The new mouse model tolerant to hIFNβ

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Promoter: Prof. Huub Schellekens

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Therapeutic proteins
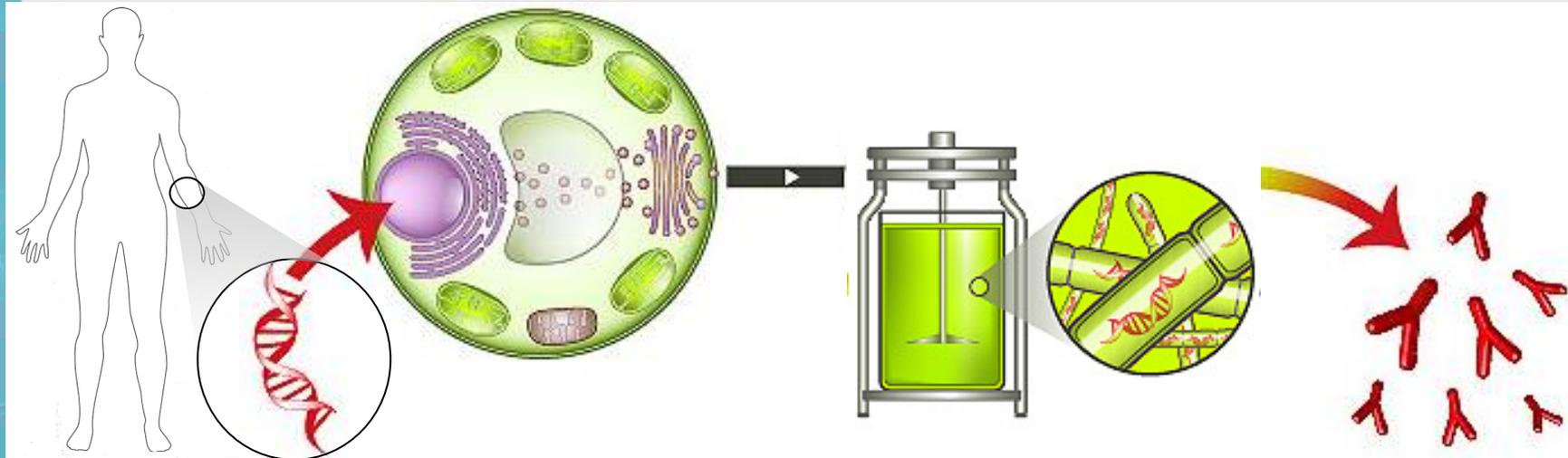
- **Pharmaceutically useful protein to cure deficiency/defect**

- **Engineered "human" protein in the living cells for pharmaceutical use**

- **Various cancer, multiple sclerosis, rheumatoid arthritis, anemia...**

- **Fast growing class drug > 30%**

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"They" Are Immunogenic....

Class	Substance	Indication	Reactivity (%)
Antibodies	Anti-CD3 (OKT3)	Immunosuppressant	<1%
	Anti-Her2	Mamma-tumor	<1%
	Anti-IgE	Allergic asthma	<1%
	Anti-Il-2R	Immunosuppressant	18%
	Anti-TNF-α	RA, M. Crohn	10%
Receptors	CD4	HIV	<1–12%
	TNF receptor	Multiple sclerosis	16%
	Il-1 receptor	Leukemia	<1%
Cytokines	Il-2	Tumor	52%
	Il-3	Tumor	>80%
	Il-12	HCV	<1%
Interferons	IFN-α2a	HCV	27–60%
	IFN-β	Multiple sclerosis	45%
Enzymes	Factor VIII	Hemophilia	10–30%
	DNase	Cystic fibrosis	9%
	Plasm.-activator	Ischemia	<1%
Hormones	Insulin	Diabetes	44–60%
	HGH	Growth	16%
	G-CSF	Neutropenia	4%
	GM-CSF	Tumor	25–80%
	EPO	Anemia	<1%

