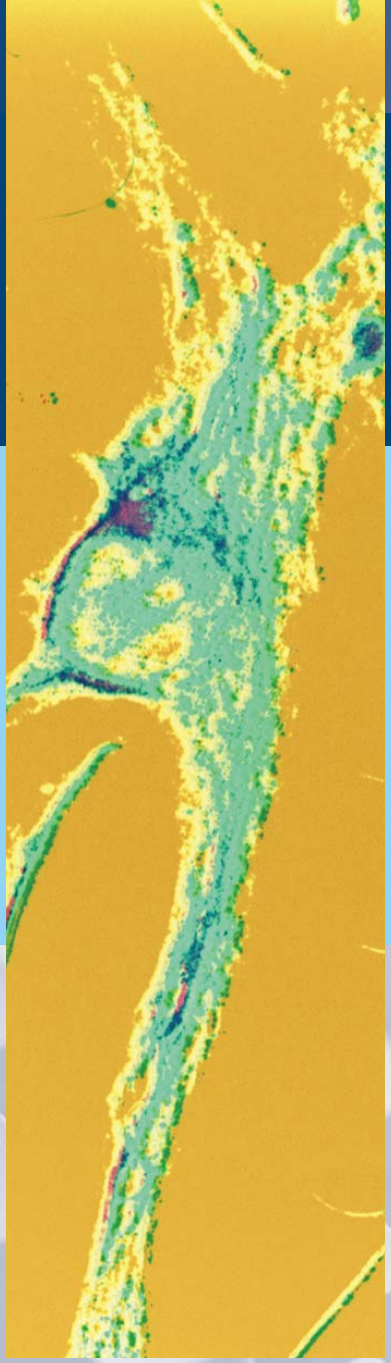

Thieme Web-Book Psychoneuroimmunology

Edited by Volker Arolt
with the Collaboration of Marion Peters

7th Expert Meeting on
Psychoneuroimmunology
Münster, June 29th-July 1st, 2001

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Unraveling the schizophrenia knot: Characterizing Immune Activation in a Subtype of Schizophrenia

Introduction

Schizophrenia is a devastating syndrome affecting 1% of the population. Schizophrenia has been considered to be a single disease despite profound between-subject (and between-pedigree) differences in illness course, symptoms, and response to medication. However, recent work documenting multiple genomic regions each of which appear to give rise to increased susceptibility to develop the syndrome (Pulver 2000) require rethinking of the question of homogeneity of the disease process. Previous work in our laboratories has provided evidence for the existence of at least three etiologically distinct forms of the syndrome (*Figure 1*):

(1) A dopamine psychosis (Garver et al. 1997), a good prognosis form of schizophrenia, in which the primary abnormality appears to be a failure of presynaptic regulation of dopamine. Here psychotic exacerbation appears abruptly, without a lengthy prodrome, and responds quickly and virtually completely following dopamine receptor blockage by the antipsychotic agents. It can be identified by essentially twice the usual levels of the dopamine catabolite, homovanillic acid (HVA), in the plasma or urine of neuroleptic-free patients during periods of psychotic exacerbation. The etiology appears to be associated with failure of the usual feedback mechanisms which confine dopamine synthesis within physiological limits.

