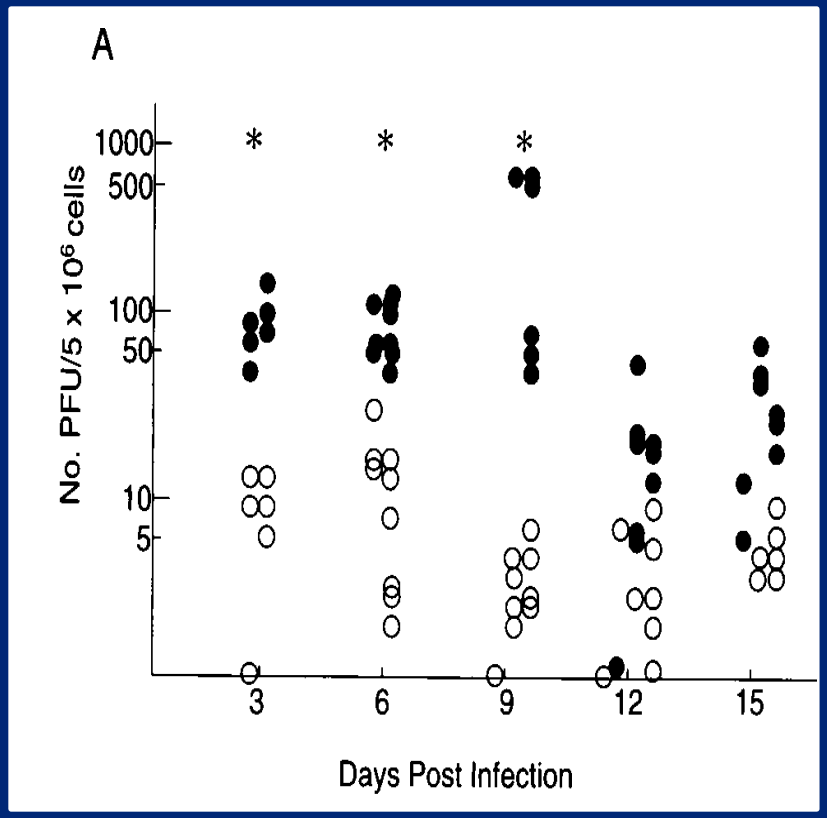
Adapted from Nature Reviews Immunology 1,135-145, 2001

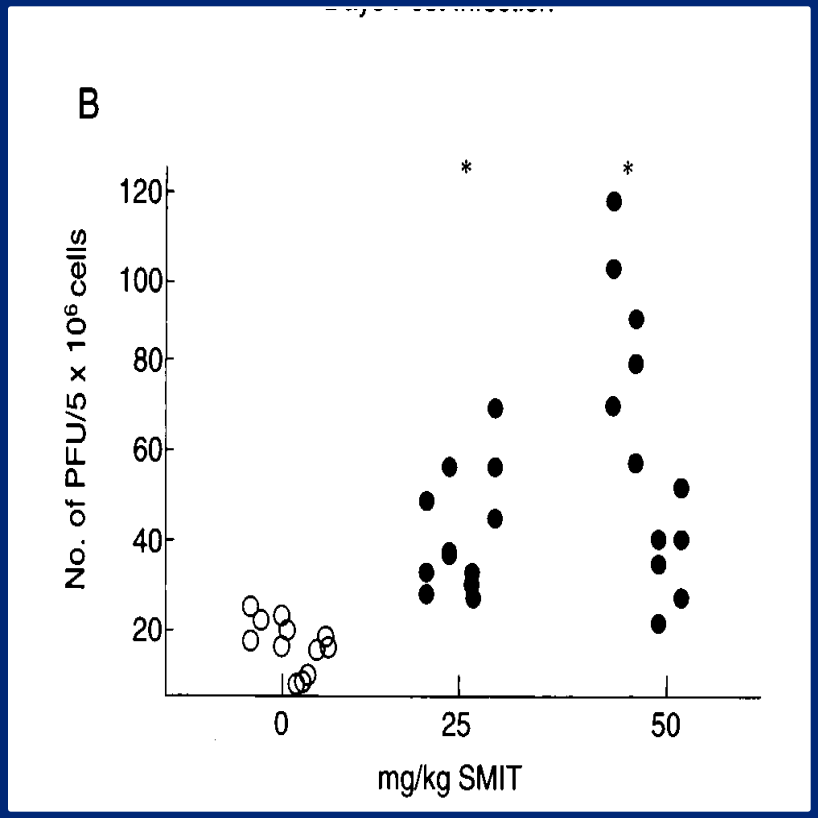
It is far more complex (mammalian models)



From Bean and Lowenthal, Avian Immunology 3th ed, p 253, 2022

Beneficial effect of Nitric Oxide (NO): inhibiting Marek's disease virus replication in chickens (Xing and Schat, JVI 74:3605, 2000)





Open circles: Control. Black circles treated with SMIT

Panel A: 25 mg/kg SMIT. Panel B: 6 days post infection

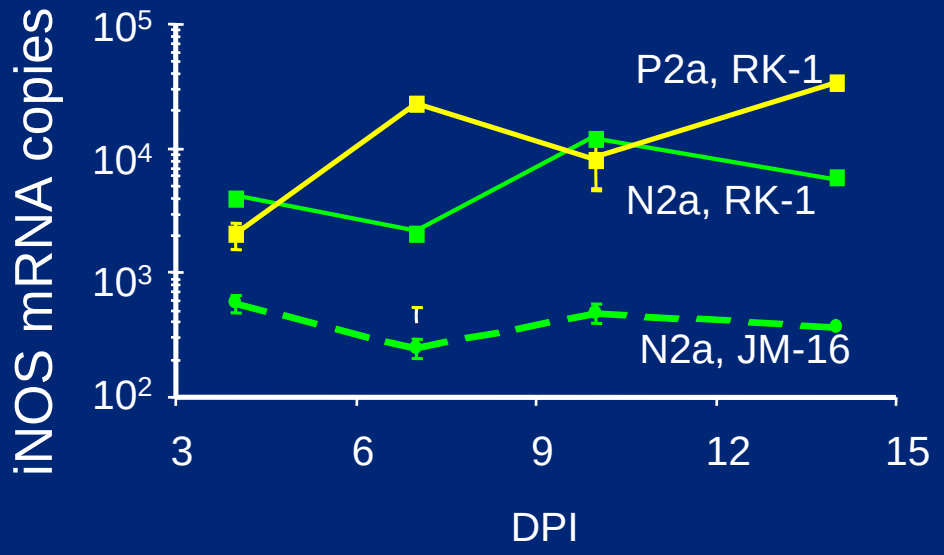
S-Methyl-Iso-Thiourea (SMIT) inhibits inducible Nitric Oxide Synthase (iNOS)

Too much NO is not good. Jarosinski et al, Viral Immunol . 2005

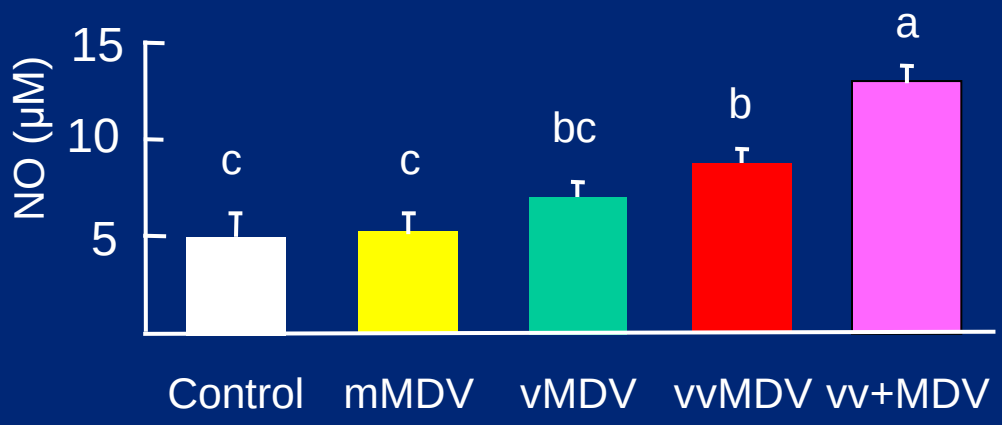
VV+ MDV (RK1 strain) causes a neural syndrome

In MD tumor susceptible and resistant lines

High levels of iNOS transcription in the brain



Levels of NO in plasma based on pathogenicity of MDV



Chicken thrombocytes

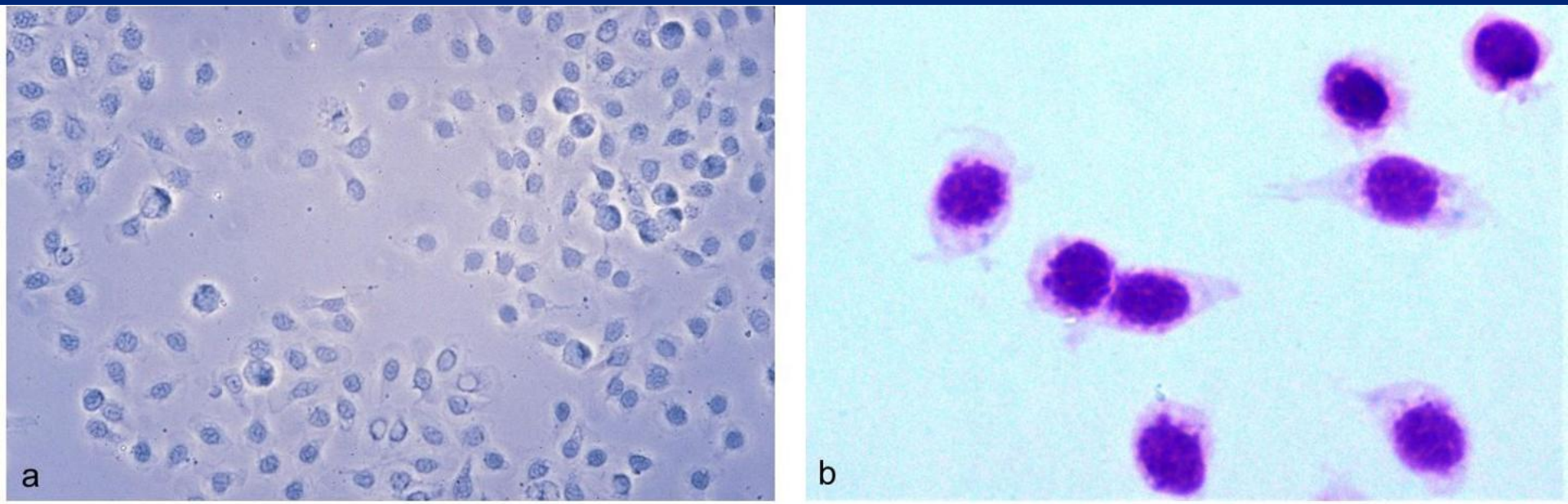


Figure 8.3.1: Images of chicken thrombocytes. (a) Ficoll-separated blood mononuclear cells were adhered to glass surface for 12 hours and non-adherent cells were removed by intensive washing. For comparison with adherent macrophages see figure 8.1.3. (b) Thrombocytes were sorted by flow-cytometry and stained with Diff-Quick staining.

