Toxin-mediated disease in the ICU

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Toxin-mediated disease
Mechanisms and management

• Superantigen-mediated toxic shock
• PVL+ Staphylococcus aureus infection
• Anthrax in IDU
Staphylococcal toxic shock

**Six markers of disease severity**
- Fever >38.9
- Rash: diffuse macular erythroderma
- Desquamation 1-2 weeks later
- Hypotension SBP<90
- Multi-system involvement (3 or more): GI; muscle; mucosae; hepatic; renal; haematologic; CNS
- Negative cultures blood, csf, throat; negative serology for measles, lepto, RMSF

Confirmed case 6/6  
Probable case 5/6  
No req for *S. aureus* culture
Staphylococcal toxic shock

- Either associated with menstruation/tampon usage in young women (mTSS) or non-menstrual (nmTSS)
- TSST-1 producing strains in >90% cases of mTSS
- Antibody to TSST-1 protective (95%) in mTSS
- mTSS usually non-invasive infection
- Mortality 1%
- Dramatic decline 1980-1990 - changes in high absorbency tampons
Non-menstrual staph TSS

- Mortality has not altered in 20y: 6-8% in US (20% in recent French study)
- No decline in incidence
- Now accounts for 50% of all TSS
- Excess incidence of females
- Often follows surgical infections
- Strains may be tsst1+ve or any other toxin
Family of Staphylococcus aureus bacterial superantigens

Phage or mobile genetic element encoded

- SEA
- SEB
- SEC
- SED
- SEE
- TSST1

- SEG
- SEH
- SEI
- SEJ
- SEK
- SEL
- SEM
- SEN
- SEO
- SEP
- SEQ
- SFR

‘SET’s or ‘SSL’s
Superantigens form a promiscuous trimolecular complex with the T cell receptor (TCR) and MHC class II.

Diagram showing the interaction between superantigens, T cell receptor, and MHC class II.

Antigen presenting cell

Antigen

Super antigen

T cell receptor

MHC Class II

T lymphocyte

TNF, IL-2

Antigen presenting cell

T cell

TNF

IL-2

SHOCK
Superantigen interaction with MHC and TCR

- Each bacterial strain may have 4-5 SAg and each superantigen may bind/activate 4-5 different Vβ subsets in vitro.

- Superantigens activate a higher proportion of T cells than a single antigen (up to 20%).

- Hard to study clinically- paradoxical results due to timing of samples and expression of multiple sometimes unknown superantigens.

- Preferential binding to human HLAII; hard to study in mice due to MHCII.
Superantigen binding to HLA class II. HLA transgenic mice are more responsive than wild type mice.

Bacterial superantigens show poor binding to murine class II molecules. Can compensate using HLA II transgenics. Promiscuous binding yet some preference eg SEB binds to DR and DQ > DP.
Humanised model: effect of bolus SEB exposure in HLA-DR1 mice

2ug SEB

Cytokine burst wholly reliant on TCR ab cells and HLA II
Source of TNF is T lymphocytes in spleen
Female responses= 2 x male

Non-menstrual SEB toxic shock

Clinical pictures removed on author’s request

• 24y female: minor surgery (mole removal) 5d ago
• Severe sepsis; erythema, conjunctivitis, shock
• Progression despite iv antibiotics, fluids, oxygen
• Source not identifiable for 3d

• MSSA seb+ from pus in subcutaneous collection
• Profound hypotension, lactic acidosis, ICU

• Why so profoundly unwell?
1. Patient lacked specific anti-toxin antibody and bacterium still actively producing SEB

- Despite high dose antibiotics- viable S. aureus++
- Source control not possible till d3
- seb+ strain:- SEB 43.8 ng/mL in pus
- No neutralising antibody to SEB- given IVIG

Davies, F. et al, 2011.
2. SEB and LPS synergise in release of TNF by human monocytes

Signaling via HLAII leads to
- upregulation of TLR4 mRNA and TLR4 expression
- Functional upregulation of TLR4 ligand signaling
- Upregulation of TLR2 surface expression but not mRNA or functional signaling

Interaction between SAg and TLR ligand signaling at multiple levels in human monocytes
3. Superantigen internal conserved dodecapeptide ligates T cell CD28 directly

