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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



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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*



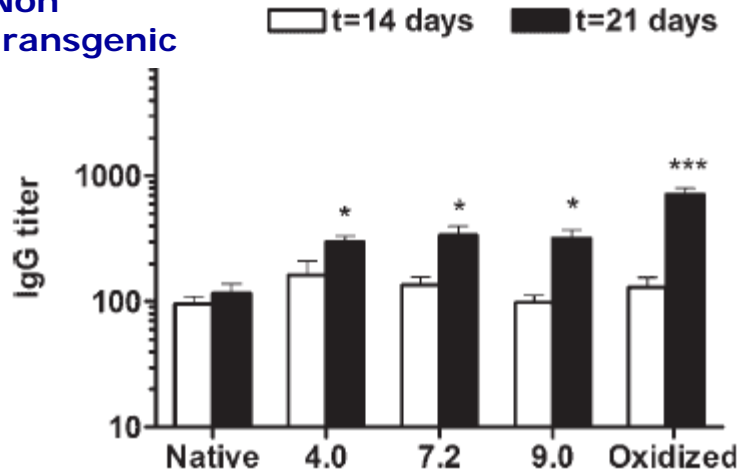
Type of aggregates

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

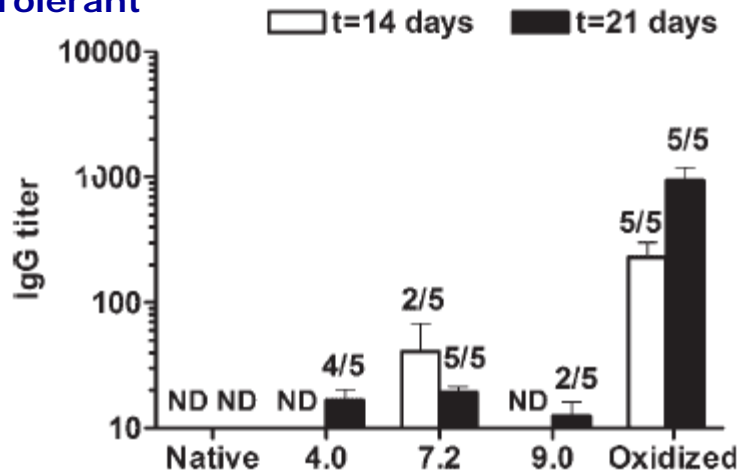


Interferon alpha

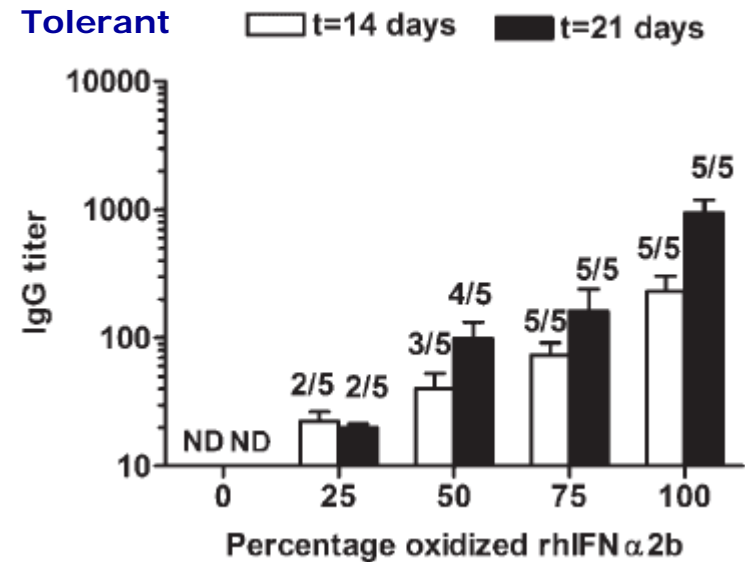
Non transgenic



Tolerant



Tolerant



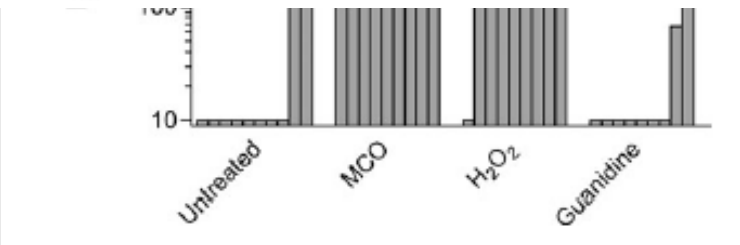
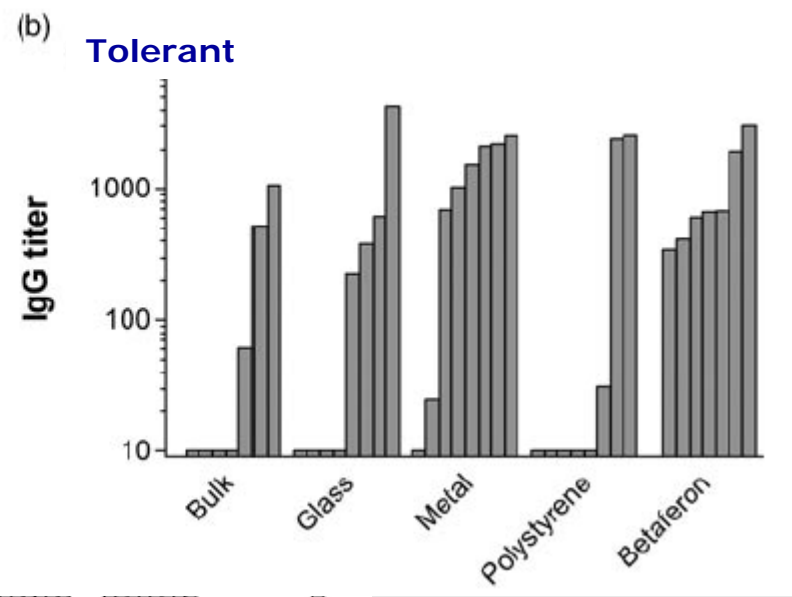
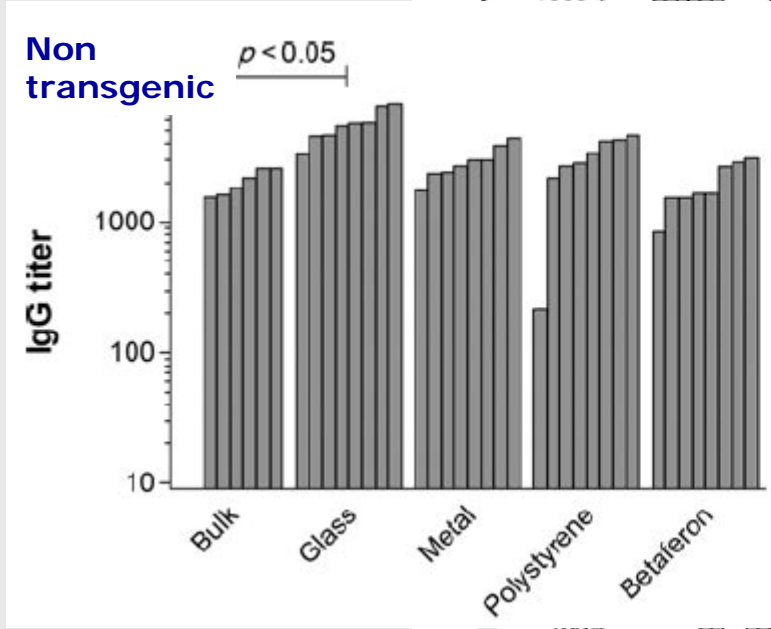
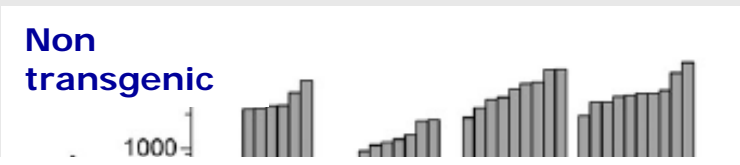
Hermeling et al., 2006

