THE ROLE OF BACTERIA IN ACNE

P. acnes or *P. bystander*?

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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 v 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?

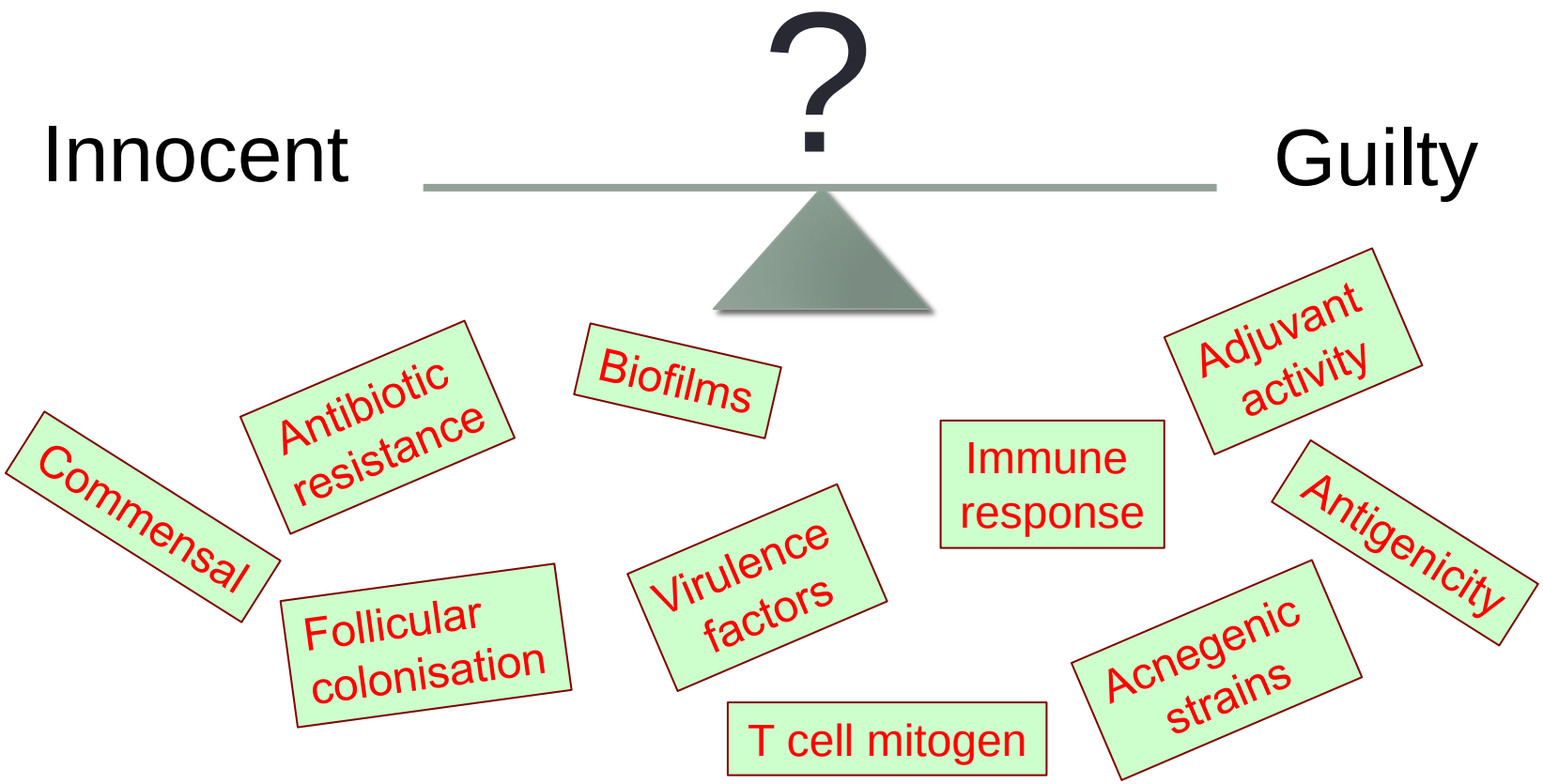
Assumptions

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

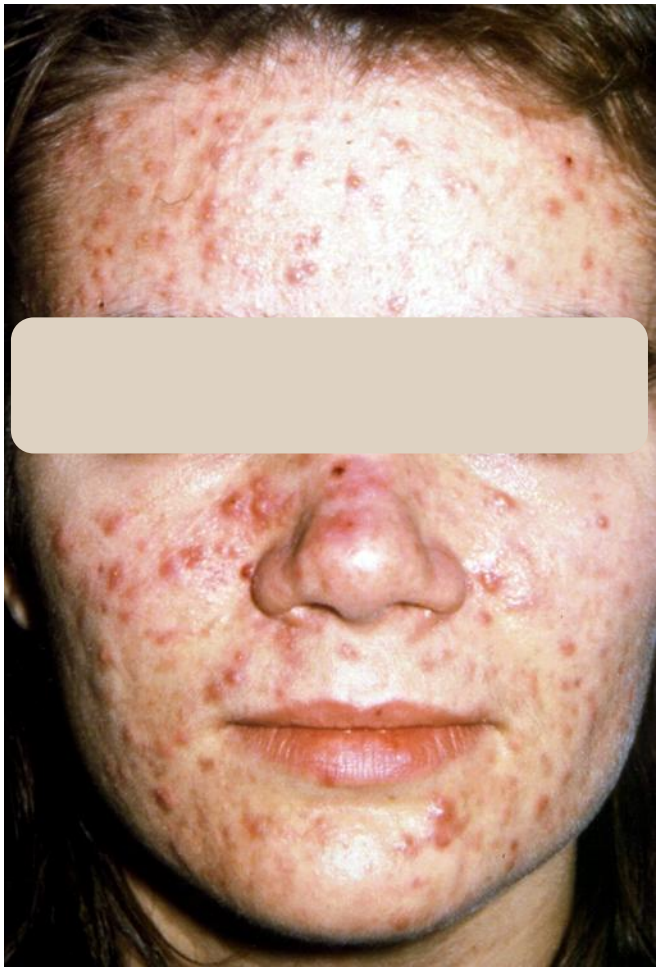
- Distribution & population densities on skin in acne and non-acne reflect location and number of sebaceous glands
  - Predominant micro-organism on face and trunk
  - Still present when acne regresses
- A closely related organism, *P. granulosum*, has a similar distribution but lower prevalence
- *P.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

