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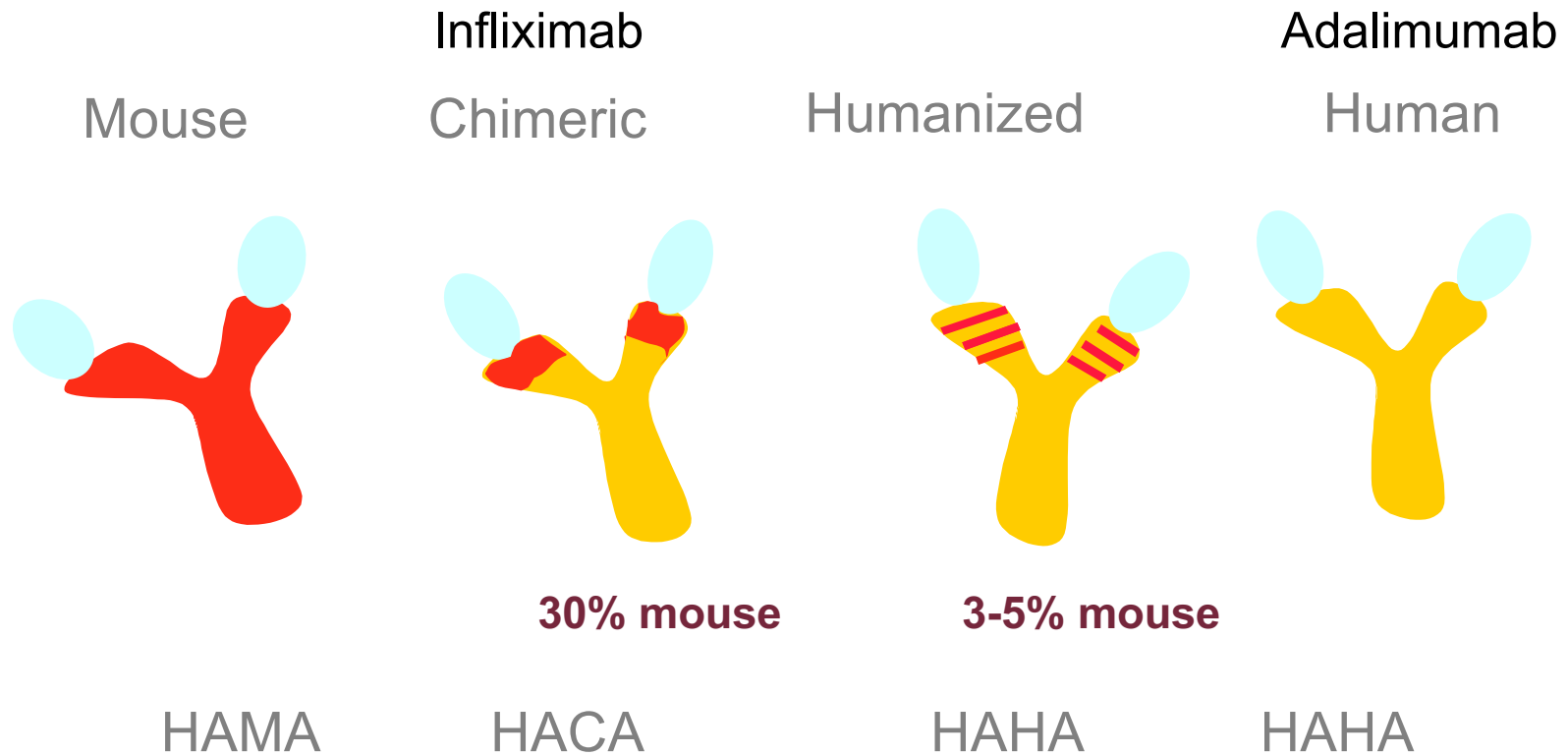


ADA to ada

**Functional analysis of the anti-adalimumab
response using patient-derived monoclonal
antibodies**

Theo Rispens

Immunogenicity therapeutic antibodies



Immunogenicity:

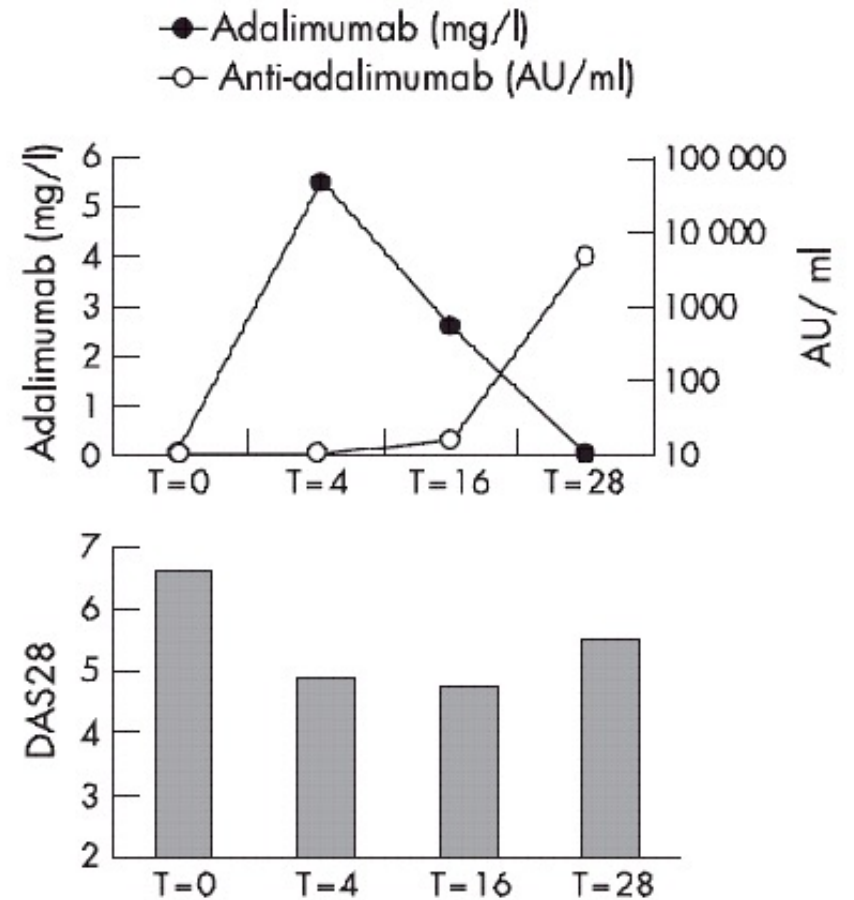


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Formation of antibodies to adalimumab

Treatment of rheumatoid arthritis with adalimumab:

- effective in 60-70% of patients
- Free anti-drug antibodies (ADA) in 17% of patients after 6 months of treatment
- ADA formation leads to impaired treatment efficacy

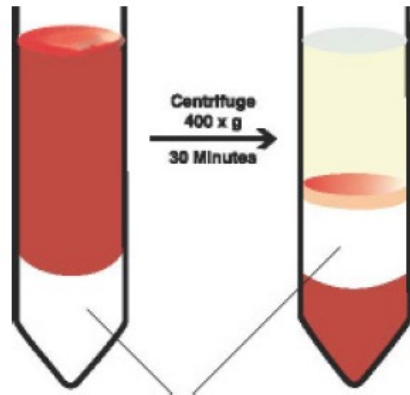


ADA issues to address using mAbs

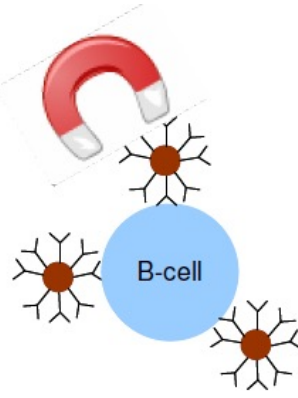
- clonality of the response
- B cell epitopes
- Immune complex formation
- Neutralization
- Standardization