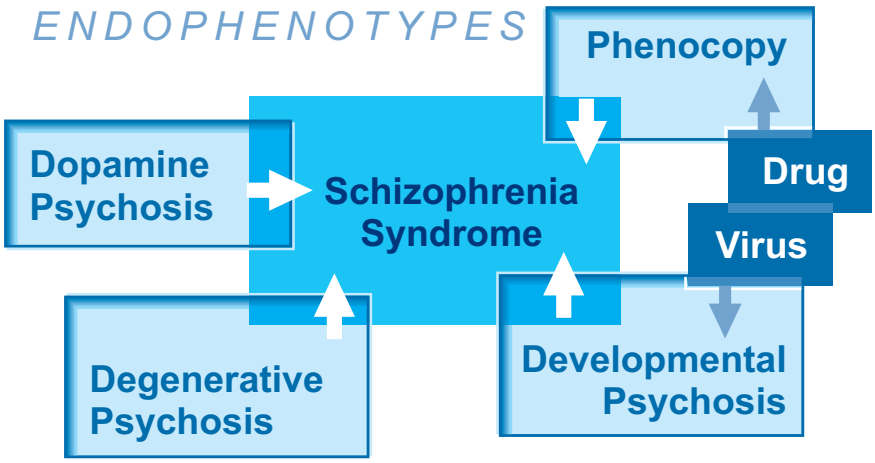

Figure 1.:
The schizophrenia syndrome and its putative component disorders

(2) A neurodevelopmental psychosis (Garver et al. 1999) has been described by a series of authors. The primary abnormalities underlying the neurodevelopmental psychosis appear to occur during early brain development. In the early trimesters of fetal development, proliferating neuroblasts migrate upon radial glia from periventricular origins through the subcortical plate, into the developing cortex. When neuroblast proliferation or migration partially fails, neurons fail to rest in characteristic cortical lamina. As cellular connections develop, absence of some cortical cells or irregular positioning of others result in the development of anomalous circuitry, which is unable to provide for stable physiological processes including detection, filtering and processing of information. Psychotic patients with such anomalous circuitry are largely resistant to the therapeutic effects of the antipsychotic drugs: patients with neurodevelopmental psychoses gain little benefit from dopamine blockade of receptors (Garver, Christensen et al. 2000). Yet the condition is non-progressive, with the primary neuro-pathology established during fetal development.

(3) An unstable or degenerative psychosis (Garver et al. 1999) has been described as an active process. The active process is observed in the progressive development of withdrawal, and of impairment of interests and of peer relationships which often precedes first episodes of psychosis by 3-5 years. Such impairment of interests and withdrawal are a „trait“ of this group of schizophrenics, even during periods of relative freedom from hallucinations, delusions and though disorder. Antipsychotic response occurs, but is progressively delayed following additional episodes of psychotic exacerbation (Garver, Christensen et al. 2000). The progression of illness appears to be punctuated by state-related changes in ventricle and brain volumes: brain swelling during periods of exacerbation and shrinkage during periods of psychosis remission (Garver, Nair et al. 2000). It is this latter unstable „endophenotype“ of the schizophrenias which appears to be associated with evidence

of membrane phospholipid degeneration and immune activation during periods of psychotic exacerbation that is the subject of this communication.

Methods

Excluding the patients with dopamine psychosis (elevated pHVA), neuroleptic-free patients with DSM-IV schizophrenia, who were in the midst of exacerbation of psychosis following mediation noncompliance, and following MRI volumetric assessments (Garver, Nair et al. 2000), underwent examinations of cerebrospinal fluid (CSF) prior to initiation of the antipsychotic, haloperidol. Antipsychotic response was assessed by serial Schedule for Assessment of Positive symptoms (SAPS) over a period of 6 months. Control subjects, free both of medications and of psychosis in themselves and in first degree family members underwent CSF examinations under identical conditions in hospital. CSF and serum cytokines were quantitatively assessed using ELISA assays6 (Rapaport et al. 1997). CSF malonyldialdehyde, a catabolite of membrane phospholipids, was measured using the TBARS reaction (Ohkawa et al. 1979).

Results

Antipsychotic response was found to cluster into two patterns: (1) a cluster of patients showing 61.9±20.5% reduction of SAPS scores from week 2 to 6 of treatment, and (2) a cluster of patients showing -6.5 ±28.8% (non-response) during the same period of treatment despite comparable 10 mg/day haloperidol dose and comparable haloperidol blood levels (Garver et al. 1999). [As previously reported (Garver, Nair et al. 2000), cluster #1 schizophrenics showed expansion of brain volumes and shrinkage of ventricle volumes during periods of psychotic exacerbation; brain volumes shrank and ventricle volumes enlarged during periods of remission of positive symptoms. No such volumetric changes were observed in the cluster #2 patients across serial scans.] Cluster #1 patients evidenced twice the concentration of malonyldialdehyde as assessed with the TBARS assessments during psychotic exacerbation as did controls (1.21±0.23 vs 0.6±0.05, $p<0.05$); no differences were found between cluster #2 patients and controls. CSF interleukin-6 (IL-6), a proinflammatory cytokine, was significantly elevated in the CSF of cluster #1 schizophrenics as compared to both normal controls ($p<0.01$) or to cluster #2 schizophrenics ($p=0.02$) (Fig 2). No such elevation of IL-6 was found in the sera in either cluster of patients.

Similarly, IL-2 and IL-4 were higher in CSF of cluster #1 patients than in cluster #2 patients ($p<0.03$ and $p<0.04$, respectively).