P. acnes & inflammatory acne
The balance of probability



Epidemiological evidence

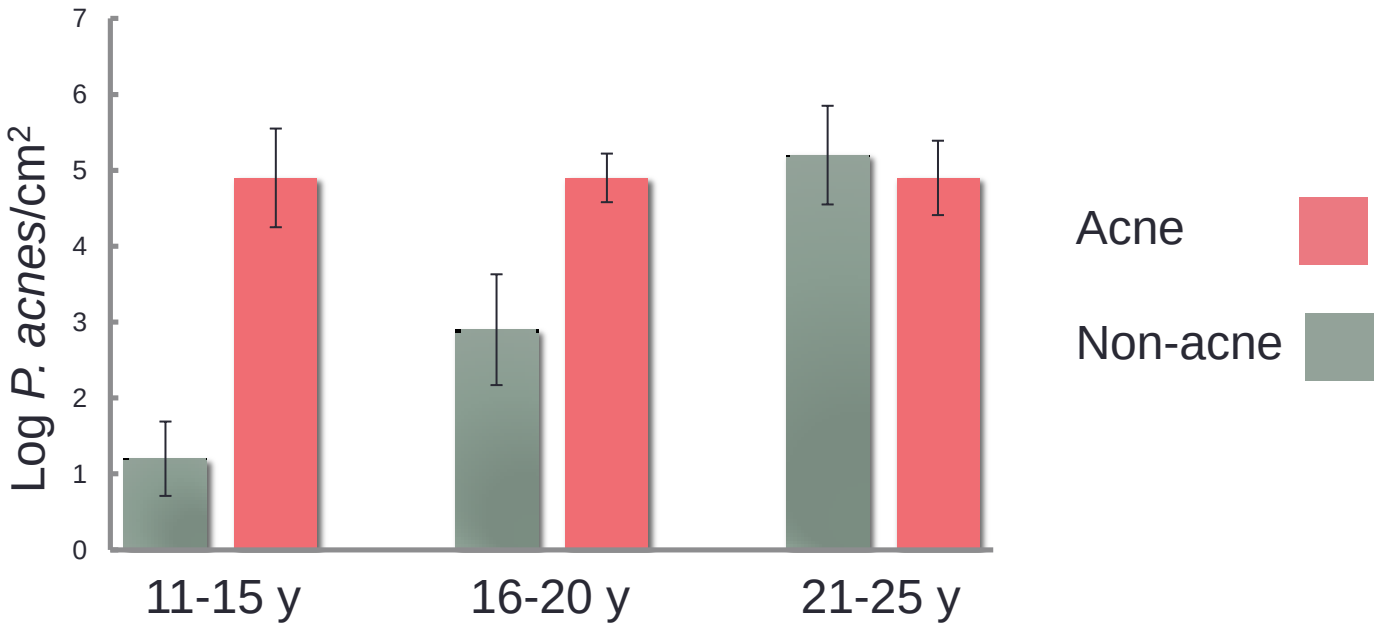
- Acne is a uniquely human disease
- Pilosebaceous follicles are unique to human skin
- *P. acnes* may be a uniquely human skin bacterium
- *P. acnes* utilises glycerol obtained by hydrolysis of sebum triglycerides (TG) as a carbon & energy source
  - Human sebum contain more TG than any other species



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

Healthy follicles (n = 77)		Uninvolved follicles acne (n = 138)		Closed comedones (n = 28)		Open comedones (n = 28)		1-day papules (n = 50)		3-day papules (n = 19)	
% colonised	Geometric mean count	% colonised	Geometric mean count	% colonised	Geometric mean count	% colonised	Geometric mean count	% colonised	Geometric mean count	% colonised	Geometric mean count
91	1.1×10^5	17	8.0×10^4	46	1.2×10^5	75	2.9×10^5	68	2.7×10^5	79	5.5×10^5

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

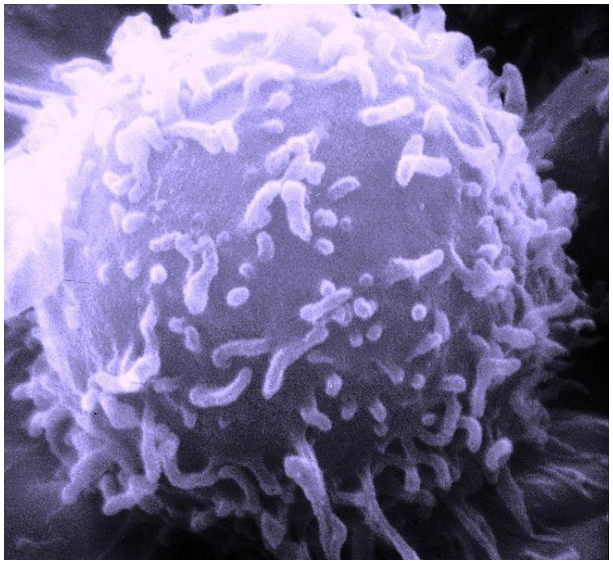
Microbiological evidence III

- 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)

