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Tolerized animal models and immunogenicity

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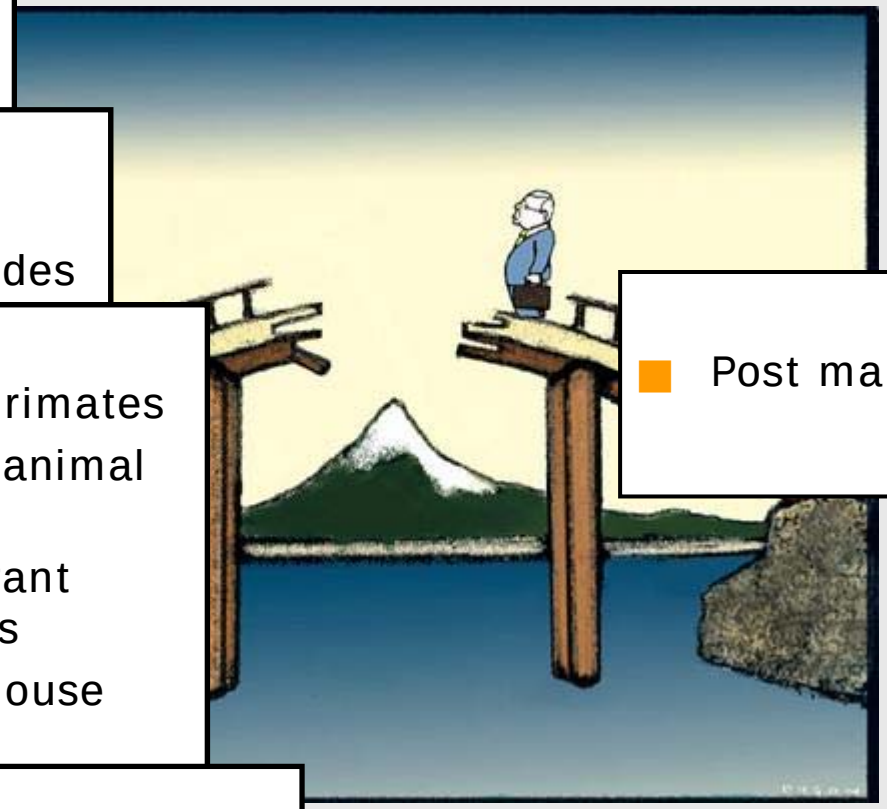
Immunogenicity research
"Bridging the gap"

- *In silico*
  - CD4 T cell epitopes
  - (B cell epitopes)
- *In vitro*
  - T cell activation
  - Artificial lymph nodes
- Animal models
  - Non-human primates
  - Conventional animal models
  - Immune tolerant mouse models
  - Humanized mouse models
- Patients
  - Phase I-III



- Post marketing

Bridging which gap?

- Predicting immunogenicity
  - Who will develop an antidrug antibody response when
  - Incidence of immunogenicity in patients
  - Clinical effect of antidrug antibodies
  - Breaking of tolerance
  - Relative immunogenicity between products
  - Effect of different formulation
  - Different treatment schedule: dose, route, frequency
  - Presence of (neo) epitopes
  - Effect of pre-existing antibodies
- Determining the underlying immune mechanisms
  - Identification of biomarkers
  - Improving existing predictive models
  - Risk factors (treatment, product, patient)
  - How to lower immunogenicity (treatment, product, patient)

Focus on animal models

Use of animal models in immunogenicity

- Prediction
- Studying underlying immune mechanisms

  - Non human primates
    - Tolerant for most human proteins
    - Ethical limitations
  - Conventional animal models (mice/rats)
    - Immune response against foreign protein
    - Mouse/rat immune system
  - Immune tolerant mouse models
    - Breaking of tolerance
    - Mouse immune system
  - Humanized mouse models
    - Immune response against foreign protein
    - “Human” immune system

Which animal model to use?