Photo courtesy Drs. Härtle and Kaspers. From chapter 8.3 in Avian Immunology, 3th Ed.

The importance of thrombocytes in addition to blood clothing

Thrombocytes have a nucleus and express many surface markers involved in immune responses.

Thrombocytes are phagocytic

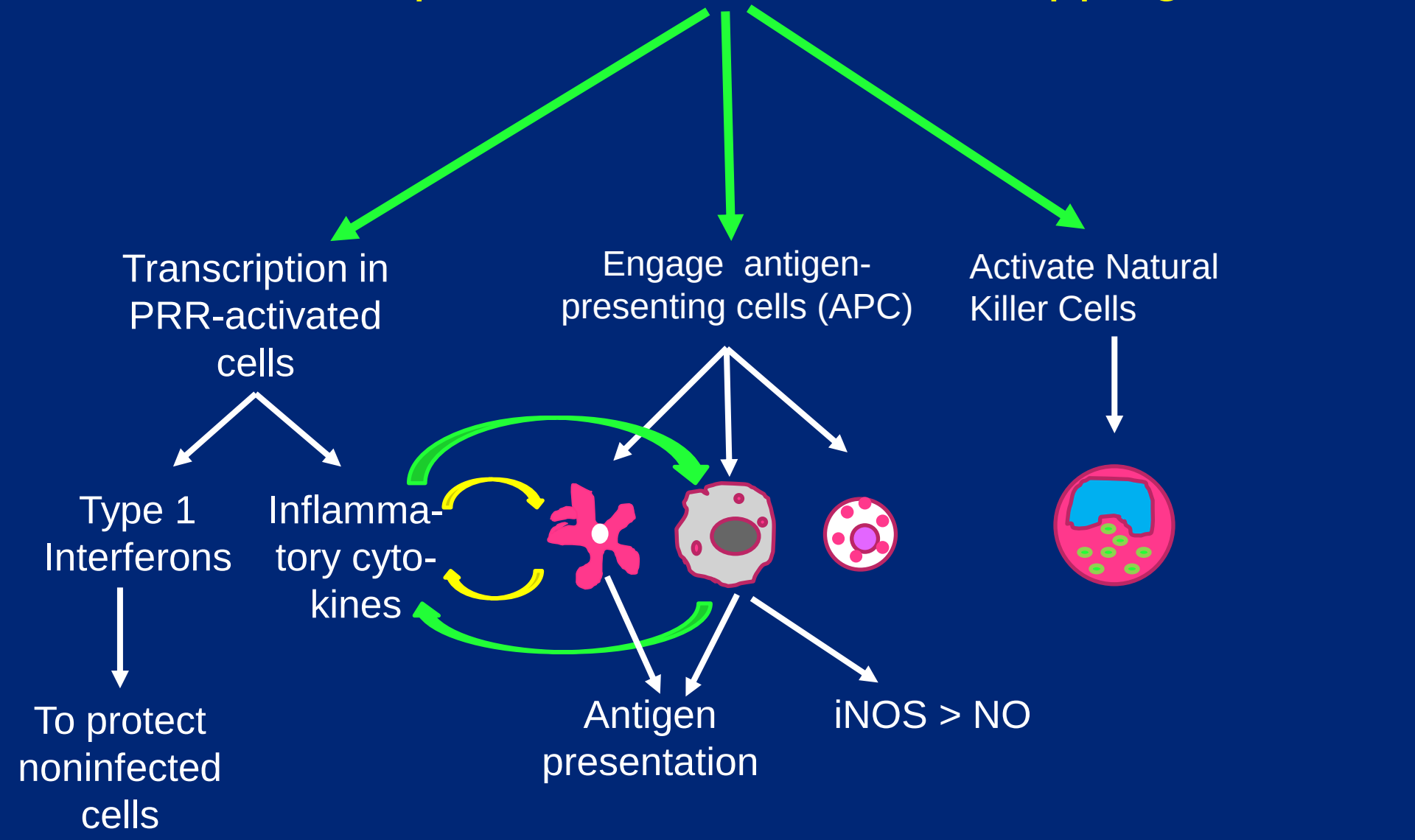
Thrombocytes contain preformed cytokines like IL6 and can produce cytokines on stimulation.

Thrombocytes express several PRRs like TLRs

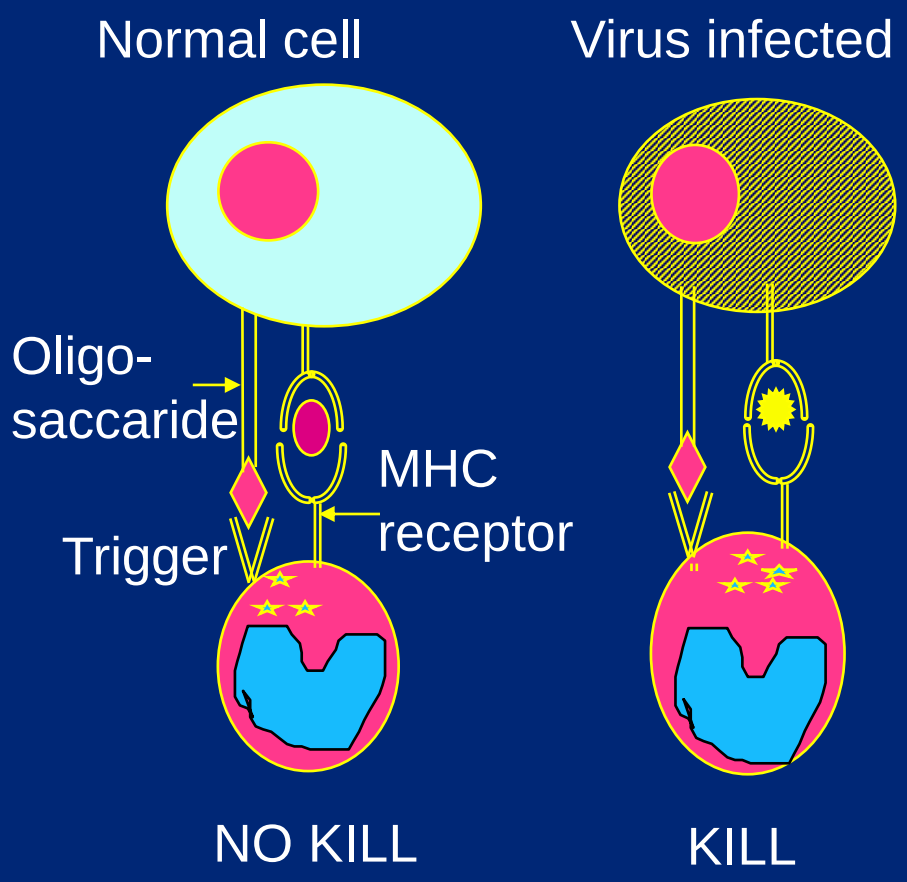
Stimulation of thrombocytes with LPS increases transcription of IL1β, IL6, IL8 and iNOS genes and functional IL6 is released

AIV infection upregulates adhesion molecules and PRR pathways

Once PRR are engaged many events start to occur in rapid succession and overlapping



Express yourself or die: NK cells in action (Kärre, Science 267:978, 1995). Other models have also been proposed