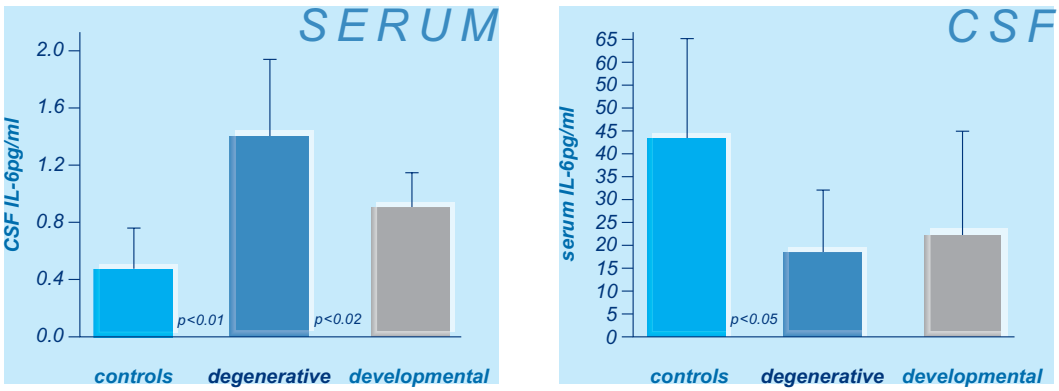

Figure 2.:
Serum and CSF IL-6 in controls, in degenerative psychosis, and in developmental psychosis. (controls, n=6; degenerative psychosis, n=22; developmenmental psychosis, n=16)

Discussion

During periods of psychotic exacerbation, one of the clusters of schizophrenic patients demonstrates brain swelling accompanied in the CSF by excess of byproducts of phospholipid membrane degeneration. Such patients could be identified clinically by delayed antipsychotic response following antipsychotic initiation. It is this subgroup of the schizophrenias for which evidence is accumulating that there may be a central immune activation, with elevations of the pro-inflammatory cytokines such as IL-6, IL-2 and IL-4. However, there is evidence that this immune activation is limited to the central compartment, as no evidence of cytokine elevation is present in the periphery (in the sera).

It is presently unclear whether the central immune activation is *primary* in origin, or whether it is *secondary* to another active process within the central compartment. The suggestion of linkage within the HLA region of chromosome 6 (Nurnberger et al. 1999) might suggest a primary abnormality associated with the immune system. On the other hand, reports of the presence of fragments of retrovirus within the CSF of some schizophrenic patients during psychotic exacerbation (Karlsson et al. 2001), and/or of a paucity of protective neurotrophic factors in brain (Durany et al. 2001) suggest that the immune response might be a (secondary) response to other pathology.

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S100B as indicator for ongoing neuroplastic activity in schizophrenia

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S100B as indicator for ongoing neuroplastic activity in schizophrenia

Introduction

There is continuing discussion on the neurodegenerative mechanisms which contribute to the etiopathogenesis of schizophrenia. Support for this hypothesis comes from postmortem studies and especially from recent volumetric MRI studies indicating that ventricular enlargement and hemispheric volumetric reductions may have a progressive component in patients with schizophrenia (for review: DeLisi 1999). Changes in brain structure and volume appear to arise from a reduction of neuritic processes (such as dendrites and synapses) rather than from a loss of neuronal or glial cell bodies (for review: McGlashan et al. 2000). A missing link for this hypothesis is a biochemical marker for cellular integrity in the brain that can be measured during acute psychotic episodes of schizophrenic patients. S100B has the potential to serve as such a marker.

S100B is synthesized mainly by astrocytes and evolves paracrine and autocrine effects on neurons and glia (Griffin et al. 1998). In adult brains it plays a role in neuronal plasticity and long term potentiation. Lower concentrations of extracellular S100B act on glial and neuronal cells as a growth differentiating factor, while higher concentrations induce apoptosis (Fano et al. 1995).

S100B as indicator for ongoing neuroplastic activity in schizophrenia

Increased CSF and plasma levels of S100B have been detected after traumatic brain injury, toxic or ischemic brain damage, and in multiple sclerosis. S100B is also elevated in several kinds of dementia, especially Alzheimer´s disease. Two studies showed increased S100B plasma levels in medicated patients suffering from acute schizophrenic psychosis (Wiesmann et al. 1999; Lara et al. 2001), whereas Gattaz and colleagues reported decreased S100B concentrations in medicated patients with chronic schizophrenia (Gattaz et al. 2000).

In the present study we were able to investigate unmedicated or even drug naive acutely psychotic schizophrenic patients. The patients were reinvestigated after 6 weeks of neuroleptic treatment.

