Hubertus Himmerich

Are cytokines biomarkers of PTSD and its therapy?

Focus: TNF-α and PTSD

Photo: Psychologie Heute 2012
Emil Kraepelin (1856-1926)

- Foundation of the classifications of mental disorders
- Publication-based habilitation 1882 at the University of Leipzig
- Connection between acute inflammatory disorders and psychiatric symptoms

Julius Wagner von Jauregg (1857-1940)

- Austrian psychiatrist
- 1927 Nobel Prize for medicine: malaria vaccine for the treatment of progressive paralysis
- About the effects of febrile diseases on psychoses (1887)
- Evaluation of cases in which fever had occurred during mental illness:

Post-Traumatic Stress Disorder (PTSD)

• Mental disorder
• May develop following exposure to traumatic events
• Specific criteria according to **ICD-10**
  – Exposure to a stressful event of threatening or catastrophic nature
  – Persistent remembering or "reliving"
  – Avoidance of circumstances resembling or associated with the stressor
  – Inability to recall important aspects
  – Psychological sensitivity and arousal
  – Symptoms continue for more than a month after the occurrence of a traumatic event

World Health Organization 1992
Post-Traumatic Stress Disorder (PTSD)

• Specific criteria according to DSM-5
  – Exposure to a traumatic event
  – Symptoms from each of four symptom clusters:
    • Intrusion
    • Avoidance
    • Negative alterations in cognitions and mood
    • Alterations in arousal and reactivity
  – Duration of symptoms
  – Functioning
  – Symptoms not attributable to a substance or co-occurring medical condition

Diagnostic Instruments for PTSD

- SCID-I
- PCL
- CAPS
- LASC
- PDS
- SRIP
- ....
Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I)

- Diagnosis according to DSM-IV criteria for PTSD
- Degree of severity not assessed

PTSD Checklist for DSM-5 (PCL-5)

- 20-item self-report measure
- Rating scale is 0-4 for each symptom
- Assesses the 20 DSM-5 symptoms of PTSD
- Allows assessment of severity

PCL for DSM-IV

- 3 different versions
  - PCL-M (military)
  - PCL-C (civilian)
  - PCL-S (specific)

Weathers et al. 2013
Clinician-Administered PTSD Scale for DSM-5 (CAPS-5)

• Gold standard in PTSD assessment
• 30-item structured interview to:
  – Make current (past month) diagnosis of PTSD
  – Make lifetime diagnosis of PTSD
  – Assess PTSD symptoms over the past week
• Covers
  – Onset and duration of symptoms
  – Subjective distress
  – Impact of symptoms on social and occupational functioning
  – Overall PTSD severity
Los Angeles Symptom Checklist (LASC)

- The LASC is a 43-item self-report measure of PTSD
- Covers DSM-IV criteria for PTSD
- Used to quantify re-experiencing, avoidance, and arousal symptoms of PTSD
- **Measure of PTSD symptom severity**: summing the ratings of the 17 items corresponding to the symptoms of PTSD
- A sum of all 43 items: *global assessment of distress and adjustment problems*

King et al: Assessments 1995
Post-traumatic Stress Diagnostic Scale (PDS)

- Brief, reliable, self-reported measure of PTSD
- Use in clinical and research settings
- Can be completed in 10-15 minutes
- Test items mirror the DSM-IV criteria for PTSD
- Assesses symptom severity


Self-Rating Inventory for PTSD (SRIP)

- Assesses PTSD symptom severity over the past month
- Consists of 3 subscales representing the DSM-IV PTSD symptom clusters: re-experiencing, avoidance and hyperarousal.
- Summed score of all items provides a total PTSD score
- Good reliability and good concurrent validity with other diagnostic interviews for PTSD, such as the CAPS

Causal Factors

- Traumatic exposure, type and duration of trauma
- Psychological factors
  - Previous trauma
  - Personality
- Biological Factors
  - Gender
  - Genes, epigenetics
  - Brain structure and function, e.g. hippocampus, amygdala, prefrontal cortex, hypothalamus
  - Neurotransmitter systems, e.g. serotonin
  - Endocrine system, e.g. cortisol, glucocorticoid receptors
  - Immune system, e.g. cytokines
- Social surrounding field

Hippocampus and PTSD

- MRI studies: significantly **smaller hippocampi** in subjects with PTSD compared to trauma-exposed and non-trauma-exposed subjects without PTSD\(^1\)
- **Neuronal reduction** in the hippocampus of patients with PTSD\(^2\)
- Small hippocampal volume seems to be a **pre-trauma risk factor** for PTSD\(^3\)
- Non-PTSD, trauma-exposed subjects have smaller hippocampi than non-PTSD, non-trauma-exposed subjects\(^4\)
- **Trauma seems to reduce hippocampal volume** regardless of the development of subsequent PTSD\(^5\)