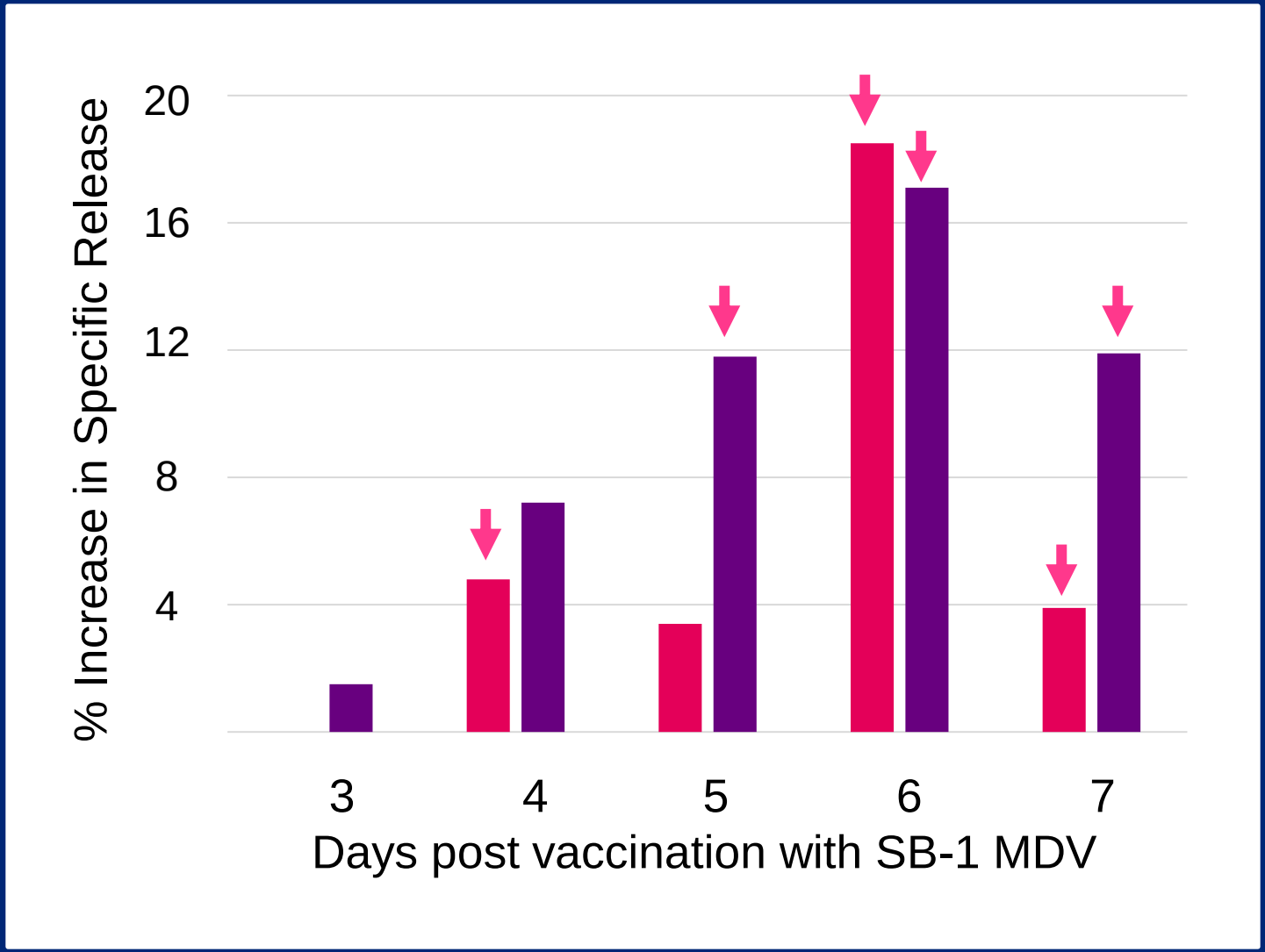
NK cells in chickens

Functional assays described NK-like activity against LSCC-RP9 in spleen and PBL

Virus-infections increased the NK-like activity. E.g., Marek's disease field strains and vaccines

Lacking monoclonal antibodies to uniquely identify NK cells in chickens (Göbel, 2022).

Typical NK cell assay: Chromium 51 release assay using spleen cells against RP9 cells (Heller and Schat, Avian Pathology 1987.



Acquired immune responses: Cytotoxic T Cells (CTL)

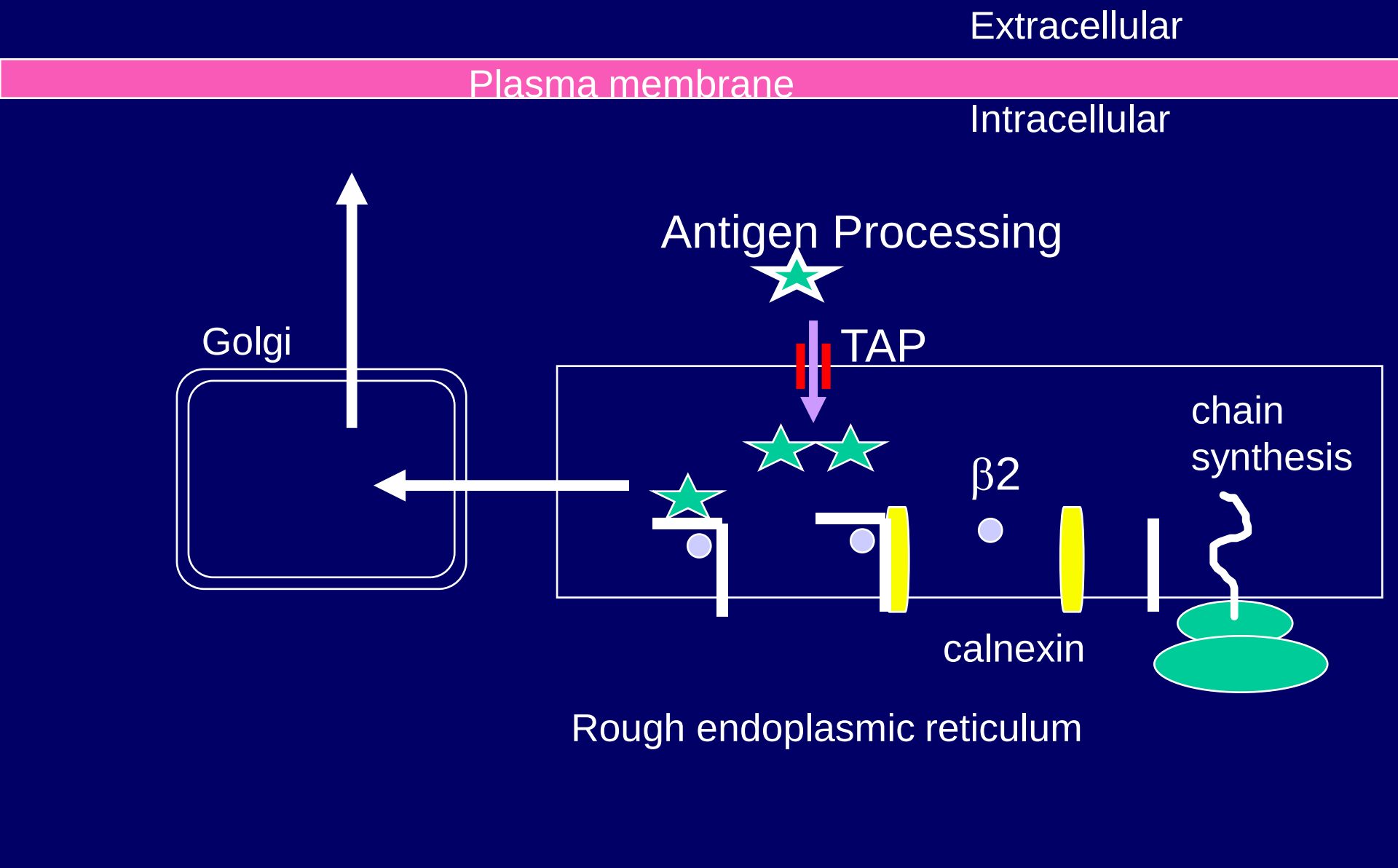
Avian CTL are expressing CD8αβ heterodimers and one of the 3 T cell receptors (TCR): TCRαβ1, TCRαβ2 or TCRγδ.

Classical experiments by Omar and Schat (1997) showed that the MD vaccine strain SB1 induced specific lysis of REV-transformed cell lines expressing specific MDV proteins.

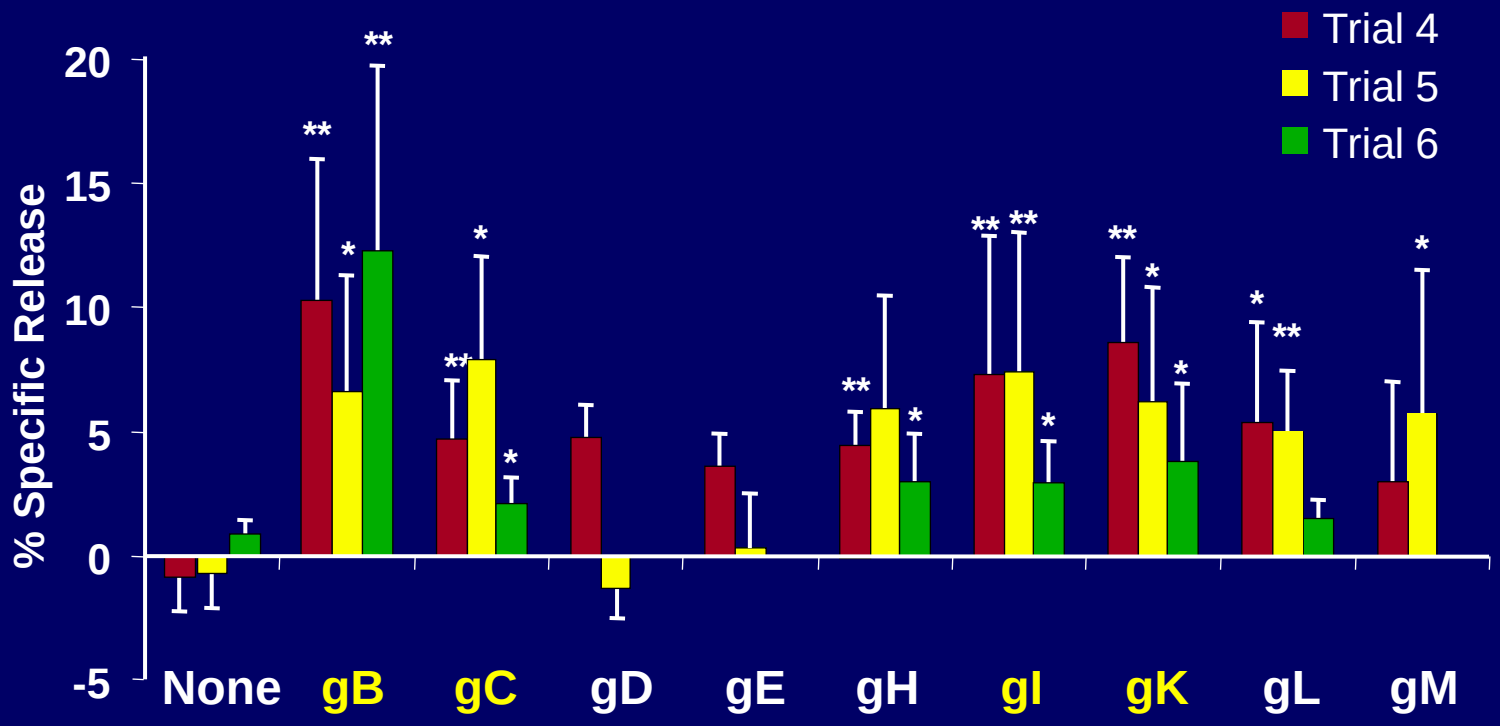
The CTL recognized the MDV proteins only when syngeneic combinations of effector and target cells were used.

Any infected cell expressing MHC-class I on the cell surface can present viral antigens to the CTL after a complex process in the infected cell.

