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Immune mechanisms underlying immunogenicity of therapeutic proteins in immune tolerant mice.

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26-02-2013**

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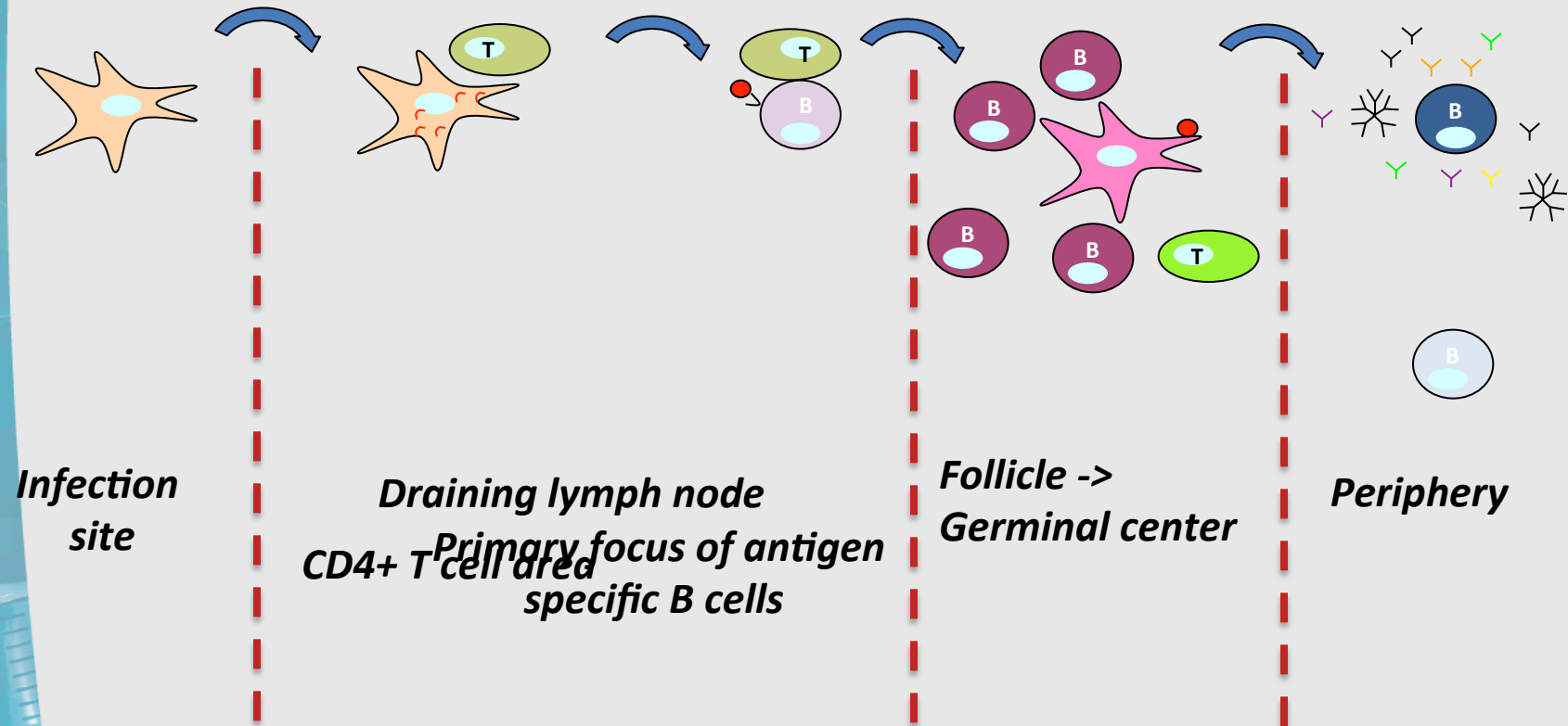
Mechanism of immunogenicity - background.



