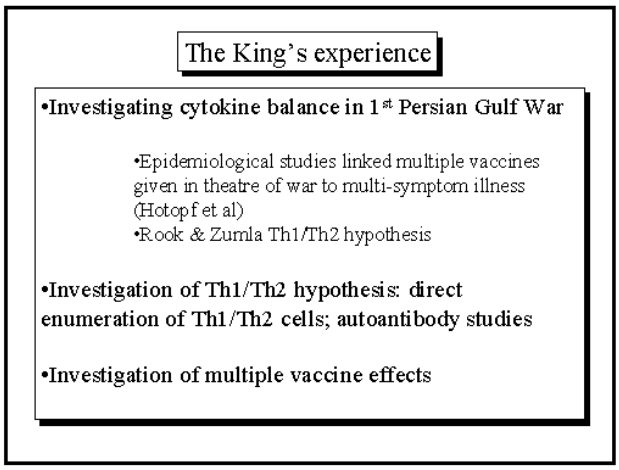
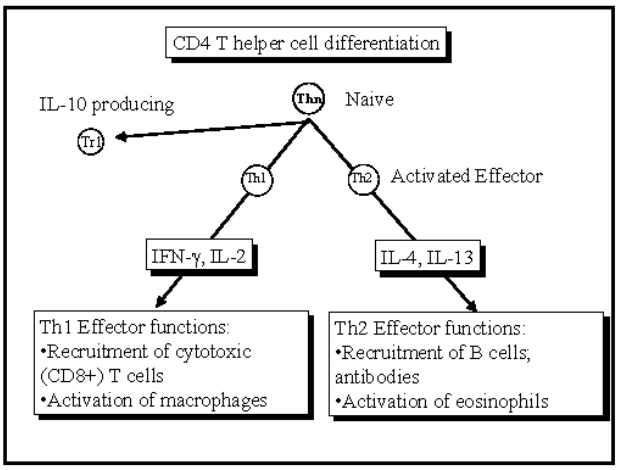
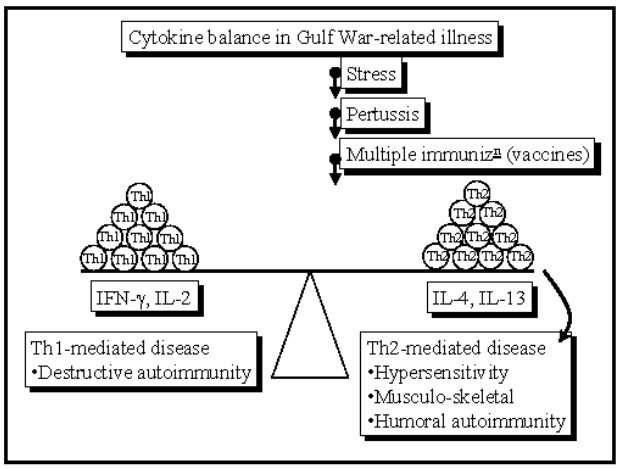
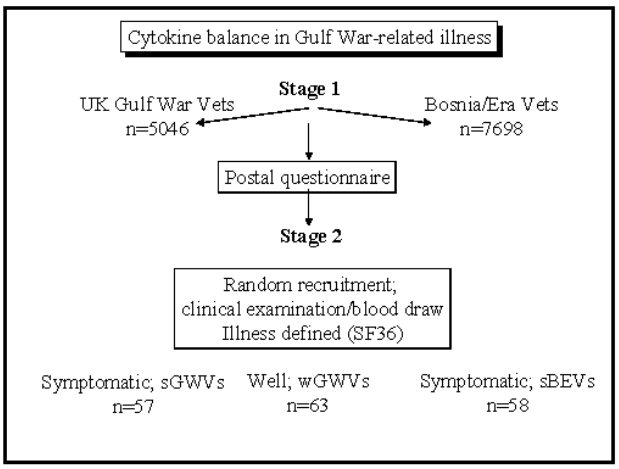
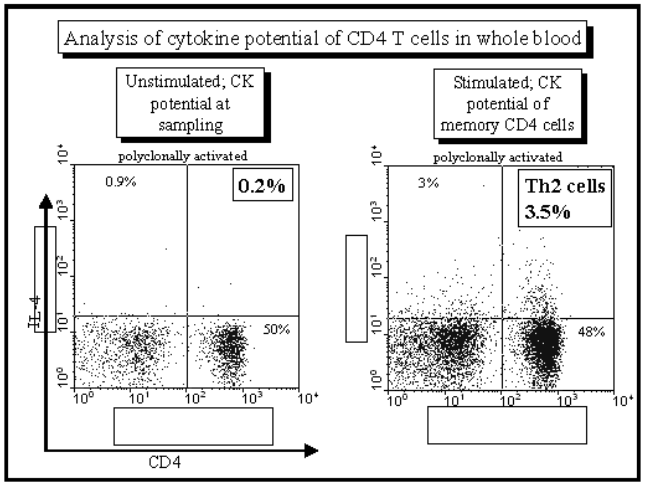
Presentation 9 - Mark Peakman