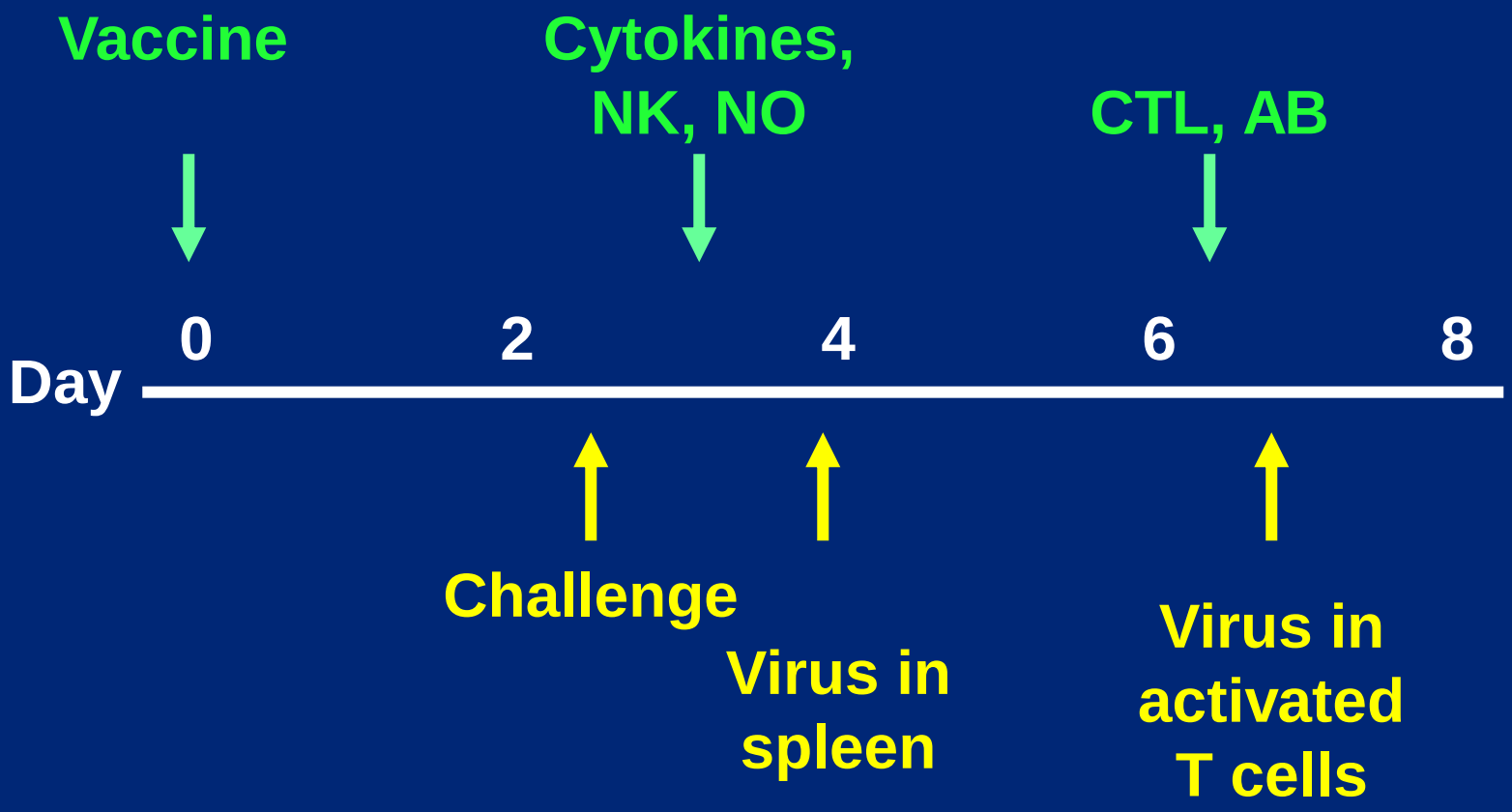
Maturation of MHC class I molecules



Lysis of REV cell lines expressing MDV glycoproteins by MDV-specific CTL from N2a ($B^{21}B^{21}$) chickens



Marek's disease immune responses in vaccinated chickens



Avian influenza and the immune responses

Immune responses to low pathogenic (LP) AIV in chickens and ducks

Immune responses to HP AIV in chickens and ducks

Differences in cell tropism of HP AIV between chickens and ducks

A proposed model to explain the rapid mortality in chickens infected with HP AIV

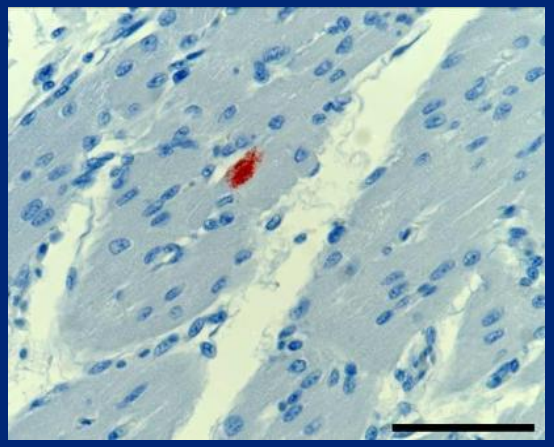
Infection of chickens with LP AIV

Mostly a limited infection in intestinal or respiratory tract

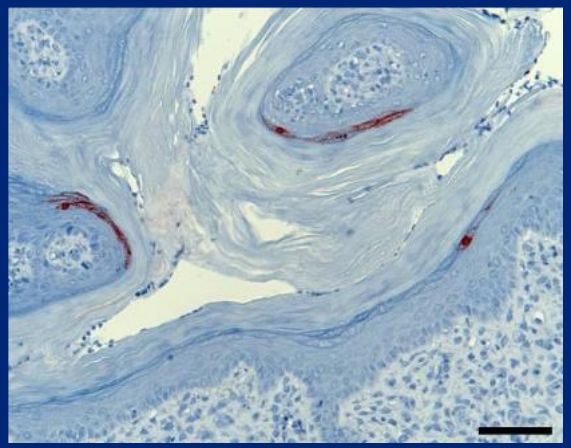
Limited infection may occur in other tissues

Schat et al (PLOS-One, 2012) infected chickens with rgH5N1 VN1203 with LP HA cleavage site

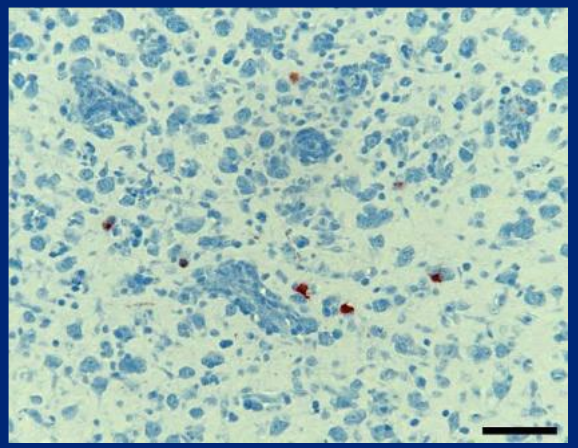
Chickens **seroconverted** and showed very limited infection in heart, comb and brain.



Heart 5 dpi



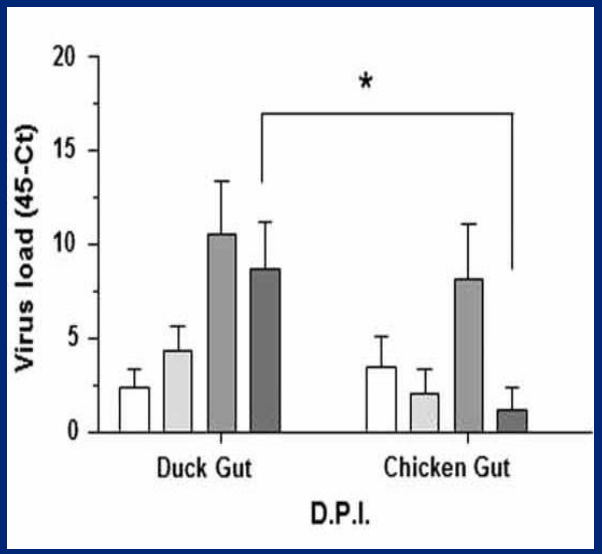
Comb 13 dpi

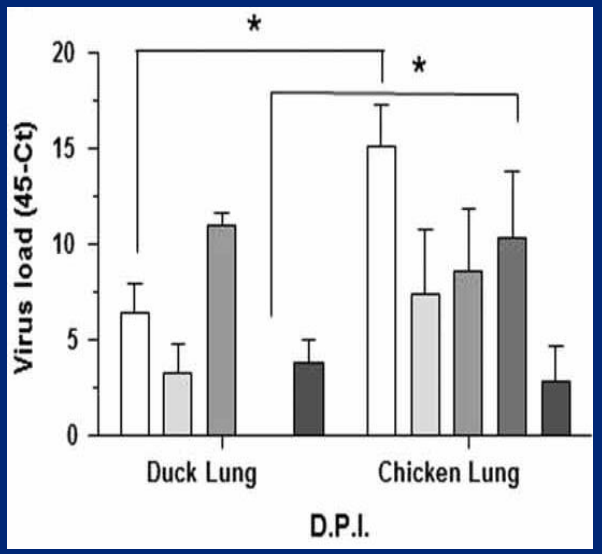


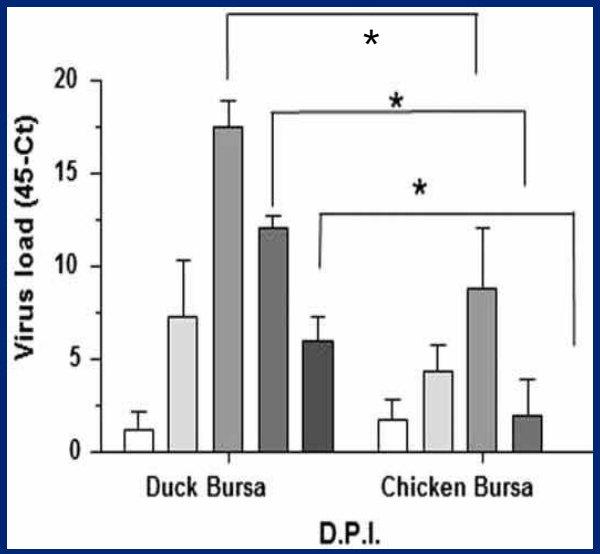
Brain 13 dpi

Cornelissen et al 2012. Compared infection with LP H7N1 in chickens and ducks. Avian Pathol. 41:519, 2012.