- Depends on how similar the processes involved in immunogenicity are compared to patients

- Possible mechanisms

  - Immune response against foreign protein
    - When therapeutic proteins differs from endogenous protein or when endogenous protein is absent
    - Factor VIII
    - Alpha galactosidase

  - Breaking of tolerance
    - When therapeutic protein is similar to endogenous protein

Immune tolerant mouse models

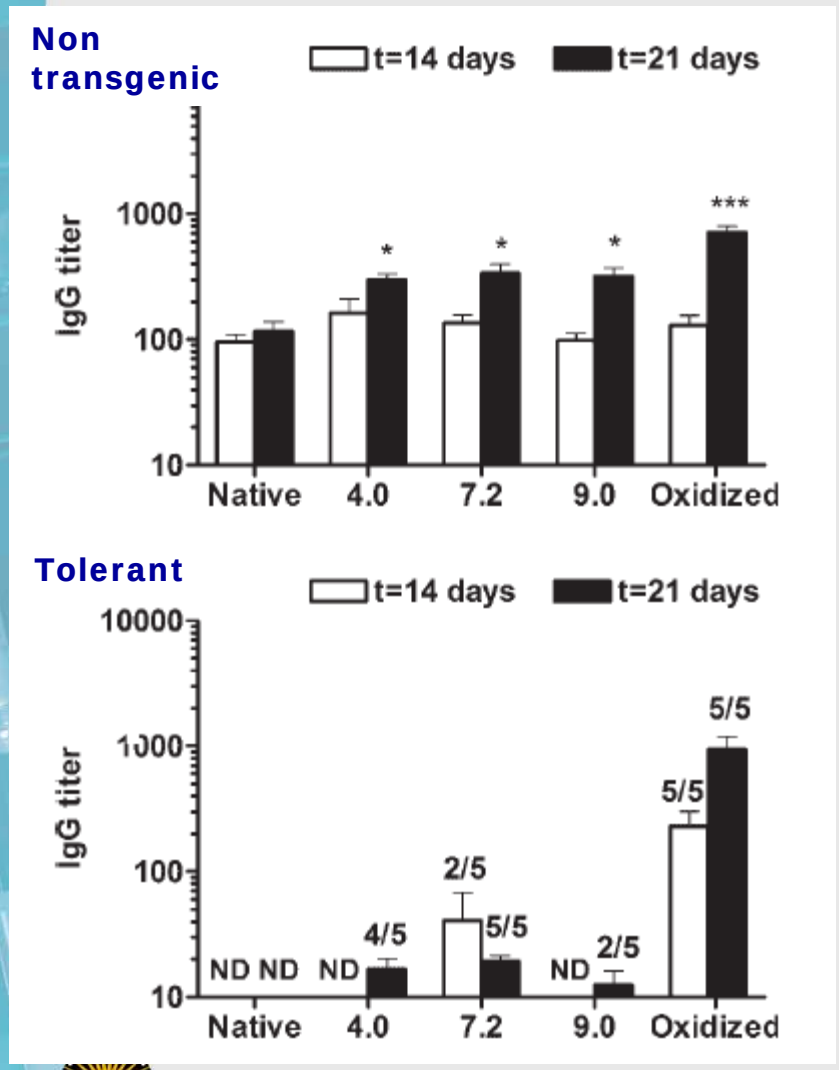
- Insertion of human gene in mouse genome (transgenic)
  - Like humans tolerant for human protein
  - Breaking of tolerance by therapeutic protein
- Prediction
  - Breaking of tolerance
  - Relative immunogenicity between products/formulations
  - Neo epitopes
- Immune mechanism
  - Identification of aggregates as risk factor

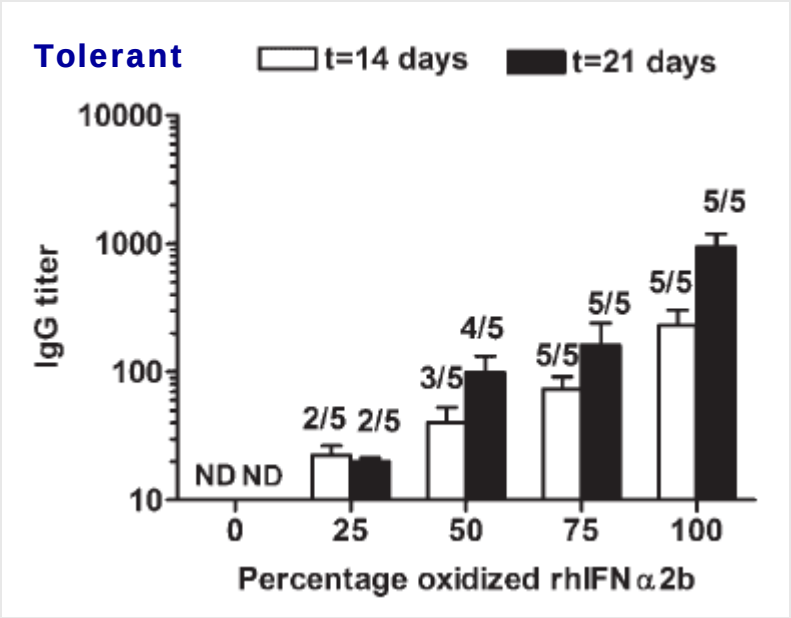
  - Which aggregates/concentration?
  - Effect of dose, number of injections, frequency, route?
  - Immune cells involved?
  - Genes/pathways involved?
  - Kinetics of aggregates *in vivo*

