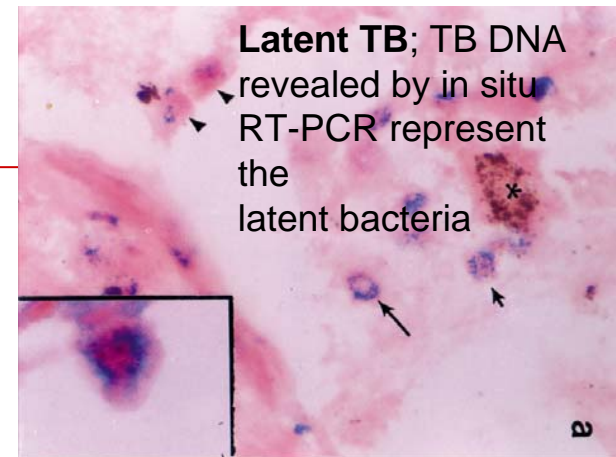


IFN- γ release assays and treatment of TB infection: what can we learn from these data?



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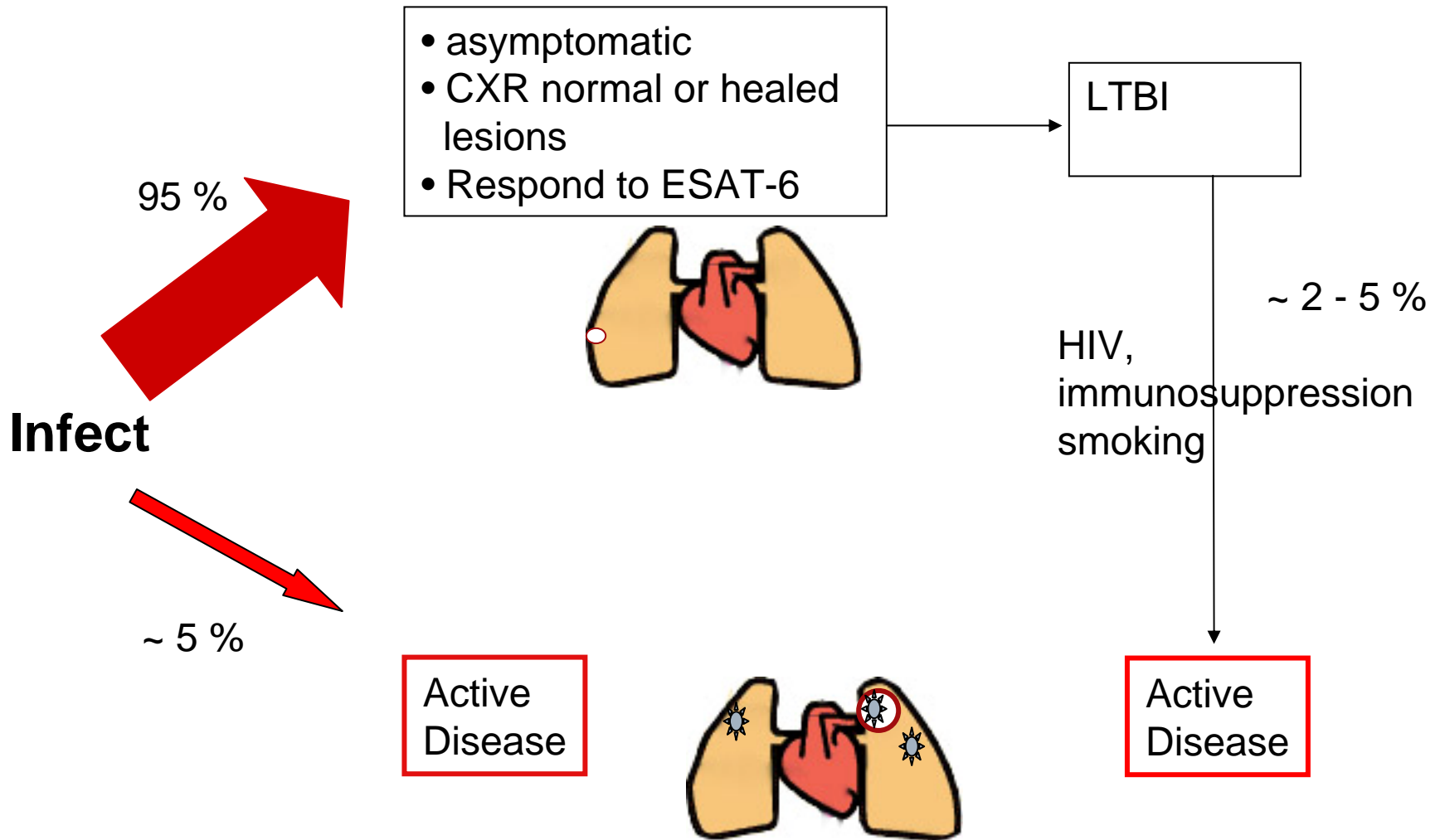
Email: k.dheda@ucl.ac.uk