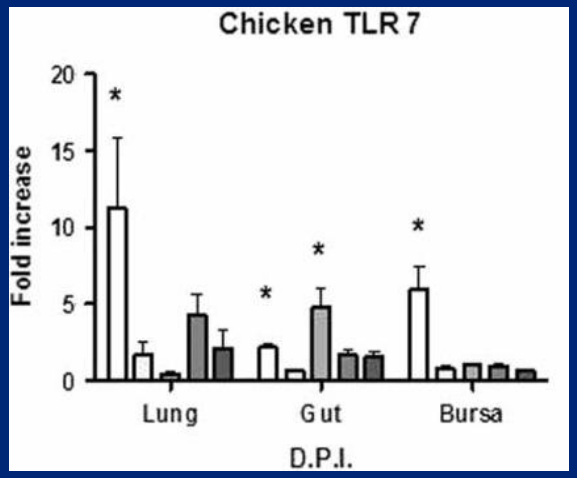
All ducks and chickens remained clinical normal, showed limited viral replication and all chickens seroconverted

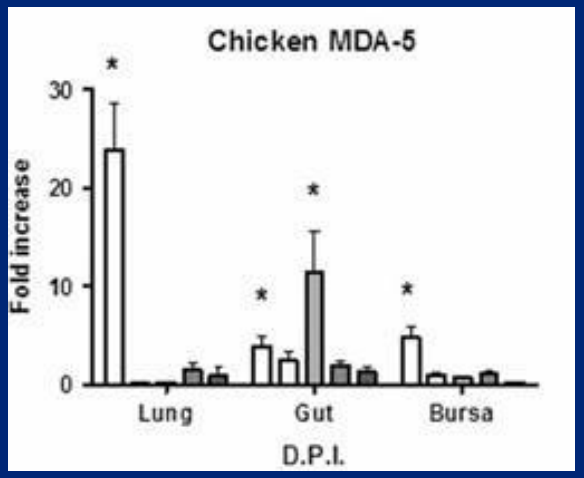


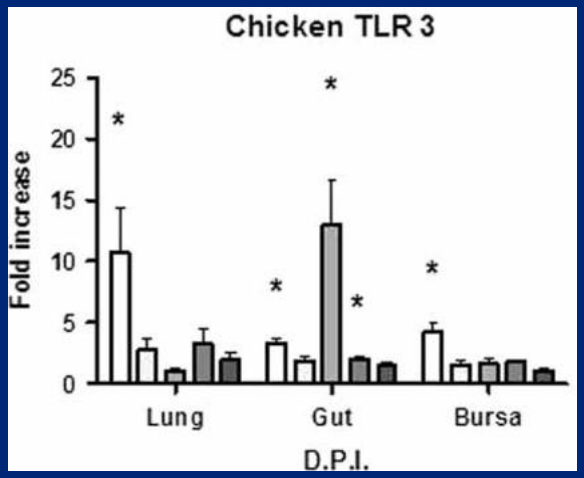


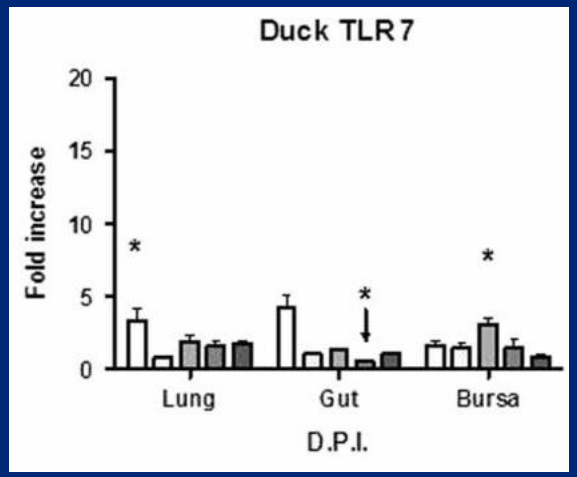


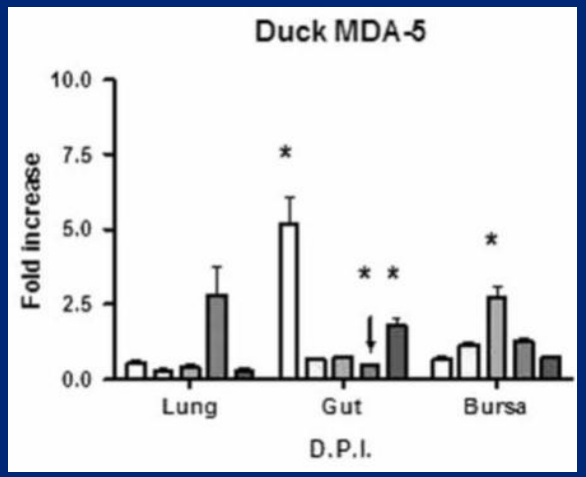
Activation of PRR in chickens and ducks by LP AIV

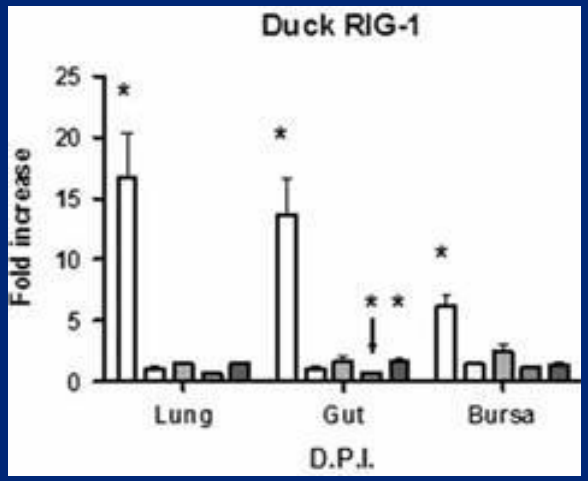






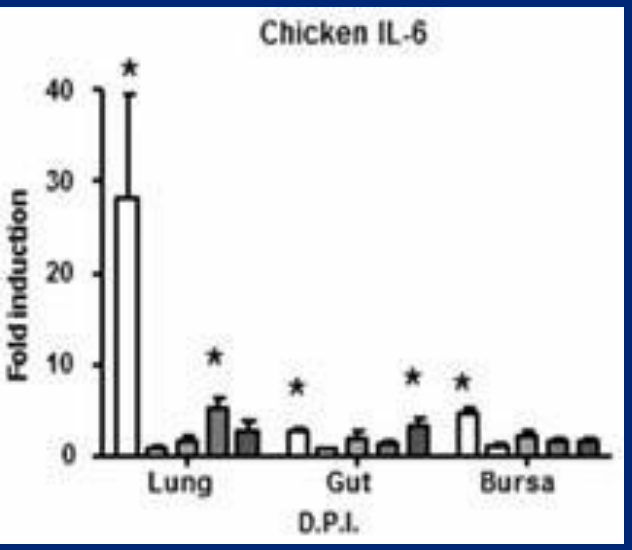


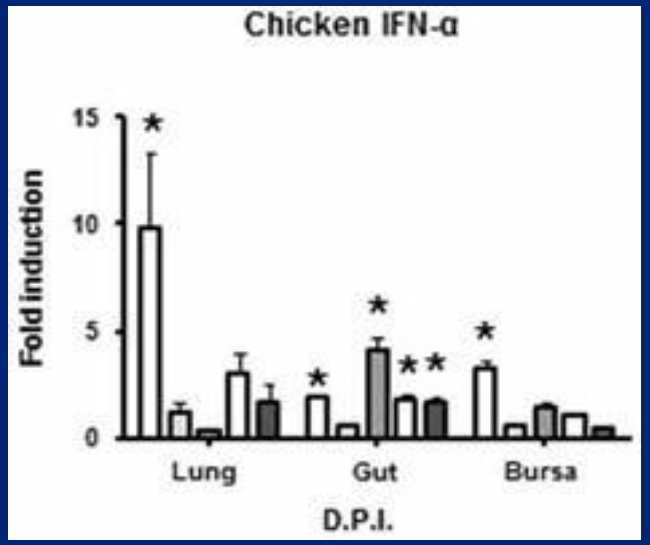


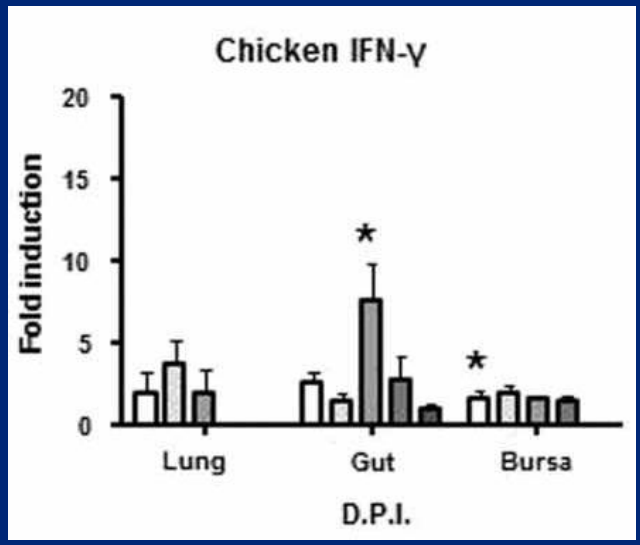


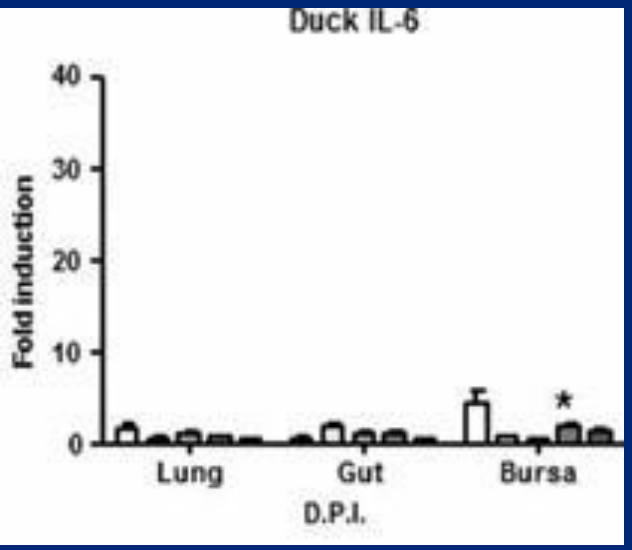
Cornellisen et al Avian Pathol 2012

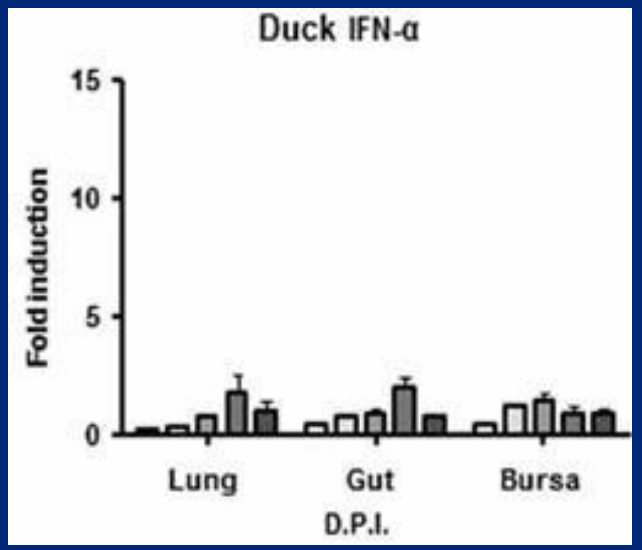
Cornelissen et al 2012: Activation of IL-6 and interferons