The mean PANSS (Positive and Negative Syndrome Scale) total score upon admission was 86.7±17.9, ranging from 55 to 127. After 6 weeks of neuroleptic treatment the mean PANSS score was 66.7±17.8 (range: 36 to 97), indicating a significant improvement of psychopathology (p<=0.0001). The positive and general psychopathology subscales showed a significant decrease after 6 weeks of treatment (p<=0.0001; p=0.003, respectively), while the negative subscale showed no significant change (p=0.29).

Upon admission, the S100B plasma level in schizophrenic patients was significantly higher compared to the matched healthy controls (p=0.001, Figure 1). After 6 weeks of treatment the level of significance was no longer reached (p=0.056). There was a significant positive correlation between the negative subscale score and the S100B concentration after 6 weeks (p=0.02). Furthermore, intraindividual differences between negative subscale scores upon admission and after treatment were significantly correlated with the S100B concentration after

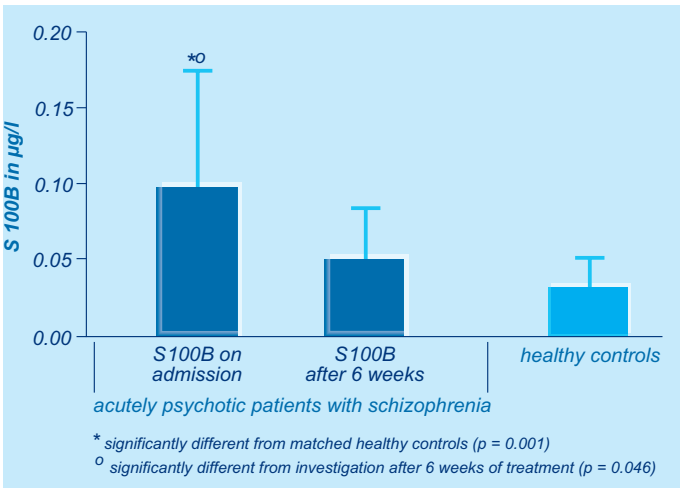

Figure 1.:
S100B blood levels (mean plus standard deviation) in acutely psychotic unmedicated schizophrenic patients and after 6 weeks of treatment compared with healthy controls.

S100B as indicator for ongoing neuroplastic activity in schizophrenia

6 weeks, indicating that little change or even deterioration of the negative symptomatology was associated with high S100B levels ($p<=0.0001$). Patients with initially high S100B plasma levels showed significantly higher PANSS negative scores after six weeks of treatment than patients who upon admission showed S100B levels closer to normal. Also, patients with high S100B levels after six weeks of treatment showed no change in the PANSS negative subscale scores, while those patients whose S100B levels returned to normal experienced a significant improvement in negative psychopathology.

In this study we were able to show that S100B plasma levels are increased in unmedicated acutely psychotic schizophrenic patients as previously shown for medicated patients (Wiesmann et al. 1999; Lara et al. 2001). Increased S100B plasma levels in schizophrenic patients are obviously not an artifact caused by neuroleptic medication. After six weeks of neuroleptic treatment, the S100B levels in the total group of patients in our study did not significantly differ from healthy controls anymore. S100B plasma levels may serve as a marker for the acute stage of schizophrenic psychosis and might normalize or even decrease in a chronic stage of disease.

Amongst patients suffering from more severe negative symptoms, astroglial integrity is affected to a greater degree not only in an acute stage of the disease but negative also after six weeks of treatment. A loss of astroglial intactness appears to be associated with the development of symptoms rather than positive or general psychopathology symptoms. A restoration of astroglial integrity seems to be associated with improvement of negative symptoms.

Further details and a complete reference list can be retrieved from:
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Schizophrenic Patients' Neutrophils Inflammatory Responses: Parallel Studies of Chemotaxis, Superoxide Release and Bactericidal Activity

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Keywords:
schizophrenia, chemotaxis, phagocytosis, superoxide anion, free radicals, reactive oxygen species.

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Schizophrenic patients' neutrophils inflammatory responses: parallel studies of chemotaxis, superoxide release and bactericidal activity

Abstract

Background: Defective neutrophil phagocytosis and an excess of oxygen free radicals in schizophrenic patients were reported in the literature.

Objective: To investigate neutrophil functions in schizophrenic patients.

Methods: Neutrophil responses of phagocytosis, chemotaxis and superoxide release were investigated in eighteen schizophrenic patients and 15 healthy controls.