How cytokines can influence the brain

Capuron L & Miller AH: Pharmacol Ther 2011
## Cytokines and their receptors in the brain

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Production in the brain</th>
<th>Receptor in the brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-α</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IFN-β</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TNF-α</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-1α</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-1β</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-2</td>
<td>+</td>
<td>+</td>
</tr>
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<td>IL-6</td>
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<td>IL-8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-10</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Capuron L, Miller AH: Pharmacol Ther 2011
**Tumour Necrosis Factor-α (TNF)-α**

- inflammatory, pleiotropic cytokine
- Blood-brain barrier is permeable to peripherally produced cytokines
- Receptors on the surface of neurons and glial cells
  - TNF-α acts via two types of receptor: **TNF-R p55** and **TNF-R p75**
  - Literature suggests association with psychiatric disorders
    - Depression, Schizophrenia, Alzheimer’s disease

TNF-α and its soluble receptor levels in subjects with or without depression

- Never depressed subjects: N = 523
- Subjects with history of depression: N = 35
- Depressed inpatients without any infectious or inflammatory disease: N = 70
TNF-α serum levels are age- and BMI-dependent

Boxplots of TNF-α levels in relation to age and BMI [kg/m²]

Himmerich et al.: Eur Cytokine Netw 2006
Immunological side effects of antidepressants: Mirtazapine

- Increase of TNF-α levels
- Neutropenia
- Thrombocytopenia

Tumor necrosis factor-α serum levels in healthy smokers and non-smokers

• 43 healthy smokers and 19 healthy non-smokers (the control group)
• A clinical and paraclinical evaluation was performed in both groups, without any evidence of infection or COPD

Table 1 The characteristics of our smokers and nonsmokers group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Smokers</th>
<th>Nonsmokers</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52.06 ± 5.86</td>
<td>55.21 ± 8.5</td>
<td>NS</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>100</td>
<td>100</td>
<td>NS</td>
</tr>
<tr>
<td>Pack-year</td>
<td>30.16 ± 10.02</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pack/day</td>
<td>1.07 ± 0.37</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>22.01 ± 1.64</td>
<td>21.78 ± 1.84</td>
<td>NS</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>27.53 ± 10.92</td>
<td>24 ± 11.73</td>
<td>NS</td>
</tr>
<tr>
<td>(g/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>12.81 ± 9.72</td>
<td>8.42 ± 5.87</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Note: Mean ± SD.
Abbreviations: NS, not significant; BMI, body mass index; CRP, C-reactive protein.
Tumor necrosis factor-α serum levels in healthy smokers and nonsmokers
Tumor necrosis factor-α serum levels in healthy smokers and nonsmokers

Petrescu et al., Int J Chron Obstruct Pulmon Dis. 2010
Serum concentrations of TNF-α, sTNF-R p55 and p75 and post-traumatic stress in German soldiers

Hubertus Himmerich¹,²,⁵, Gerd D. Willmund³, Peter Zimmermann¹, Jörg-Egbert Wolf³, Antje H. Bühler¹, Lesca M. Holdt³, Daniel Teupser³, Kenneth C. Kirkby⁴, Ulrich Wesemann¹

¹ Department of Psychiatry, Psychotherapy and Psychotraumatology, Bundeswehr Hospital, Berlin, Germany
² Department of Psychiatry and Psychotherapy, University Hospital Leipzig, Leipzig, Germany
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⁴ Department of Psychiatry, University of Tasmania, Hobart, Tasmania, Australia
⁵ Institute of Psychiatry, Psychology and Neuroscience, King’s College London, London, UK

- 135 male German soldiers
- 70 deployed abroad, 65 deployed in Germany only
- Of the 70 deployed abroad, 38 with PTSD and 32 without PTSD
- PTSD symptoms measurement: PDS
- Serum levels of TNF-α, sTNF-R p55 and sTNF-R p75
- No significant Spearman rank correlations between PDS scores and serum levels of TNF-α, sTNF-R p55 or sTNF-R p75
- No significant differences between soldiers with or without a PDS-derived diagnosis of PTSD after controlling for age, smoking and body mass index (BMI)
Serum concentrations of TNF-α, sTNF-R p55 and p75 and post-traumatic stress in German soldiers

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Table 1

Spearman rank correlations (r: correlation coefficient; p: 2-tailed level of significance) between TNF-α, sTNF-R p55 and sTNF-R p75 serum levels (pg/mL) and PDS and TICS scores. None of the tested correlations was significant according to the corrected level of significance of p* = .008.

<table>
<thead>
<tr>
<th></th>
<th>Whole group (N = 135)</th>
<th>Soldiers deployed abroad (N = 70)</th>
<th>Soldiers deployed in Germany (N = 65)</th>
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<tbody>
<tr>
<td></td>
<td>PDS</td>
<td>TICS</td>
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<td>TNF-α</td>
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<tr>
<td>r</td>
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<td>-.078</td>
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<tr>
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<td>sTNF-R p75</td>
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Himmerich et al.: Eur Cytokine Netw 2015
Methods

- 38 male German soldiers with combat-related PTSD
- Patients randomized: inpatient psychotherapy (N=21) including eye movement desensitization and reprocessing (EMDR) or outpatient clinical management (N=17)
- Symptoms of PTSD were measured using the Post-traumatic Stress Diagnostic Scale (PDS)
- Before and after treatment: serum levels of TNF-α, sTNF-R p55 and sTNF-R p75
Results

- The PDS score significantly decreased across time in both groups
- Serum concentrations of TNF-α increased significantly
- sTNF-R p55 and sTNF-R p75 levels decreased significantly
- No significant differences regarding TNF-α, sTNF-R p55 or sTNF-R p75 changes between the inpatient psychotherapy group and the outpatient clinical management control group
- Results controlled for medication
Discussion

- Changes in PTSD symptoms possibly associated with changes in TNF-α system
- TNF-α may increase as a result of psychotherapy
Critical remarks about our research

• Focus on TNF-α and its receptors in the serum is a too narrow approach, other cytokines also exert important roles
• N=38 is a small sample size for a longitudinal study
• We did not control for persons feigning PTSD
• Replication in a well assessed sample necessary
• First study on TNF receptors in PTSD
• First study on TNF-α and TNF receptors during psychotherapy for PTSD
Systematic review: TNF-α and PTSD

- Systematic PubMed review according to the PRISMA guidelines
- (("post-traumatic stress disorder"[Title/Abstract]) OR ("PTSD"[Title/Abstract])) AND (("tumor necrosis factor-alpha"[Title/Abstract]) OR ("tumor necrosis factor"[Title/Abstract]) OR ("TNF-alpha"[Title/Abstract]) OR ("TNF"[Title/Abstract]))
- Date: 15th November 2016
- Inclusion:
  - Original research articles referring to PTSD and TNF-α
- Exclusion:
  - Letters, reviews
  - Inadequate trauma (e.g. cancer diagnosis)
  - Trauma only, but not PTSD
  - Genetic data only, TNF receptors only, no measurement of TNF-α (protein or mRNA)
  - Studies only investigating the effect of dexamethasone on stimulated TNF-α production to study glucocorticoid receptor sensitivity
Results: Articles obtained

Records identified by PubMed search \((n=40)\)

Articles without applicable data \((n=17)\)
- Reviews \((n=4)\)
- No TNF-\(\alpha\) data or TNF-\(\alpha\) data not related to PTSD \((n=7)\)
- Studies on GR sensitivity \((n=3)\)
- No PTSD or inadequate trauma \((n=3)\)

Eligible original research articles \((n=23)\)

Articles about animal studies \((n=3)\)

Articles about human studies \((n=20)\)
- Articles with cross-sectional data about serum, plasma or CSF levels of TNF-\(\alpha\) \((n=18)\)
- Articles with cross-sectional data about TNF-\(\alpha\) production in-vitro \((n=3)\)
- Articles with longitudinal data \((n=4)\)
• Rats
• Exposed or unexposed to stress
• Stress: Placing the animals on well-soiled cat litter (in used by the cat for 2 days) for 10 min
• TNF-α concentrations in hippocampus
• Experimental treatment: minocycline

Levkowitz et al.: Eur Neuropsychopharmacology 2015
- Rats
- Exposed or unexposed to stress:
  - Stress: Placing the animals on well-soiled cat litter (in use by the cat for 2 days) for 10 min
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• Rats
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• Experimental treatment: minocycline
3 Animal studies

- Levkowitz et al. 2015:
  - TNF-α↑ in hippocampus, but not in frontal cortex or hypothalamus after stress in rats
  - minocycline lead to normalised TNF-α concentration and improvement in EPM
- Lee et al. 2016:
  - TNF-α mRNA↑ in hippocampus after stress in rats
  - Alleviated anxiety symptoms and normalisation of TNF-α mRNA after ibuprofen.
- Liu et al. 2015:
  - no difference in TNF-α concentrations in hippocampus after stress in rats
  - TNF-α seems to be elevated in hippocampus of rats after stress
  - Current data situation: insufficient and inconsistent
18 Cross-Sectional Studies: 
**TNF-α in Serum, Plasma or CSF**