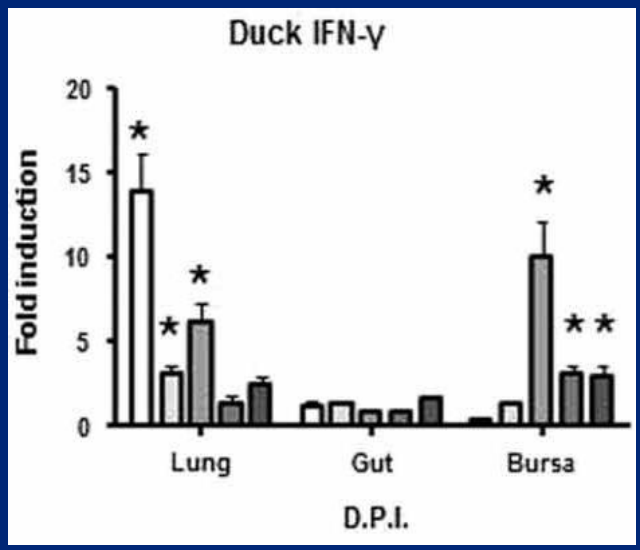












Vaccine studies

Chickens vaccinated with killed or recombinant vaccines develop VN (and HI) antibodies that are protective against HA matching AIVs.

Conclusions

There is no inherent immunological deficit in chickens preventing innate and acquired immune responses to LP AIV.

Question

What happens after infection with HP AIV in ducks and chickens?

Cytokine responses in chickens after infection with HP AIV
Karpala et al J Inf Cytokine Res 31:393, 2011

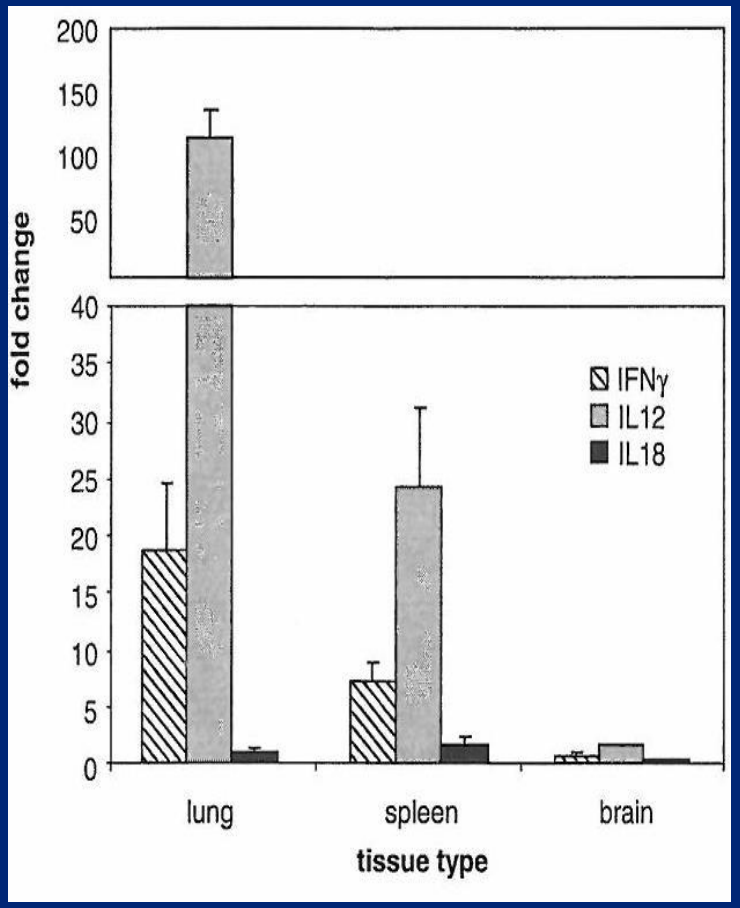
Infected 4-week-old chickens with HP or LP rgVN1203 H5N1.

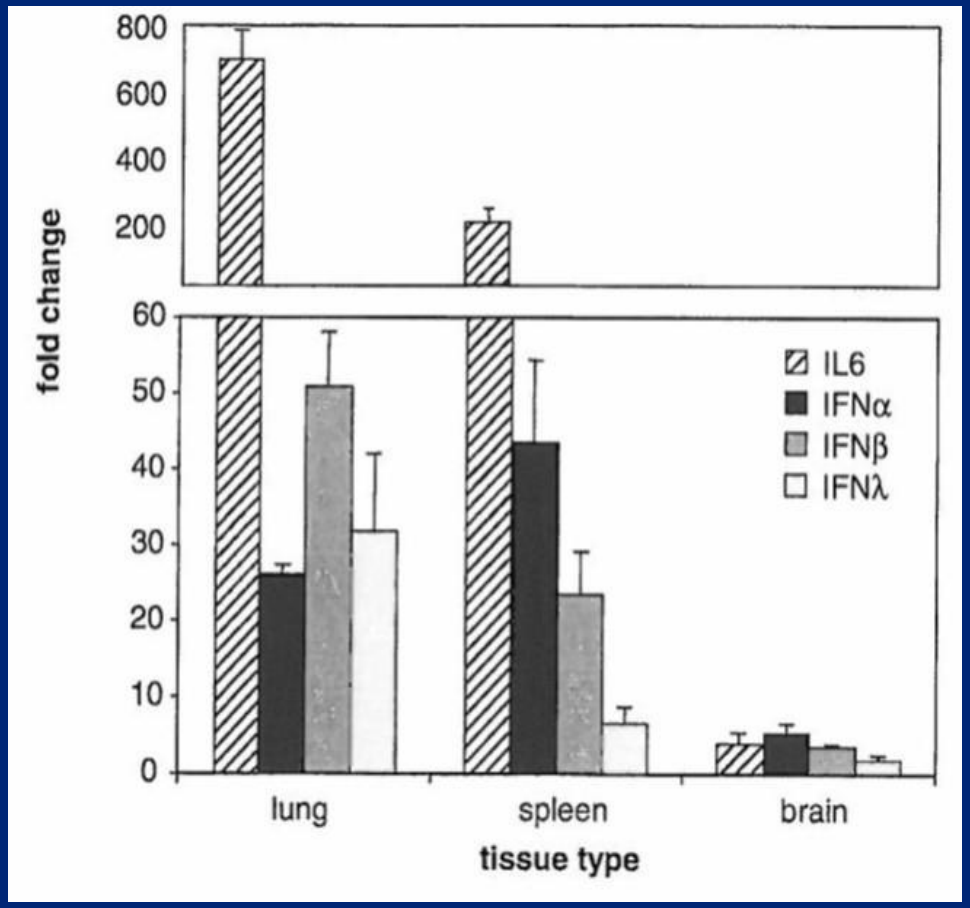
Cytokine and interferon mRNAs were measured at 36 hours post infection in lung, spleen and brain tissues.

Levels of proinflammatory cytokine mRNAs were similar to controls after infection with the LP rgVN1203.

Th2 cytokine responses were not changed after infection with HP rgVN1203.

An example of rapid induction of interferons and proinflammatory cytokines. Karpala et al, 2012.



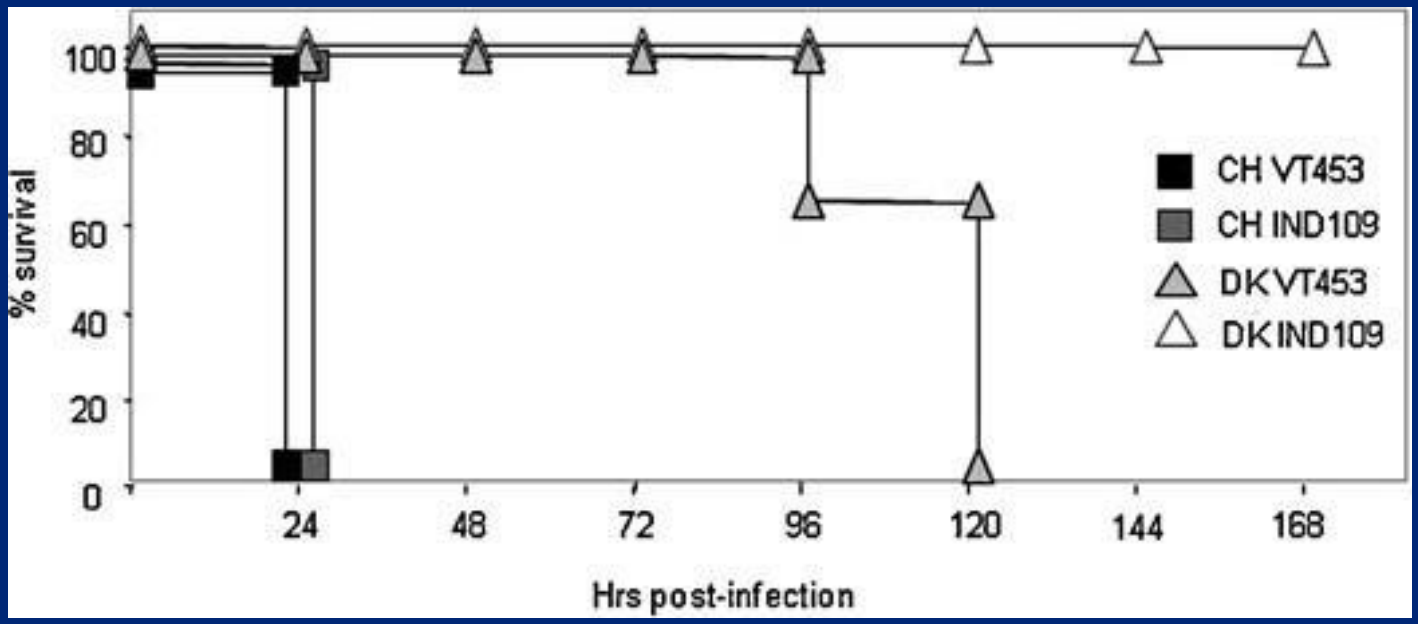


HP rgVN1203 causes a Th1 cytokine storm within 36 hrs pi.

