

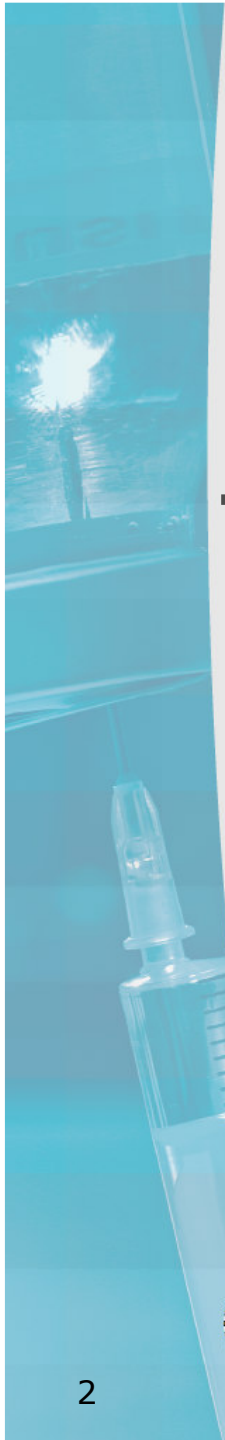


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Immunogenicity of recombinant human therapeutics – characterization of the underlying immune response

Melody Sauerborn, PhD student
Department of Pharmaceutics
2nd EIP symposium
Leiden, 18th November 2009

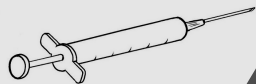


Immunogenicity – The potential of a rh protein therapeutic to elicit an immune response leading to formation of anti-drug antibodies (ADAs)



Factors influencing immunogenicity

Route of administration

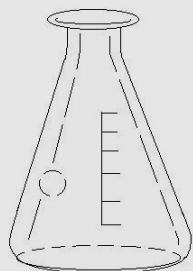


Aggregation!

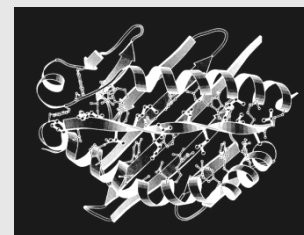
Duration of treatment



Formulation



Genetic profile/HLA



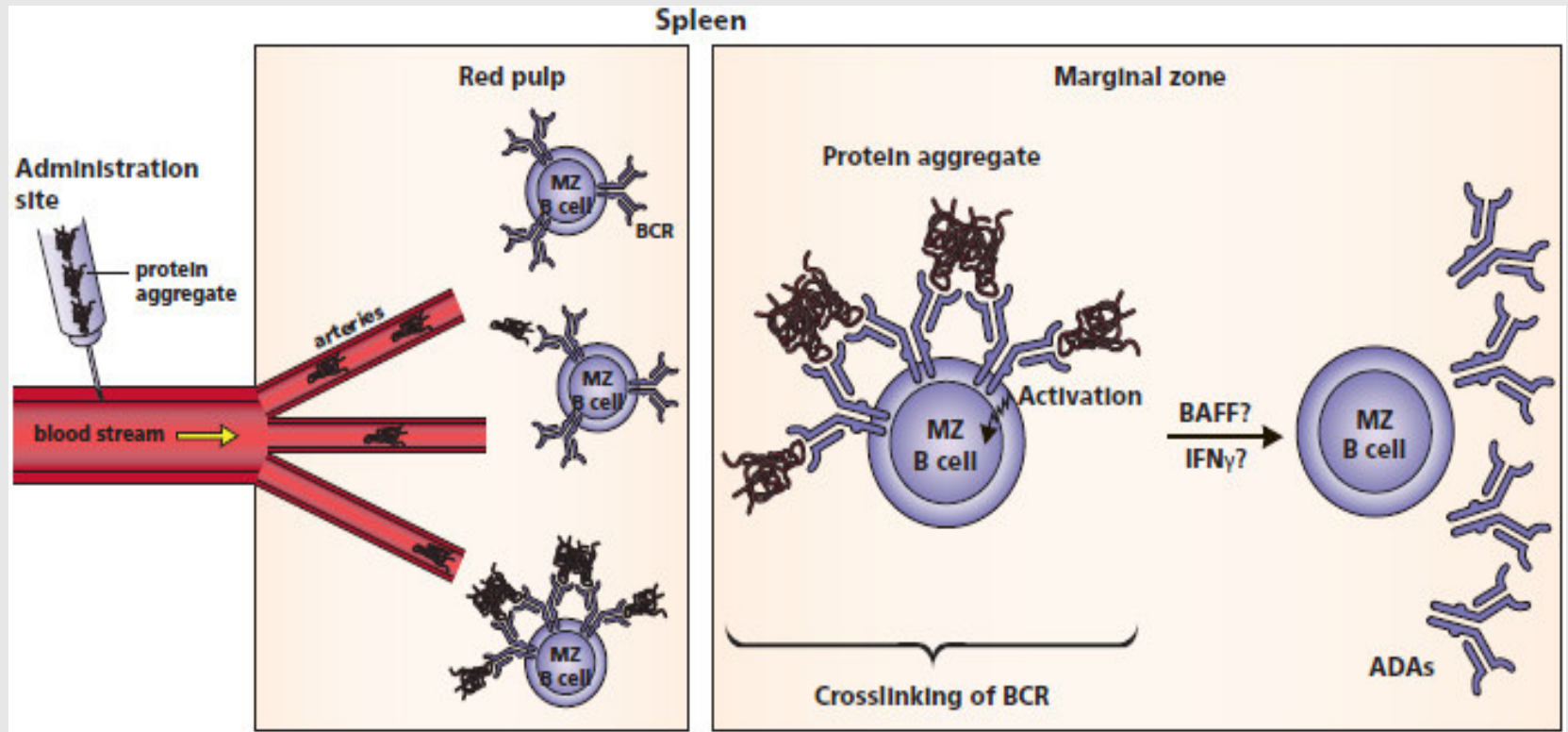
...and many more!