Overview

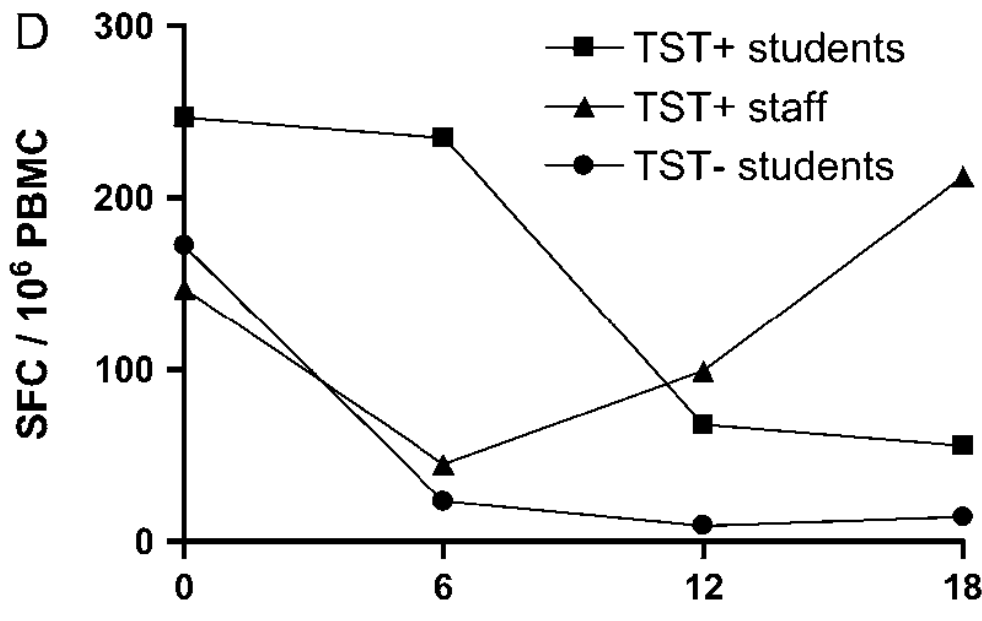
1. Once infected; always infected: how true is this adage?
 2. What are the kinetics of ESAT-6 production and of antigen-specific T cells in peripheral blood?
(Can ESAT-6 secretion be intermittent and are T cells always in peripheral blood compartment in LTBI?)
 3. Does anti-TB treatment effect sterilisation and hence abrogate IGRA responses?
(How specific is the IGRA assay and can we use it as a proxy marker of disease activity or cure ?).
 4. Data on IGRAs during and after completion of TB treatment are discrepant; how do we explain this?
(How does the environment, host and strain modulate IGRA responses?)
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Life cycle of *M. tuberculosis*



Once infected; always infected: how true is this adage?

- Only 30 – 50% of close contacts (TST or IGRA) have likely TB infection
 - N= 735 HC of index cases; 41% TST+ and 30% IGRA+
Hill PC et al; CID; 2004 (The Gambia)
 - N= 942 HC; 50% TST + and 42% IGRA +
Soysal A et al; Lancet, 2005 (Turkey)
 - N= 111; 28% TST+ and 32% IGRA +
Shams H et al, AJRCCM (USA)
 - Ferebee SH, Am Rev Resp Dis, 1962 (US PHS)
Veening GJJ, Bull Int Union Tuberc, 1968
 - presumably innate effector mechanisms may prevent establishment of infection in a significant majority
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N= 14 TST – IGRA+ students (•)

Ewer et al,
AJRCCM, 2006

□ spontaneous reversion may suggest that some subjects have ‘acute resolving infection’

IGRA: Pai et al, AJRCCM, 2006 (IGRA)

Hassan M et al, 1st Global IGRA Conference, 2007 (abstract; IGRA)