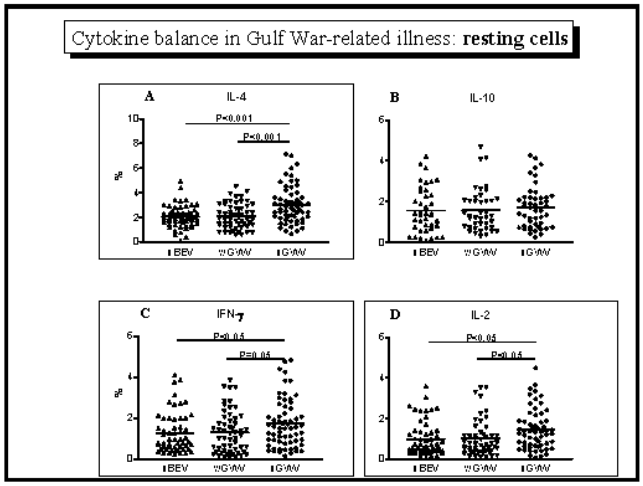






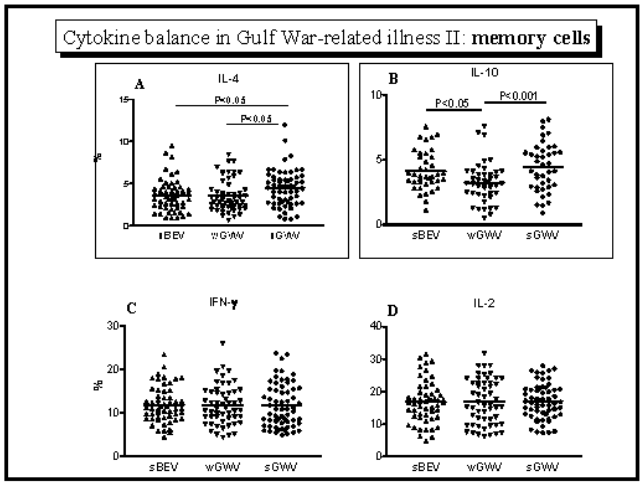






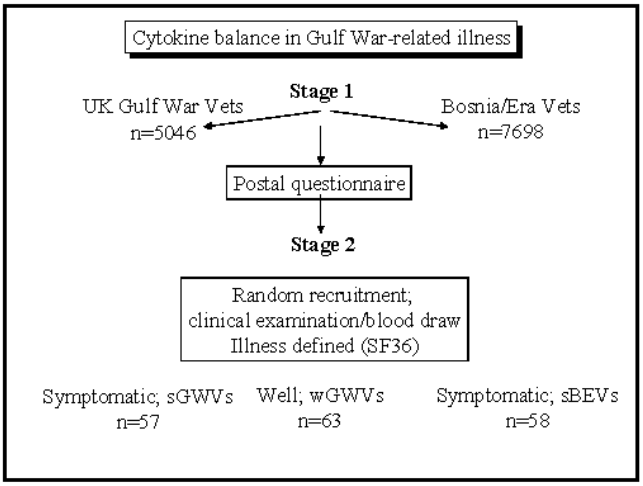
Potential confounders:
Cytokine balance in Gulf War-related illness: **resting cells**

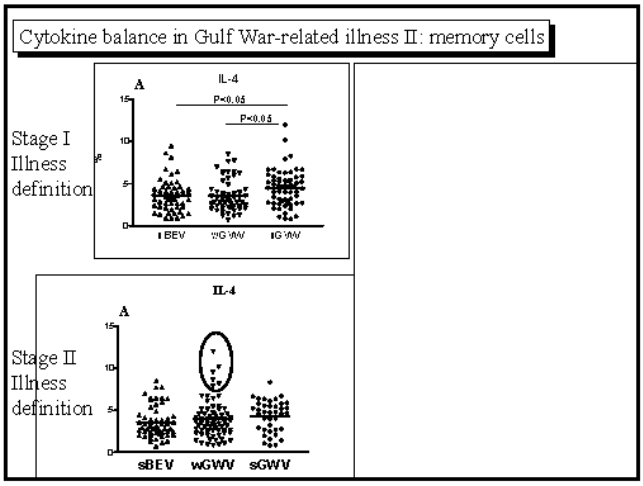
Th type	sGWV (%)	wGWV (%)	Mean diff (corrected for age, sex)	Mean diff (corrected for age, sex, vaccines, depression, atopy)
Th2				
IL-4	3.0	2.4	0.6 **(p=0.04)**	0.3 (p=0.33)
Tr1				
IL-10	1.7	1.6	0.1 (p=0.6)	0.2 (p=0.4)
Th1				
IFN-γ	1.9	1.4	0.5 **(p=0.03)**	0.7 **(p=0.01)**
IL-2	1.6	1.1	0.5 **(p=0.008)**	0.8 **(p=0.001)**