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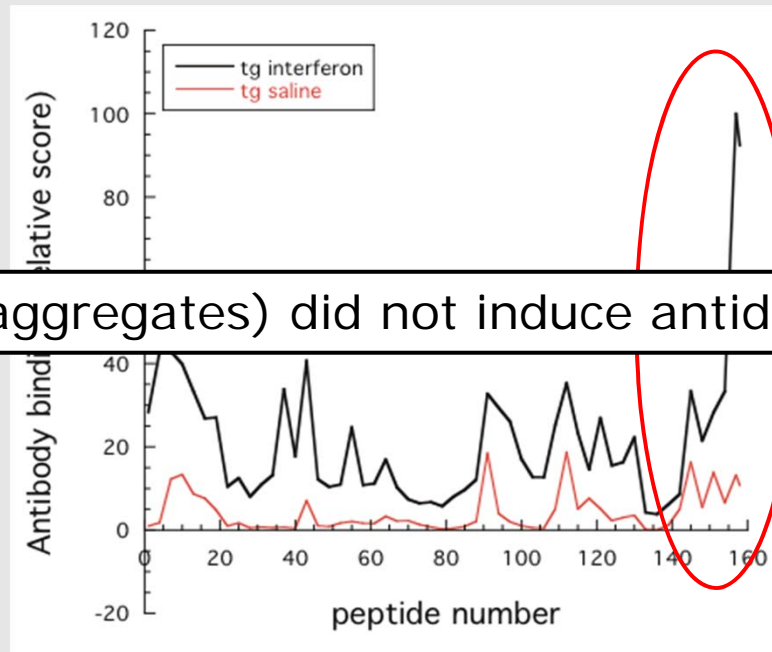
Interferon beta



Van Beers et al., 2011

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Interferon beta peptide aggregates