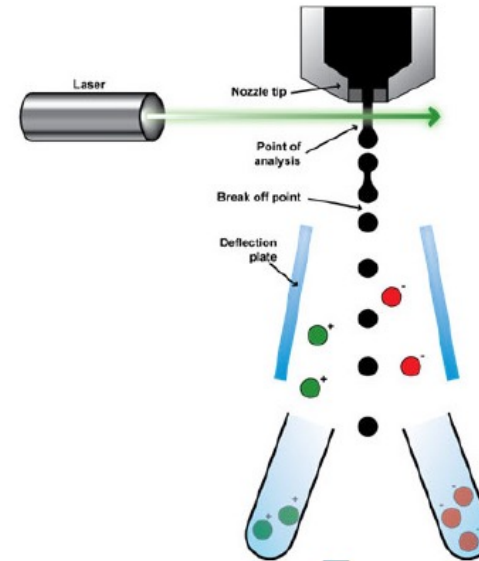
Making human monoclonal antibodies



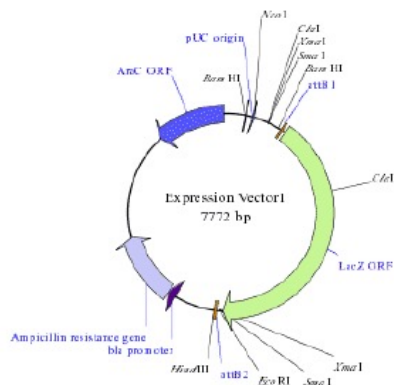
isolate PBMCs



isolate B cells



sort antigen-specific cells



recombinant expression monoclonal antibodies

...AGGCATATCGA...

isolate RNA, determine sequence for VH/VL



culture 1 cell/well; screening



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Schouwenburg et al., ARD 2012

**All monoclonal antibodies are derived from
different precursor B-cells**



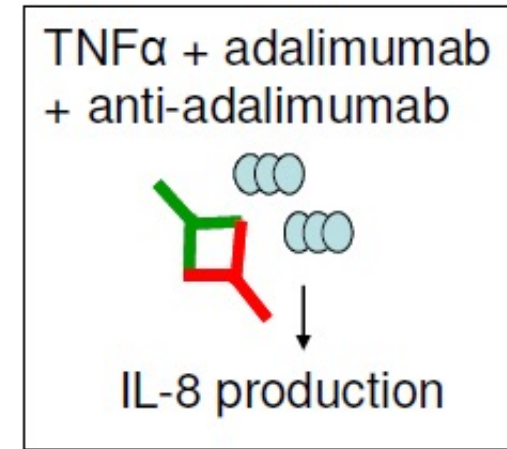
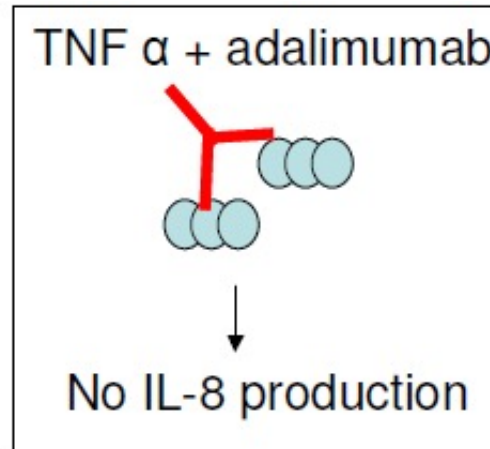
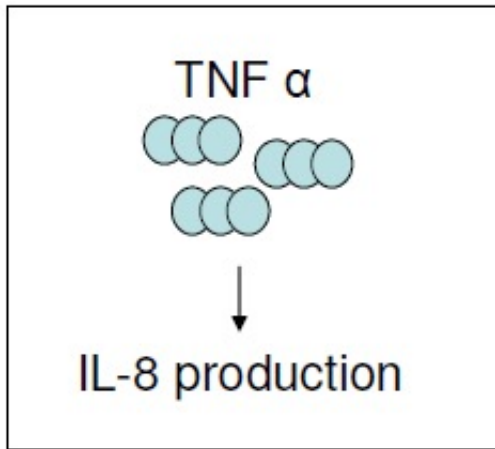
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All mAbs bind to an overlapping epitope