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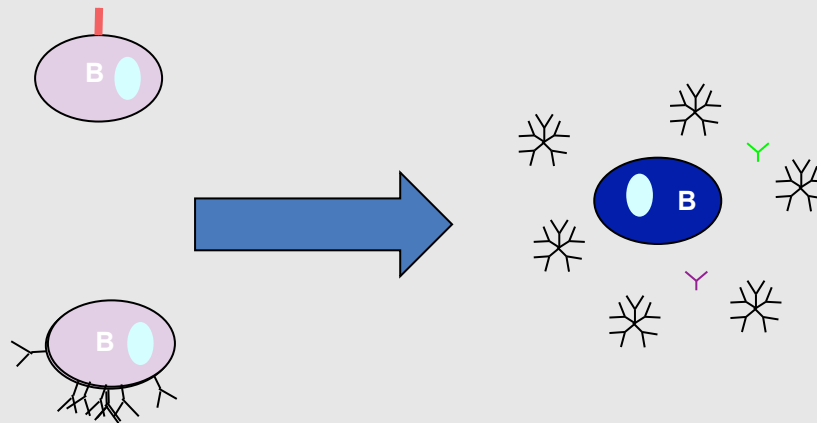
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T cell dependent immune response.



T cell independent immune response.

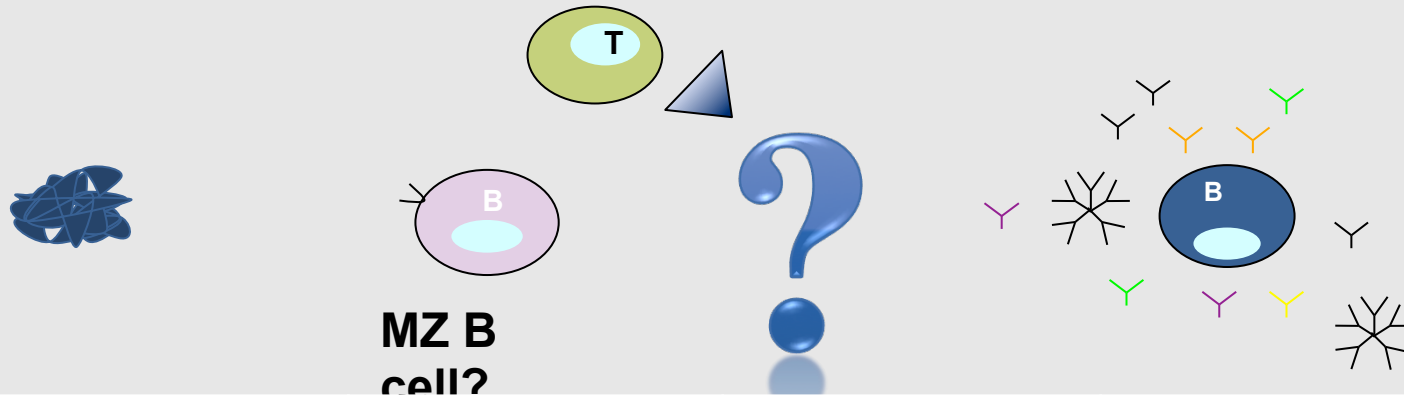
CpG,
LPS



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Anti recombinant human protein antibody response.



	TD	TI	Immunogenicity
<i>Antigen</i>	Foreign protein	Bacterial/Viral molecules	Rh proteins (aggregated)
<i>Abs development</i>	Within days	Within days	From days to months
<i>MZ B cells</i>	Not crucial	Crucial	Important
<i>Isotype switching</i>	Yes	Limited	Yes
<i>Affinity maturation</i>	Yes	No	Yes (neutralizing antibodies)
<i>GCs formation</i>	Yes	Impaired	?
<i>(Direct) Involvement of CD4⁺ T cells</i>	Yes	No	Yes (?)
<i>Memory formation</i>	Yes	No	No (?)
<i>Susceptibility to adjuvants</i>	Yes	No	?

Aim of study: To better understand the processes undergoing during immunogenicity reaction.



Animal and protein model.