Potential confounders:
Cytokine balance in Gulf War-related illness: **memory cells**

Th type	sGWV (%)	wGWV (%)	Mean diff (corrected for age, sex)	Mean diff (corrected for age, sex, vaccines, depression, atopy)
Th2				
IL-4	4.34	3.6	**1.0 (p=0.05)**	0.7 (p=0.1)
Tr1				
IL-10	4.5	3.2	**1.2 (p=0.003)**	**1.0 (p=0.03)**
Th1				
IFN-γ	11.6	11.9	0.4 (p=0.7)	1.6 (p=0.1)
IL-2	17.3	16.7	1.2 (p=0.3)	1.7 (p=0.2)

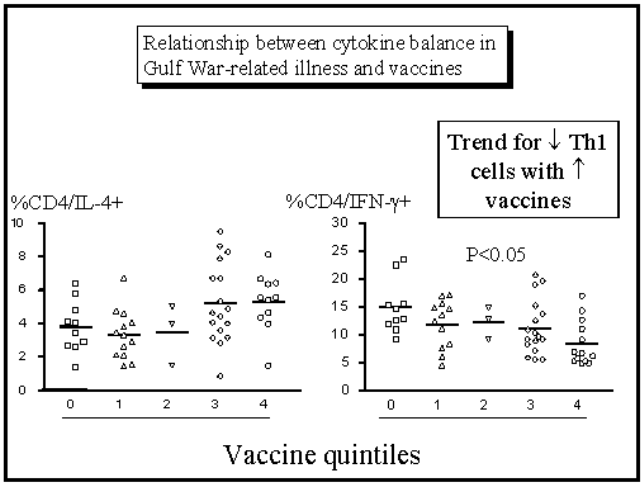




Cytokine balance in Gulf War-related illness: **memory cells (Stage II analysis)**

Th type	sGWV (%)	wGWV (%)	Mean diff (corrected for age, sex)	Mean diff (corrected for age, sex, vaccines, depression, atopy)
Th2				
IL-4	4.3	3.9	0.4 (p=0.3)	0.4 (p=0.4)
Tr1				
IL-10	5.1	3.2	**1.8 (p<0.001)**	**1.9 (p<0.001)**
Th1				
IFN-γ	12.0	11.6	0.6 (p=0.6)	1.6 (p=0.1)
IL-2	17.4	16.4	1.2 (p=0.3)	1.8 (p=0.2)

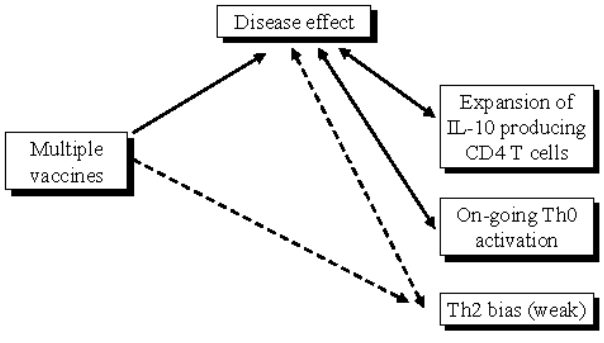
Relationship between cytokine balance in Gulf War-related illness and vaccines



Conclusions I

- **CD4 T cell cytokine balance abnormal in GW-related illness**
  - On-going Th0 (Th1>Th2) activation
  - ↑ IL-10 production by memory cells
  - Th2 activity:- results equivocal
  - Relationship to vaccines: complex

Summary of Th1/Th2 analyses: can we close the loop?