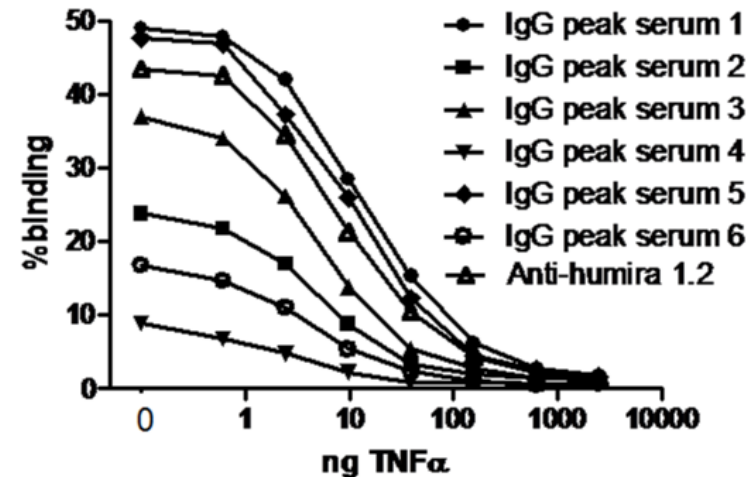
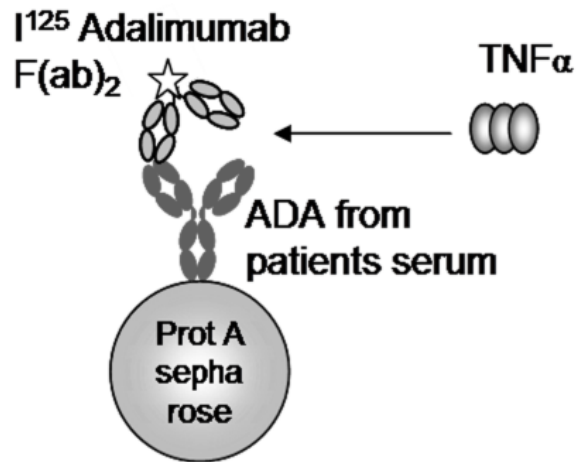