Factors Influencing Immunogenicity
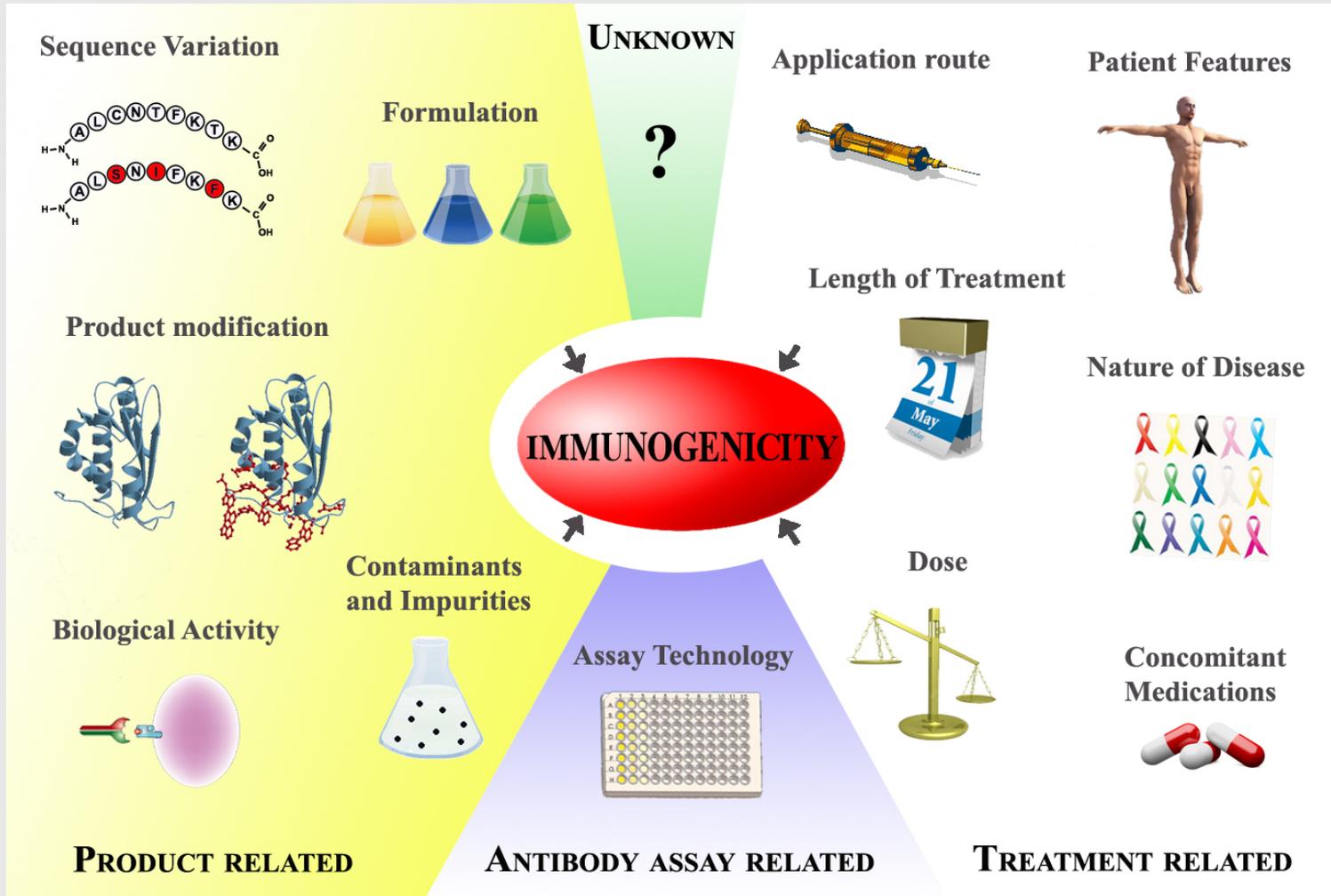
Possible mechanisms induce Ab response...

Classical Immune Response	Breaking of Tolerance
■ Immune response against foreign proteins	■ Therapeutic protein is similar to endogenous protein
■ Formation of immunological memory (vaccination – like effect)	■ Absence of immunological memory
■ Rapid Ab formation	■ Slow antibody formation
■ Long half-life	■ Initiated after repeated exposure
	■ Disappear after (during) treatment

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Recombinant Human Interferon Beta (rhIFNβ) Products

