Clinical implications of immunogenicity of TNF inhibitors

Theo Rispens
Disclosure

In relation to this presentation, I declare the following, real or perceived conflicts of interest:
- received fees for lectures from Pfizer, AbbVie, Regeneron

A conflict of interest is any situation in which a speaker or immediate family members have interests, and those may cause a conflict with the current presentation. Conflicts of interest do not preclude the delivery of the talk, but should be explicitly declared. These may include financial interests (eg. owning stocks of a related company, having received honoraria, consultancy fees), research interests (research support by grants or otherwise), organisational interests and gifts.
TNF blockers

Inflammation in e.g.
- Rheumatoid arthritis
- Crohn’s disease
- Psoriasis

can be suppressed by blocking TNF:

- Infliximab 1998
- Etanercept 1998
- Adalimumab 2002
- Golimumab 2008
- Certoluzimab 2009
Specificity: cross-reactive & pre-formed antibodies

complementarity determining regions

framework regions

idiotopes & xenotopes

glycans

allotopes

Fab

Fc

van Schie et al. mAbs, 2015, 7:4, 662-671
Immunogenicity therapeutic antibodies

- Mouse
- Infliximab Chimeric
- Humanized
- Adalimumab Human

Immunogenicity:
- 30% mouse
- 3-5% mouse
Antigen binding test (ABT)

- capture IgG from serum (Prot. A Sepharose)
- wash out unbound material
- incubate with radiolabeled adalimumab F(ab’)2
- wash out unbound label
- measure radioactivity

-Does not measure rheumatoid factors
Adalimumab: Cohort 272 RA patients

- 3 year follow-up
- Long term measurement of adalimumab levels, ADA and disease activity.

Bartelds et al., JAMA April 2011
Remission (DAS28 < 2.6) is associated with absence of ADA

Gecorrigeerd voor MTX dosis, bezinking en CRP (HR: 3.6; 95%CI: 1.8-7.2, p<0.0001)

Bartelds et al., JAMA April 2011
Infliximab RA

22/51 pt (43%) develops antibodies within 1 year

Anti-adalimumab measured in bridging ELISA and antigen binding test

- different numbers of ADA+ patients in different assays

Hart et al., JIM 2011
Relation between drug levels and ADA

- bridging elisa: detects ADA only if no adalimumab is detected

![Graphs showing the relationship between drug levels and ADA](image)

Hart et al., JIM 2011
1. acid
2. neutralize / anti-ADL Fab

1. acid
2. neutralize / ADL F(ab)_2-bt

ADL F(ab)_2-bt / 37 °C / 16 hr

1. acid
2. neutralize / ADL-bt / ADL-sulfo-tag

Detection

Prot. A Sepharose

PIA

ARIA

TRIA

ECL

ADA

adalimumab (ADL)

rabbit anti-ADL Fab

Bloem et al, J Immunol Methods 2015
drug-tolerant ADA: analysis in ADL-treated RA patients

<table>
<thead>
<tr>
<th></th>
<th>ABT</th>
<th>ARIA</th>
<th>TRIA</th>
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<td>66.0</td>
<td>57.4</td>
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</table>

Bloem et al, J Immunol Methods 2015
Clinical response vs drug-tolerant ADA assay
RA patients/adalimumab

Sustained remission (DAS28 < 2.6)

- PIA neg (n=46)
- PIA pos (n=53)

Percent of patients

Time (wk)

van Schouwenburg et al. ARD 2013
PK assay

Anti-idiotype

adalimumab

TNF

Anti-TNF
Drug levels vs anti-drug antibodies: a balance

16 or 28 weeks after start adalimumab

ABT vs PK

PIA vs PK

Sanquin
Detection of anti-drug-antibodies (ADA)

**Anti-TNF antibody levels**

<table>
<thead>
<tr>
<th></th>
<th>Free anti-TNF agent</th>
<th>ADA-drug complexes</th>
<th>Free ADA</th>
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**ADA detection method**

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<th>Free anti-TNF agent</th>
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<td>ELISA</td>
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<tr>
<td>PIA</td>
<td>–</td>
<td>+/-</td>
<td>+</td>
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<tr>
<td>Pharmacokinetic assay (TNF capture)</td>
<td>++</td>
<td>+</td>
<td>+/-</td>
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</tbody>
</table>
Concentration-effect curve (adalimumab/RA)