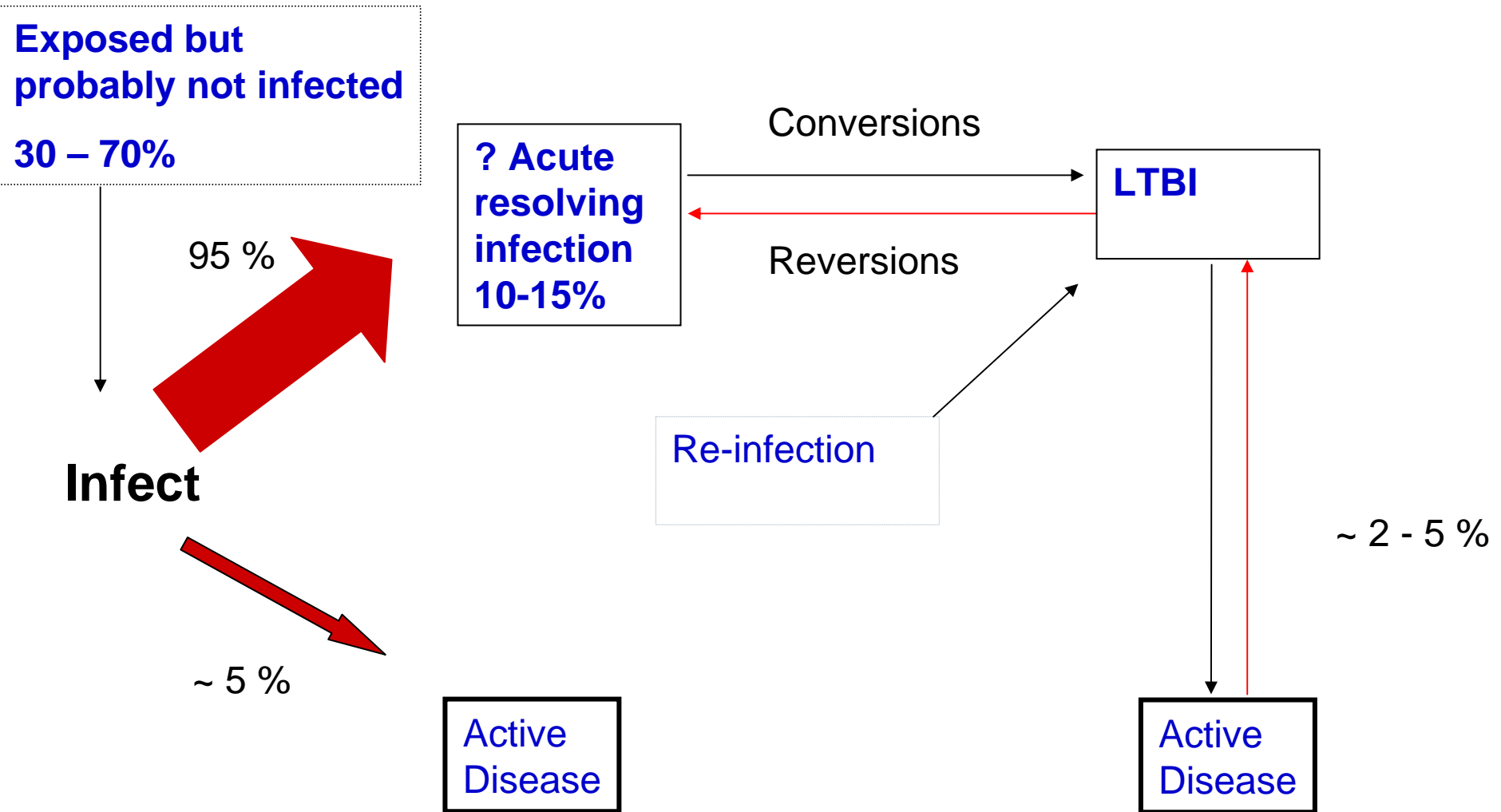
TST: Menzies D, AJRCCM, 1999 & Fine PE, IJTLD, 1999

Animal studies: Vordermeier HM, Infect & Imm, 2002

-
- ❑ Alternative explanation: LTBI but the circulating T cells are below the detection limit of the ELISPOT assay
 - ❑ Compartment-specific effect
Tully G, J Immunol, 2005
 - ❑ Shift in cytokine profile with loss of IFN- γ production
 - ❑ Change in the antigen secretion profile of mycobacteria
Haile Y, Microbiology, 2002

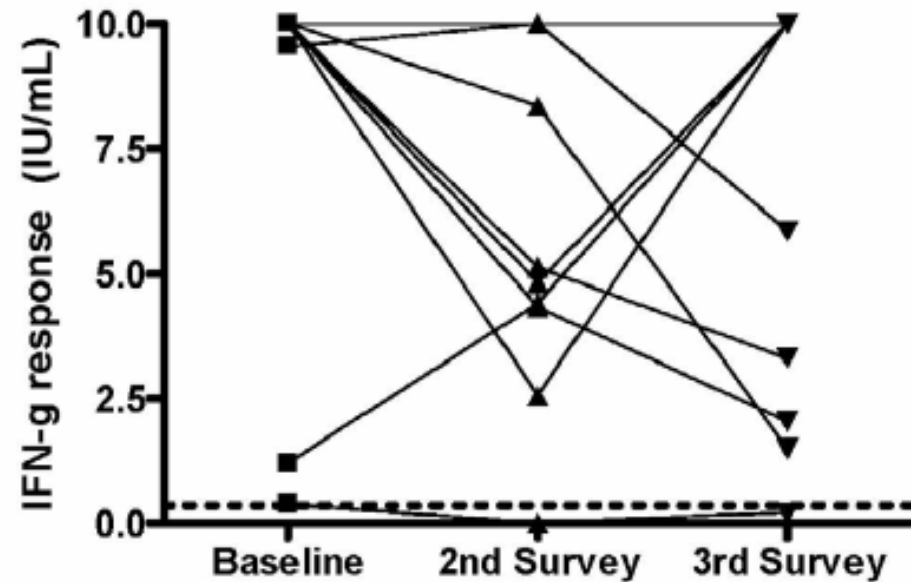
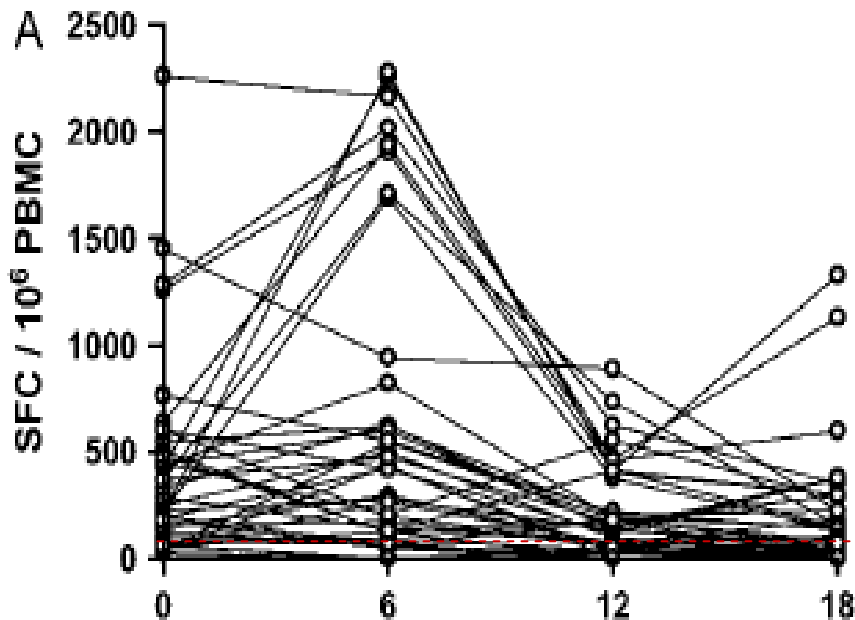
LONGITUDINAL STUDIES REQUIRED TO DELINEATE THESE POSSIBILITIES