■ First line treatment for relapsing remitting multiple sclerosis

■ Betaferon : 50% NAb
■ Avonex : >5% NAb
■ Rebif : 30% NAb

Treatment effectiveness
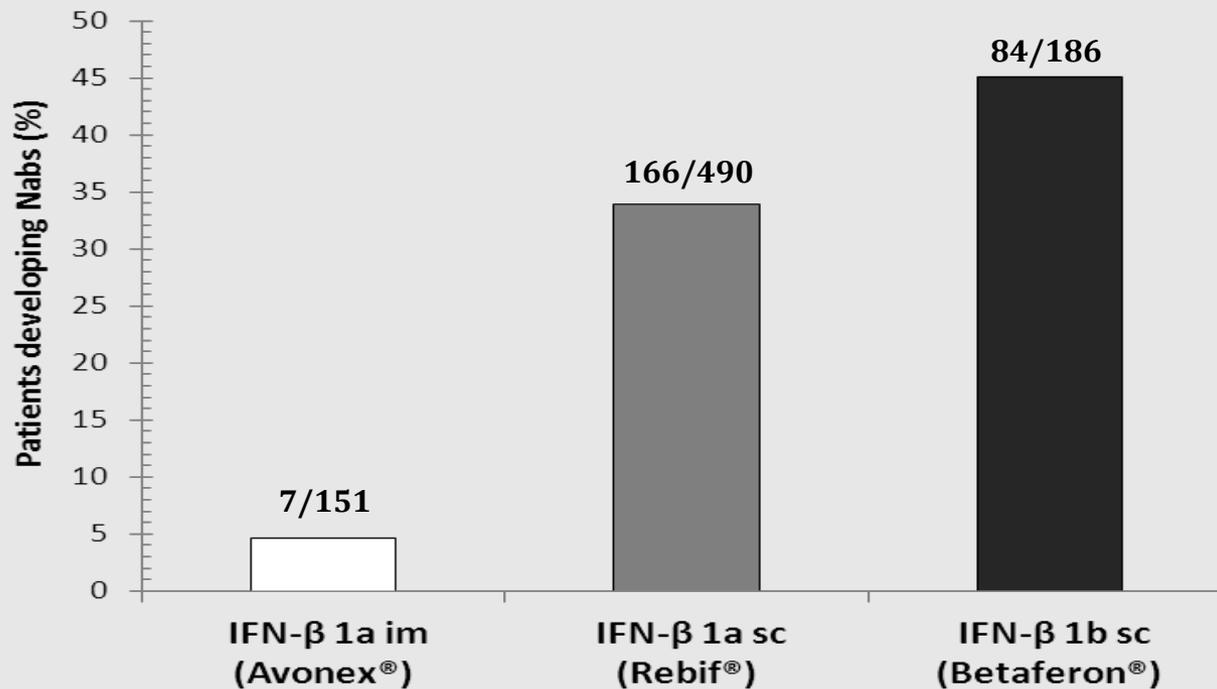
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Previous data with the transgenic mouse model……

■ Immunogenicity of
- Rebif
- Avonex
- Betaseron

Rebif   ### Avonex   ### Betaseron

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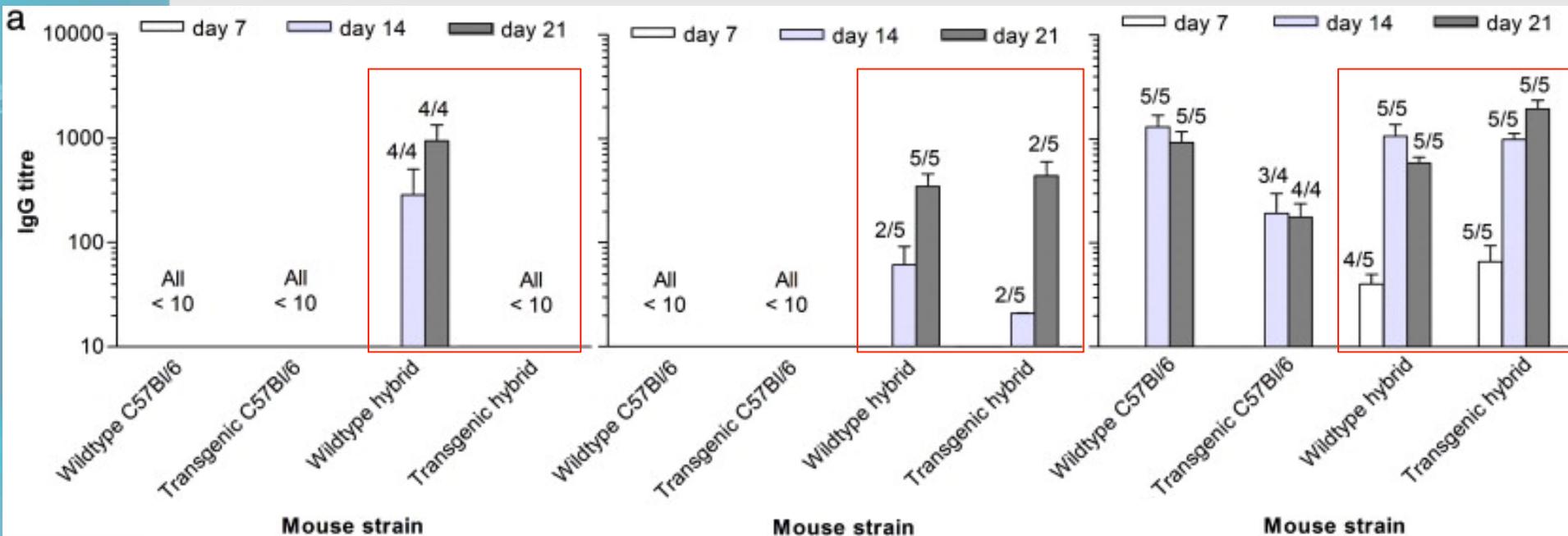
The current mouse models