Comparison of infection in chickens and ducks with two HP AIV strains. Burggraaf et al. Virus Res 185:23, 2014

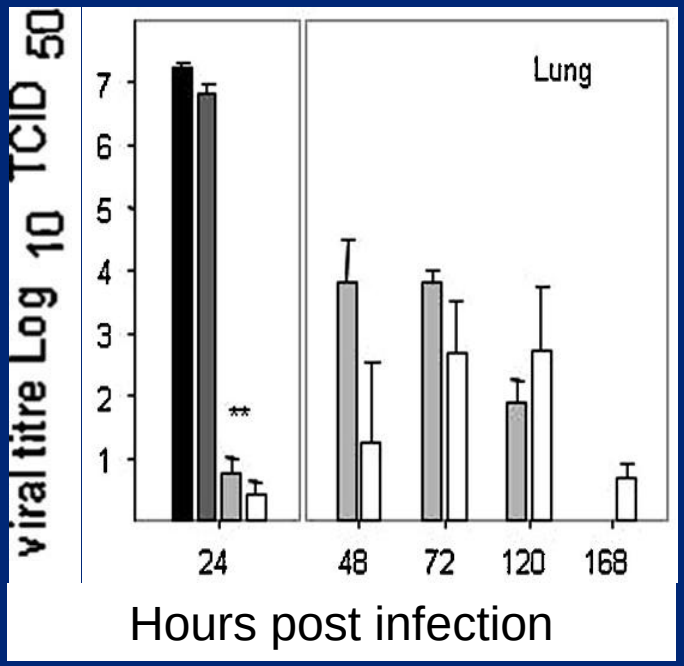
Chickens and ducks were infected with HP H5N1 VN.453, HP H5N1 Ind109 or LP Vc1462.

Chickens died or were euthanized within 24 hours pi

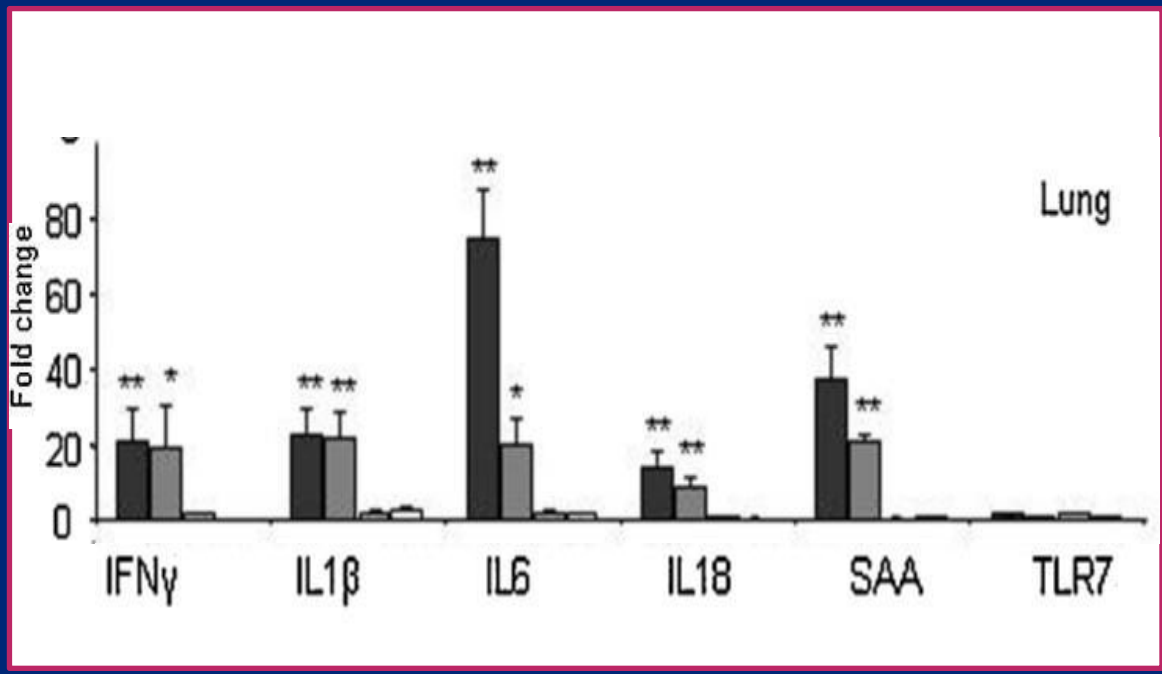


Burggraaf et al, 2014

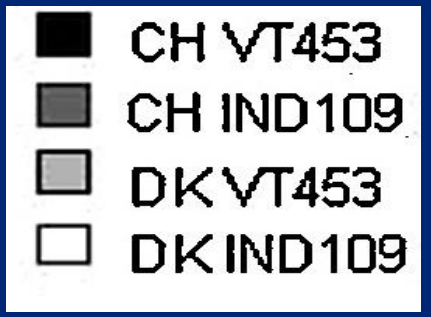
Virus replication



Cytokine responses at 24 hrs PI



Similar results were obtained for spleen, brain and heart



Studies in ducks infected with HP or LP AIV. Fleming-Canepa et al. Vet Microbiol 228:101, 2019.

rgVN1203: RIG-1, IFNβ, and downstream IF stimulated genes (ISGs) were highly upregulated in spleen and lung at 1 dpi

PA mutant T515A of VN1203 replicated at similar levels as VN1203 but induced significantly lower RIG-1, IFNβ, and ISGs responses

Fold increase of transcripts at dpi 1 compared to controls

Virus	RIG-1 Lung	RIG-1 Spleen	IFNβ Lung	IFNβ Spleen
VN1203	157.9	11.1	178.0	17.9
PA T515A	47.3	9.2	27.4	5.7
Significance	p<0.0001	P<0.05	p<0.0001	ns

Conclusions. I.

Infection with LP AIV in ducks and chickens:

- Causes in general a limited infection in respiratory and or intestinal tract
- In general no or limited innate immune responses
- Infected birds develop HI and VN antibodies

Infection with HP AIV in ducks:

- Causes generalized infection with mortality depending on the virus strain.
- Significant innate immune responses as early as 1 dpi
- Survivors develop antibodies

Conclusions. II.

Infection with HP AIV in chickens:

- A very significant increase in innate immune responses
- Infection causes 100% mortality within 24 to 48 hours after experimental infection

Questions

- Why do chickens die and ducks survive?
- Are there differences in the pathogenesis of HP AIV infection between ducks and chickens?

HP AIV infects endothelial cells in chickens

Schat et al (2012) infected 4-week-old chickens with HP rgVN1203. Tissues harvested within 48 hours post infection

Schat et al PLoS ONE 7(2): e30960 and unpublished photos.

Infection in endothelial cells

Endothelial cells are associated with HP H5N1 AIV in chickens

LP H5N1 AIV does not infect endothelial cells in chickens

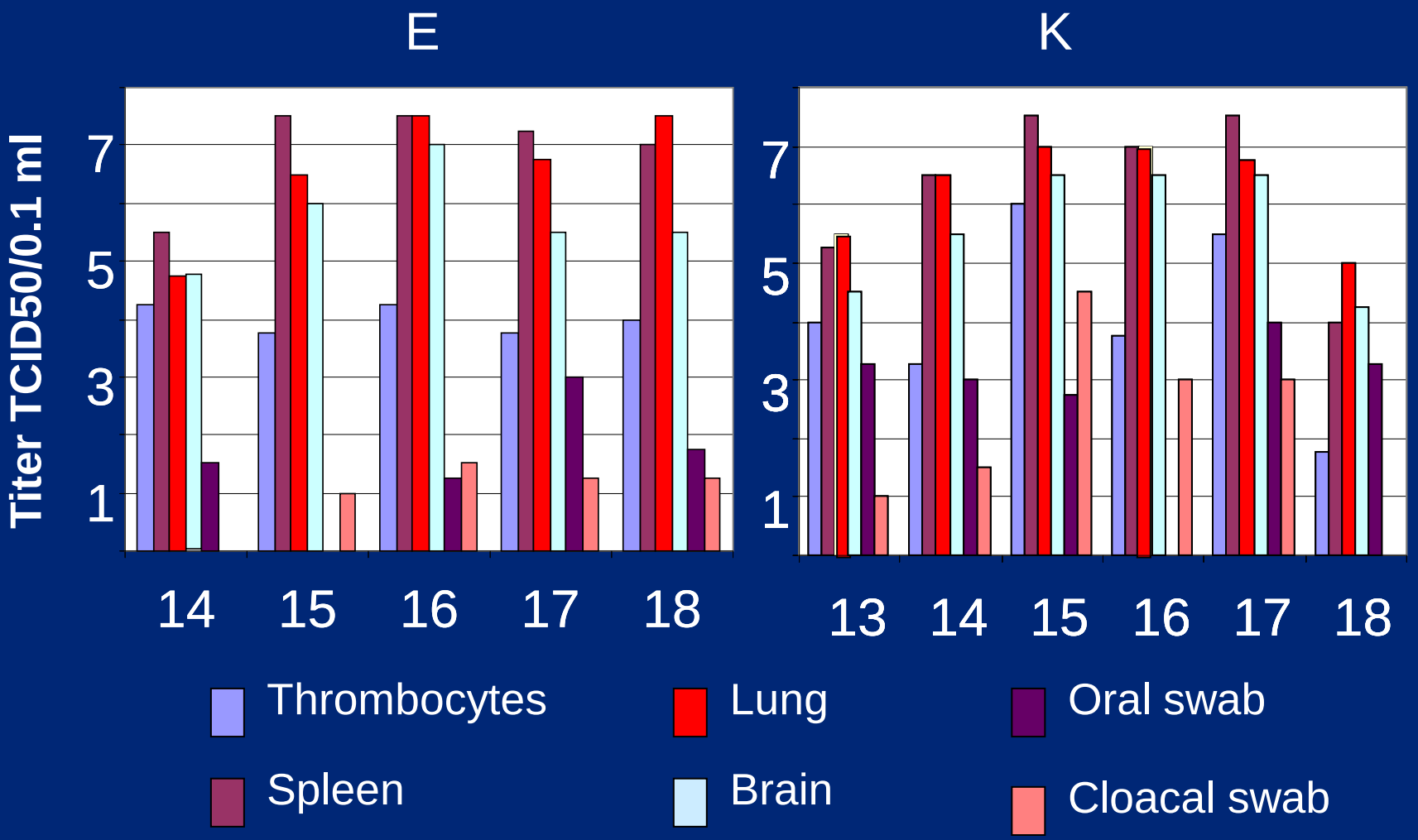
HP H5N1 AIV infect parenchymal cells in white Pekin ducks and not endothelial cells