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mAbs neutralize adalimumab

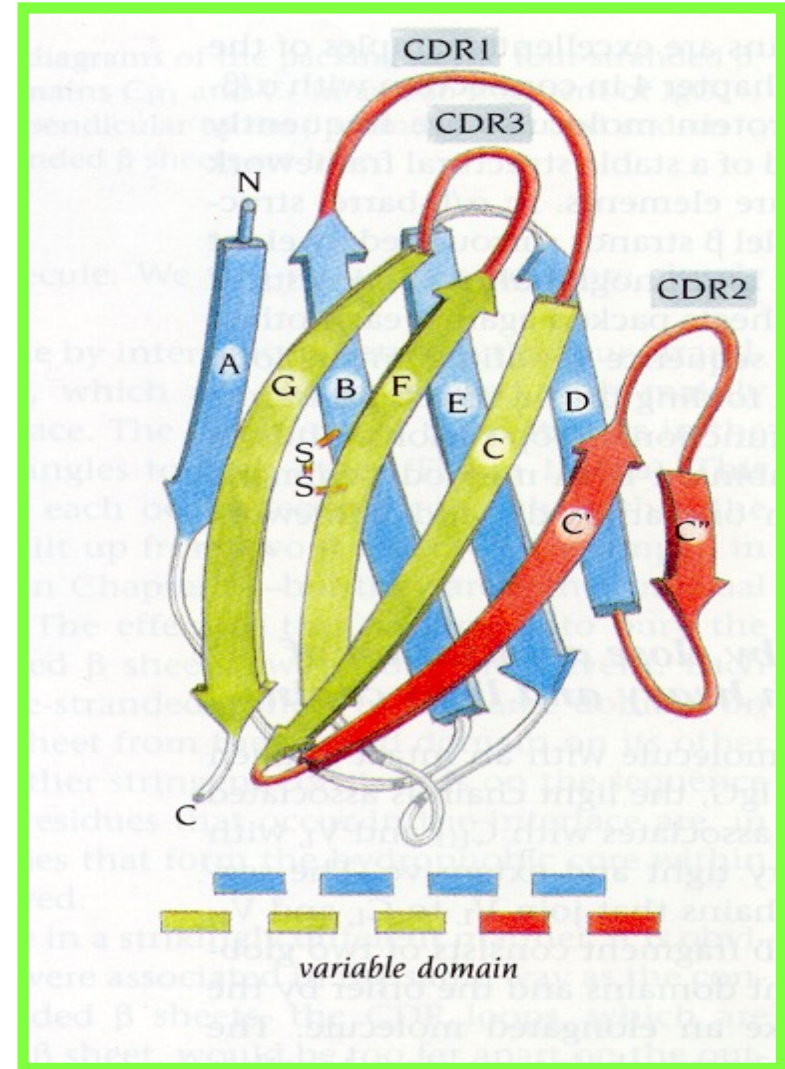
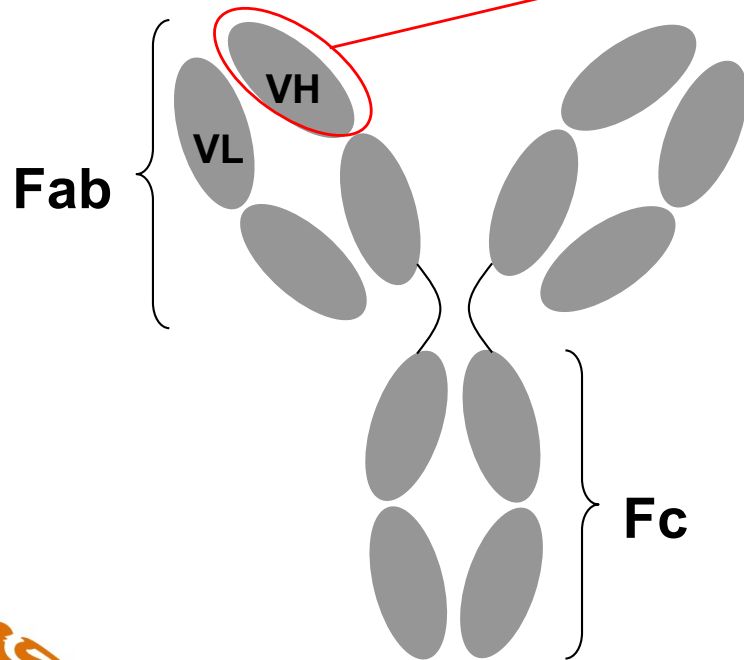