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Formation of ADAs after administration of aggregated rh therapeutic

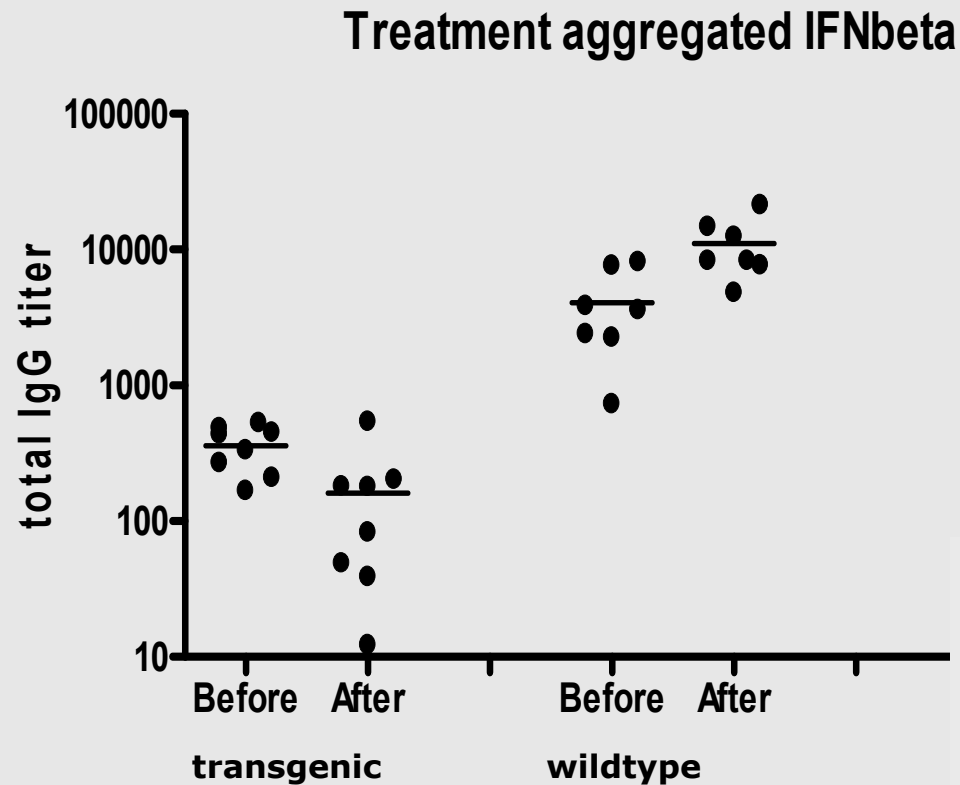


Experimental data obtained with aggregated rh IFNbeta to support theory

1. Memory formation after re-challenge with both non-aggregated (previous presentation, Miranda van Beers) and aggregated rh IFNbeta
2. T cell dependency
3. MZ B cell involvement