Type of aggregates

-mice tolerant for human interferon alpha/beta-
-mice tolerant for human Ig-

Interferon alpha





Hermeling et al., 2006

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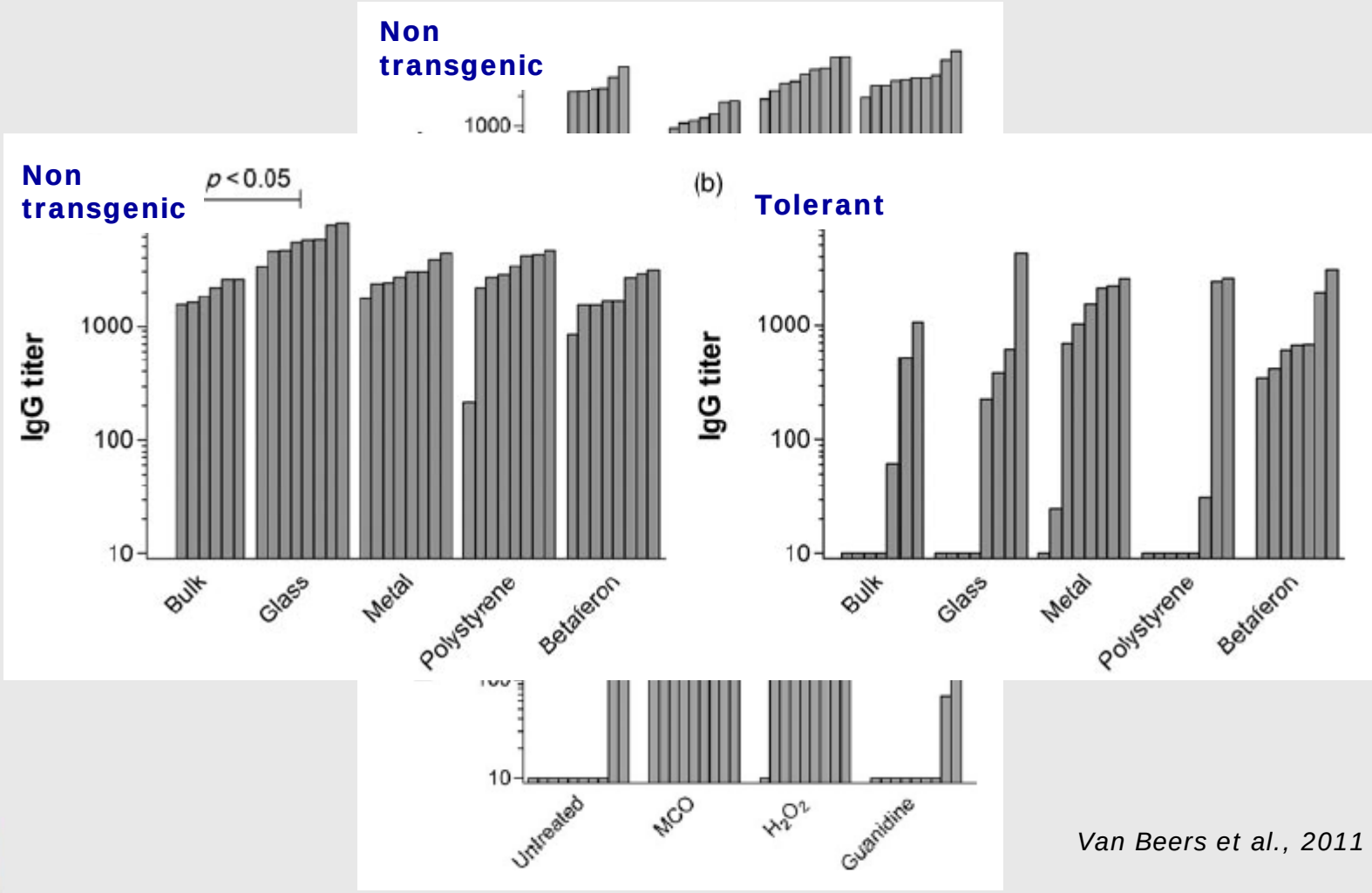
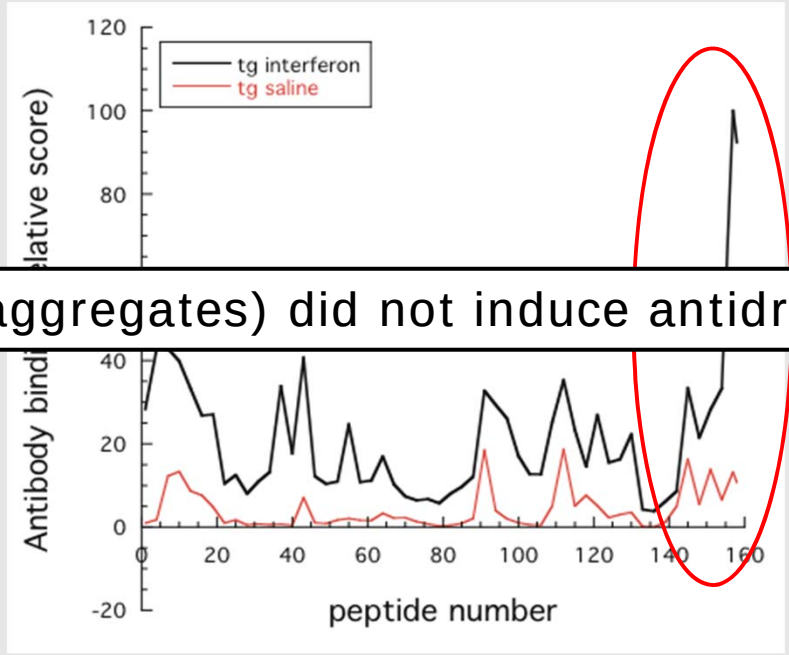
Interferon beta