Peptide (aggregates) did not induce antidrug antibodies

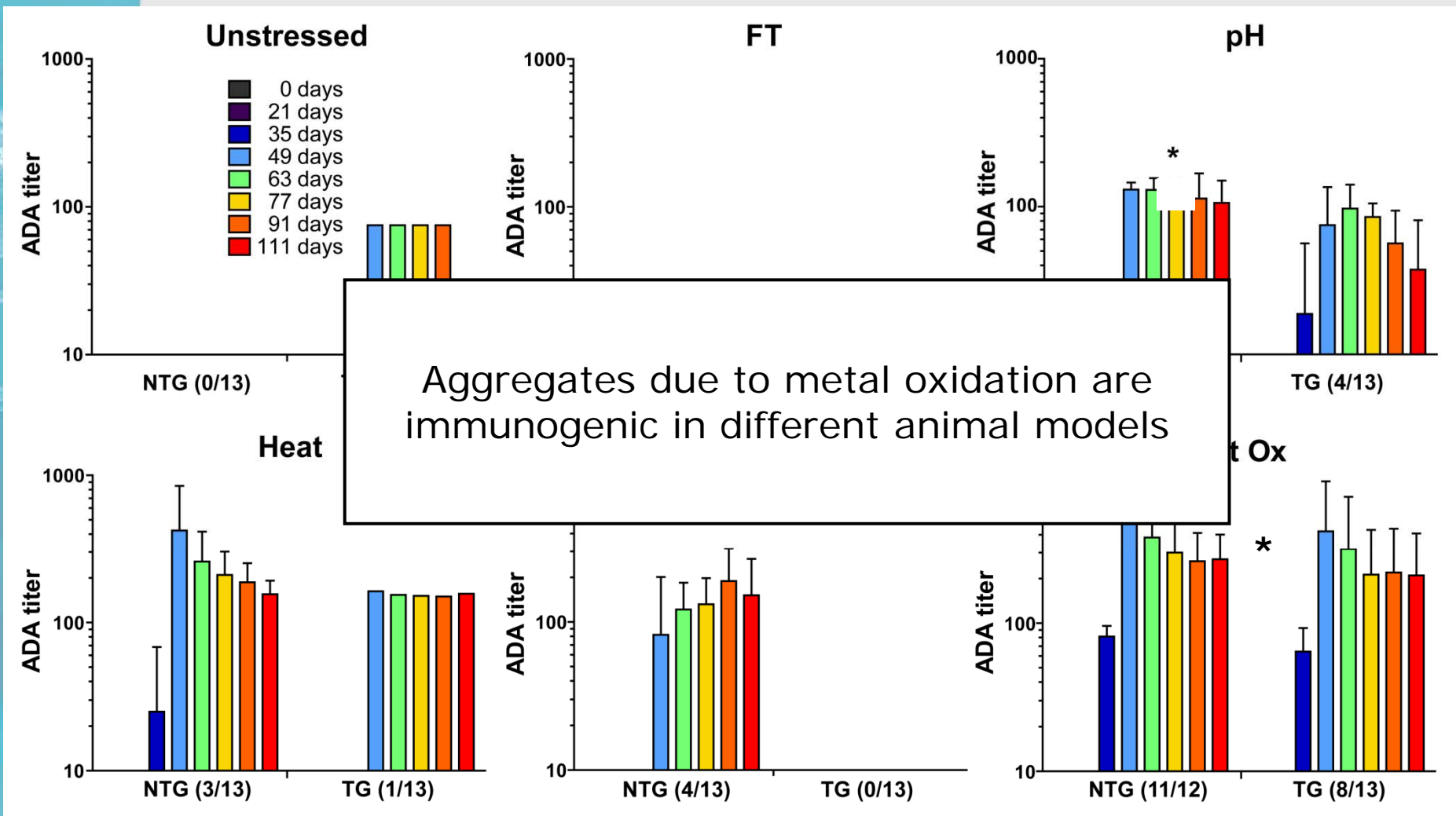
Unpublished data



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IgG1





Treatment-related factors affecting immunogenicity