Life cycle of *M. tuberculosis*



What are the kinetics antigen-specific T cells and ESAT-6 in peripheral blood?

Treated for LTBI

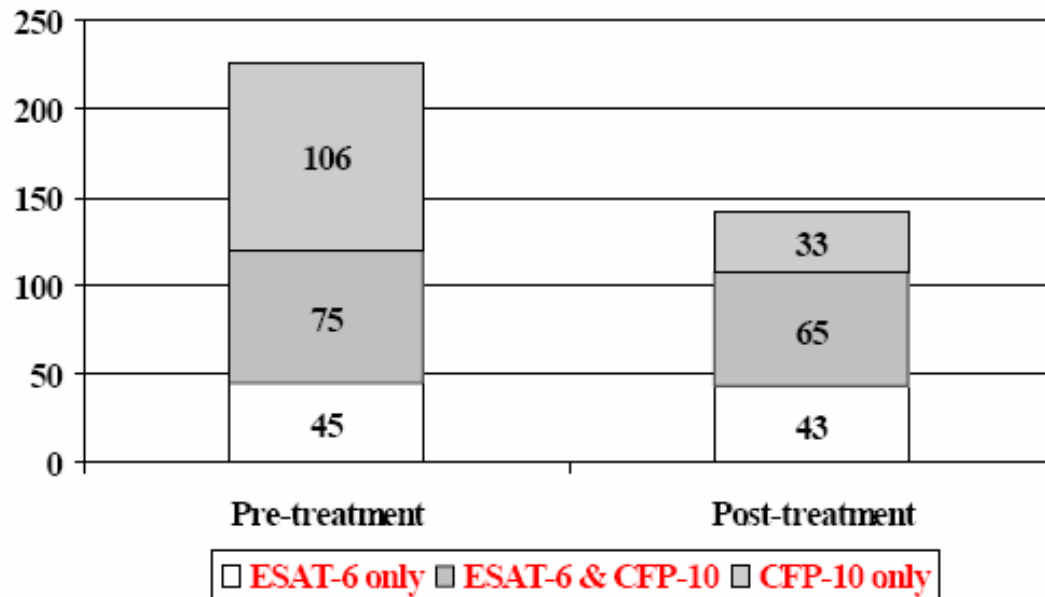


□ Longitudinal IGRA responses over 18 months in N= 32

Ewer et al, AJRCCM, 2006

□ Over 22 months in N= 9
Pai et al, J Occ Med & Tox, 2006

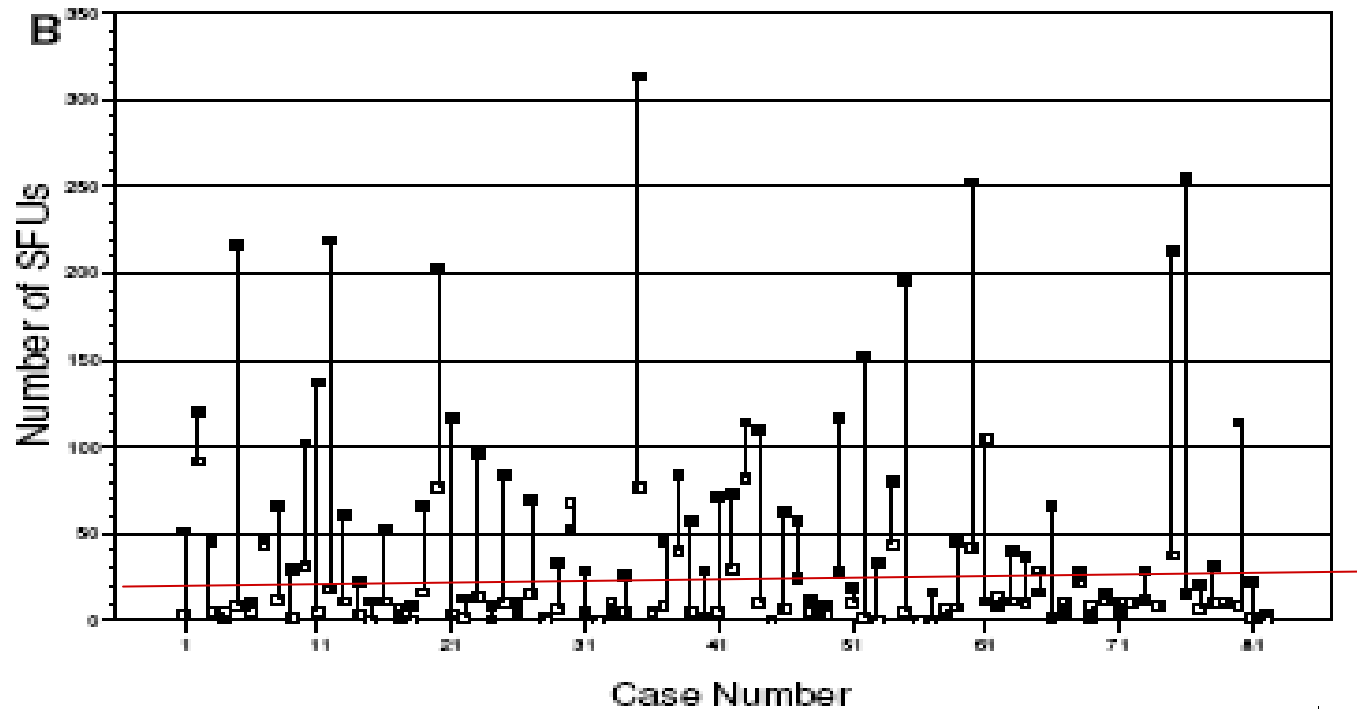
Number of contacts with response to ESAT-6 only, CFP-10 only and both ESAT-6 and CFP-10 antigens
Pre- and post-LTBI treatment



Chee C et al,