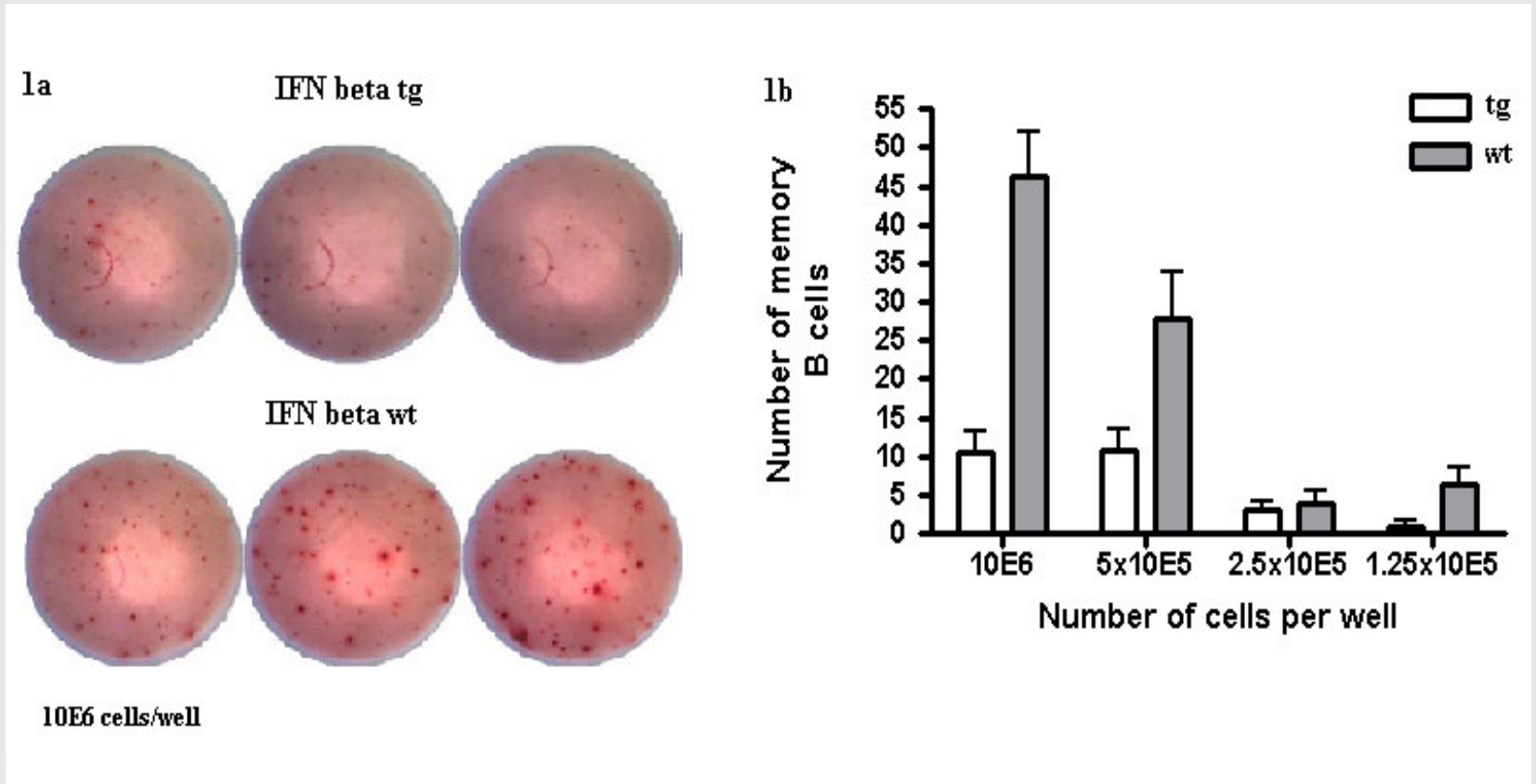
Memory formation after re-challenge with aggregated rh IFNbeta