Van Beers et al., 2011

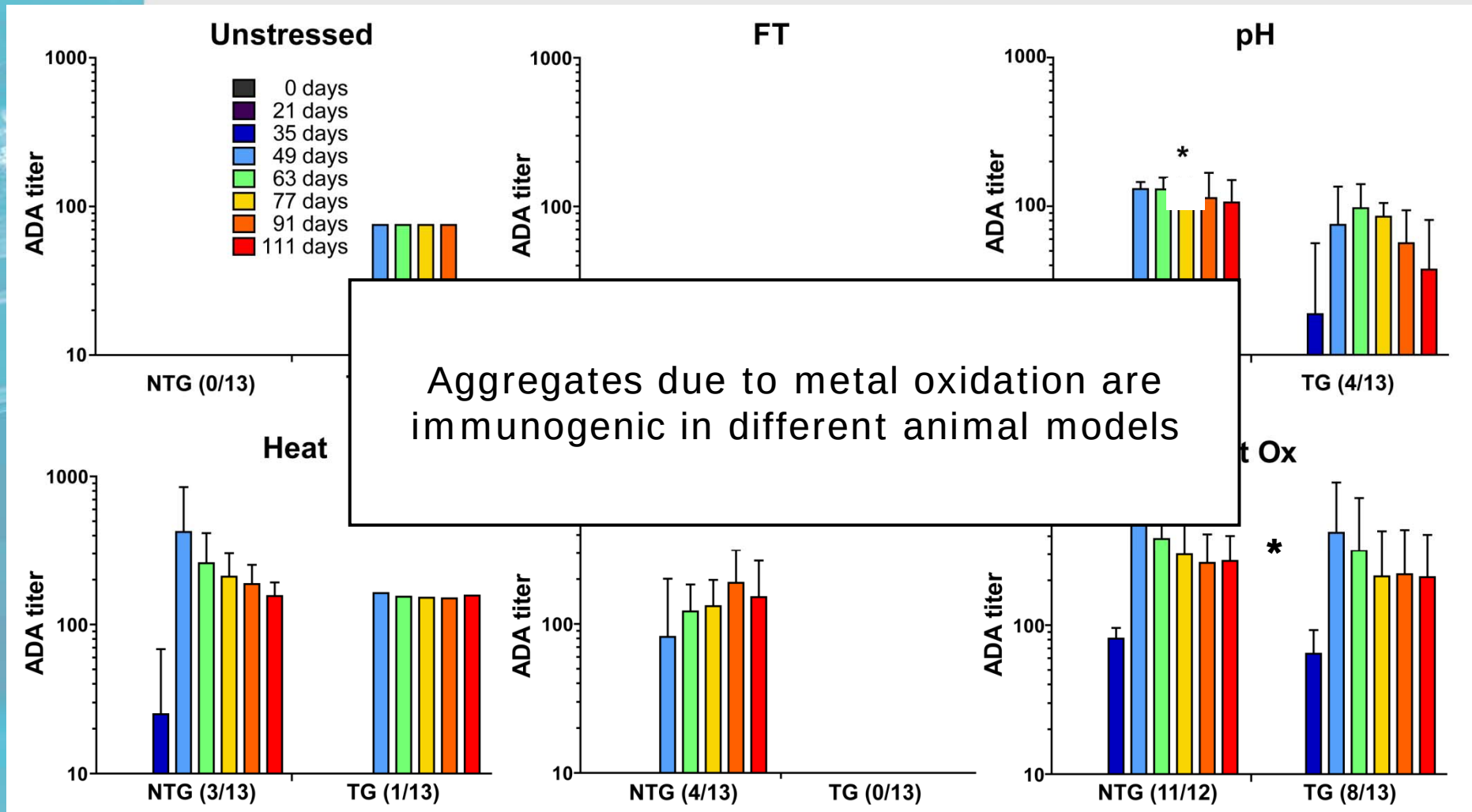
Interferon beta peptide aggregates



Peptide (aggregates) did not induce antidrug antibodies

Unpublished data

IgG1



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Filipe et al., in preparation