Cytokine Storm in a Phase 1 Trial of the Anti-CD28 Monoclonal Antibody TGN1412


SUMMARY

Six healthy young male volunteers at a contract research organization were enrolled in the first phase 1 clinical trial of TGN1412, a novel superagonist anti-CD28 monoclonal antibody that directly stimulates T cells. Within 90 minutes after receiving a single intravenous dose of the drug, all six volunteers had a systemic inflammatory response characterized by a rapid induction of proinflammatory cytokines and accompanied by headache, myalgias, nausea, diarrhea, erythema, vasodilatation, and hypotension. Within 12 to 16 hours after infusion, they became critically ill, with pulmonary infiltrates and liver injury, renal failure, and disseminated intravascular coagulation. Severe and unexpected depletion of lymphocytes and monocytes occurred within 24 hours after infusion. All six patients were transferred to the care of the authors at
Case: management

- Plastic surgery review—further pus drainage after sutures removed
- Pooled human IVIG 2g/kg for TSS
- Antibiotics continued 2 weeks total
Treatment recommendations for staphylococcal TSS

- Anticipatory management of multisystem organ failure - FLUIDS
- Source control essential - SURGERY/RADIOLOGY
- Parenteral antimicrobial therapy at maximum doses
  - Kill organism with bactericidal anti-staphylococcal antimicrobial agent)
  - Stop enzyme, toxin, or cytokine production with protein synthesis inhibitor (clindamycin or other agent)
- Unresponsive to first line treatment for selected cases: Acute use IVIG 2g/kg followed by 2nd dose if response observed
Rationale for IVIG: in vitro neutralisation of superantigens

RCT evaluation of IVIG in streptococcal toxic shock

- No RCT in staphylococcal TSS
- Darenberg, 2003; multi-centre RCT in severe GAS disease
  - 10 IVIG 1/10 died
  - 11 control (albumin) 4/11 died
- Did not reach significance in 1st endpoint (28d m)
- Trial stopped because of slow recruitment
- SOFA score reduced in IVIG group days 2 and 3
- 2008 DoH revised guidance on use of IVIG for TSS
## Immunoglobulin use in UK for infection

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>$n$</th>
<th>Volume used (g)</th>
<th>Average dose (g/patient)</th>
</tr>
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<tbody>
<tr>
<td>Severe invasive group A streptococcal disease</td>
<td>31</td>
<td>4451</td>
<td>144</td>
</tr>
<tr>
<td>Necrotising (PVL-associated) staphylococcal sepsis</td>
<td>20</td>
<td>2673</td>
<td>134</td>
</tr>
<tr>
<td>Severe or recurrent <em>Clostridium difficile</em> colitis</td>
<td>67</td>
<td>2297</td>
<td>34</td>
</tr>
<tr>
<td>Staphylococcal toxic shock syndrome</td>
<td>11</td>
<td>1388</td>
<td>126</td>
</tr>
<tr>
<td>Toxin-related infection in paediatric intensive care</td>
<td>19</td>
<td>719</td>
<td>38</td>
</tr>
<tr>
<td>Sepsis in the intensive care unit not related to specific toxins or <em>Clostridium difficile</em></td>
<td>3</td>
<td>384</td>
<td>128</td>
</tr>
<tr>
<td>Neonatal sepsis (prevention or treatment)</td>
<td>2</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Other (Infectious diseases)</td>
<td>31</td>
<td>4096</td>
<td>132</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>184</strong></td>
<td><strong>16,018</strong></td>
<td></td>
</tr>
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</table>

Immunoglobulin database, 2010, Dept Health
Panton Valentine Leukocidin made by S. aureus

Community MRSA in the U.S.; severe cases all assoc with a non-hospital mec gene and PVL toxin
Necrotising skin infection

Clinical pictures removed on author’s request
PVL toxin can be made by MSSA or MRSA.

Phages carry the genes between strains
MRSA pvl strains have largely evolved from MSSA pvl strains
Situation in UK:- HPA Reference data

UK- multiple lineages carrying PVL, mostly MSSA not MRSA

Study at Imperial: 9% of community MSSA strains carry genes for PVL (Turner C, unpublished).

http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1218699411960
Returning traveller with CA-MRSA and PVL

Clinical pictures removed on author’s request

Had been sharing house with friends
2\textsuperscript{nd} abscess at site of ‘insect bite’; presented to hospital as feeling short of breath.
PVL+ MRSA isolated from arm and blood cultures.
Managed using Linezolid and Clindamycin: 2 agents for 3 weeks then 1 agent for 3 weeks
Skin clearance regimen
Mechanisms of disease

Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients

Yves Gillet, Bertrand Issartel, Philippe Vanhems, Jean-Christophe Fournel, Gerard Leul, Michele Bes, Francoise Vandenesch, Yves Peimont, Nicole Brousse, Daniel Floret, Jerome Etienne

Summary

Background: Between 1986 and 1998, eight cases of community-acquired pneumonia due to *Staphylococcus aureus* strains carrying the gene for the Panton-Valentine leukocidin (PVL) were recorded in France, six of which were fatal. We aimed to assess the clinical features of these eight cases and those of other cases identified prospectively, and to compare them with the characteristics of patients with pneumonia caused by *S. aureus* without PVL.