- ✓ NAs assay on day 77 showed NAs in wildtype but not in transgenic animals



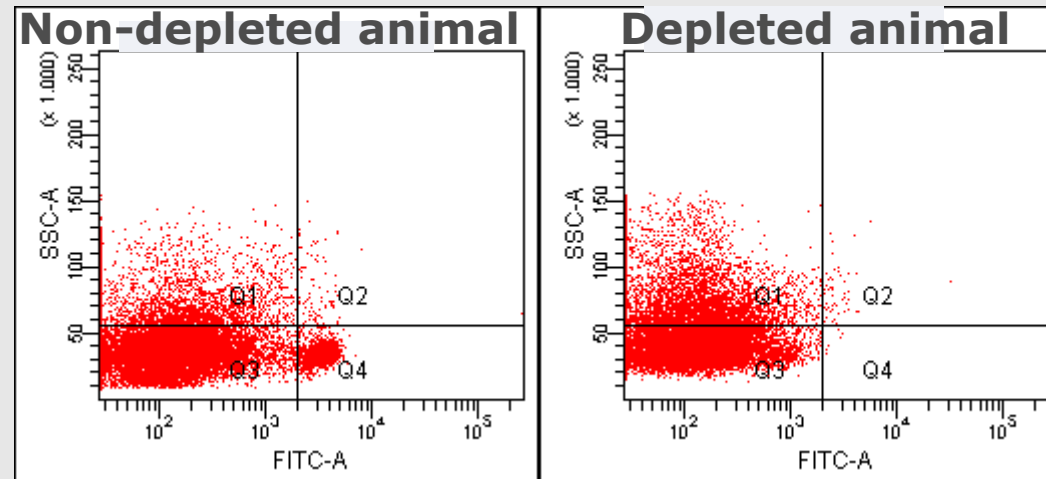
EliSpot for detection of memory B cells after re-challenge experiment (day 77)



Formation of ADAs after depletion of CD4+ T cells (T cell dependency)

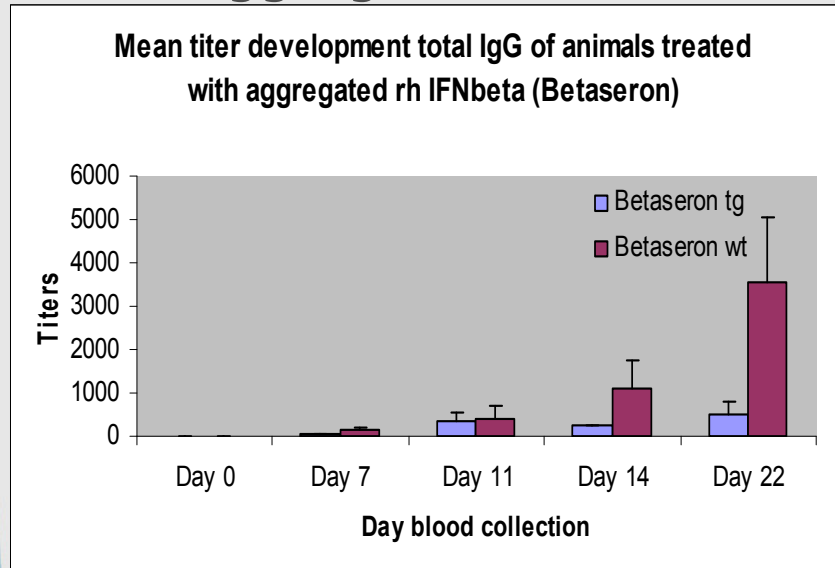
✓ 3 week immunization; 15 inj.; Mon - Fri