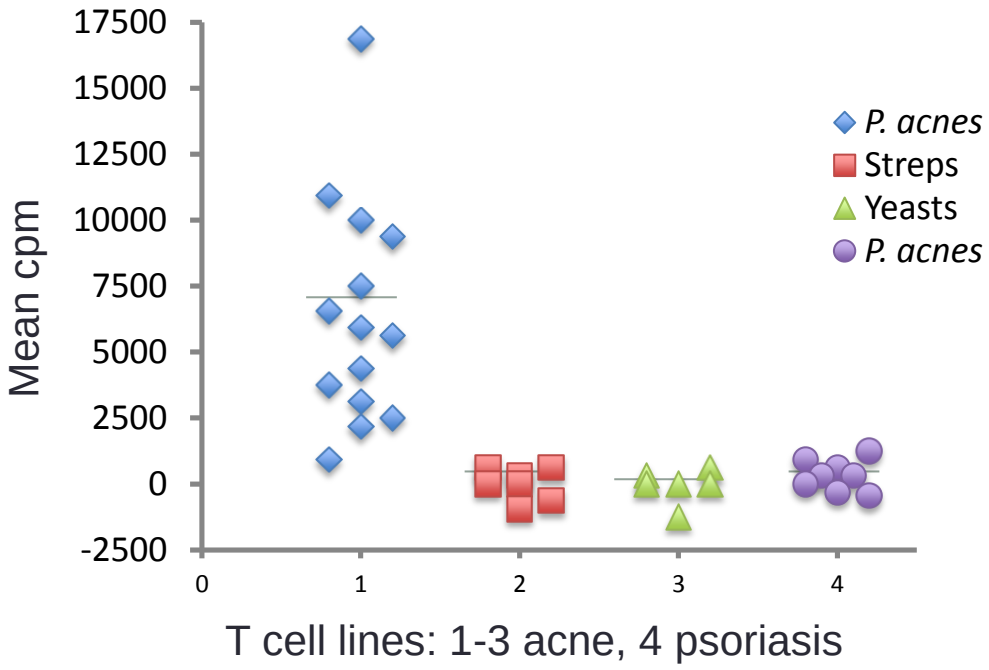
Evidence from the immune response I

- Acne is characterised by a perifollicular infiltrate in which $CD4^+$ T_h1 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)