1) Transgenic C57Bl/6 (Hermeling et al. 2005)

- Immune tolerant for hIFNβ
- Not Suitable to study breaking of tolerance

2) Transgenic C57Bl/6 mice x wild type FVB/N mice (van Beers et al. 2010)

- Immune tolerant for hIFNβ
- Suitable to study breaking of tolerance
- Genetic variability between sibling
- Not sensitive enough to study alterations in structure, formulation or aggregation

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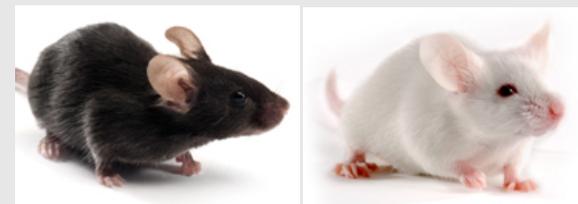
Transgenic FVB/N mice

Generation	Backcross	% of genome
F1	tg ♂ x wt ♀	50% C57Bl/6-50%FVB/N
F2	tg ♂ F1 x wt ♀	25% C57Bl/6- 75%FVB/N
F3	tg ♂ F2 x wt ♀	12.5% C57Bl/6- 87.5%FVB/N
F4	tg ♂ F3 x wt ♀	6.25% C57Bl/6- 93.75%FVB/N
F5	tg ♂ F4 x wt ♀	3.12% C57Bl/6- 96.88%FVB/N
F6	tg ♂ F5 x wt ♀	1.56% C57Bl/6- 98.44%FVB/N
F7	tg ♂ F6 x wt ♀	0.78% C57Bl/6- 99.22%FVB/N
F8	tg ♂ F7 x wt ♀	0.39% C57Bl/6- 99.61%FVB/N
F9	tg ♂ F8 x wt ♀	0.19% C57Bl/6- 99.81%FVB/N
F10	tg ♂ F9 x wt ♀	0.1% C57Bl/6- 99.9%FVB/N