	Non-depleted	Depleted
Betaseron	A	B
OVA	C	D
TNP Pneumo23	E	F

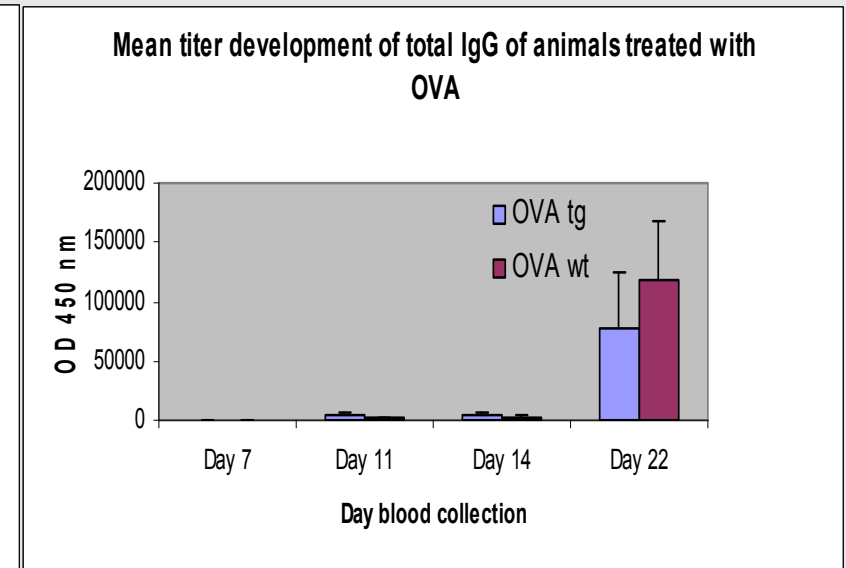


Total IgG titers of non-depleted animals

Aggregated IFNbeta



Ovalbumin



- ✓ TNP / Pneumo23 Elisa in progress; preliminary data suggests no difference between depleted and non-depleted animals



The road so far...

- ✓ Significant differences in onset and magnitude of antibody response between wildtype and transgenic
- ✓ Breakage of tolerance against aggregated IFNbeta
 - NO MEMORY, NO NAs
- ✓ EliSpot reveals ,unconventional` B cell population in transgenic animals
 - ✓ T CELL DEPENDENT
 - ❖ BUT - depletion of ALL CD4+ bearing cells; also Tregs



Are marginal zone B cells the source of ADAs?

- ✓ Since no memory formation was detected a suppressing mechanism could exist
 - suppressor T cells?
- ✓ The immune response against human therapeutics requires the presence of CD4 bearing cells
- ✓ The involvement of different B cell subsets, such as MZ B cells, might explain the existence of plasma cells but the absence of memory B cells – different develop and survival mechanisms than B2 cells (Seen in EliSpot?)
- literature states that MZ B cells are excluded from GC and thereby also from hypermutation into memory cells



Formation of ADAs after depletion of MZ B cells

✓ 3 week immunization; 15 inj.; Mon - Fri

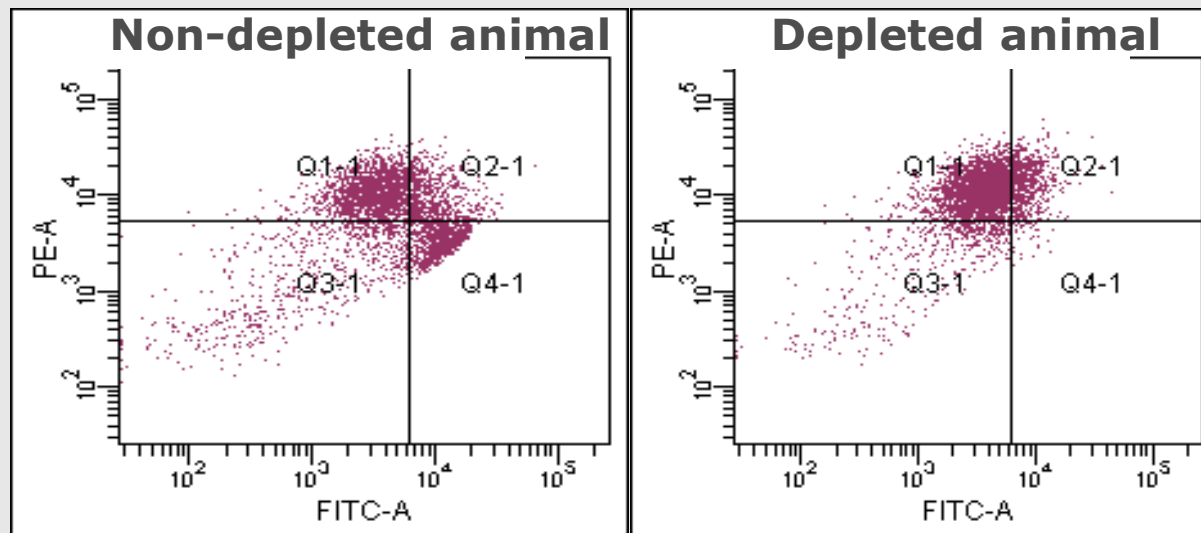
	Non-depleted	Depleted
Betaseron	A	B
OVA	C	D
TNP Pneumo23	E	F