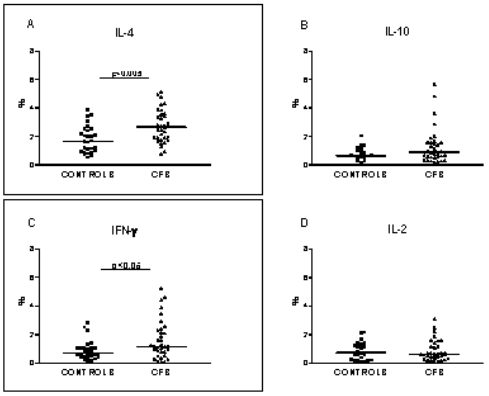
Conclusions II

Expansion of memory IL-10 producing cells:

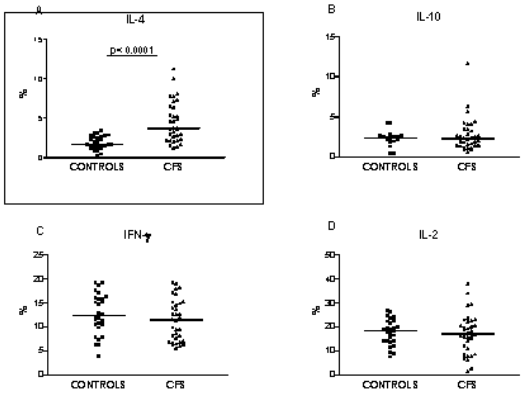
- IL-10 is a major immunoregulatory cytokine
- Inhibits activation and function of T cells and APCs
- Potent negative regulator of Th1 cells
- Mechanisms of IL-10 effect?
- Consequences of IL-10 production for vaccination?

Comparison with chronic fatigue syndrome patients

Cytokine producing cells in chronic fatigue syndrome: **resting CD4**



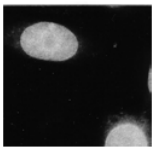
Cytokine producing cells in chronic fatigue syndrome: **memory CD4**



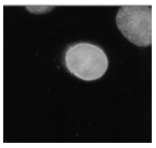
Other potential markers of "Th2-ness":

Analysis of anti-nuclear autontibodsies in GW veterans and CFS patients

Homogenous ANA



Nuclear Envelope (NE) ANA
(Eng Tan, Scripps, 1996)



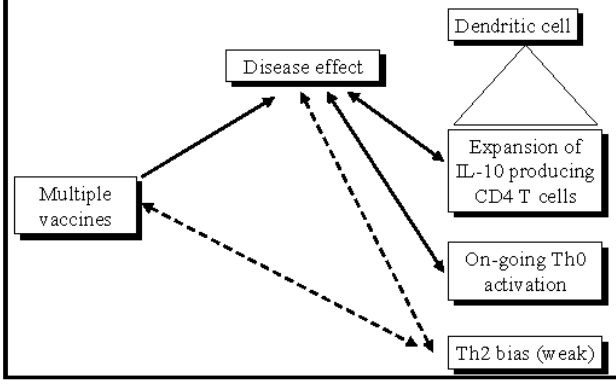
Prevalence of anti-nuclear autoantibodies in Gulf War-related illness

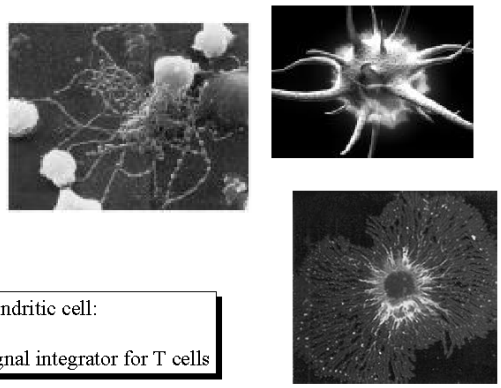
	Homog	Speckled	Nucleolar	Total (%)
sGWV (n=130)	4	3	3	10 (8)
wGWV (n=90)	0	1	2	3 (3)
sBEV (n=128)	7	2	2	11 (9)
Controls (n=51)	2	2	2	6 (12)
CFS (n=100)	0	9	9	18 (18)
Controls (n=111)	7	6	5	18 (16)

Conclusions III

- **There is some resemblance between the Th1/Th2 profile in GW veterans and CFS patients**
  - If primary, suggests common pathogenesis
  - Immune changes could be secondary
- **No evidence for increased anti-nuclear autoimmunity in GW veterans**

Summary of Th1/Th2 analyses: can we close the loop?

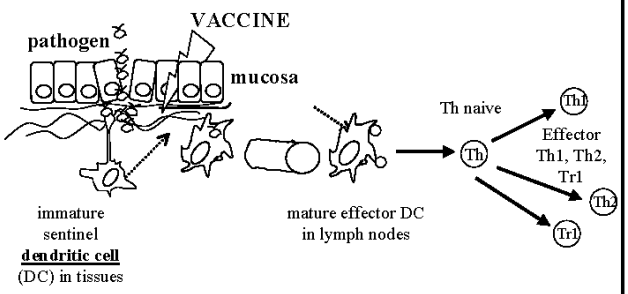




Dendritic cell:

Signal integrator for T cells

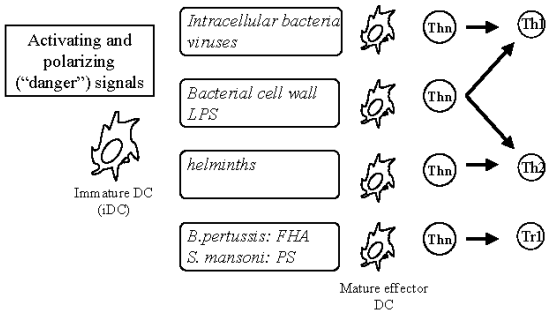
Vaccine/pathogen interactions with the innate immune system I



DCs acquire:
1. antigen
2. activating signals
3. polarizing signals

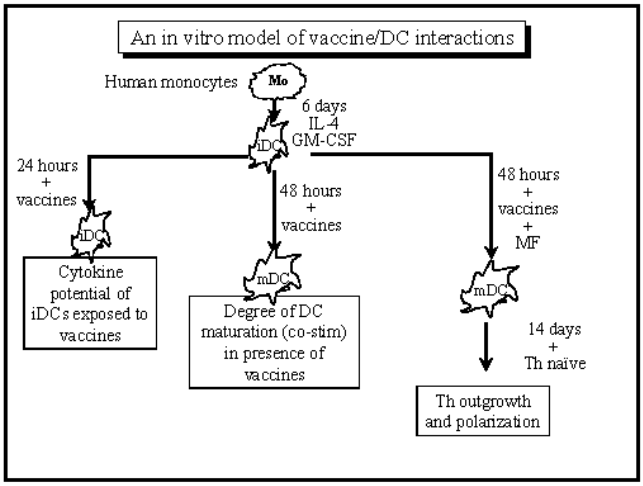
Pathogen-specific signals to DCs
signal 1: antigen
signal 2: Co-stimulatory molecules
signal 3: Polarization (cytokines)

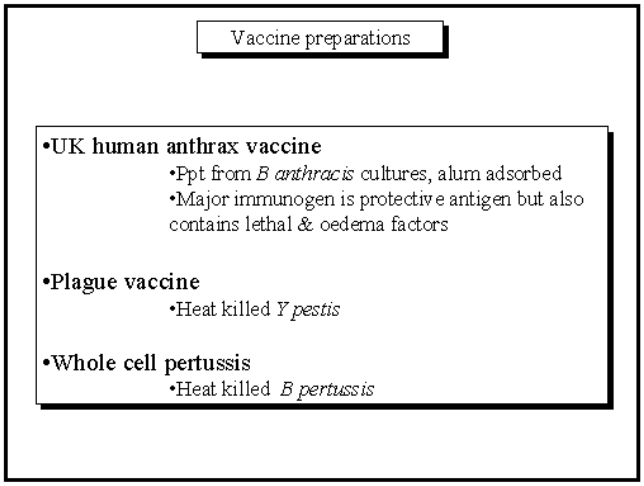
Vaccine/pathogen interactions with the innate immune system II

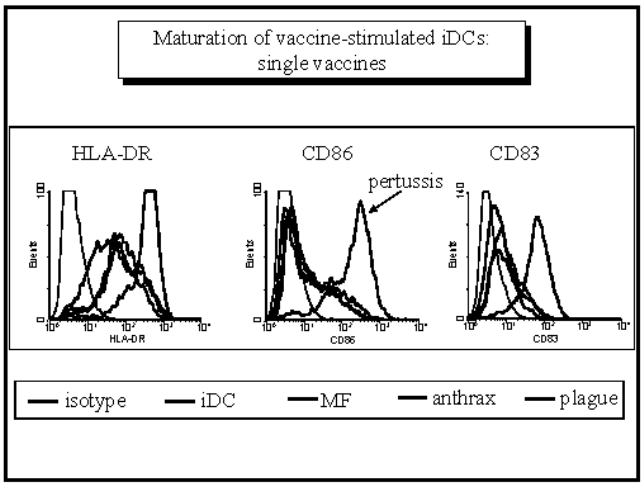


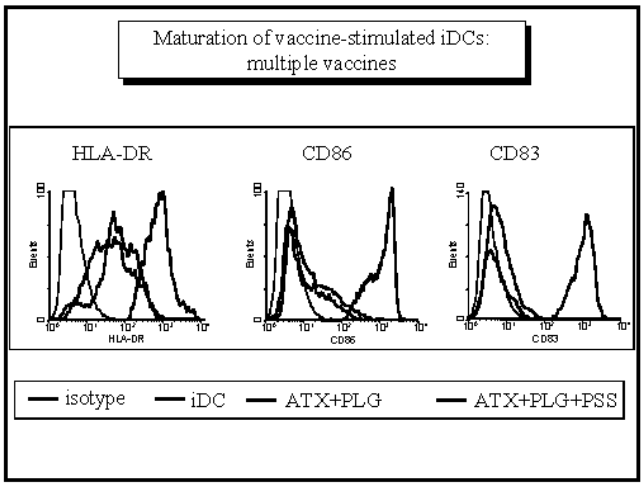
Studying vaccine interactions with the innate immune system

