Prozone effect can be specific to single antigen bead kit manufacturers

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SAB Assay – HLA ab detection

Single antigen bead (SAB) assays for the detection of HLA antibody

- Highly sensitive
- Accurate analysis of complex HLA specific antibody profiles
- Important questions raised regarding sensitivity
  - Various reports of ‘blocking’ mechanisms which can produce false negative
  - Hidden specificities reportedly revealed after dilution

Previous observations

Detection of Immunoglobulin G Human Leukocyte Antigen-Specific Antibodies in Renal Transplant Patients Using Single-Antigen-Beads is Compromised by the Presence of Immunoglobulin M Human Leukocyte Antigen-Specific Antibodies

Elisabeth Schwaiger,1 Markus Wahrmann,1 Gregor Bond,1 Farsad Eskandary,1 and Georg A. Böhmig1,2

Dilution of samples reveals masked HLA antibodies - DTT resolves and suspected cause is IgM

Identify complement component as key blocking mechanism

Complement Component C3 Activation: The Leading Cause of the Prozone Phenomenon Affecting HLA Antibody Detection on Single-Antigen Beads

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So what’s going on?

Assay interference has been attributed to

- Blocking by complement factors (C1, C3, C4)
- IgM antibodies
- Prozone / High Dose hook effect

- Note – blocking by IgM and Complement blocking are not a true Prozone effect!
- Prozone – blocking by high titre antibody

Complement interference

Major cause - mimic’s a prozone effect and often reported as such

- Antibodies undetectable in neat sample become detectable after dilution
- Complement C1 split products prevents binding of secondary IgG
- Commonly eliminated through addition of EDTA

- Represents the most common mechanism of blocking interference in the literature

Proposed mechanism – complement blocking

Diagram showing single antigen bead with HLA-A1 and secondary conjugate Anti-HLA-A1.
Proposed mechanism – complement blocking

EDTA – complement blocking

• Addition of agents known to interfere with C1 integrity increase MFI in affected sera

EDTA – complement blocking

• Some researchers have demonstrated a role for C3 – proposing that C1 complex initiates classical cascade resulting in dense C3b/d deposition (Schwaiger et al, 2014 Transplantation)

• In this study cobra venom was used to fragment C3 while leaving C1 integrity unaffected
  • Prozoning was removed
Is complement to blame for all blocking effects?

A few reports in the literature describing a blocking effect when EDTA and other agents targeting complement were used.

- Tambar et al AJT 2015
  “We further showed that EDTA treatment (6%) does not always remove all inhibitory factors compared with titration studies”

- Schwaiger et al. 2014
  “Remarkably, DTT treatment and heat inactivation also uncovered a second population of low-level SA reactions that also fulfilled our defining criteria of prozoning”

Blocking effects and MFI levels

Dilution to monitor HLA Epitopes
Edinburgh HLA antibody specificity analysis

Use two suppliers kits – Immucor, Lifecodes Single Antigen and One Lambda SAB. Alternate between kits

- Different HLA epitopes profiles are present in the panel composition of these kits
- Helps resolve HLA epitopes in reactive sera
- The ratio of beads to serum within the Immucor and One Lambda kits is different
  - Effectively this allows two test dilutions ‘neat’ and ‘diluted’ to be set up.

Different HLA epitope composition

- Some currently defined HLA epitopes are absent from the panels of both kits.
- Using two kits covers all currently described epitopes
- Increases ability to define epitopes / awareness of intra / inter locus epitopes

Light grey = OL, Dark grey = Immucor
Hidden HLA antibody reactivity
Case report

When alternating manufacturers we observed a prozone effect which was manufacturer specific

- The case – a renal transplant patient with a failing graft (due to AMR)
- Monitoring HLA antibodies during graft failure / for relisting
  - Consistent approach when monitoring so one manufacturer is used (i.e. Immucor or One Lambda)
- One Lambda detected HLA specificities seemed to disappear within 8 weeks of testing

A genuine change in the profile seemed unlikely

- Patient was sensitised to specific mismatches on the failing allograft
- Reactivity towards these seemed to be increasing on previous testing
- No known change to treatment strategy
  - IS had previously been withdrawn and no Plasma ex had been commenced
- Further testing performed
  - Immucor assay set up
  - Dilution on One Lambda
  - Rpt sample requested

Reactivity profile

Kit 1 = OL, Kit 2 = Immu
EDTA routinely added to test serum
True Prozone effect?

Proposed mechanism relates to the high density on the beads.

(a) at a low density only univalent binding is possible
(b) High antigen density enables bivalent binding – increases antigen/antibody stability
(c) Very high density may lead to tightly packed antibodies which interfere with binding of secondary antibody

Weinstock et al. 2013 IJI

Summary

Other causes
• Cannot exclude IgM blocking as EDTA treatment does not fragment IgM
• The increasing level of antibody over time is suggestive of a true prozone effect
• It is clear that addition of EDTA does not remove all blocking mechanisms

Summary

Alternating between suppliers kits for SAB has two key advantages
• Increases the panel of antigens – testing panels epitope composition are different
• Cost effective way to incorporate a dilution effect into routine testing