AJRCCM, 2006

		CFP-10			
		Fall	Unchanged	Rise	Total
ESAT-6	Fall	88	8	14	110
	Unchanged	20	0	8	28
	Rise	52	6	30	88
	Total	160	14	52	226

Treatment of active TB



- At 12 months post-treatment N= 38/82 (45%) ELISPOT positive
Aiken et al, AJRCCM, 2006 (The Gambia)

How do we explain these variable patterns?

- T cell-related factors
 - Kinetics of ESAT-6 production
 - Environmental factors
-

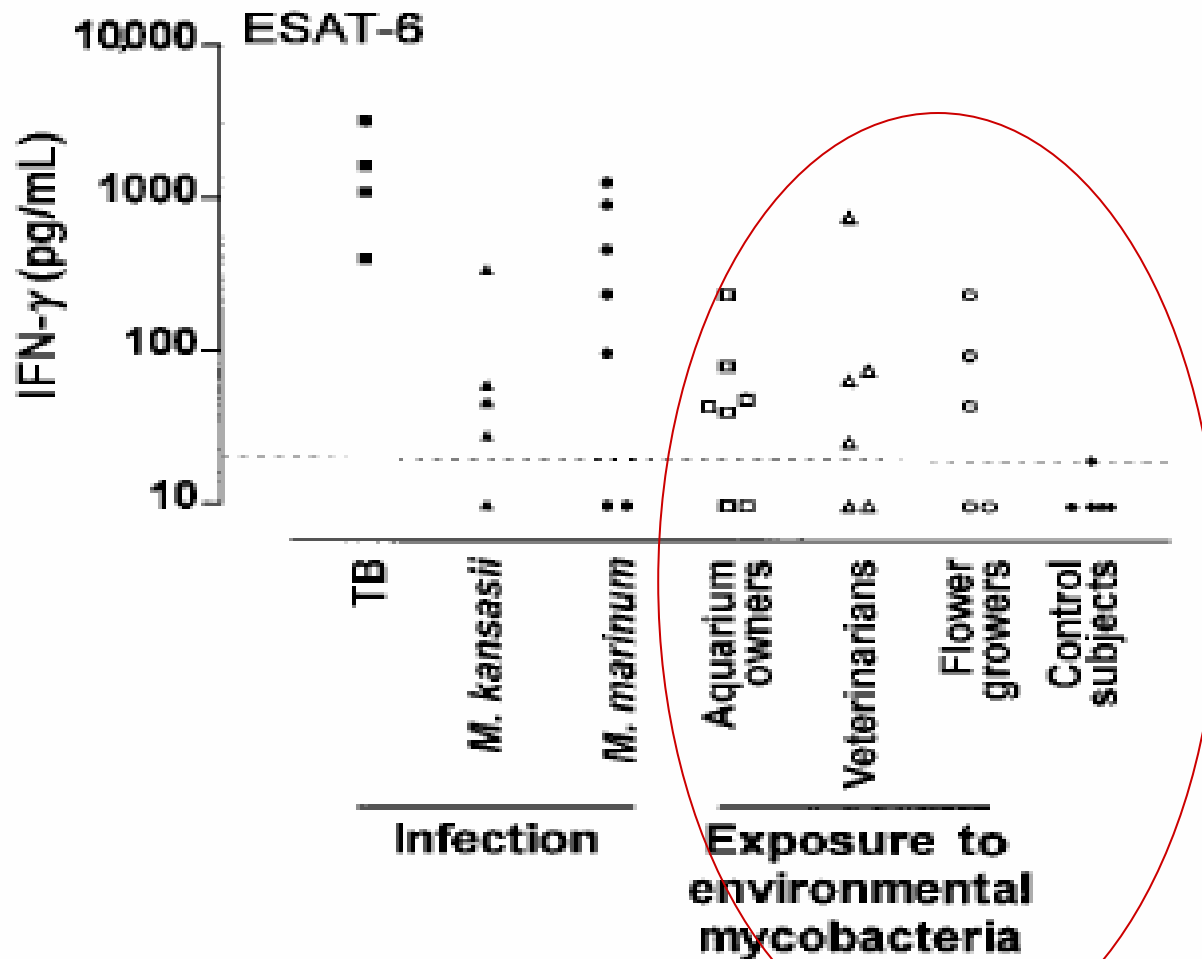
(i) The persistently + responses despite treatment