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Animal experiment

DAY

| 1 | 2 | 3 | 4 | 5 | | 8 | 9 | 10 | 11 | 12 | | 15 | 16 | 17 | 18 | 19 | | 22 |
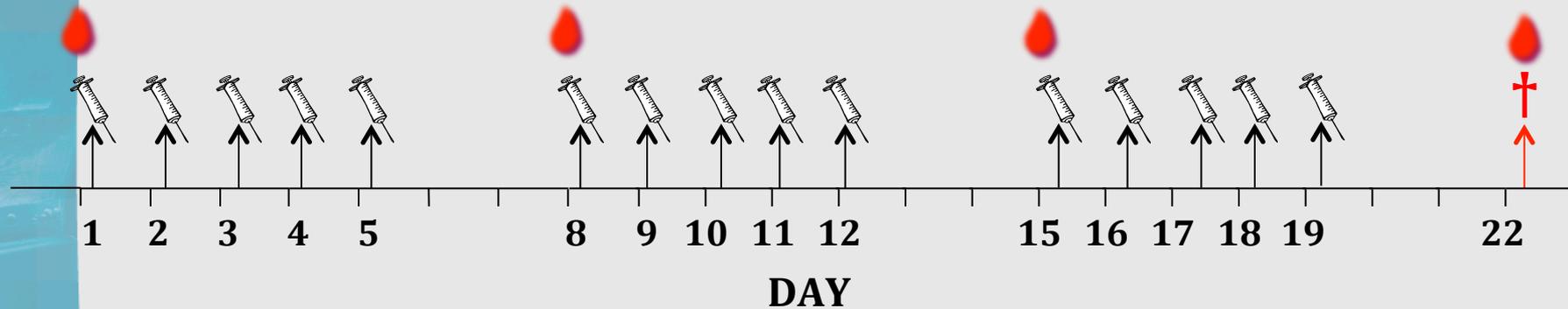
Animal:

n=4   **C57Bl/6**

            C57Bl/6 x FVB/N

            FVB/N

Treatment:

Both Tg and wild type(control) were treated with:

    Betaseron   **: 5 μg IFNβ + 166.5 μg HSA / i.p. injection**

    Avonex   **: 5 μg IFNβ / i.p. injection**

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Wild type / Avonex

Tg / Avonex

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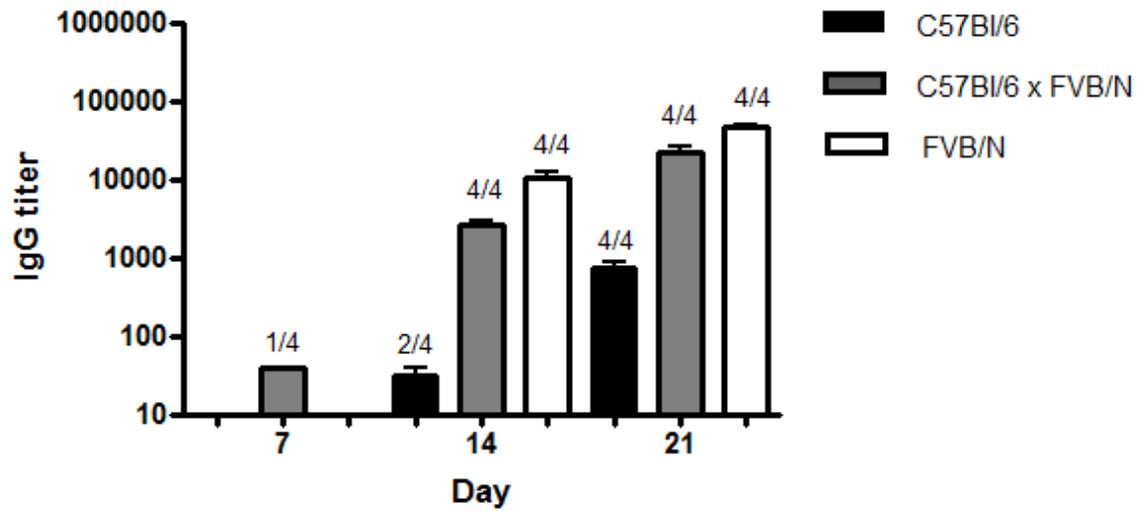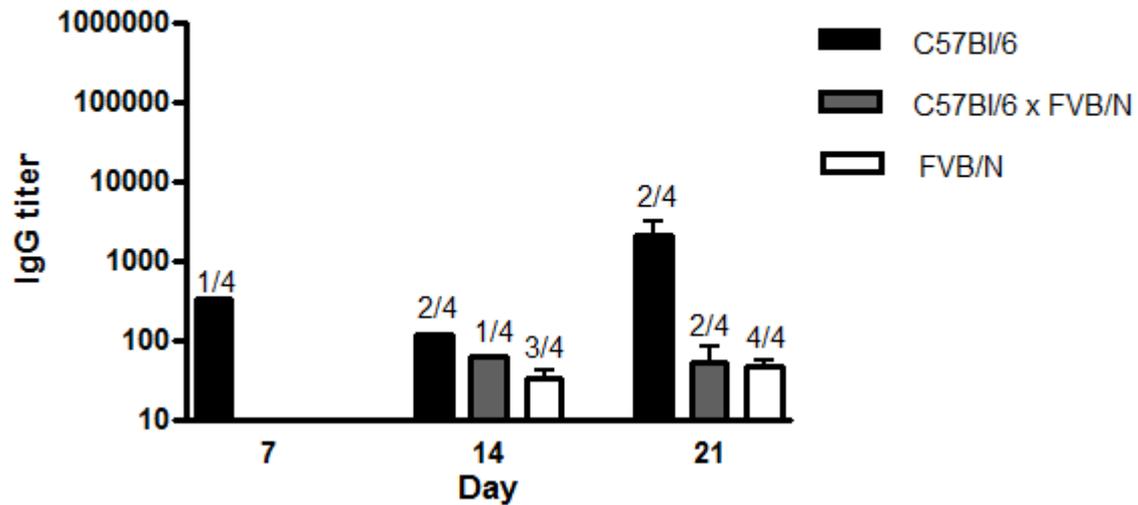