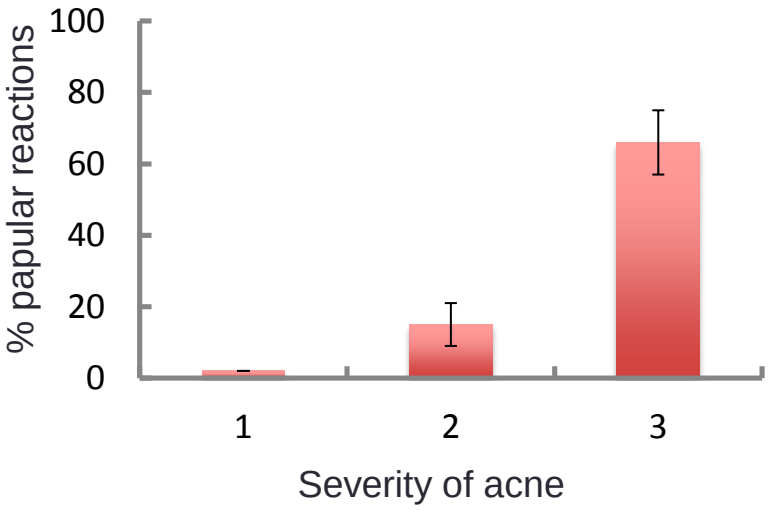


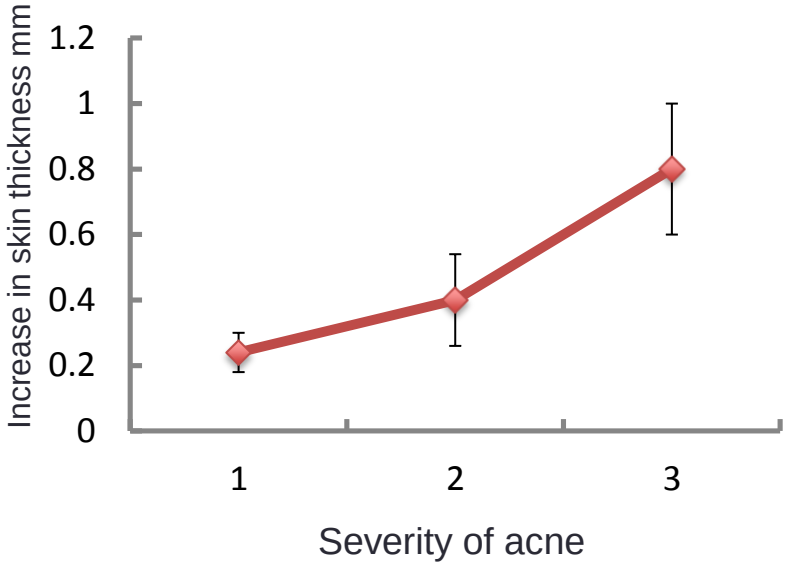
P. acnes induces T_h1 responses

- *P. acnes* immunotherapy effects a T_h2 to T_h1 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* ⟶ T_h1 driven inflammatory response
- *P. acnes* microparticle adjuvant (cell wall skeleton + DNA fragments) promotes T_h1 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-inflamed ones, are coated with IgG
- Primed for complement activation & phagocytosis

	Mean titre (anti-immunoglobulin test)		
	IgM	IgA	IgG
Comedones	<1	<1	3.6 ± 2.9
Pustules	<1	<1	4.8 ± 2.6

	Immunofluorescence mean intensity (0-4 scale)		
	IgM	IgA	IgG
Comedones	1	1	3.3
Pustules	1	1	2.8

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 ≥4 mo) on oral erythromycin (Eady et al 1989)

Prevalence of skin carriage of erythromycin resistant propionibacteria		
Responders (n = 21)	Non-responders (n = 30)	Untreated controls (n = 100)
24%	70%	3%

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)

Phylotype	Major clonal complexes	Acne % of 105 isolates	Skin % of 77 isolates
IA_1	**CC1**, CC3, CC4	73.3	40.1
IA_2	CC2	9.5	18.2
IB	CC5	6.6	7.8
IC	Novel	3.8	0
II	CC6, CC72	7.6	15.6
III	CC77	0	18.2

