THE ROLE OF BACTERIA IN ACNE

P. acnes or P. bystander?

E Anne Eady
Evidence based dermatology & basic research

- No equivalent of the Cochrane Skin Group & associated systems for systematic identification/critical appraisal of evidence
  - Reviewers rarely rate evidence quality or challenge authors’ conclusions
- Tendency to focus on what’s new
- Few studies build on those that have gone before
  - It’s more fun to be first & easier to get funding for novel ideas
- This review is ‘work in progress’ & a snapshot is presented here
Identifying & appraising relevant articles

PubMed [08 May 12]

- 3689 *P. acnes*
  - 776 (21%) *Propionibacterium AND acne*
- 4077 *C. parvum*

- 400 articles addressed the role of *P. acnes* in acne
- ~150 selected for detailed review

- Emphasis on in vivo data
- Focus on topics addressed by at least 2 independent studies
- No date limit
- Similar numbers dealt with microbiological vs immunological aspects

- Also searched for alternative hypotheses
Proving a link between a microbe and a disease

• Koch’s postulates
  • the microorganism should be present in every case of the disease
  • it should be isolated in pure culture & re-create the same symptoms of disease when inoculated into a new host
  • the isolate recovered from the new host should be indistinguishable from the original isolate

• Problems
  • *P. acnes* is a member of the resident commensal flora of healthy skin (& mucous membranes)
  • Is it reasonable to expect every lesion to be colonised?
Assumptions

1. Not all follicles are colonisable
   - SER exceeds maximum growth rate of *P. acnes*
   - Water activity too low
   - Niche already occupied
   - Antimicrobials of cellular origin may be present in inhibitory concs
   - Immune response may already be activated

2. Immune response is heterogeneous
   - More than one antigen
   - Innate & adaptive mechanisms involved & linked

3. Inflammation can occur via more than one pathway
P. acnes: indisputable facts

- Distribution & population densities on skin in acne and non-acne reflect location and number of sebaceous glands
  - Predominant micro-organism on face and trunk
  - Still present when acne regresses
- A closely related organism, P. granulosum, has a similar distribution but lower prevalence
- P. acnes is a potent adjuvant
  - Non-specifically up-regulates cellular immune responses to unrelated antigens
P. acnes & inflammatory acne
The balance of probability

Innocent

?  Guilty

- Commensal
- Antibiotic resistance
- Follicular colonisation
- Biofilms
- Virulence factors
- T cell mitogen
- Adjuvant activity
- Immune response
- Antigenicity
- Acnegenic strains
Epidemiological evidence

- Acne is a uniquely human disease
- Pilosebaceous follicles are unique to human skin
- *P. acnes* may be a uniquely human skin bacterium
- *P. acnes* utilises glycerol obtained by hydrolysis of sebum triglycerides (TG) as a carbon & energy source
  - Human sebum contain more TG than any other species
Microbiological evidence I

Age-related changes in propionibacterial numbers on the skin surface: Leyden et al 1975

- Never repeated
- Acne may be due to colonisation of immature follicles
**Microbiological evidence II**

### Follicular distribution & population densities

<table>
<thead>
<tr>
<th></th>
<th>Healthy follicles (n = 77)</th>
<th>Uninvolved follicles acne (n = 138)</th>
<th>Closed comedones (n = 28)</th>
<th>Open comedones (n = 28)</th>
<th>1-day papules (n = 50)</th>
<th>3-day papules (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% colonised</td>
<td>91</td>
<td>17</td>
<td>46</td>
<td>75</td>
<td>68</td>
<td>79</td>
</tr>
<tr>
<td>Geometric mean count</td>
<td>$1.1 \times 10^5$</td>
<td>$8.0 \times 10^4$</td>
<td>$1.2 \times 10^5$</td>
<td>$2.9 \times 10^5$</td>
<td>$2.7 \times 10^5$</td>
<td>$5.5 \times 10^5$</td>
</tr>
</tbody>
</table>

Data from Puhvel et al 1975 and Leeming et al 1988; Leeming’s data confirmed by Till et al 2002

10 other studies (1900 – 1979) examined lesions (mainly pustules & comedones) by histology or culture; prevalence of propionibacteria 64 – 100%
Recent study using MAbs (Jahns et al 2012) found lower prevalence in acne (38%) but did not distinguish between uninvolved follicles & lesions
Microbiological evidence III

- Absence of propionibacteria in comedones from prepubertal children (Lavker et al 1981) using
  - Culture
  - Light microscopy
  - EM

- Pivotal & highly influential study *BUT*
- Never repeated despite small numbers (28 comedones from 5 children aged 9-11 y)
Evidence from the immune response I

- Acne is characterised by a perifollicular infiltrate in which CD4\(^+\) T\(_h\)1 cells & macrophages predominate
  - Severe acne is TB-like – characterised by granulomatous inflammation
  - Infiltrate consistent with DTH response to a persistent intracellular pathogen
- *P. acnes* persists within macrophages (and keratinocytes) both in vivo & in vitro
Evidence from the immune response II

- T cells have been cloned from acne lesions, shown to be reactive to *P. acnes* & express a Th1 cytokine profile (Mouser et al 2003)

![Graph showing immune response to different types of microbes in various T cell lines]
**P. acnes induces T\textsubscript{h}1 responses**