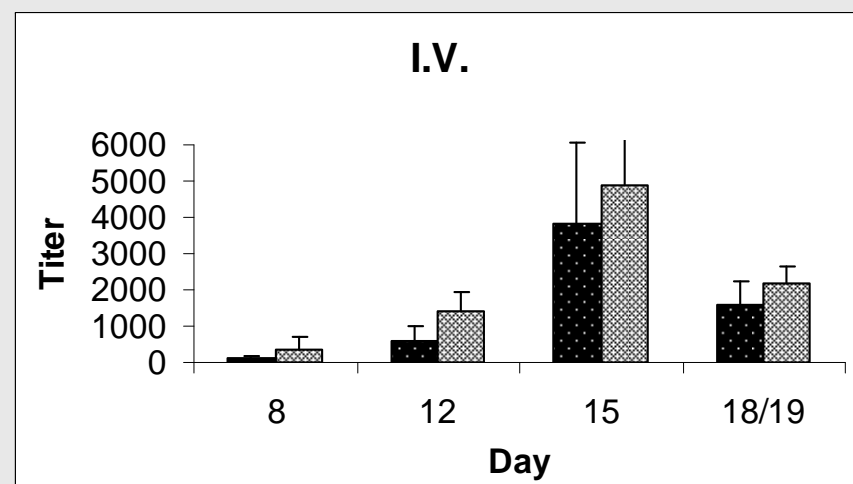
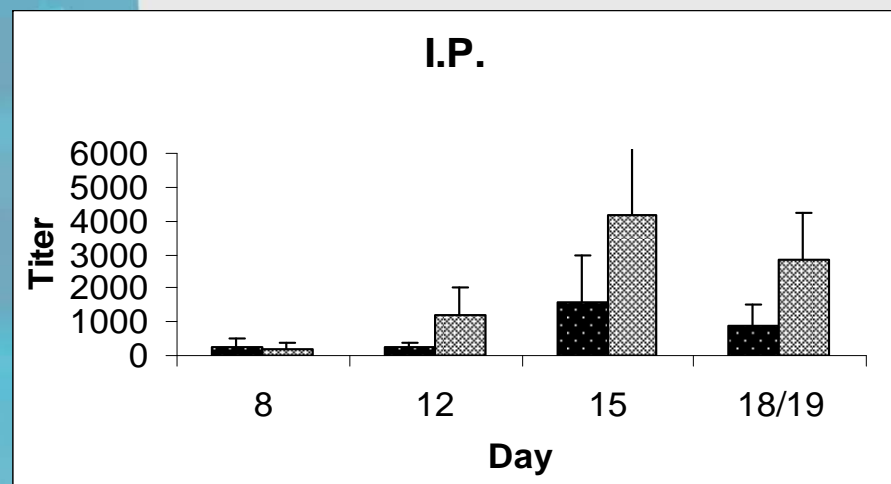
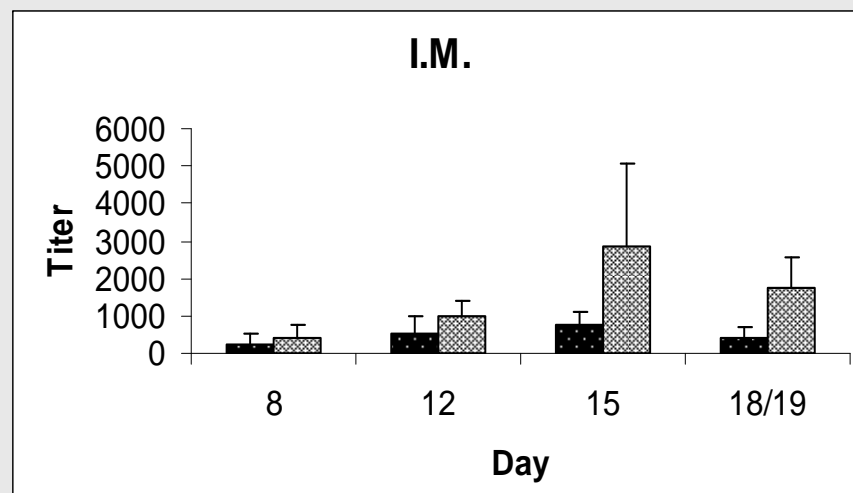
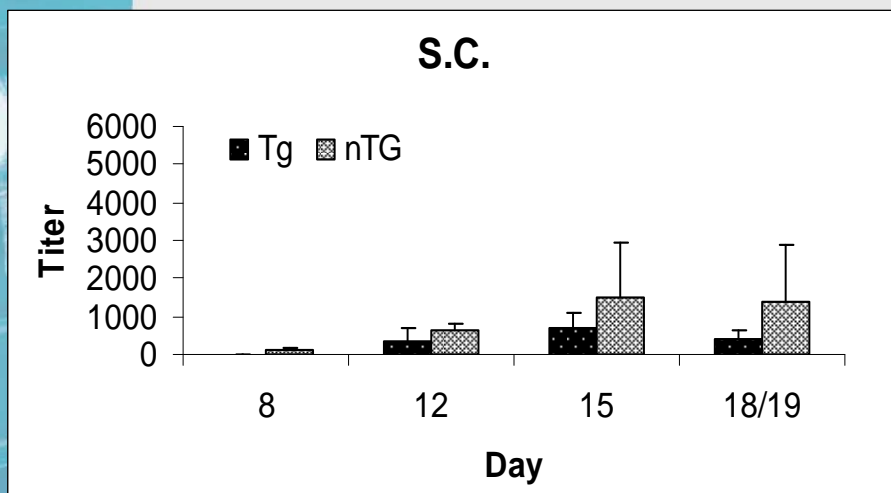
-mice tolerant for human interferon beta-