Each dot is mean of 20 patients

Pouw et al. ARD 2015
Characterization of anti-adalimumab antibodies
Making human monoclonal antibodies

1. Isolate PBMCs
2. Isolate B cells
3. Recombinant expression monoclonal antibodies
4. Isolate RNA, determine sequence for VH/VL
5. Sort antigen-specific cells
6. Culture 1 cell/well; screening

Schouwenburg et al., JBC 2014
All monoclonal antibodies are derived from different precursor B-cells

<table>
<thead>
<tr>
<th>clone</th>
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<th>V</th>
<th>D</th>
<th>J</th>
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Adalimumab

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Infliximab

Schouwenburg et al., JBC 2014
IgG4 anti-adalimumab

adalimumab-treated RA patients in patients without detectable drug levels: significant part is IgG4

Mabs underwent extensive somatic hypermutation

Schouwenburg et al., JBC 2014
mAbs neutralize adalimumab

- TNF
- TNF + ADL
- TNF + ADL + ADA

IL-8
- IL-8
- no IL-8
- IL-8

IL-8 (%)

Schouwenburg et al., JBC 2014
Neutralization by ADA
Adalimumab: TNF inhibition

125I adalimumab Fab → TNFα

ADA from patients serum → Prot A sephrose

% AU inhibited

% AU

Schie et al., ARD 2015
Infliximab: potential epitopes

adapted from Liang et al. JBC 2013
Infliximab: TNF inhibition

\[ % \text{AU inhibited} \]

\[ % \text{AU} \]

\[ \text{ng TNF\textsubscript{\(\alpha\)}} \]

Schie et al., ARD 2015
correlation
WEHI assay – anti-infliximab RIA

Schie et al., ARD 2015
• ADA to anti-TNF antibodies are predominantly neutralizing:
  > 98% for adalimumab (21 pt)
  > 90% for infliximab (34 pt)
  > 97% for certolizumab (9 pt)

• paratope is immunodominant?

• No need for bioassays assessing neutralizing capacity
Immune complexes
Serum analysis anti-adalimumab complexes sucrose gradients

- Most sera contain complexes
- Complexes are small
- These complexes are not rapidly cleared
Immune complexes are formed upon infliximab administration

ADA-positive patients that receive infliximab will develop immune complexes

Various sizes of immune complexes can be formed

van der Laken et al., Ann Rheum Dis, 2007
Infusion reactions correlate with ADA titer

- Infusion reactions are allergic-like reactions upon infliximab infusion
  - Symptoms: flushing, chest tightness, dizziness, bronchospasms
- 7% of patients experience an infusion reaction
- We find little anti-IFX IgE in patient sera
- High ADA titer increases chance of infusion reactions

Pascual-Salcedo et al., Rheumatology, 2011
Neutralizing monoclonal antibodies show diversity in complex formation

data unpublished, available upon request
t.rispens@sanquin.nl

van Schie, unpublished
Immune complex size is concentration dependent

data unpublished, available upon request
t.rispens@sanquin.nl
influence ratio infliximab/anti-infliximab

data unpublished, available upon request

t.rispens@sanquin.nl

van Schie, unpublished
Immune complex formation: spiking labeled infliximab in ADA+ sera

high ADA titers increase propensity to form large IC

data unpublished, available upon requist
t.rispens@sanquin.nl

van Schie, unpublished
Very large immune complexes can be formed using high concentrations of ADA and drug

data unpublished, available upon request
t.rispens@sanquin.nl

van Schie, unpublished
Neutrophil activation depends on complex size

data unpublished, available upon request
t.rispens@sanquin.nl

van Schie, unpublished
measurement of IgE anti-infliximab

data unpublished, available upon request
t.rispens@sanquin.nl
• Small immune complexes can be detected weeks after last administration of adalimumab

• Larger immune complexes can be formed *in vitro* from ADA in patients sera
Take home

Anti-drug antibodies (ADA) to therapeutic Abs:

- can lead to *loss of efficacy*
- occasionally may induce hypersensitivity
  - probably immune complex mediated
- *neutralization* leads to non-response
- *Quantity* of ADA is relevant: balance ADA/drug
- clinical relevance of ADA is best interpreted in conjunction with PK
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