Introduction: *Staphylococcus aureus* is responsible for about 2% of cases of community-acquired pneumonia, and at least 10% of cases of nosocomial pneumonia. Most patients with *S. aureus* pneumonia are elderly and have serious underlying disorders such as cardiovascular disease, malignant disease, chronic pulmonary disease, and diabetes mellitus. Panton-Valentine leukocidin (PVL) is an extracellular pyrogenic toxin detected in fewer than 20% of
Panton Valentine Leukocidin: neutrophil destroyer

lukF

lukS

Heptameric pore

Neutrophil
Does PVL actually cause damage in real infections?- Mouse studies

*Staphylococcus aureus*

Panton-Valentine Leukocidin Causes Necrotizing Pneumonia

Maria Labandeira-Rey, Florence Couzon, Sandrine Boisset, Eric L. Brown, Michele Bes, Yvonne Benito, Elena M. Barbu, Vanessa Vazquez, Magnus Höök, Jerome Etienne, François Vandenesch, M. Gabriela Bowden

The *Staphylococcus aureus* Panton-Valentine leukocidin (PVL) is a pore-forming toxin secreted by strains epidemiologically associated with the current outbreak of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) and with the often-lethal necrotizing pneumonia. To investigate the role of PVL in pulmonary disease, we tested the pathogenicity of clinical isolates, isogenic PVL-negative and PVL-positive *S. aureus* strains, as well as purified PVL, in a mouse acute pneumonia model. Here we show that PVL is sufficient to cause pneumonia and that the expression of this leukotoxin induces global changes in transcriptional levels of genes encoding secreted and cell wall-anchored staphylococcal proteins, including the lung inflammatory factor staphylococcal protein A (Spa).

*Science. 2007 Feb 23;315(5815):1082-3.*
Species matters: rabbits are closer to humans than monkeys or mice

1. Only rabbit and human neutrophils sensitive to PVL apoptosis. Mouse neutrophils are NOT
2. Phenol soluble modulins cause most of the neutrophil lysis observed in other species

Rabbit models to assess PVL toxicity

- Lung model.

- Osteomyelitis

- Bacteremic seeding of kidneys—moderate effect only in first 48h

- Soft tissue infection
  - Abscess size correlated with PSM expression and neutrophil lysis but NOT PVL expression. A PVL negative (non-isogenic) strain had similar virulence. Isogenic strains not yet tested
Recommended therapy for severe *S. aureus* infection associated with pvl+ strains

- For minor infections incision and drainage may suffice with clearance regimen
- CA-MRSA susceptibilities vary but often sensitive to clindamycin unlike eMRSA

- Severe (HPA): high dose appropriate antibiotic therapy with protein synthesis inhibitors rather than flucloxacillin eg
  - Clindamycin/rifampicin
  - Linezolid/rifampicin
  - Vancomycin/rifampicin
  - Vancomycin/clindamycin
  - Clindamycin/linezolid/rifampicin (HPA working group recommendation for necrotising pneumonia)

- Intensive care support
- Source control where possible
- If unresponsive to std first line Rx use IVIG 2g/kg. 2\textsuperscript{nd} dose of 1g/kg if response observed (In vitro data only: Gauduchon J Infect Dis. 2004;189:346-53)
Is flucloxacillin bad for PVL expression?


Pattern of PVL production by UK MSSA strains

Need to evaluate impact of antibiotics on MSSA and MRSA strains in vitro and in vivo incl combinations

MSSA HSS156

For clinical MSSA PVL strains, flucloxacillin does not increase transcription of lukF-PV.

Anthrax in UK

<table>
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<tr>
<th></th>
<th>Confirmed Cases</th>
<th>Deaths</th>
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</thead>
<tbody>
<tr>
<td>Scotland</td>
<td>47</td>
<td>13</td>
</tr>
<tr>
<td>England</td>
<td>5 (6)</td>
<td>4</td>
</tr>
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</table>
SSTI and sepsis in injecting drug users

Clinical pictures removed on author’s request

Which one has anthrax?