TNF- α completely inhibits binding of ADA from patient sera



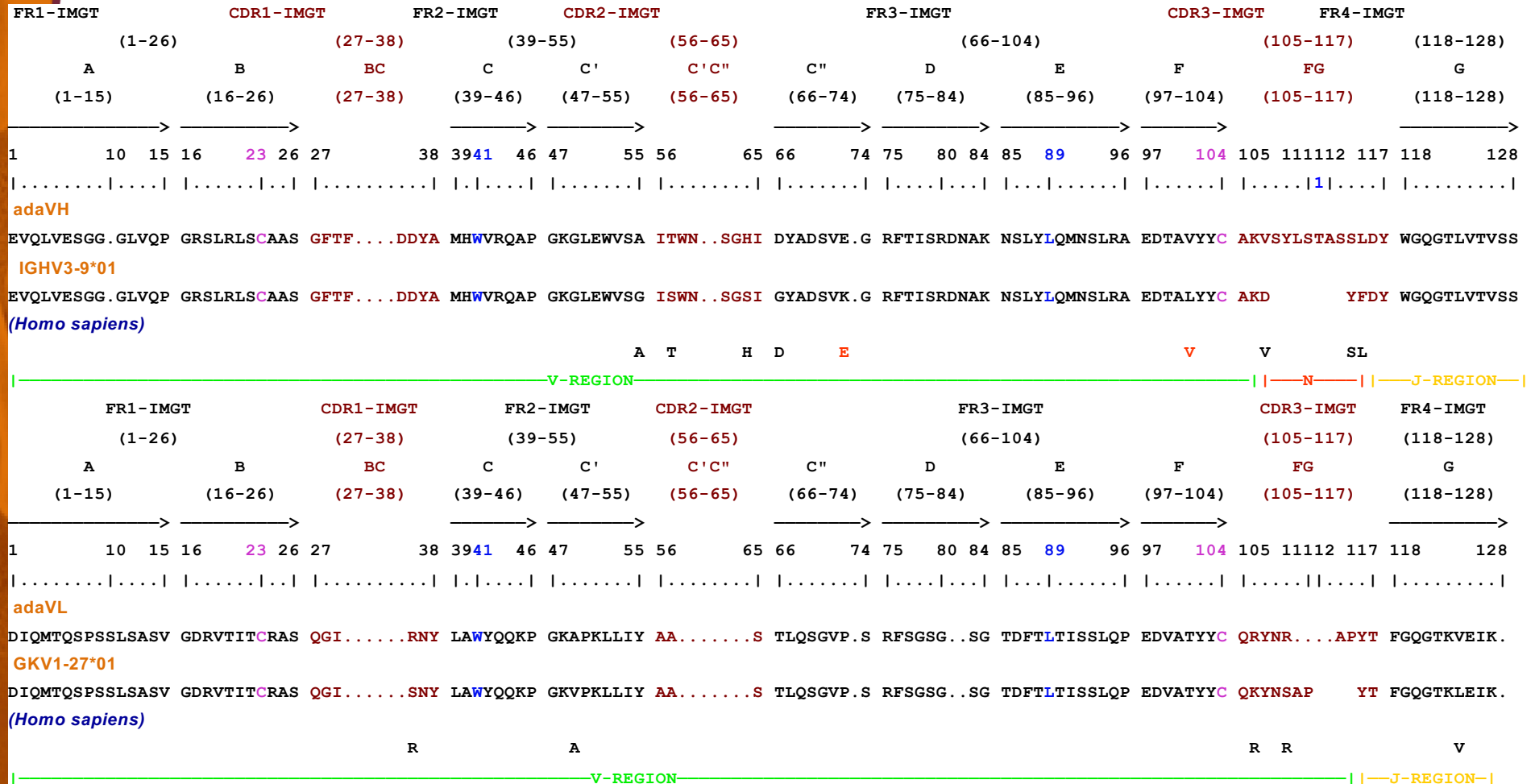
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Antibody structure



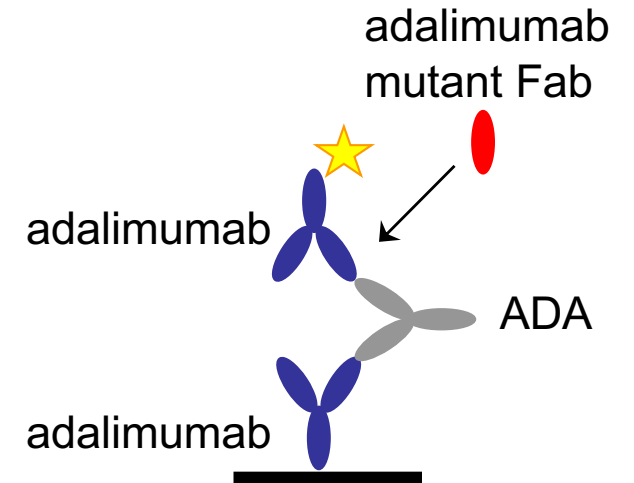
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Adalimumab: possible antigenic determinants