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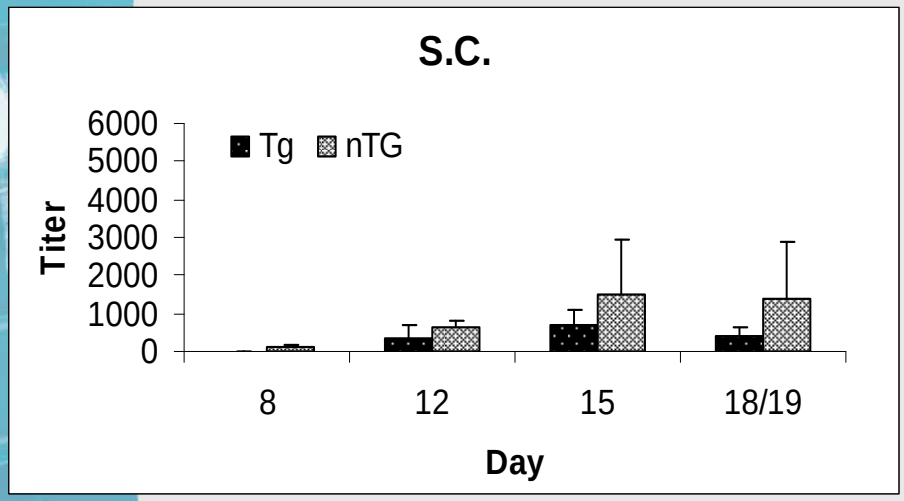
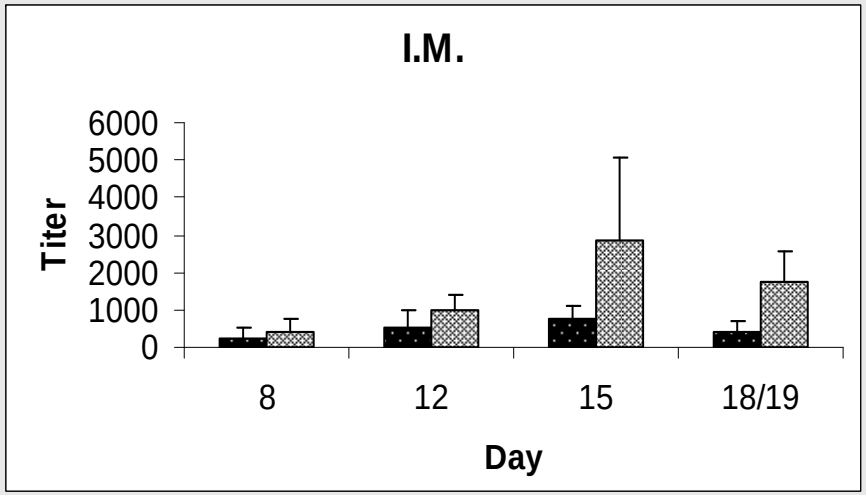
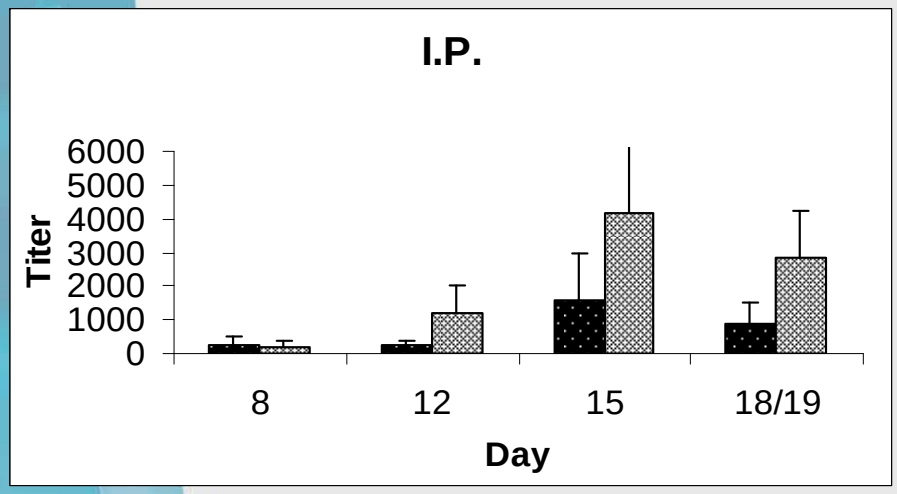
Treatment-related factors affecting immunogenicity