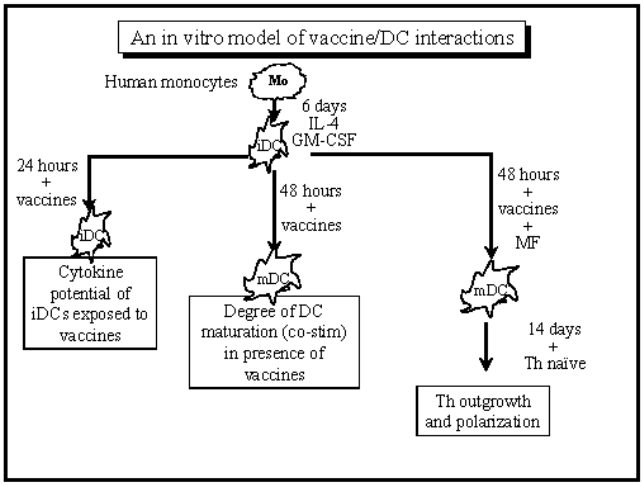
- In vitro model of vaccine interactions with the innate immune system
- Use of model to examine multiple vaccine effects
- Assessment of in vivo T cell immunity to vaccines


An in vitro model of vaccine/DC interactions
Human monocytes
Mo
6 days
IL-4
GM-CSF
iDC
24 hours
+
vaccines
48 hours
+
vaccines
48 hours
+
vaccines
+
MF
iDC
mDC
mDC
Cytokine potential of iDCs exposed to vaccines
Degree of DC maturation (co-stim) in presence of vaccines
14 days
+
Th naïve
Th outgrowth and polarization


Vaccine preparations
•UK human anthrax vaccine
•Ppt from B anthracis cultures, alum adsorbed
•Major immunogen is protective antigen but also contains lethal & oedema factors
•Plague vaccine
•Heat killed Y pestis
•Whole cell pertussis
•Heat killed B pertussis


Maturation of vaccine-stimulated iDCs: single vaccines
HLA-DR
CD86
CD83
pertussis
isotype
iDC
MF
anthrax
plague


Maturation of vaccine-stimulated iDCs: multiple vaccines
HLA-DR
CD86
CD83
isotype
iDC
ATX+PLG
ATX+PLG+PSS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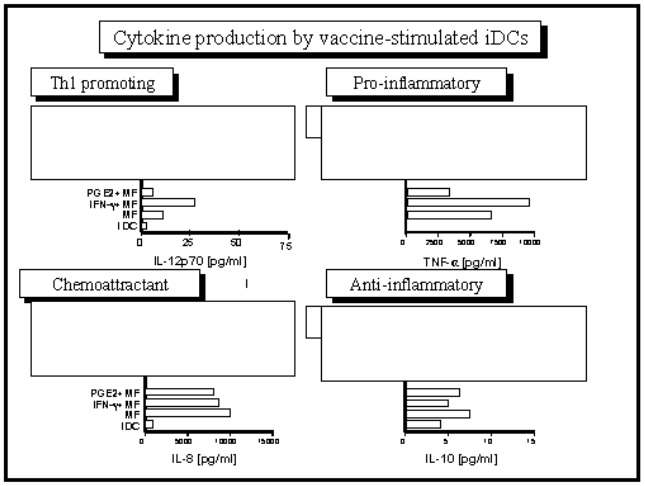
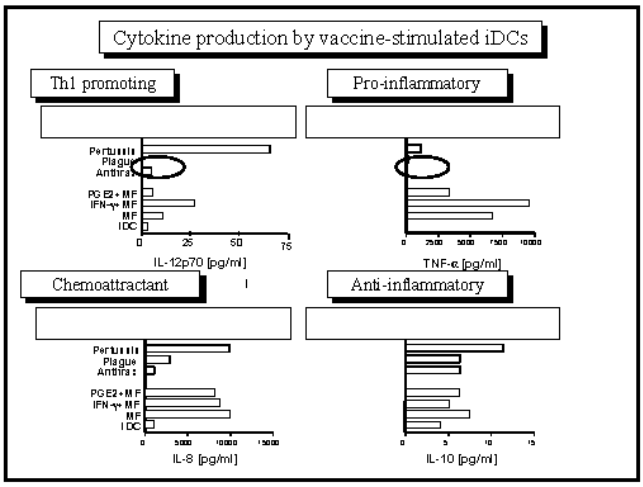

Cytokine production by vaccine-stimulated iDCs
Th1 promoting
Pro-inflammatory
PGE2+MF
IFN-γ+MF
MF
IDC
IL-12p70 [pg/ml]
TNF-α [pg/ml]
Chemoattractant
Anti-inflammatory
IL-8 [pg/ml]
IL-10 [pg/ml]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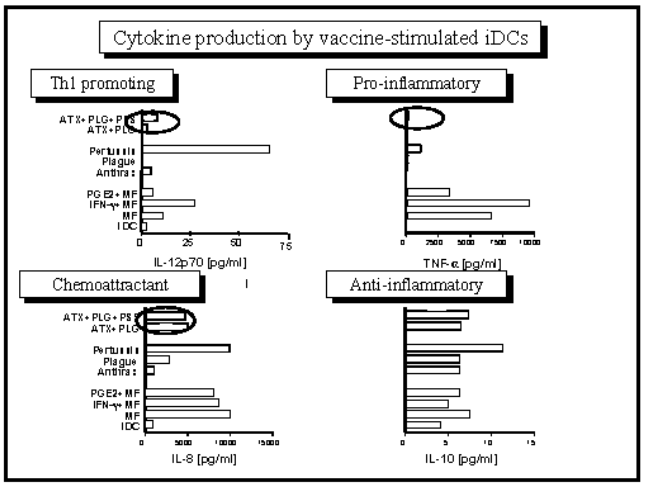
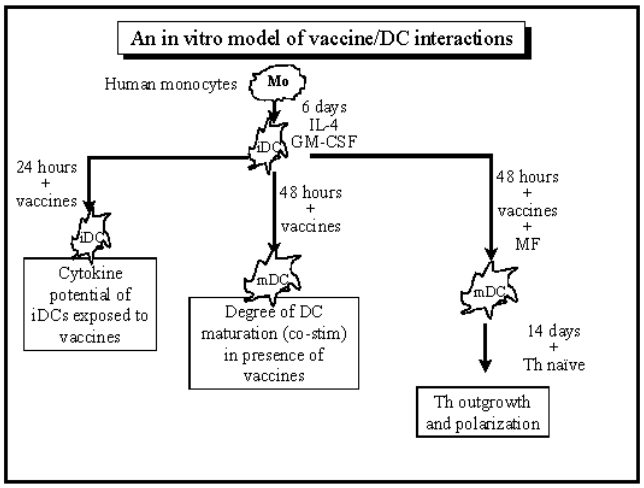
Pro-inflammatory
Pertussis
Plague
Anthrax
PGE2+MF
IFN-γ+MF
MF
IDC
IL-12p70 [pg/ml]
TNF-α [pg/ml]
Chemoattractant
Anti-inflammatory
IL-8 [pg/ml]
IL-10 [pg/ml]