✓ First in vivo study in September failed due to incapacity of depleting compound to deplete MZ B cells
In our transgenic mouse model

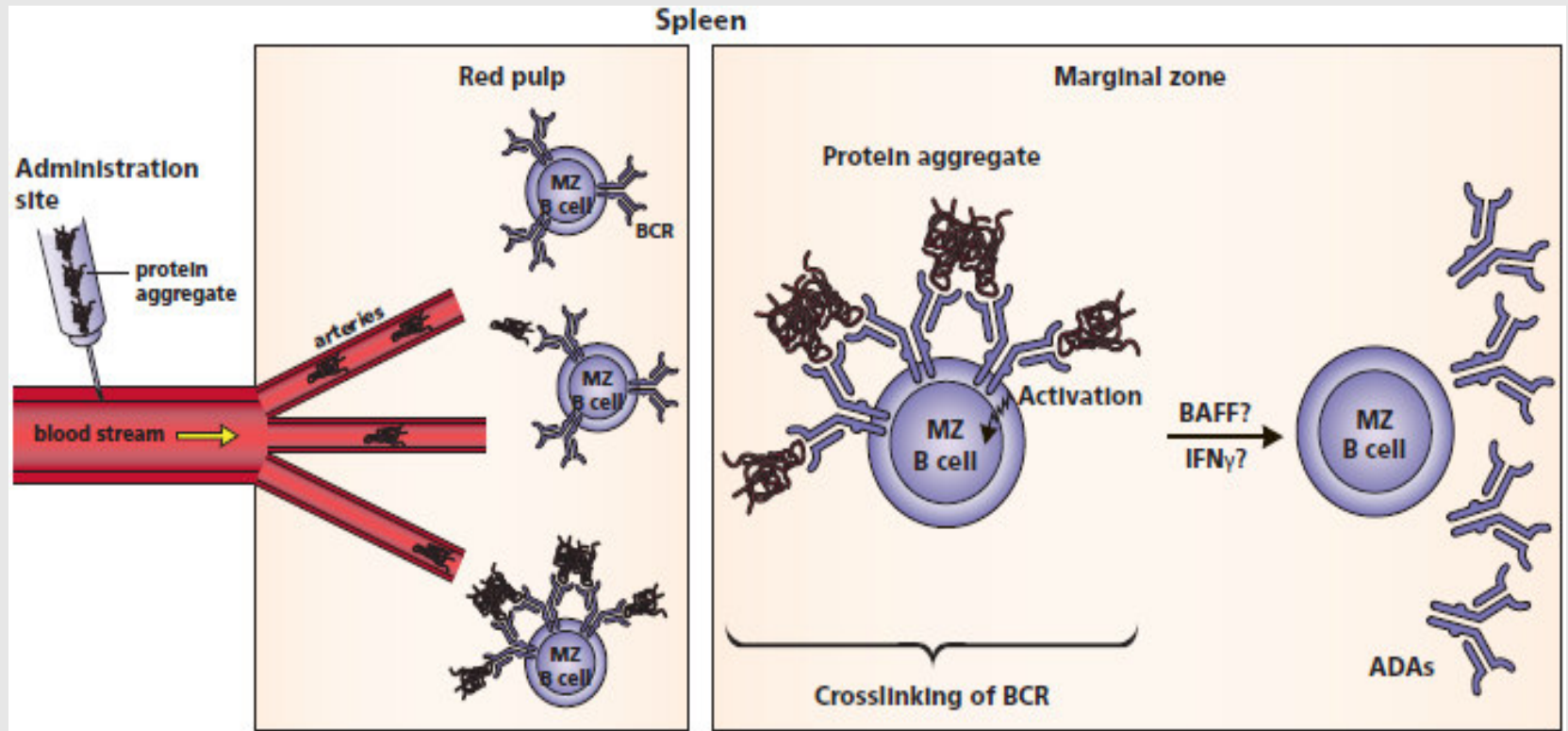


Formation of ADAs after depletion of MZ B cells II

- ✓ Usage of two mAbs directed against integrins
- ✓ 'Pushes' the MZ B cells out of the MZ of the spleen
 - ✓ loss of function
 - ✓ Pilot successful