Chicken aortic endothelial cell lines display a more pro-inflammatory response to HP H5N1 AIV and to polyI:C than white Pekin duck aorta endothelial cell lines (Tong et al. Genes 12:201, 2021)

Duck or swan species experiencing rapid mortality after HP H5N1 infection often show virus replication in endothelial cells

Chicken but not duck thrombocytes are infected with HP AIV (Schat et al, 2012)

Virus isolation at 21 hrs PI with rgVN/1203/04_627E (E at PB2-627) and 24 hrs PI with rgVN/1203/04 (K at PB2-627)



Conclusions

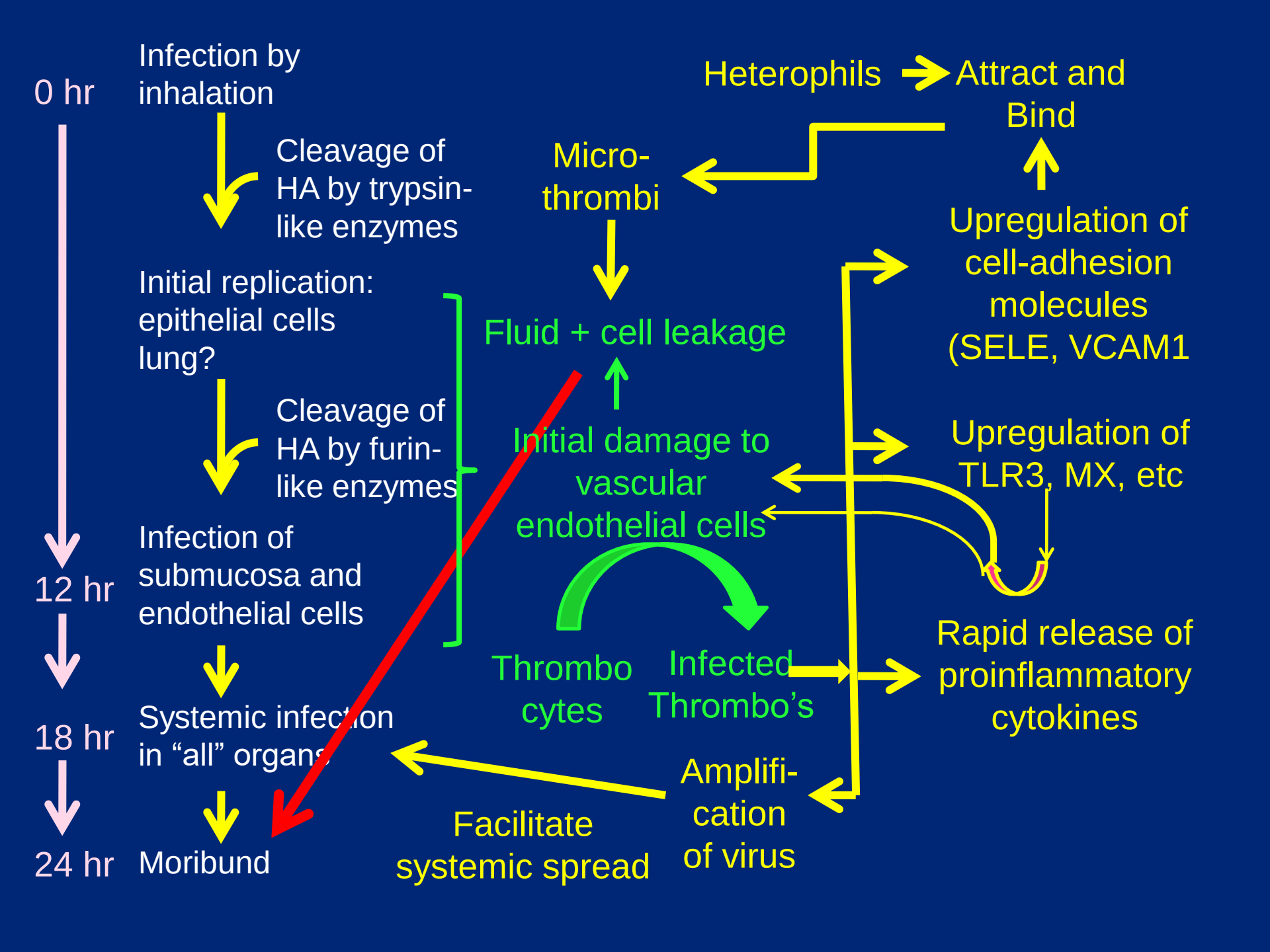
There are 4 important differences between infections between ducks and chickens when infected with HP H5N1 AIV

1. Endothelial cells are a prime target in chickens and Australian Black swans, but not in mallard ducks and white Pekin ducks

2. Chicken thrombocytes become infected but duck thrombocytes are not a main target for infection

3. Different cytokine responses

4. Acute mortality in chickens and black swans

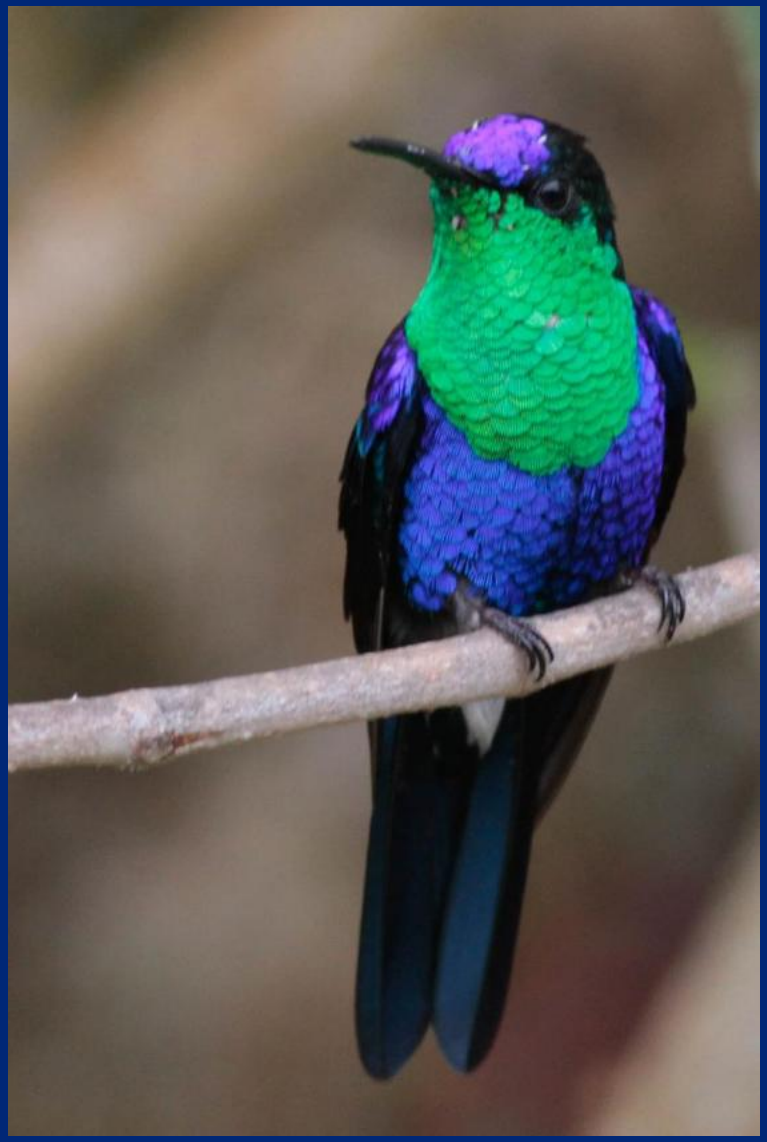

0 hr
Infection by inhalation
Cleavage of HA by trypsin-like enzymes
Initial replication: epithelial cells lung?
Cleavage of HA by furin-like enzymes
12 hr
Infection of submucosa and endothelial cells
18 hr
Systemic infection in “all” organs
24 hr
Moribund
Heterophils
Attract and Bind
Micro-thrombi
Upregulation of cell-adhesion molecules (SELE, VCAM1
Fluid + cell leakage
Initial damage to vascular endothelial cells
Upregulation of TLR3, MX, etc
Thrombo cytes
Infected Thrombo’s
Rapid release of proinflammatory cytokines
Amplifi-cation of virus
Facilitate systemic spread

FINAL CONCLUSION

The proposed model explains the rapid mortality in chickens and the absence of rapid mortality in mallard and white Pekin ducks

I thank the organizers of the BVPA for the invitation to present this lecture

QUESTIONS



Violet-crowned wood nymph. Photo Ton Schat