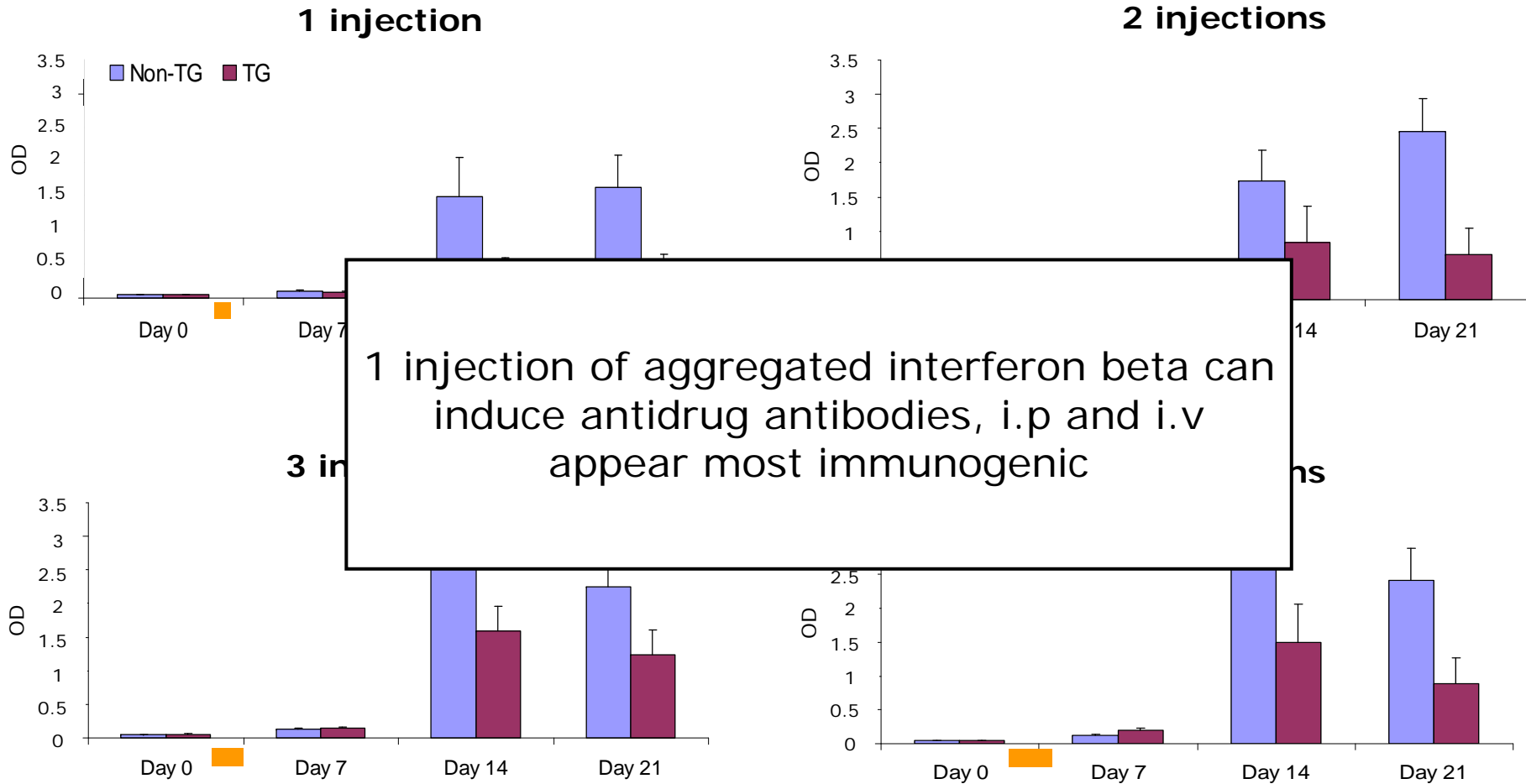
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Route of administration