Formation of ADAs after administration of aggregated rh therapeutic



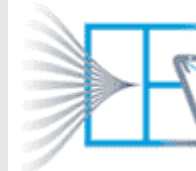
Acknowledgments

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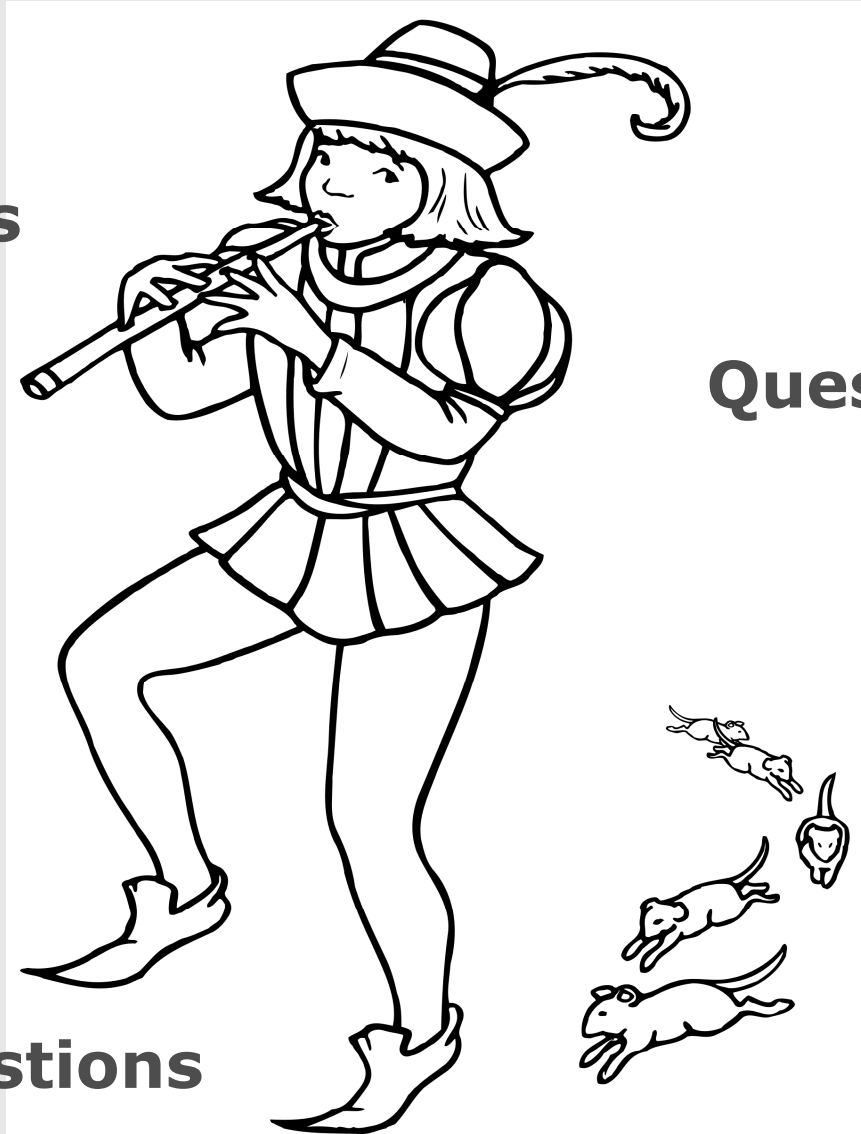
Harald Kropshofer
Franziska Regenass



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Questions



Questions

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