Wild type / Betaferon®
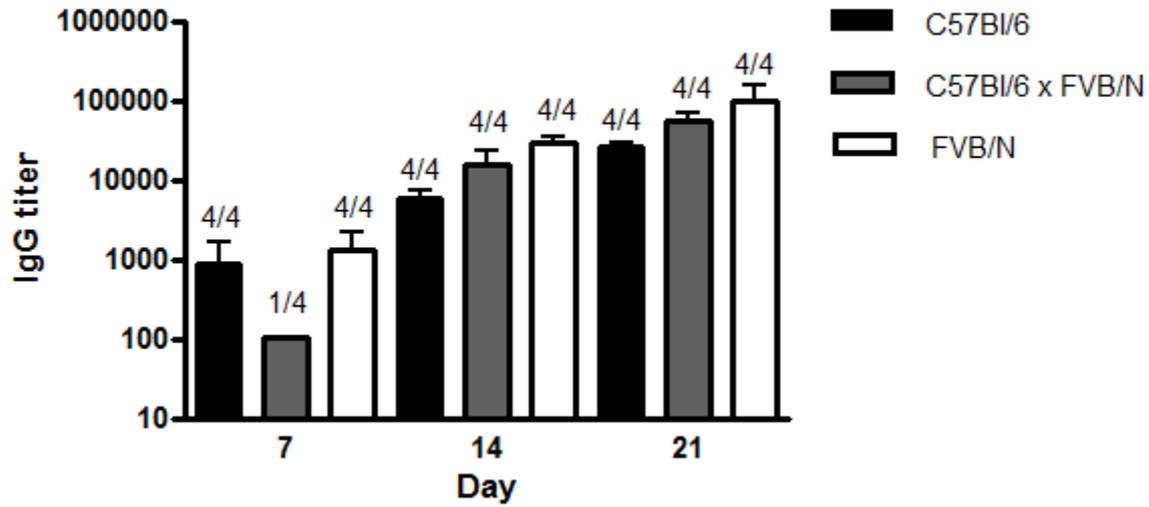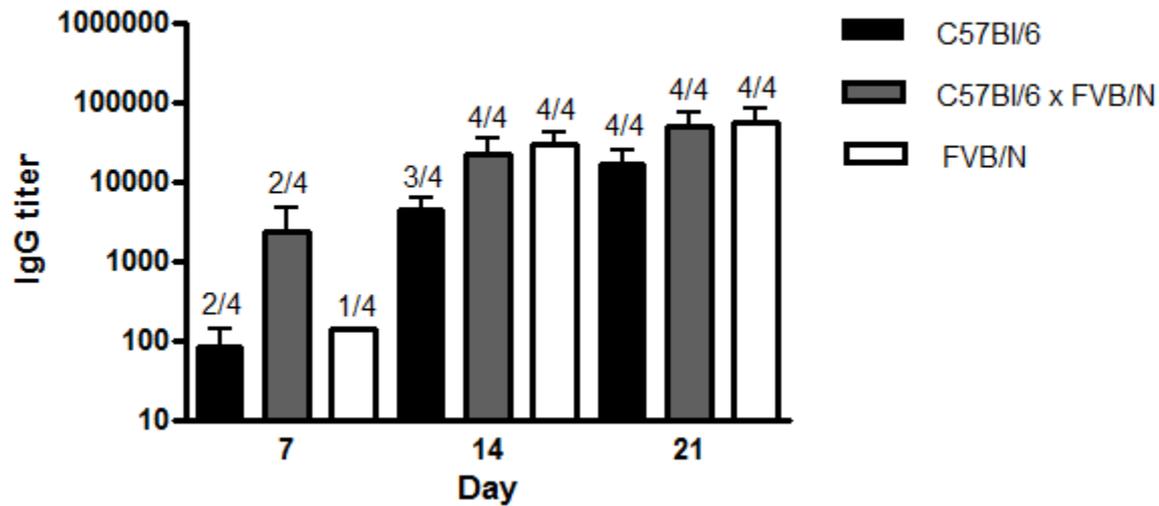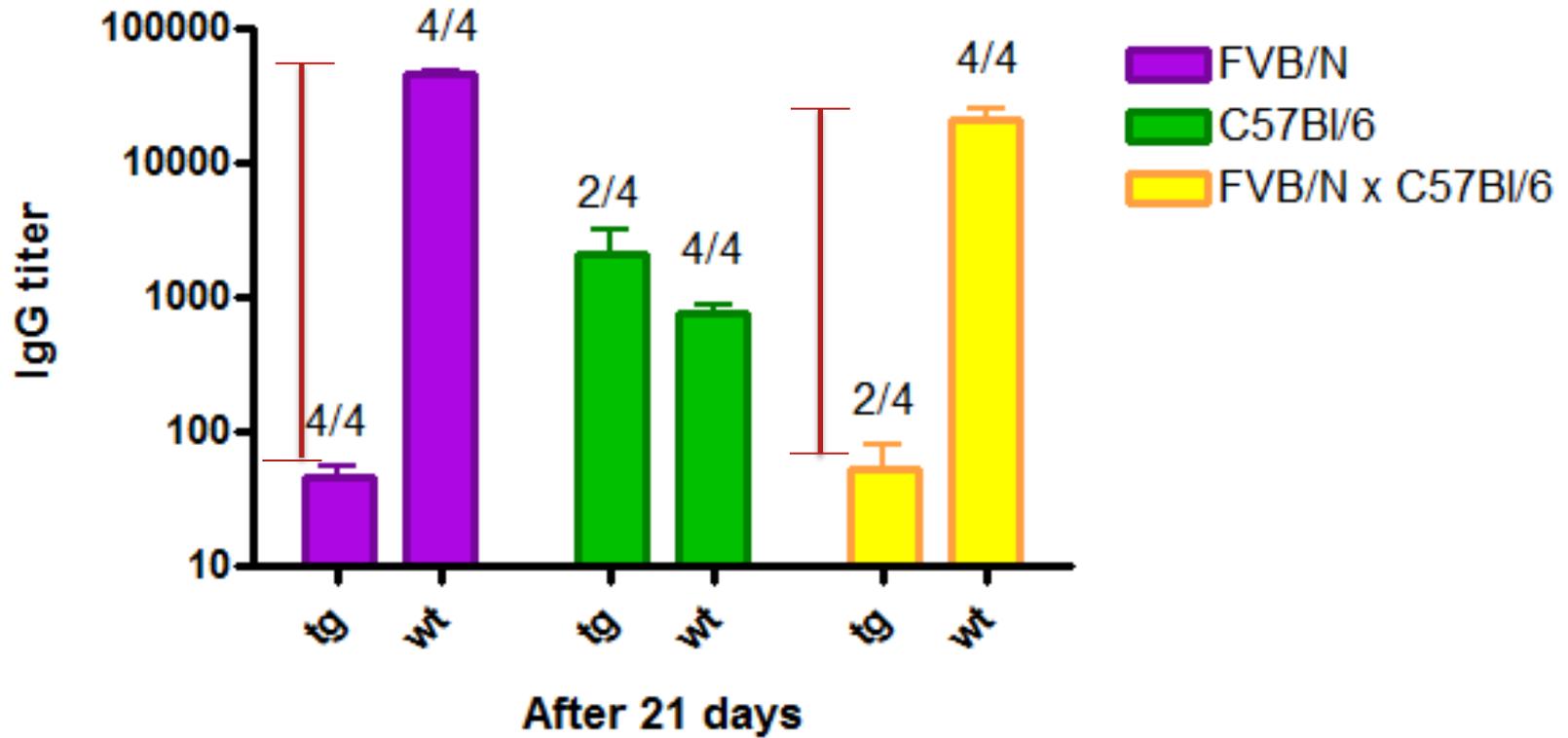
Tg / Betaferon®
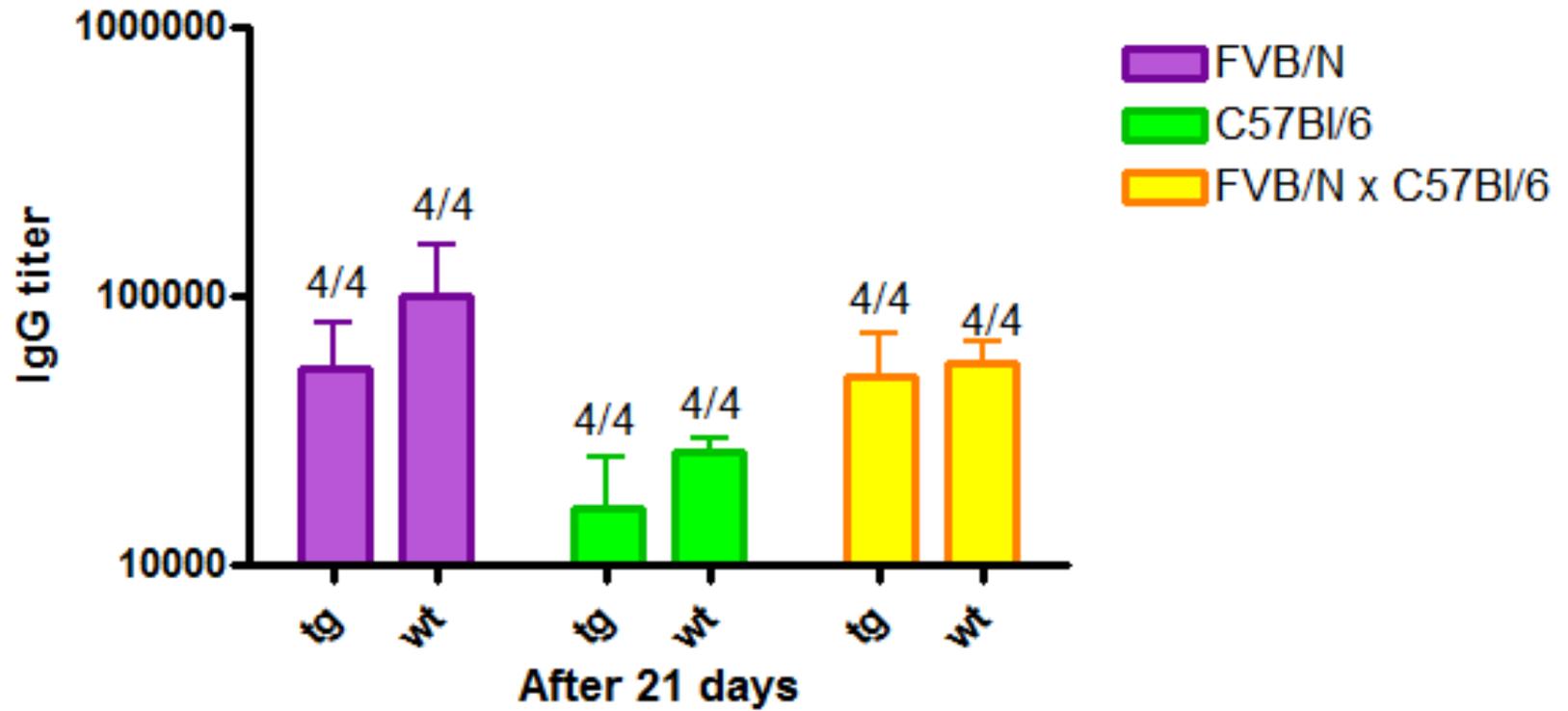
Transgenic FVB/N mice

- Suitable to study breaking of tolerance

- Therapeutic interferon beta is biologically inactive

→ Makes it possible to study immunogenicity without many

  confounding factors

- Sensitive enough to study alterations in structure, formulation

  or aggregation

- The same antibody response between sibling

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