Photos courtesy Dr. Aula Abbara
Anthrax Toxin mechanisms of action on cells

PA binds ATR
PA cleaved
Forms pore through which EF and LF enter

Anthrax: diagnostic clues

- Fasciitis
  - Disproportionate oedema, usually painless
  - No clear delineation of dead tissue
  - Normal WBC and CRP

- Septicaemia
  - Rapid progression
  - May present with intracranial haemorrhage

- Progress
  - Often biphasic with interval of recovery

Clinical pictures removed on author’s request
CXR at Day 3

- Severe “sepsis” on admission
- High inotrope requirement
  - May continue for several days
- Platelet fall heralds relapse
  - Death can be rapid
- Rapid debridement and antibiotics essential to remove toxin

Clinical pictures removed on author’s request
Diagnosis

- Blood culture
- Tissue culture: often positive even if blood is negative
  
  PCR on blood or tissue for 3 genes
  
  - Cap
  - Lef
  - Bag

- Toxin and antibody assays: pleural fluid useful

- In practice diagnosed by:
  - 1/3 by culture and PCR ± toxin
  - 1/3 by PCR alone ± toxin
  - 1/3 by seroconversion
Toxin & antibody testing

- Toxin levels correlate with bacteraemia
  - PA and LF measured by ELISA
  - Toxin also seen in serum in tissue infection
  - High toxin found in all serious cases: outcome poor

- Antibodies appear as patient recovers
  - Anti-PA and anti-LF measured by ELISA
  - Patients who have recovered have high levels
  - Some patients without full array of symptoms have seroconverted
  - Non-lethal infection obviously occurs

Source: Tim Brooks, Special Pathogens Unit, HPA Porton Down
Management

• Take blood cultures and EDTA for PCR
• Give antibiotics
• Take to theatre: take samples for culture/PCR
• Debridement and removal of pus essential
• ICU if appropriate
• Consider Anthrax Immune Globulin
Antibiotics

- Up to 5 antibiotics

- **Mandatory:**
  - Clindamycin – for tissue penetration
  - Ciprofloxacin: for tissue and CSF penetration (15% penR)

- **Consider also:**
  - Beta lactam eg ceftriaxone or flucloxacillin
  - MRSA treatment
  - Carbapenem in case of ESBL
  - Metronidazole
Anthrax immune globulin: from those vaccinated with US anthrax vaccine

<table>
<thead>
<tr>
<th>Variable</th>
<th>Anthrax immunoglobulin</th>
<th>Raxibacumab</th>
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</thead>
<tbody>
<tr>
<td>Brand name</td>
<td>None</td>
<td>ABthrax</td>
</tr>
<tr>
<td>Synonyms</td>
<td>Anthrax immunoglobulin</td>
<td>...</td>
</tr>
<tr>
<td>Specificity</td>
<td>Polyclonal</td>
<td>Monoclonal IgG1</td>
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<tr>
<td>Manufacturer</td>
<td>CanGene</td>
<td>Human Genome Sciences</td>
</tr>
<tr>
<td>License status</td>
<td>Investigational new drug.</td>
<td>Investigational new drug</td>
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<tr>
<td>Route of administration</td>
<td>Intravenous</td>
<td>Parenteral (intramuscular and intravenous described)</td>
</tr>
<tr>
<td>Source</td>
<td>Human plasma</td>
<td>Mouse myeloma cell line</td>
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<tr>
<td>Notes</td>
<td>Emergent BioSolutions is also developing a polyclonal human anthrax immunoglobulin</td>
<td>Other monoclonal anthrax immunoglobulins in development include Anthim (Elusys Therapeutics) and Valtorim (developed via a partnership between Medarex and PharmAthene)</td>
</tr>
</tbody>
</table>
Fully human mAb can protect in two animal models...but is not available

Toxin mediated disease

- Superantigen-mediated toxic shock
  - Novel mechanisms of interaction with leukocytes
  - Possible role of IVIG

- PVL+ Staphylococcus aureus infection
  - Suppurative infection
  - Potential for rapid deterioration-lung
  - Antibiotics to inhibit toxin and consider MRSA
  - Possible role of IVIG

- Anthrax in IDU
  - Think about it and test for it
  - Antibiotic combinations and surgery
  - Uncertain role of AIG
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