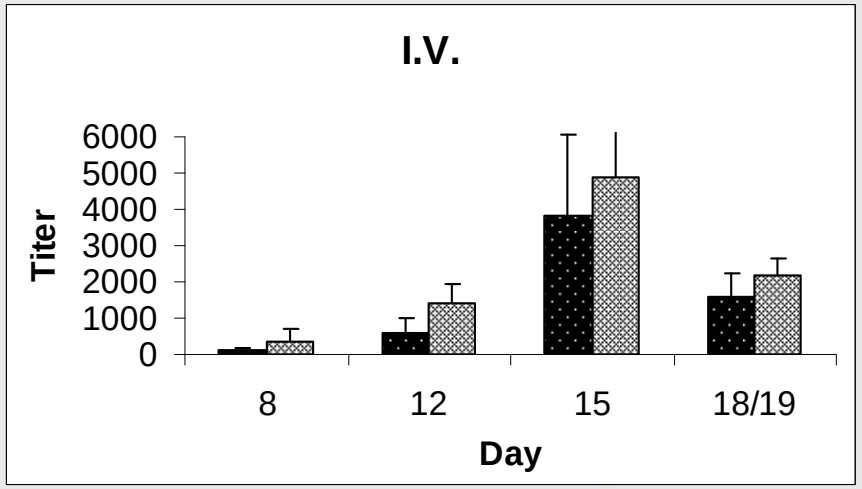
-mice tolerant for human interferon beta-

Route of administration









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Kijanka et al., in preparation