Results: A significant statistical increase of superoxide anion release was found in schizophrenic patients as compared to controls (mean±SEM patients: 6.89±0.30 nmol O_2^- / 10^6 cells/min, controls: 5.13±0.55 nmol O_2^- / 10^6 cells/min, p=0.015). We were unable to detect differences in chemotaxis and phagocytosis between healthy controls and schizophrenic patients.

Conclusions: In schizophrenic patients there is over production of free radicals. Further studies are required to establish the role of oxidative stress in the ethiopathogenesis of schizophrenia.

Schizophrenic Patients' Neutrophils Inflammatory Responses: Parallel Studies of Chemotaxis, Superoxide Release and Bactericidal Activity

Introduction

An association between schizophrenia and altered immunity has been suggested but has not been consistently demonstrated (Firer et al. 1994; Schattner et al. 1996; Sirota 1990; Sirota et al. 1993a; Sirota et al. 1993b; Sirota et al. 1995). Neutrophils phagocytose, destroy microorganisms and play a role in natural immunity to tumors (Murphy 1976; Roitt, Brostoff & Male 1985). Neutrophil phagocytosis could be studied at the known different steps, including adherence, chemotaxis, ingestion and killing of microorganisms by oxidative and non-oxidative mechanisms (Wolach, Bachner & Boxer 1982). The numbers of both monocytes and neutrophils were increased significantly along with impaired neutrophil chemotaxis, phagocytosis, and oxidative metabolism in schizophrenic patients (Cosentino et al. 1996). The present study reports our results of 18 drug-free schizophrenic patients. We determined neutrophil functions such as chemotaxis, superoxide production and bactericidal activity.

Methods

Subjects: Eighteen schizophrenic patients in acute exacerbation (14 males and 4 females) aged 21-45 (mean ± SD: 36.2 ± 5.8 years) and 15 healthy controls (12 males and 3 females) aged 22-46 years (36.8±5.6 years) participated in the study after their signing a consent form. All subjects were physically healthy (by medical history, physical examination and routine blood and urine tests) and had no past or present history of drug dependence or alcohol abuse. All participants adhered to a low-monoamine, alcohol-free and caffeine restricted diet. Female participants did not use contraceptive pills.

None of the participants had been treated with antipsychotics and drugs known to affect the immune system or the free radical formation, at least three months prior to entering the study: “wash-out period”. Matching between the patients and controls was done according to the following variables: age, sex, origin, smoking habits as much as possible. Care was taken to exclude heavy smokers (more than 10 cigarettes/day). The subjects were interviewed according to the guidelines of the Schedule for Affective Disorders and Schizophrenia-Lifetime version (Endicott & Spitzer 1978). They were diagnosed as suffering from schizophrenia according to DSM-3 (American Psychiatric Association 1987). Age of onset of the disease was 22±3 years (16-31 years). Disease duration was 1-10 years (mean ±SD=5.5±1.8 years). Laboratory tests were conducted in a double-blinded fashion: diagnoses were assigned blind to chemotaxis, bactericidal activity and free radical data and laboratory data were obtained on coded blood samples.

Polymorphonuclear Leukocyte (Neutrophil) Isolation

Twenty ml of heparinized peripheral blood was collected concomitantly from patients and controls between 8:00 AM and 9:00 AM. The blend was mixed with equivalent volumes of 3% dextran (T-250) in saline. Neutrophils were isolated according to Boyum (1963), and residual erythrocytes were then removed by hypotonic lysis. The neutrophils were then suspended in M199 medium for chemotaxis assays, in HBSS buffer for superoxide anion release assays, and in PBS supplemented with 0.2% D-glucose and 1% bovine serum albumin (PBS-GA) for bactericidal activity assays.

Chemotaxis Assay

A 48·well chemotactic microchamber (Neuro, Probe, Inc. Bethesda, MD) was used to determine random migration and chemotaxis of neutrophils (Falk et al. 1980). The number of migrating cells were determined in nine fields by light microscopy (x40 objective and a x10 ocular equipped with a fine grid). Net chemotaxis was calculated by subtracting the random migration (M199 medium in the bottom wells) from the FMLP-driven neutrophils. Experiments were carried out in duplicate.

Bactericidal Activity

The quantitation of maximal bactericidal activity was measured as the decrease in the number of viable bacteria after incubation of bacteria and PMNs in the presence of serum, as described previously (Clawson & Repline 1976). