Presentation 8 - John Ottenweller

Immune Dysregulation in Gulf Veterans with CFS and its Relationship with Cognitive Function and Functional Status

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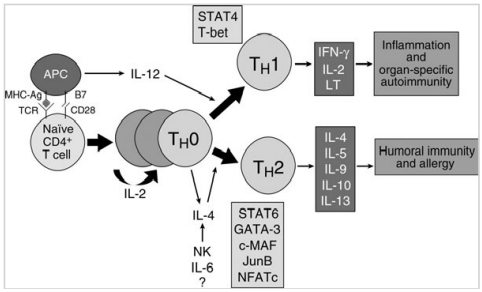
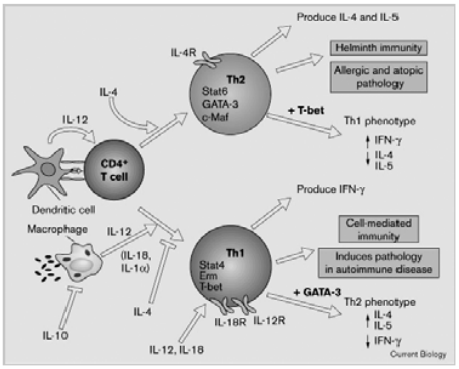
Background of our Immune Studies

- Hypothesis that CFS is due to infection and immune dysregulation.
- We have identifed CFS in GVs, and it is the only medical condition that occurs more frequently in GVs than non-Gulf era veterans.
- We examined the hypothesis that immune function is dysregulated in GVs with CFS.

1997 Rook and Zumla paper

- Hypothesis: GWI due to a switch from Th1 to Th2 profile
  - Associated with EBV reactivation
  - Vaccines with large antigenic load
    - Those given troops going to Gulf
  - Th2 particularly responsive to stress
  - Exposure to insecticides inhibits Th1 IL-2 functions
- Recent Paper by Peakman, Wessely and their colleagues shows elevated Th2-like cells in civilians with CFS





Hypothesis #1

- GVs with CFS show an up-regulated Th2 immune profile.
  - **Alternative that Th1 cytokines will be elevated**
- Differentiation to Th2 cells due to IL-4 and IL-6
- Th2 cells secrete IL-4, IL-5, IL-9, IL-10 and IL-13

Demographics for GVs

	Healthy	CFS
Total n =	34	43
Male	88%	74%
White	85%	77%
Education		
> high school	79%	56%
Axis I	18%	72%

Methodology

- Collected blood by venipuncture
- PBLs labelled with cell surface markers and counted by FACscan to give cell counts for different types of lymphocytes and their % of the total lymphocytes
- mRNA isolated from PBLs and semiquantitative RT-PCR used to estimate mRNA levels of cytokines

Immune Variables

- $CD3^+$ (Total T Cells)
- $CD3^+CD4^+$ (MHC II T Cells)
- $CD3^+CD8^+$ (MHC I T Cells)
- $CD3^-CD19^+$ (B Cells)
- $CD3^-CD[16^+56^+]$ (NK Cells)

- IL-2
- IL-4
- IL-6
- IL-10
- IL-12
- TNF-α
- INF-γ

Cell Types and Cytokines in GWVs

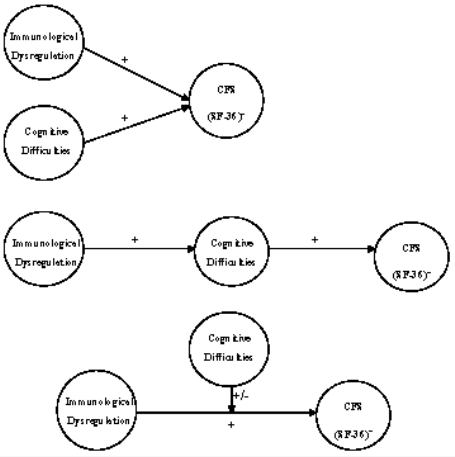
Cell Type	Healthy	CFS	P values
Lymphocytes (Counts)	1918 +/- 96	2120 +/- 104	NS
NK Cells, CD16+56+ (%)	15.5 +/- 1.1	12.2 +/- 0.9	P < 0.02
Total T Cells, CD3+ (%)	71.9 +/- 1.2	76.4 +/- 0.9	P < 0.01
MHC II Cells, CD3+ CD4+ (%)	42.4 +/- 1.4	48.3 +/- 1.0	P < 0.001
Cytokine mRNA			
IL-2	252 +/- 61	431 +/- 140	P < 0.05
IL-4	134 +/- 19	256 +/- 58	NS
IL-6	1711 +/- 337	2882 +/- 505	NS
IL-10	496 +/- 265	604 +/- 137	P < 0.02
IL-12	136 +/- 39	300 +/- 85	NS
INF-gamma	166 +/- 27	289 +/- 48	P < 0.02
TNF-alpha	632 +/- 146	1002 +/- 164	P < 0.01

Summary

- Partially supports shift to Th2 phenotype because IL-10 mRNA is elevated
- However, elevated IL-2 and IFN- γ suggest that Th1 lymphocytes are also activated in GWs with CFS

Hypotheses 2 & 3

- GVs with CFS would be more likely to show cognitive difficulties than the healthy controls.
- The impact of immune up-regulation on CFS caseness would be either independent of, mediated or moderated by cognitive difficulties.



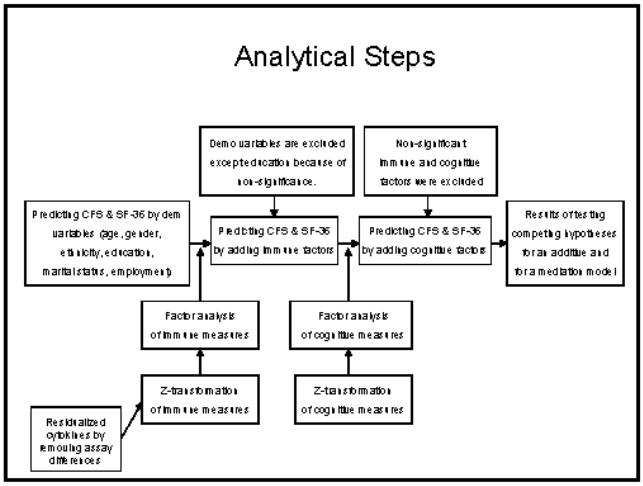
11 Cognitive Tests

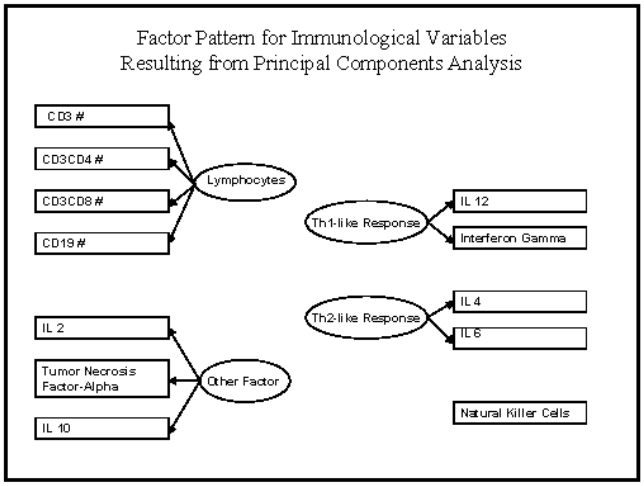
- NES simple and complex reaction time tests
- Paced Auditory Serial Addition Test (PASAT)
- WAIS-R Digit Span subtest
- Category Test
- Verbal Fluency Test
- Rey-Osterrieth Complex Figure Test

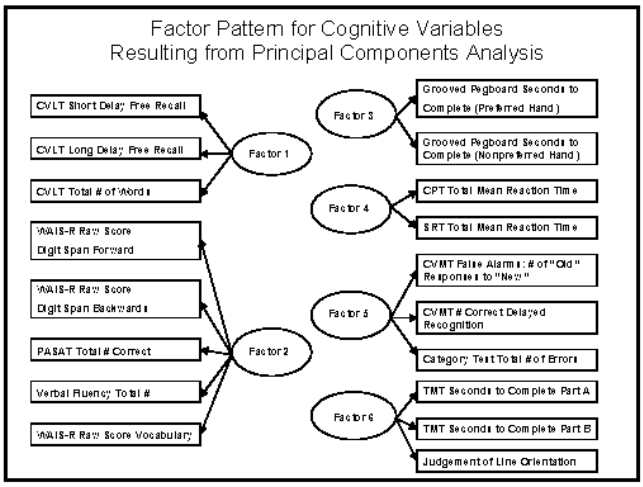
- California Verbal Learning Test
- Continuous Visual Memory Test
- Judgement of Line Orientation
- Wais-R Block Design subtest
- Grooved Pegboard Test

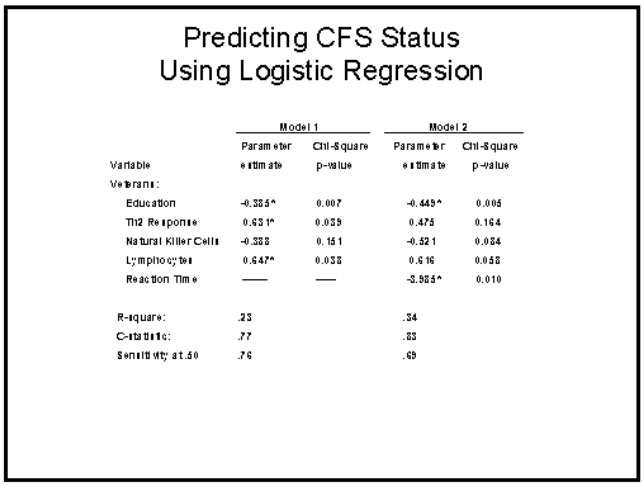
Reasons for using Factor Analysis

- Data reduction in view of small sample size and large number of immune and cognitive variables
- Allows us to see if discrete Th1 and Th2 clusters emerge from raw data









	Model 1		Model 2	
Variable	Parameter estimate	Chi-Square p-value	Parameter estimate	Chi-Square p-value
Veterans:				
Education	-0.535^	0.007	-0.449^	0.005
Th2 Response	0.631^	0.039	0.475	0.164
Natural Killer Cells	-0.338	0.151	-0.521	0.084
Lymphocytes	0.647^	0.038	0.616	0.052
Reaction Time	——	——	-3.935^	0.010
R-square:	.25		.34	
C-statistic:	.77		.83	
Sensitivity at .50	.76		.69	

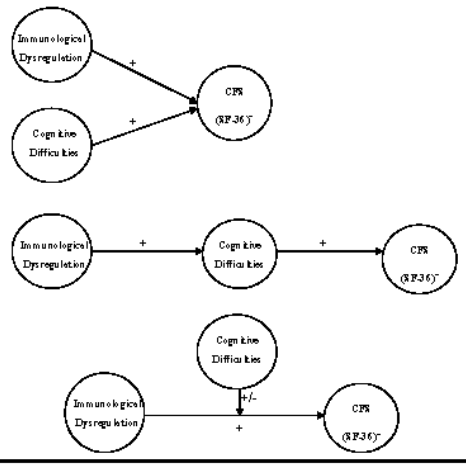
How Do Immune Factors Predict CFS Caseness?

For GVs with CFS, the Th2-like factor and the lymphocyte factor were elevated.
- Th2 result different from first analysis

Supports Rook and Zumla hypothesis that GVs with CFS will exhibit a Type 2 predominance in their cytokines

Controlling for previous predictors, do cognitive factors predict CFS caseness?

- Only one factor -- longer latencies to react -- was associated with CFS caseness
- When reaction time was added to the model, immune factors no longer predict caseness
  - Cognitive problem directly assoc with caseness
  - Immune dysregulation affects cognitive function and is only indirectly associated with caseness



Answers to Hypotheses

- #2: There is a relation between immune dysregulation and CFS for GVs
- #3: This relationship is indirect and mediated by cognitive dysfunction

Hypothesis #4

- The impact of Th2 immune up-regulation on functional status would be either independent of, mediated or moderated by cognitive difficulties.

Predicting Physical Functioning Using MANOVA

Variable	Model 1 Parameter estimate	Model 1 Pr > |t|	Model 2 Parameter estimate	Model 2 Pr > |t|
Veterans:				
Education	0.139^	0.014	0.132^	0.011
Th2 Response	-0.147^	0.035	-0.091	0.378
Natural Killer Cells	0.077	0.428	0.068	0.554
Lymphocytes	-0.303^	0.007	-0.264^	0.018
Reaction Time	—	—	0.324^	0.012

^, p-value < 0.05

Predicting Social Functioning Using MANOVA

Variable	Model 1 Parameter estimate	Model 1 Pr > |t|	Model 2 Parameter estimate	Model 2 Pr > |t|
Veterans:				
Education	0.181^	0.001	0.183^	0.001
Th2 Response	-0.212^	0.004	-0.168	0.144
Natural Killer Cells	-0.002	0.980	-0.007	0.940
Lymphocytes	-0.248^	0.003	-0.238^	0.047
Reaction Time	—	—	0.212^	0.020

^, p-value < 0.05

Predicting General Health Using MANOVA

Variable	Model 1 Parameter estimate	Model 1 Pr > |t|	Model 2 Parameter estimate	Model 2 Pr > |t|
Veterans:				
Education	0.214^	0.000	0.218^	0.000
Th2 Response	-0.184^	0.010	-0.148	0.105
Natural Killer Cells	0.031	0.774	0.033	0.747
Lymphocytes	-0.273^	0.022	-0.288^	0.004
Reaction Time	—	—	0.247	0.068

^, p-value < 0.05

Predicting Mental Health Using MANOVA

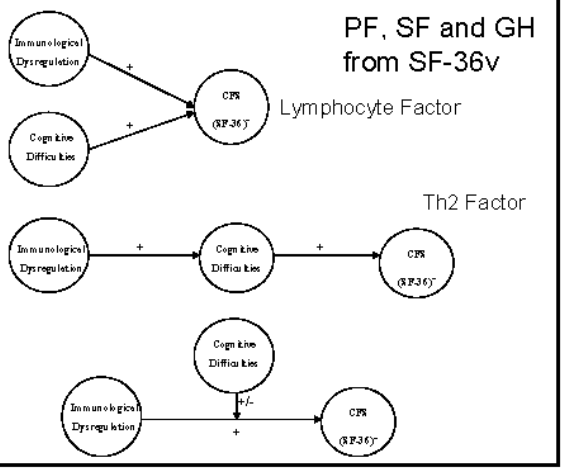
	Model 1		Model 2	
Variable	Parameter estimate	Pr > |t|	Parameter estimate	Pr > |t|
Veterans:				
Education	0.218*	0.002	0.217*	0.001
Th2 Response	-0.163	0.079	-0.043	0.719
Natural Killer Cells	0.120	0.337	0.093	0.448
Lymphocytes	-0.161	0.243	-0.123	0.373
Reaction Time	——	——	0.343*	0.033

* p-value < 0.05

Immune and Cognitive Factors and the SF-36

- Th2-like and lymphocyte factors are associated with poorer general health, and physical and social functioning in GVs
  - Not associated with poorer mental health
- Reaction time, when added to model, predicted functional disability for GVs
- Impact of Th2-like factor on function in GVs was mediated by reaction time
  - Lymphocyte factor remained independent of reaction time

PF, SF and GH from SF-36v



Answers to Hypotheses about Functional Status

- #4: There is a relation between immune dysregulation and functional abilities in GVs
- #4: There is an indirect relationship between the Th2-like factor and functional abilities that is mediated by cognitive dysfunction
  - There is a direct relationship between the lymphocyte factor and functional abilities for GVs

Conclusions

- Mediation model was partially supported in GVs
- Based on factor analyzed immune measures, we found elevated Th2-like and lymphocyte factors in GVs with CFS
  - Supports Rook and Zumla hypothesis
- Effect of elevated immune factors on CFS caseness is mediated by reaction time factor

- Elevated Th2-like factor was indirectly associated with poor functional status in GVs with CFS
  - Effect mediated by reaction time factor
- Elevated lymphocyte factor was directly associated with poor functional status in these veterans
- Reaction time factor was associated with poor functional status for GVs with CFS

National Health Survey of Gulf Era Veterans and their Spouses

- Phase III
  - Representative subsample of NHS Phases I/II
  - Comprehensive exams including H&P, psych evaluation, blood samples, etc.
  - 3250 serum samples
    - About 1000 each from GVs, EVs and Spouses
    - We received 300 microliter aliquots of all samples

Funded Pilot Project

- Measured Cortisol, Paraoxonase and Butyrylcholinesterase in all 3,250 samples
- Completed assays January, 2003
- Statistical analyses of cortisol completed

Cytokine Study in NHS Samples

- Selected totally random samples of 71 GVs and 69 EVs for measurement of plasma cytokine levels
- No consideration of health status or demographics
- Used Luminex technology to measure 9 cytokines simultaneously in 50 microliters serum

Preliminary Plasma Cytokine Levels in 71 Gulf Vets and 69 non-Gulf Era Veterans

Cytokine	Non-Detectable[1]	Published Levels[1]	Overall Levels[2]	EVs*	GVs*
IL-1β	14 (11.4%)	1.1 (0- 44) n=22	13.7 (9.7- 19.7)	17.4 ± 13.0	18.9 ± 29.9
IL-2	11 (7.8%)	12.0 (0-518) n= 9	34.5 (23.2- 87.8)	41.7 ± 45.3	40.7 ± 45.3
IL-4	9 (6.4%)	5.4 (0- 17) n=10	35.2 (23.4- 51.4)	41.8 ± 28.5	35.0 ± 19.9
IL-6	8 (5.7%)	2.0 (0-233) n=30	14.8 (11.1- 27.4)	22.3 ± 17.8	19.8 ± 13.2
IL-8	3 (2.1%)	5.0 (0-224) n=21	18.1 (11.4- 23.2)	31.4 ± 22.5	45.4 ± 29.8
IL-10	8 (5.7%)	3.2 (0- 48) n=24	7.8 (3.2- 11.4)	9.2 ± 9.9	8.3 ± 6.0
IL-12	20 (14.3%)	24.0 (0-234) n=14	39.2 (28.9- 53.8)	58.4 ± 81.1	39.3 ± 31.4
IL-13	Not Assayed	20.0 (1-119) n= 5			
IFN-γ	14 (10.0%)	17.4 (0-388) n=12	111.4 (70.4-130.5)	119.8 ± 74.5	113.1 ± 45.9
TNF-α	14 (10.0%)	4.2 (0- 90) n=32	3.3 (1.4- 5.3)	5.2 ± 7.3	3.7 ± 3.2

[1] Published levels are medians of the reported means or medians for control values, the range is in parentheses, and n = the number of studies consulted. All levels are in pg/ml.
[2] Levels for the 140 Veteran Samples: medians (interquartile ranges).
* Cytokine Levels for EVs (n=69) and GVs (n=71): means ± standard deviations.

Summary

- Plasma Th2 cytokines declined (P's ~ 0.1)
  - IL-4 by 16% and IL-6 by 11%
- A plasma Th1 cytokine declined (P ~ 0.1)
  - IL-12 by 33%
- Not the same as mRNA changes, but earlier studies compared sick and healthy GVs
- In this study, GVs and EVs randomly chosen
- For systemic actions, plasma cytokines are the effectors

Factor Analysis of Plasma Cytokines

Model explained 81% of variance.

- Factor 1 (38% of variance)
  - Th2 Cytokines: TNF, IL-10, IL-4, IL-6
- Factor 2 (26% of variance)
  - Th1 Cytokines: IL-2, IFN
- Factor 3 (17% of variance)
  - Other Cytokines: IL-1, IL-8

Vaccination Rates in GVs and EVs

Vaccination	GVs (%)[1]	EVs (%)[1]	P< [2]	GVs (%)[3]	EVs (%)[3]	P< [4]
Anthrax	43.7	4.7	0.001	44.5	10.3	0.001
Typhoid	40.7	44.3	0.001	54.3	50.0	NS
Botulism	13.4	3.2	0.001	12.7	2.9	0.05
Plagus	24.4	13.9	0.001	28.2	22.1	NS
Meningitis	14.5	6.4	0.001	28.2	7.4	0.01
Gamma Globulin	63.4	23.0	0.001	60.6	33.8	0.01

[1] %'s out of approximate 1000 depending on the number who answered each question.
[2] Comparison of rates in columns 2 and 3 using χ^2-tests.
[3] %'s out of 71 GVs and 69 EVs.
[4] Comparison of rates in columns 5 and 6 using χ^2-tests.

Cytokines and Vaccinations

- Anthrax
  - Lower IL-12 (Th1)
- Typhoid
  - Elevated IL-2 and IFN (Th1)

Logistic regression predicting vaccinations, P's <0.05

Summary of Plasma Cytokine Results

- Plasma levels of IL-4, IL-6, IL-12 and TNF may be lower in GVs compare with EVs.
- Plasma cytokines cluster into groups of Th1 and Th2 cytokines.
- Decreased levels of IL-12 and increased levels of IL-2 and IFN may be associated with self-reported vaccinations
- Based on only 7% of the NHS Phase III samples

Hypotheses that can be tested with measurement of cytokines in all NHS samples

- GVs will have higher levels of Th2 cytokines and lower levels of Th1 cytokines than EVs.
  - Alternatively, Th1 cytokines may be higher in GVs.
- Th2 cytokines will be higher in GVs with poorer physical or mental functioning (SF-36v).
- Th2 cytokines will be higher in GVs with more vaccinations.
- Stress will increase Th2 cytokines and decrease Th1 cytokines.
- Cognitive Impairment will be associated with higher Th2 cytokines in GVs with CMI, but not healthy GVs or EVs.