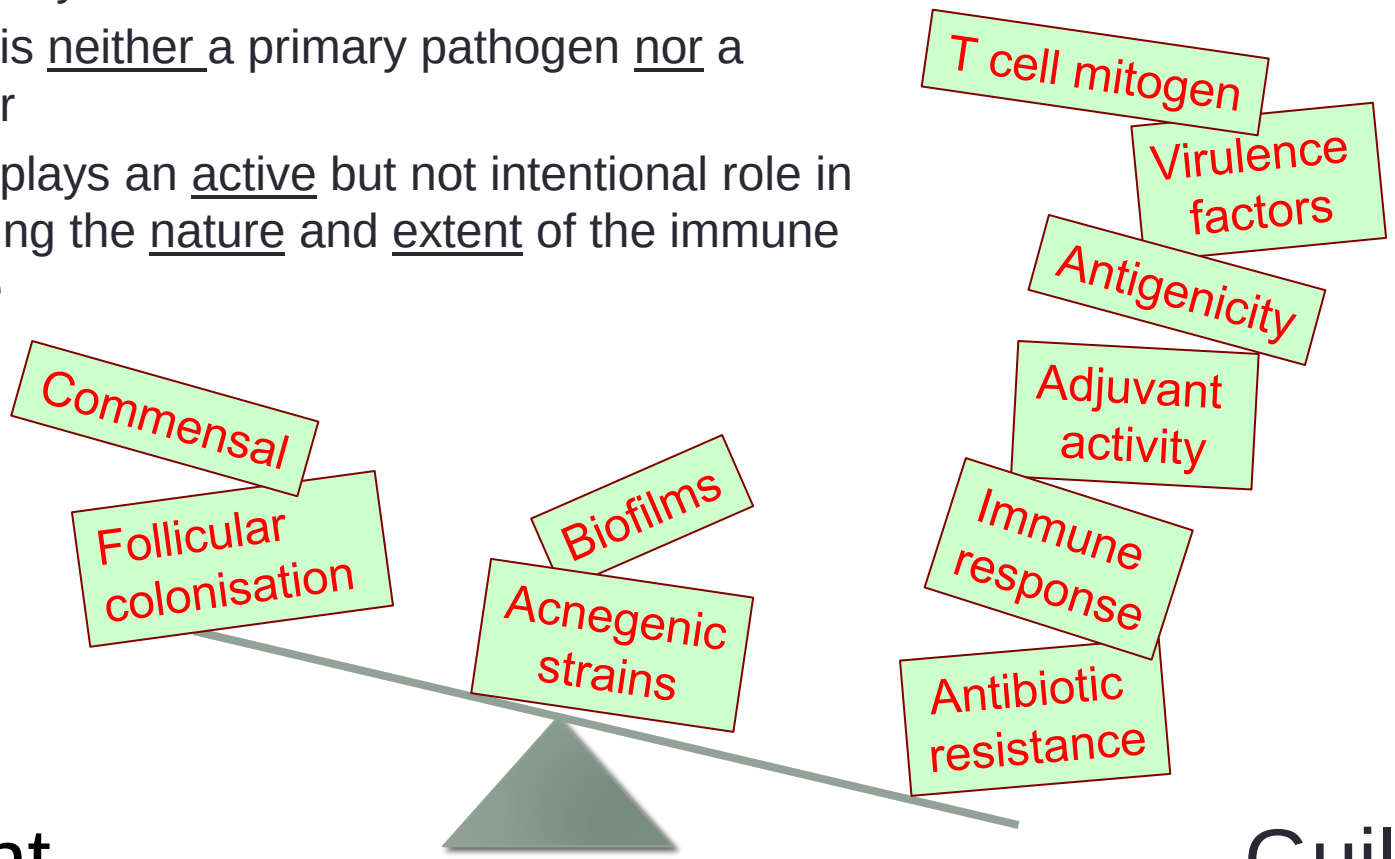
- 17 articles report strain differences in effects on immune cells, keratinocytes, sebocytes, secretome or transcriptome
- Differences appear not to reflect traits associated with specific clones

Alternative hypotheses: inflammatory acne

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

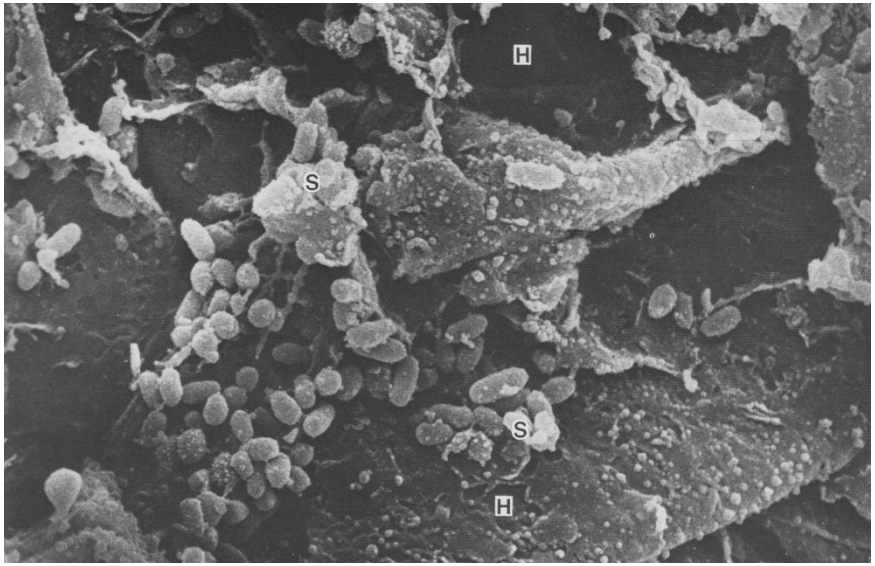
Conclusions I

- 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