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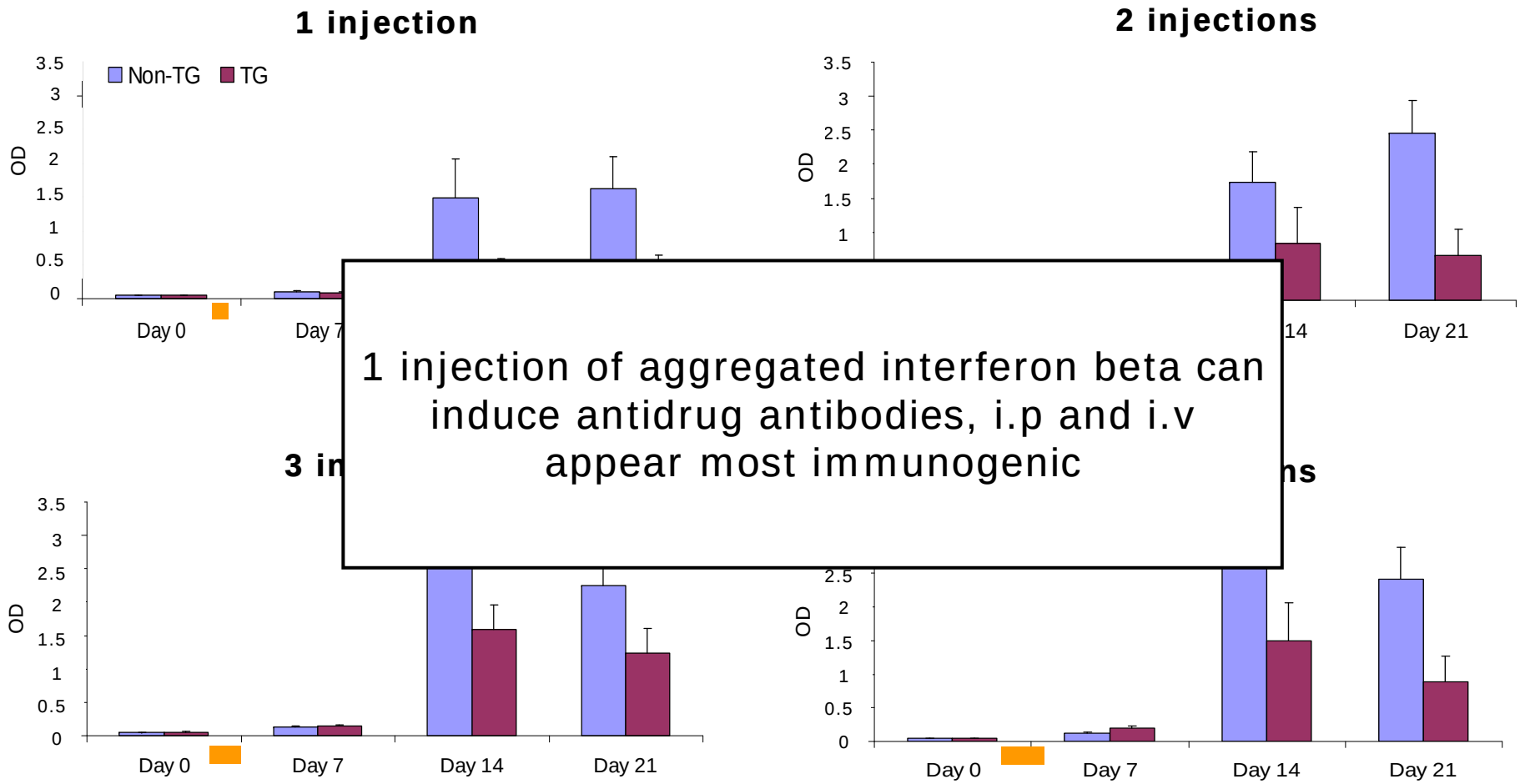
Number of injections



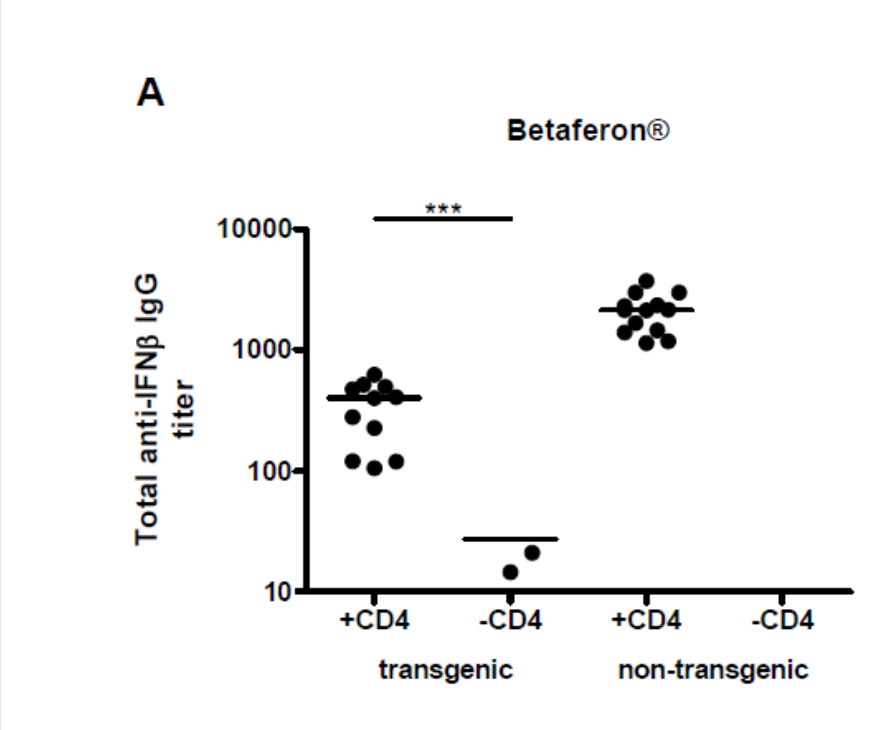
1 injection of aggregated interferon beta can induce antidrug antibodies, i.p and i.v appear most immunogenic