Number of injections



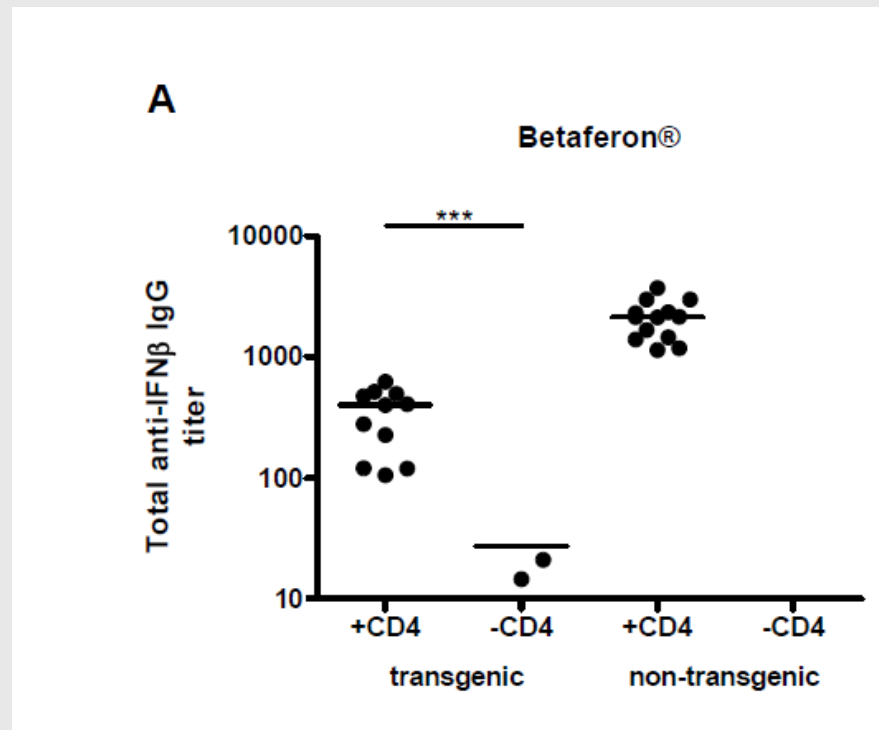


Immune cells involved

-mice tolerant for human interferon beta-



Involvement of CD4 T-cells



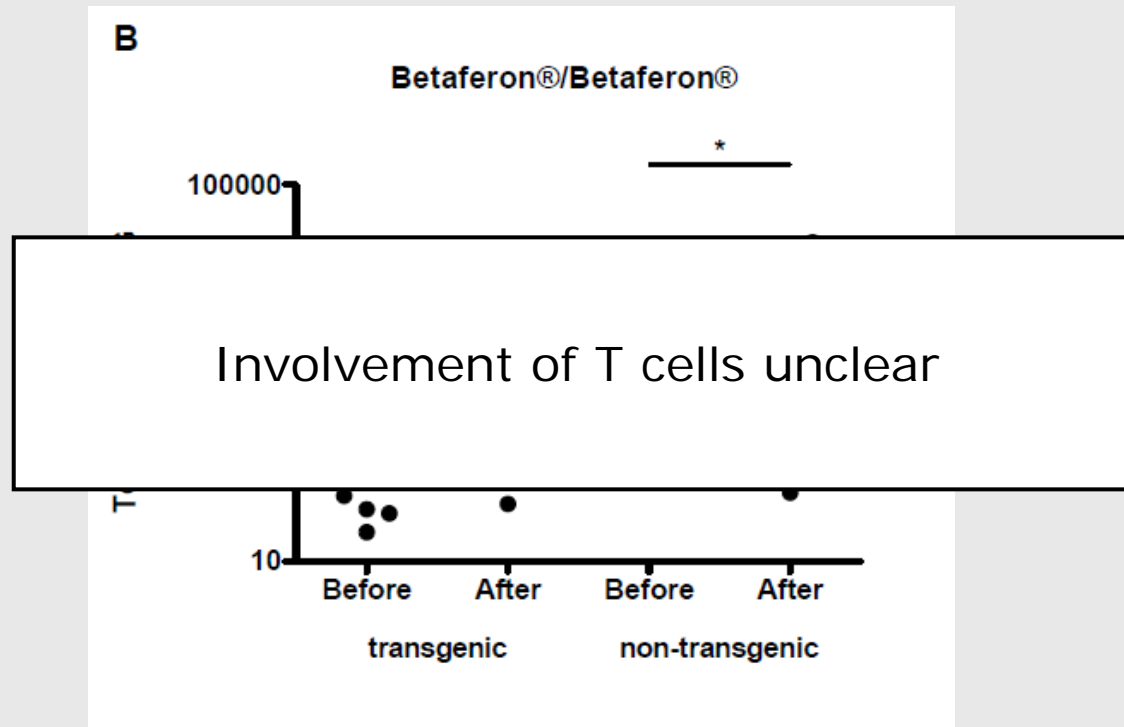
Sauerborn et al., in preparation



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Formation of immunological memory



Sauerborn et al., in preparation



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Genes/Pathways involved

-mice tolerant for human interferon beta-



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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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