- *P. acnes* immunotherapy effects a T\textsubscript{h}2 to T\textsubscript{h}1 switch in:
  - Atopic dermatitis (mouse model, Kitagawa et al 2011)
  - Malignant melanoma (mouse model, Tsuda et al 2011)
  - Focal segmental glomerulosclerosis (mouse model, Reis et al 2011 [epub])
  - *Trypanosoma cruzi* infection (mouse model, Mussalem et al 2006)
- *P. acnes* induces hepatic granuloma in a widely used mouse model
  - LPS alone has no effect
  - LPS + *P. acnes* \(\rightarrow\) T\textsubscript{h}1 driven inflammatory response
- *P. acnes* microparticle adjuvant (cell wall skeleton + DNA fragments) promotes T\textsubscript{h}1 responses (Girvan et al 2011)
Evidence from the immune response III

- Intradermal injection of killed *P. acnes* induces delayed inflammatory response, the extent of which correlates with number of bacteria injected & severity of acne (Kersey et al 1980)

Similar result observed by Puhvel & Sakamoto (1977) for acne v non-acne: infiltrate shown to be predominantly lymphocytic
Evidence from the immune response IV

- *P. acnes* cells in acne lesions, even non-inflammatory ones, are coated with IgG
- Primed for complement activation & phagocytosis

<table>
<thead>
<tr>
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<th>Mean titre (anti-immunoglobulin test)</th>
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<tr>
<td></td>
<td>IgM</td>
</tr>
<tr>
<td>Comedones</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Pustules</td>
<td>&lt;1</td>
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<table>
<thead>
<tr>
<th></th>
<th>Immunofluorescence mean intensity (0-4 scale)</th>
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<tr>
<td></td>
<td>IgM</td>
</tr>
<tr>
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<td>1</td>
</tr>
<tr>
<td>Pustules</td>
<td>1</td>
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</table>

From Knop et al 1983
Evidence from treatment outcomes

- Antimicrobials which target *P. acnes* are clinically effective
- Skin colonisation with Ery\(^R\) propionibacteria associated with poor therapeutic outcome (unchanged or worse after \(\geq 4\) mo) on oral erythromycin (Eady et al 1989)

<table>
<thead>
<tr>
<th>Prevalence of skin carriage of erythromycin resistant propionibacteria</th>
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<tr>
<td>Responders (n = 21)</td>
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<tr>
<td>24%</td>
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P<0.001 responders v non-responders

Criticisms: surface not follicular samples, small numbers
Evidence for acnegenic *P. acnes* strains

- Multilocus sequence typing (MLST, McDowell et al 2012)

<table>
<thead>
<tr>
<th>Phylotype</th>
<th>Major clonal complexes</th>
<th>Acne % of 105 isolates</th>
<th>Skin % of 77 isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA&lt;sub&gt;1&lt;/sub&gt;</td>
<td><strong>CC1, CC3, CC4</strong></td>
<td>73.3</td>
<td>40.1</td>
</tr>
<tr>
<td>IA&lt;sub&gt;2&lt;/sub&gt;</td>
<td>CC2</td>
<td>9.5</td>
<td>18.2</td>
</tr>
<tr>
<td>IB</td>
<td>CC5</td>
<td>6.6</td>
<td>7.8</td>
</tr>
<tr>
<td>IC</td>
<td>Novel</td>
<td>3.8</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>CC6, CC72</td>
<td>7.6</td>
<td>15.6</td>
</tr>
<tr>
<td>III</td>
<td>CC77</td>
<td>0</td>
<td>18.2</td>
</tr>
</tbody>
</table>

- 17 articles report strain differences in effects on immune cells, keratinocytes, sebocytes, secretome or transcriptome
- Differences appear not to reflect traits associated with specific clones
## Alternative hypotheses: inflammatory acne

<table>
<thead>
<tr>
<th>Agent</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>Marples et al 1970</td>
</tr>
<tr>
<td><em>Malassezia furfur</em></td>
<td>Marples et al 1972</td>
</tr>
<tr>
<td>Comedonal components</td>
<td>Puhvel et al 1977; Leeming et al 1988; Strauss &amp; Pochi 1965</td>
</tr>
<tr>
<td>Statum corneum (SC) &amp; keratin</td>
<td>Bladon et al 1985; Terui et al 1989</td>
</tr>
<tr>
<td>Unculturable bacteria within follicles</td>
<td>Bek-Thomsen et al 2008</td>
</tr>
<tr>
<td><em>Demodex</em> mites</td>
<td>Zhao et al 2012</td>
</tr>
<tr>
<td>IL-1α</td>
<td>Ingham et al 1992; Jeremy et al 2003</td>
</tr>
<tr>
<td>Squalene peroxides</td>
<td>Chiba et al 2000; Ottaviani et al 2006; Ottaviani et al 2010</td>
</tr>
<tr>
<td>Neurogenic inflammation</td>
<td>Toyoda et al 2002</td>
</tr>
<tr>
<td>Leukotrienes</td>
<td>Zouboulis 2009</td>
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</tbody>
</table>
Conclusions I

• Overwhelming body of evidence implicates propionibacteria, and *P. acnes* in particular, in inflammatory acne

• *P. acnes* is **neither** a primary pathogen **nor** a bystander

• *P. acnes* plays an **active** but not intentional role in determining the **nature** and **extent** of the immune response
Conclusions II

- Acne is almost certainly NOT initiated by *P. acnes*
- Ductal obstruction prevents organisms & their products escaping onto the skin surface
- Inflammation is an inevitable consequence of exposure of WBC/viable KC to a potent immuno-modulator (antigen, mitogen, adjuvant, chemoattractant)

SEM comedone × 3,170 showing *P. acnes* adhering to squames