1. Re-infection (unlikely in the UK study)
2. - Half-life of HIV-specific T cells after HAART 6 wks
Ogg GS, J of Virol, 1999
- Anti-TB treatment (\downarrow antigen load) $t_{1/2}$ T cells 8 - 10 wks
Pathan et al, JI, 2001
Carrara Et al, CID, 2004
Thus, responses may be positive several months after putative microbiological sterilisation
3. T cell phenotypes: possible that some individuals may retain a population of persisting effector memory cells in the presumed absence of residual disease
Wu-Hsieh et al, CID, 2001

How can we explain the persistently + responses despite treatment

4. Particularly in high burden settings exposure to environmental mycobacteria producing homologues of ESAT-6 may play a role

[Arend et al, JID, 2004](#)



- Arend SM, JID, 2002
- Walters WR, Clin Vaccine Imm; 2006

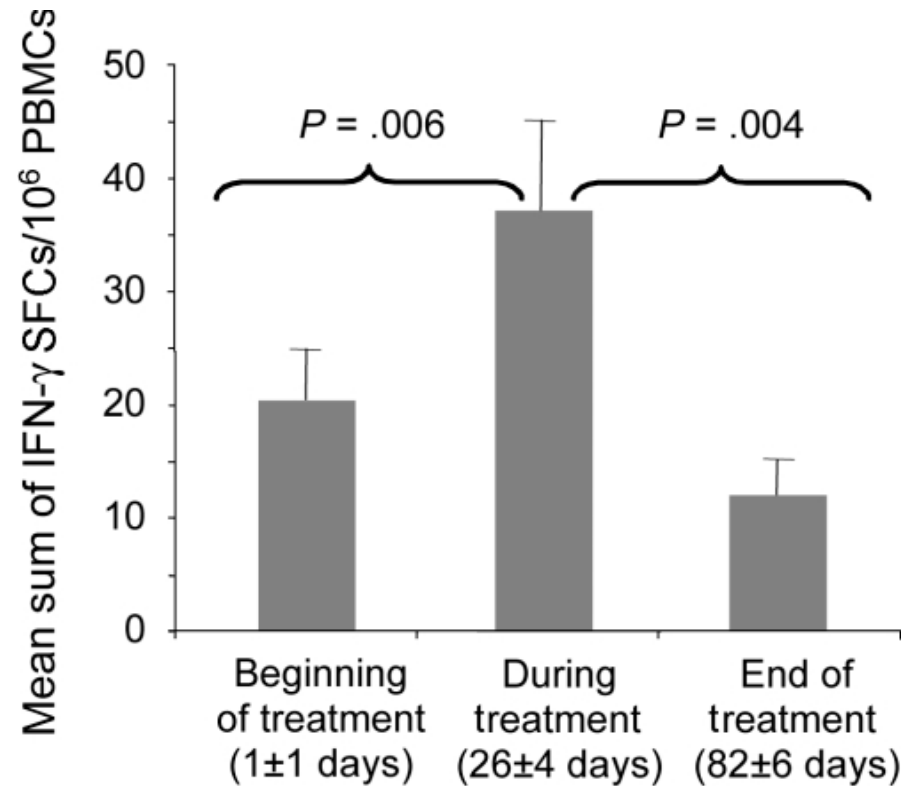
(ii) Transient increase

Wilkinson et al, J Infect Dis, 2006

Nicol M, CID, 2005,

Ewer K et al, AJRCCM, 2006

n = 23 treated for LTBI, UK,
transient increase then
declining responses



❑ cytosolic stores of ESAT-6 released with mycobacterial killing (Pym et al, Nat Med, 2003)