- Comparison of TNF-α serum/plasma/CSF levels between subjects with and without PTSD
- **Serum/plasma concentrations of TNF-α found elevated in PTSD patients in 13 studies**
  - Results became insignificant in 2 of the studies with positive result when controlled for age, BMI and smoking
- 4 studies: no difference in TNF-α levels between PTSD and control subjects
- 1 study: TNF-α found elevated in CSF of patients with PTSD after pain
- Elevated levels of IL-1β, IL-6 and IFN-γ reported in several studies
- SCID-I, PCL, CAPS, PDS, SRIP and LASC were used

- Results suggest an elevation of serum concentrations of TNF-α and other cytokines in patients with PTSD
- The differences may be partly be explained by age, BMI, smoking
- Various questionnaires used, most studies used the CAPS, studies overall but not in detail comparable
3 Cross-Sectional Studies: TNF-α production in-vitro

- 3 cross-sectional studies measuring TNF-α production in-vitro
- Increased in-vitro TNF-α production of blood cells from PTSD patients in 2/3 studies
  - Increased TNF-α production could be associated with PTSD

4 Longitudinal Studies: TNF-α serum levels

- 4 longitudinal studies about TNF-α serum levels in patients with PTSD
  - Changes in TNF-α, sTNF-R p55 and sTNF-R p75 levels during six weeks of psychotherapeutic treatment
  - Changes in TNF-α levels during treatment with FSWW08, a specially formulated fermented soy product given for 3 months
  - No changes over 3 months without standardized treatment
  - Changes in TNF-α serum levels over 6 years time
  - TNF-α serum levels could be a state marker of PTSD
  - Psychotherapy and a specific soy product influence PTSD symptoms and the TNF-α system
  - As only 2 longitudinal studies are available, long-term data is too scarce to draw firm conclusions
Summary of the systematic review

• Animal studies
  - TNF-\(\alpha\) gene expression and production seems to be increased in hippocampus after stress in rats
  - Minocycline and Ibuprofen: Restoration of normal TNF-\(\alpha\) and TNF-\(\alpha\) mRNA concentration

• Case-control studies
  - Majority of studies: Elevation of TNF-\(\alpha\) serum/plasma concentrations in patients with PTSD
  - Differences in IL-1\(\beta\), IL-6 and IFN-\(\gamma\) levels reported in several studies

• Longitudinal studies
  - Changes of TNF-\(\alpha\) levels during treatment
  - Changes of TNF-\(\alpha\) levels over longer time periods
Critical remarks about the systematic review

- Only Pubmed was used
- Focus on TNF-α alone is a narrow perspective, because TNF-α acts together with other cytokines
Meta-analysis of TNF-α: Subgroup meta-analysis with use of psychotropic medications as a predictor

Passos et al.: Lancet Psychiatry. 2015
Inflammatory markers in post-traumatic stress disorder: a systematic review, meta-analysis, and meta-regression

Meta-analysis of IL-1β: Subgroup meta-analysis with use of psychotropic medications as a predictor

Passos et al.: Lancet Psychiatry. 2015
Inflammatory markers in post-traumatic stress disorder: a systematic review, meta-analysis, and meta-regression

### Meta-analysis of IL-6: Subgroup meta-analysis with use of psychotropic medications as a predictor

Passosos et al.: Lancet Psychiatry. 2015
Conclusions

Content-related conclusions

• Elevated concentrations of TNF-α, IL-1β, IL-6 and IFN-γ in the blood as well as in the hippocampus could be an unspecific biomarker for PTSD

• Elevated TNF-α production seems to be rather a trait marker than a state marker, as it has been shown to be influenced by time and psychotherapy

• Hippocampal TNF-α production could play a role in the pathophysiology of PTSD

• Hippocampal TNF-α production can be influenced by drugs such as minocycline and ibuprofen

• Anti-inflammatory drugs: Future treatment for PTSD?
Conclusions

Research-related conclusions and future perspectives for cytokine studies

- Data regarding cytokine concentrations in important brain regions like amygdala, prefrontal cortex are scarce
- No studies relating brain structure and volume to cytokine levels
- BMI, age, medication and smoking should always be considered as control variables
- Important psychological aspects possibly influencing cytokine production should be taken into consideration:
  - Hostility¹
  - Activity²
  - Sleep³
- Long-terms studies, specifically clinical studies are needed
- Sample sizes are small: Multicentre/multinational studies are advisable⁴

Publications


Thank you

- **Bundeswehr Hospital Berlin**: Peter Zimmermann, Antje H. Bühler, Jörg-Egbert Wolf, Gerd D. Willmund, Ulrich Wesemann

- **IoPPN**: Norman Jones, Nicola Fear, Serkan Hussein, Bethan Dalton

- **King’s College Hospital**: Mohammad Ibrahim

- **Ludwig-Maximilians-University Munich**: Lesca M. Holdt, Daniel Teupser

- **University of Tasmania**: Kenneth C. Kirkby
Thank you for your attention