Animals:

C57Bl/6xFVB/N Tg immune tolerant for hIFN β

Interferon β :

- Betaferon[®] - highly aggregated, containing HSA
- Avonex[®] - low content of aggregates, HSA-free



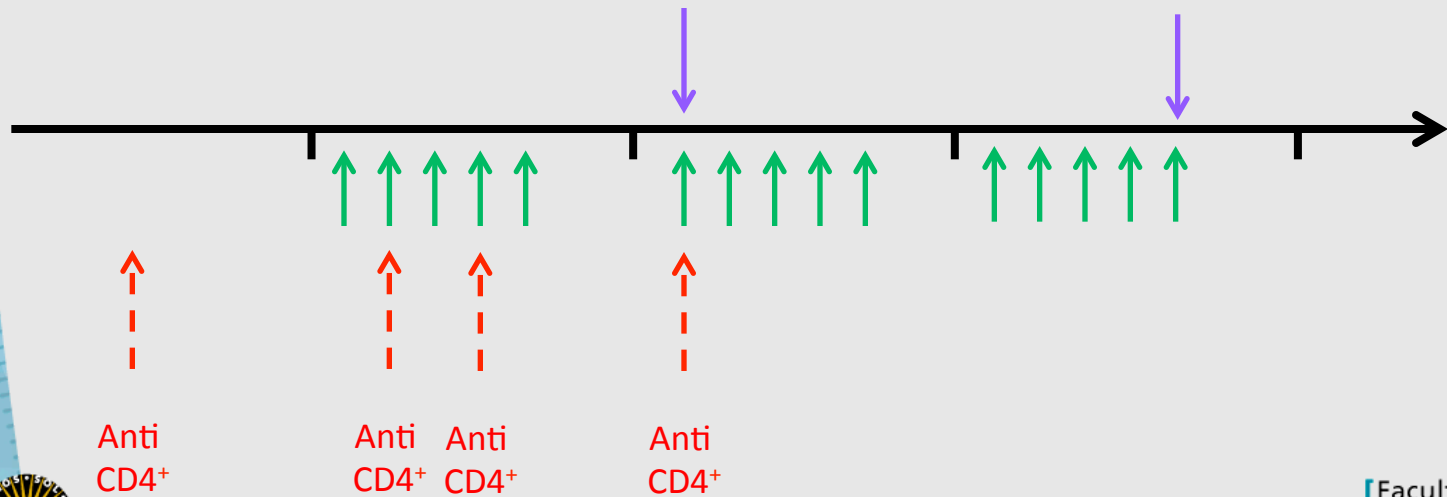
1. Time of CD4⁺ T cell recruitment in ADA production against therapeutic interferon beta

Mice:

TG and Non-TG

Treatment:

- Betaferon[®] 5 µg/injection, 5 inj/week for 3 weeks
- Anti CD4 starting at different time points