inhibition of anti-ada to wild-type adalimumab by ada Fab variants

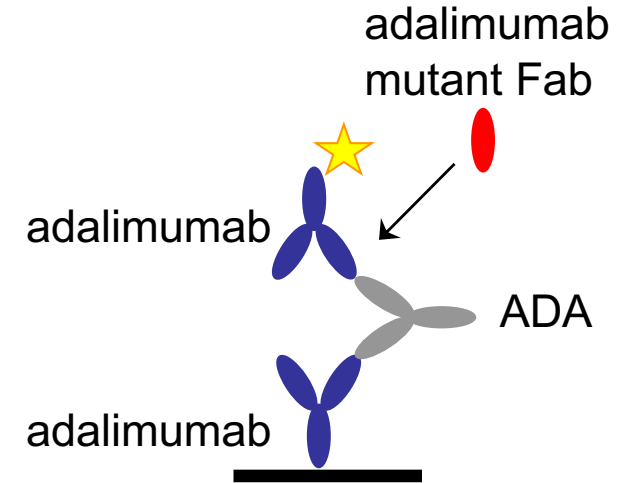
- single-point mutants of adalimumab
- selected for enhanced TNF binding



inhibition of anti-ada to wild-type adalimumab by ada Fab variants.

values represent binding relative to uninhibited:

100% = no inhibition = no binding by ada Fab variant = mutation affects binding

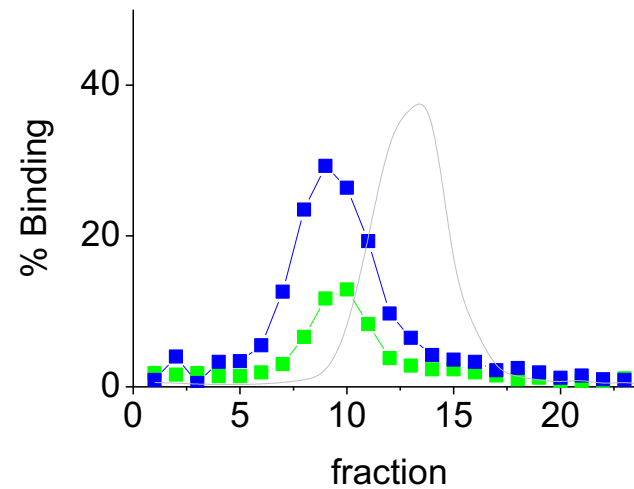


mAbs: immune complexes with adalimumab



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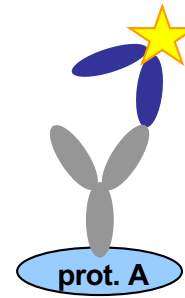
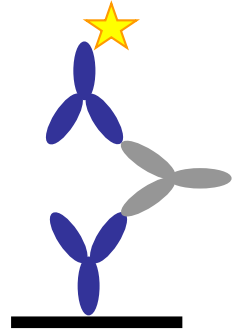
Results gradients Prot A



- Most sera contain complexes
- Complexes are small
- These complexes are not rapidly cleared



ELISA and ABT vs affinity



Conclusions

- ADA to ada is a diverse response
 - broad range of unrelated clones
 - Extensive SHM / affinity maturation
 - Multiple epitopes on adalimumab
- response is also very restricted
 - All antibodies bind competitively to overlapping epitopes
 - >98% of antibody response neutralizes TNF- α
- formation of small and larger immune complexes



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