❑ stress reaction in response to treatment

(iii) Negative post-treatment responses

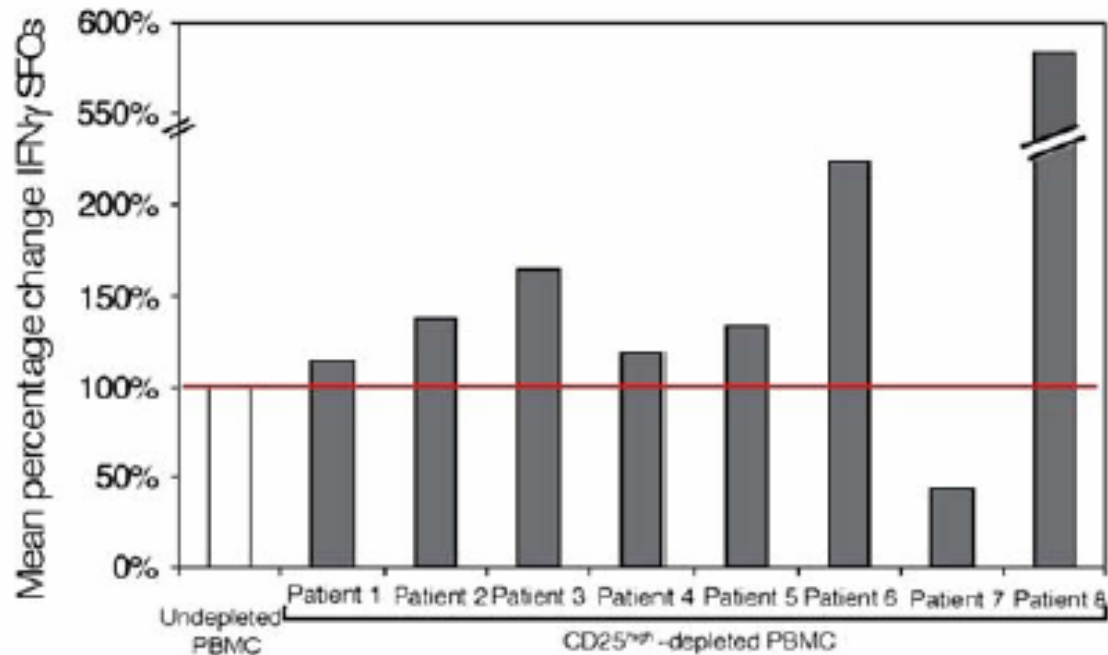
1. Likely that in many cases this reflects sterile cure of TB infection/ disease
2. Alternatively, may reflect site-specific T cell heterogeneity
Several ESAT-6 epitopes were only recognised at the site of disease (pleural fluid and lung granulomas)

[Wilkinson KA et al, CID, 2004; Tulley G, J Immunol, 2005](#)

3. The T cell frequency may be below the detection limit of the assay or around the detection threshold (T cells in the tissues)
4. Regulatory T cell profile modulate IGRA responses

[Guyot-Revol et al, AJRCCM, 2006](#)

T cell responses: Treg



- Treg: downregulate effector responses and proliferation of other T cells

Guyot-Revol V et al; AJRCCM; 2006

5. 5-20% of patients with active disease are IGRA negative and many may remain so even during the course of treatment

- 16/89 (18%) were ELISPOT negative at diagnosis and at 12 months after start of anti-TB treatment

[Aiken et al, BMC Infect Dis, 2006](#)