Cytokine production by vaccine-stimulated iDCs
Th1 promoting
Pro-inflammatory
ATX+PLG+PS
ATX+PLG
Pertussis
Plague
Anthrax
PGE2+MF
IFN-γ+MF
MF
IDC
IL-12p70 [pg/ml]
TNF-α [pg/ml]
Chemoattractant
Anti-inflammatory
IL-8 [pg/ml]
IL-10 [pg/ml]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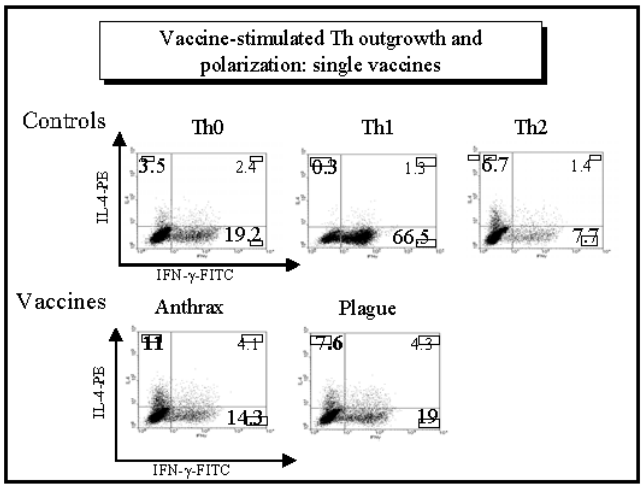
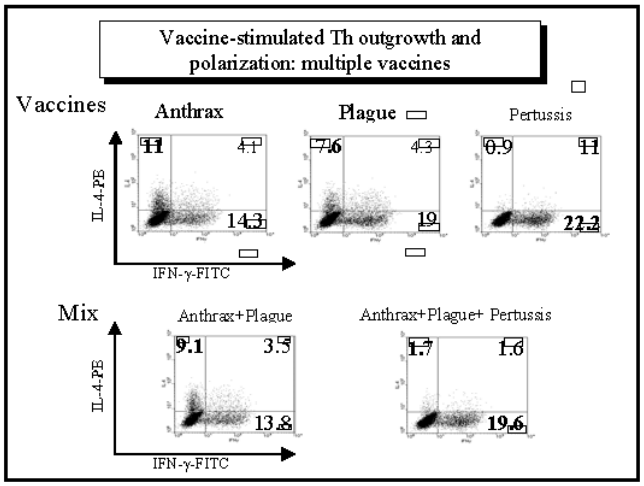
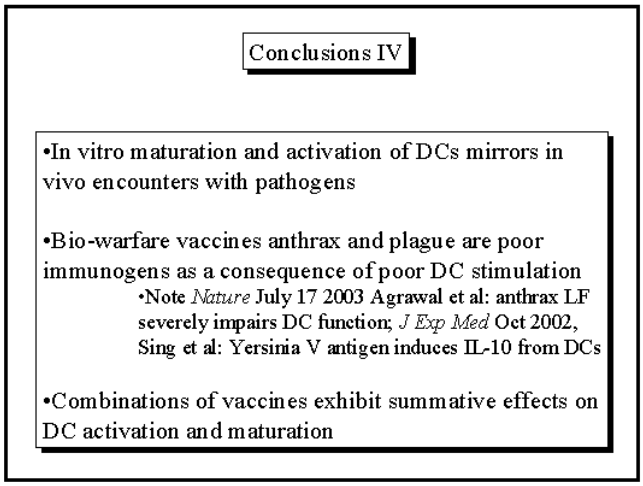
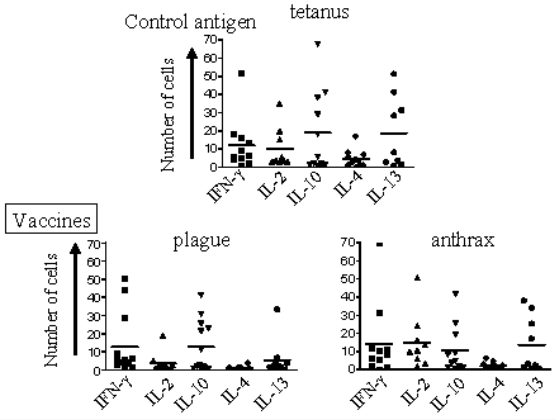
14 days
+
Th naive
Th outgrowth and polarization


Vaccine-stimulated Th outgrowth and polarization: single vaccines
Controls
Th0
Th1
Th2
3.5
2.4
19.2
0.3
1.5
66.5
6.7
1.4
7.7
IL-4-PE
IFN-γ-FITC
Vaccines
Anthrax
Plague
1.1
4.1
14.3
7.6
4.3
19
IL-4-PE
IFN-γ-FITC


Vaccine-stimulated Th outgrowth and polarization: multiple vaccines
Vaccines
Anthrax
Plague
Pertussis
1.1
4.1
14.3
7.6
4.3
19
0.9
1.1
22.2
IL-4-PE
IFN-γ-FITC
Mix
Anthrax+Plague
Anthrax+Plague+ Pertussis
9.1
3.5
13.8
1.7
1.6
19.6
IL-4-PE
IFN-γ-FITC


Conclusions IV
•In vitro maturation and activation of DCs mirrors in vivo encounters with pathogens
•Bio-warfare vaccines anthrax and plague are poor immunogens as a consequence of poor DC stimulation
•Note *Nature* July 17 2003 Agrawal et al: anthrax LF severely impairs DC function; *J Exp Med* Oct 2002, Sing et al: Yersinia V antigen induces IL-10 from DCs
•Combinations of vaccines exhibit summative effects on DC activation and maturation

T helper cell responses to bio-warfare vaccines II



Conclusions V

- Recall responses to bio-warfare vaccines are detectable in Gulf War veterans 11-12 years after vaccination
- Preliminary results reflect mixed Th1/Th2/Tr1 immunity to anthrax, poorer immunity to plague

Summary

- Evidence of cellular immune activation in GW veterans, but analyses and relationship to vaccines difficult to evaluate, presumably due to time elapsed
- In vitro model of DC activation by vaccines provides a technology for assessing single vaccine effects, and for assessing multiple agents
- Multiple agents appear to have predictable, summative effects

Key collaborators

- Simon Wessely, Matthew Hotopf, King's College London
- Gareth Griffiths, DSTL, Porton Down, UK
- Martien Kapsenberg, Esther De Jong, U Amsterdam
- Funding: Medical Research Council, UK; US DoD