Kijanka et al., in preparation

Immune cells involved

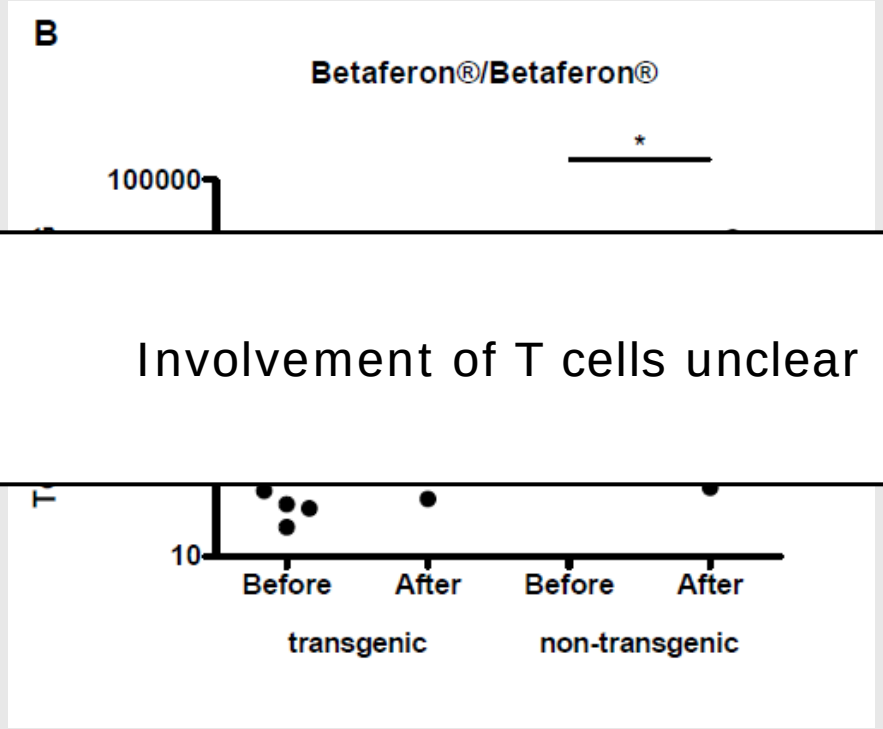
-mice tolerant for human interferon beta-

Involvement of CD4 T-cells



Sauerborn et al., in preparation

Formation of immunological memory



Involvement of T cells unclear

Sauerborn et al., in preparation

Genes / Pathways involved

-mice tolerant for human interferon beta-

Microarray experiment-*preliminary*

- Gene-expression before onset of antidrug antibody formation
  - Non transgenic and immune tolerant transgenic mice
  - 204 genes regulated
  - 13 genes regulated in immune tolerant mice
    - Innate immune system, inflammation

Kinetics of aggregates in vivo

-Will be presented by Grzegorz Kijanka-

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Summary

- Immune tolerant mouse models are used for
  - Prediction
  - Studying immune mechanisms
- Interferon alpha/beta and Ig tolerant mouse models show that:
  - (Aggregates of) “immunogenic” peptides do not give an antidrug antibody response, metal oxidized aggregates are immunogenic in different mouse models
  - 1 injection is sufficient to produce antidrug antibodies
  - i.v. and i.p. are most immunogenic
  - No classical T-cell dependent or independent mechanism
  - Gene activation points to involvement of innate immune system/ inflammation

Conclusion / Discussion

- Is the antibody response initiated by “immunogenic” peptides?
- What makes metal oxidized aggregates immunogenic?
- Breaking of tolerance
  - Aggregates are important
  - Fast
  - Effect of route of administration might be different than expected
  - Involvement of CD4 T cells in antibody production
  - Involvement of marginal B cells in antibody production
  - No apparent memory response
  - Preliminary data suggests that innate immune system might be important during initial stages (before antibody formation)

  - Involvement of CD4 T cells during initial stages of immune response? Which subsets are involved?
  - Are germinal centers formed?
  - How can the innate immune system be involved? Structural “epitopes”, dendritic cells, complement system?

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