Anti
CD4⁺

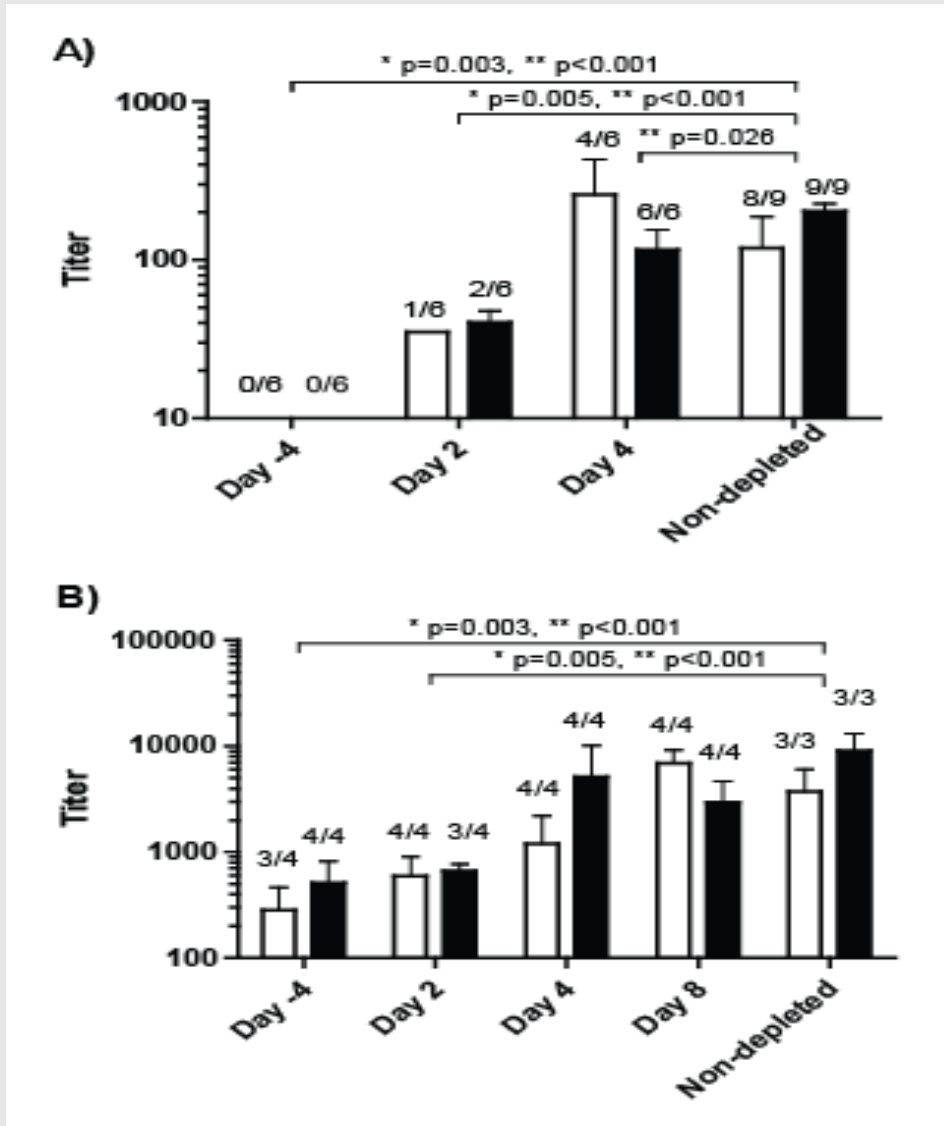
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Anti Anti
CD4⁺ CD4⁺

Anti
CD4⁺

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1. Time of CD4⁺ T cell recruitment in ADA production in immune tolerant mice.



Day 8

Day 19

* Tg
** non-Tg

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2. Formation of Germinal Centers (GCs) during ADA response.

Mice:

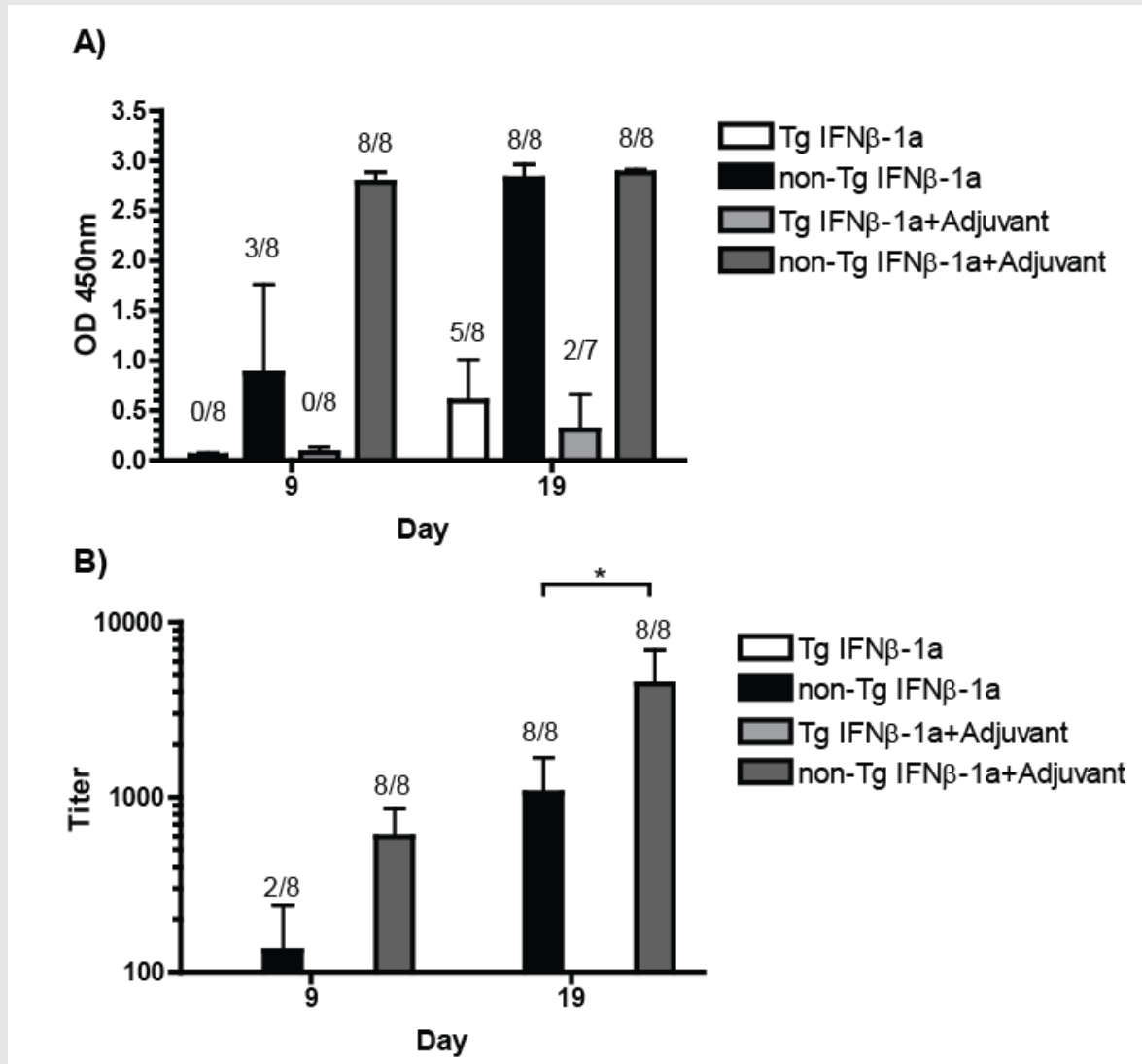
TG and non-TG

Treatment:

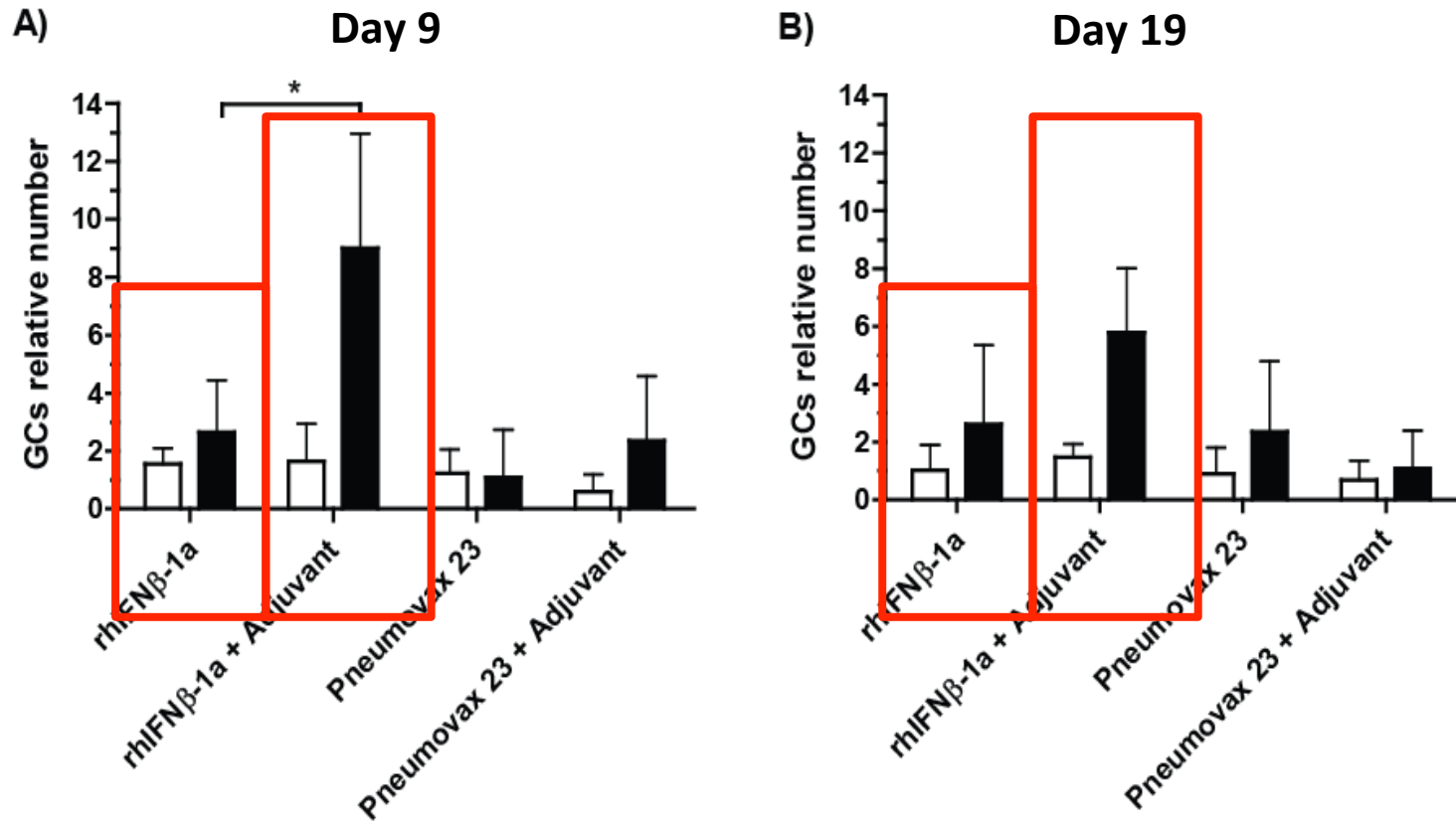
- Avonex[®] 5 µg/injection, 5 inj/week for 3 weeks
- Avonex[®] 5 µg/injection + adjuvant (Aluminum hydroxide),
- Pneumovax[®] 23 1 µg/injection, days 1 and 10
- Pneumovax[®] 23 1 µg/injection + adjuvant, days 1 and 10
- Saline



2. Formation of GCs – Antibody response.



2. Formation of GCs – GCs formation.



3. Memory formation upon enhanced exposure to protein antigen.

Mice:

TG and non-TG

Treatment:

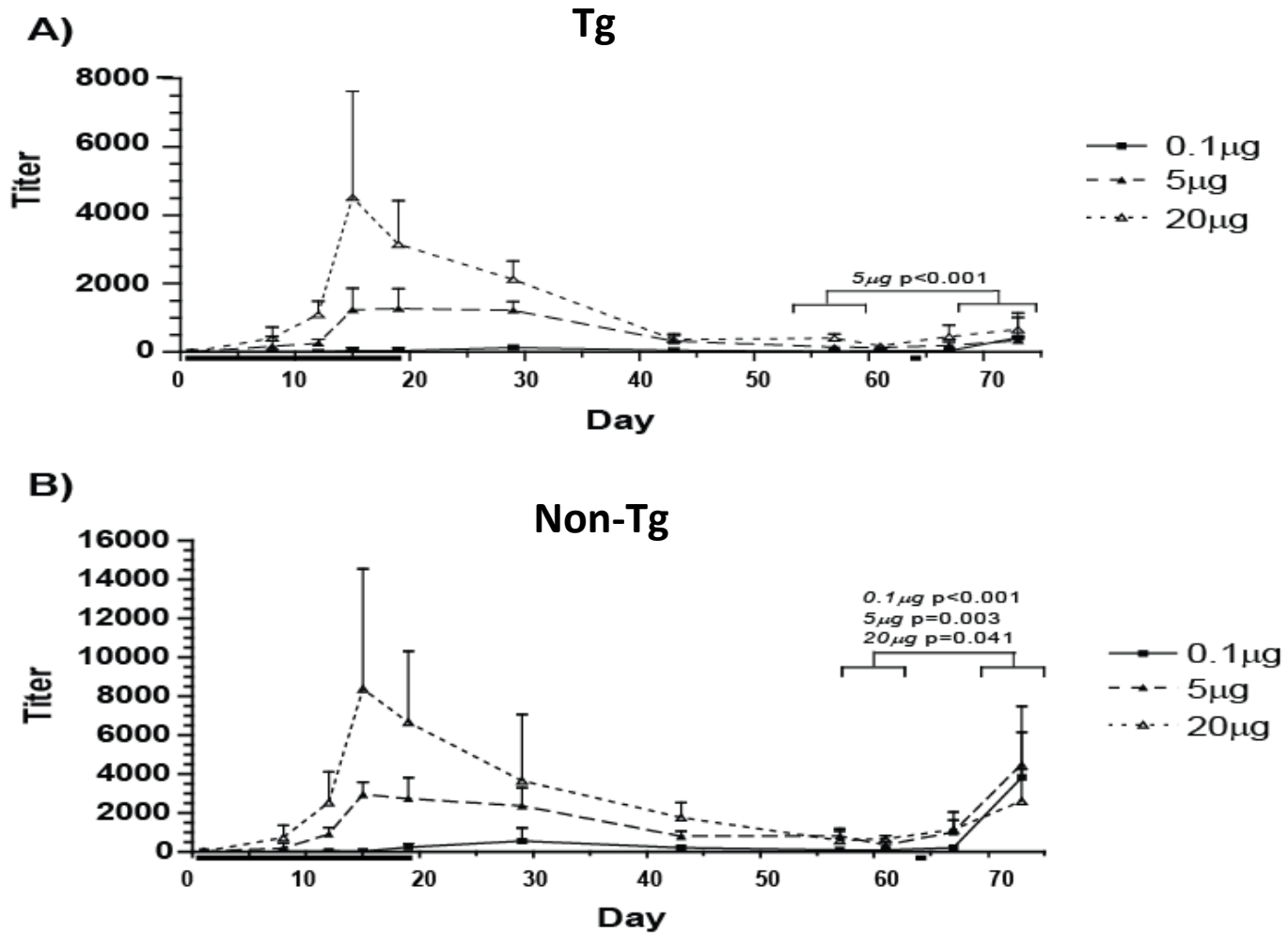
Betaferon® 0.1, 5, 20 µg/injection

Primary injections: 5 inj/week for 3 weeks

Re-challenge after 6 weeks interval: 2 inj , 2 days apart (5µg dose)



3. Memory response upon enhanced exposure to antigen.



4. The impact of adjuvants on the immunogenicity of therapeutic interferon beta.

Part 1:

Mice: TG and non-TG

Treatment: Avonex® (HSA-free), 5ug/ injection, 5 inj/week for 3 weeks

Adjuvants

- 1) CFA/IFA
- 2) Saponin
- 3) Montanide ISA 50 V2
- 4) Aluminum hydroxide
- 5) Control (IFN β without adjuvant)

Part 2:

Mice: TG and non-TG

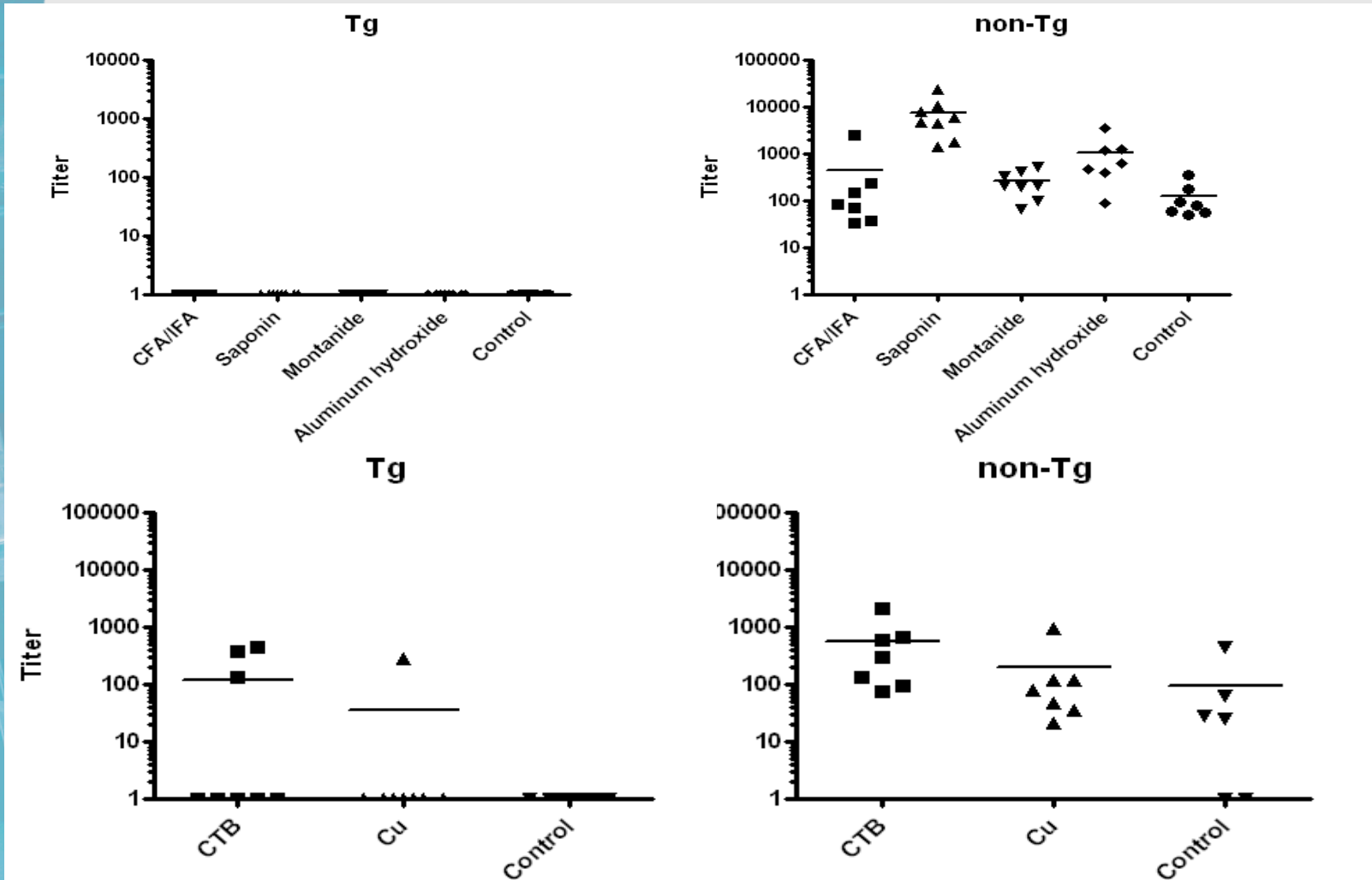
Treatment: Avonex® (HSA-free), 5ug/ injection, 5 inj/week for 3 weeks

Adjuvants

- 1) CTB
- 2) Copper 0,04M
- 3) Control



4. The impact of adjuvants on the immunogenicity of therapeutic interferon beta.



Conclusions:

1. To the point of isotype switching immunogenicity reaction seems to be very similar to T cell dependent response, but magnitude of ADA production is lower.
2. The “late” processes like memory formation seem to be inhibited.
3. Adjuvants do not increase response in Tg animals, except CTB.



Acknowledgements

■ Department of Pharmaceutics

Huub Schellekens

Vera Brinks

Andhyk Halim

Melody Sauerborn (TNO)

■ Bioceros

Louis Boon

■ GDL

Anja van der Sar

Tamara van den Heiligenberg



Thank you for attention!