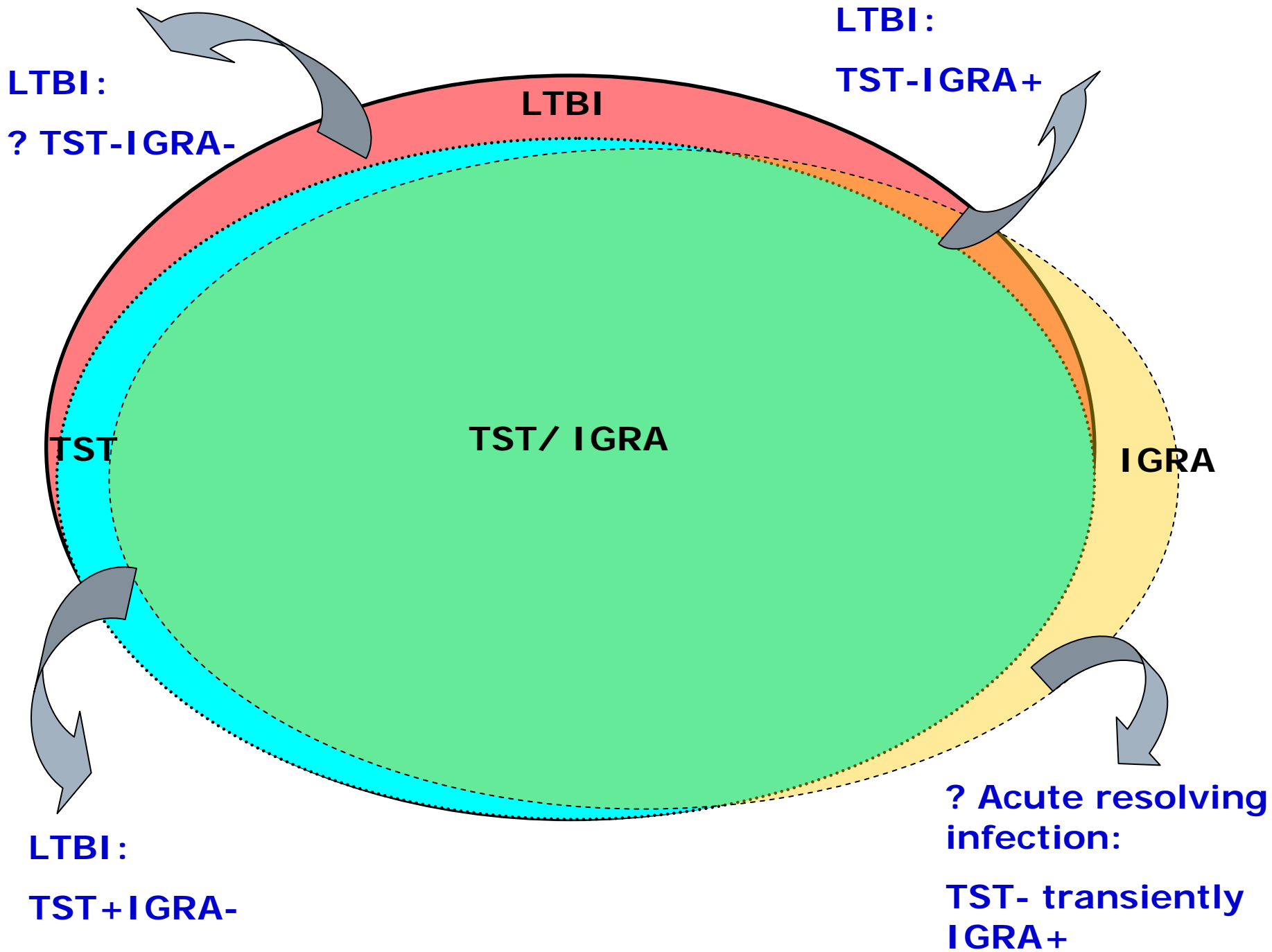
? all TB strains equally secrete ESAT-6, secretion may be intermittent, post-translational modification, or there may be holes in the TCR repertoire or HLA types that attenuate responses to ESAT-6/CFP-10

[deJong, J Infect Dis, 2005](#)

T cell/ ESAT-6 kinetics: summary

Given the likely variability in ESAT-6 kinetics and T cell kinetics/ phenotypes or heterogeneity

- LTBI may be IGRA negative
 - False + IGRA responses may occur
-



IGRA responses before, during or after anti-TB treatment for active disease

Three reasons why these studies are important:

1. Informs on the specificity of the assay
 2. Supports the notion that the assay is detecting effector cells (not memory of eradicated remote infection)
 3. Supports the use of IGRA as a proxy marker of disease activity
-

Declining responses

- Lalvani A et al, J Infect Dis, 2001 n= 5
- Pathan et al, J Immunol, 2001 n= 12
- Carrara et al, CID, 2003 n= 18
- Dheda et al, J Infect, 2007 n= 33 post-Rx
- **Aiken et al, BMC Infect Dis n= 84**

Overnight incubation, peptides

Persistently + responses

- Ulrichs T, IJTLD, 2000 n= 10
- Vekemans J, Inf & Imm, 2001 n= 18
- Wu-Hsieh BA, CID, 2001 n= 18 post-Rx
- Al-Attayah R, FEMS I M M, 2003 n= 12
- Ferrand RA, IJTLD, 2005 n= 10 post-Rx

Incubation of several days, proteins

How do we explain these discrepant results?

1. Laboratory/ technical factors
 2. - Host factors (T cell phenotypes, kinetics, TCR specificity, HIV, BCG)
 - Environmental (NTMs, re-infection)
 - Organism (strain, kinetics of RD1 antigens)
- N=17 patients with culture + TB with negative responses post-treatment: protein antigens (ESAT-6), endotoxin and prolonged incubation failed to elicit + responses
- [Dheda et al, J Infect, in press](#)
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Summary

- ❑ Significant proportion of individuals probably remain uninfected when heavily exposed
 - ❑ A significant minority of infected subjects probably have acute resolving infection
 - ❑ **Environmental** (NTMs), **host** (T cell kinetics and phenotypes) and the **organism** (ESAT-6 kinetics and strain) all modulate IGRA responses: hence it is possible that false + and false - IGRA responses occur
 - ❑ Further longitudinal data and the evaluation of other novel antigens are required to overcome these drawbacks
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Acknowledgments

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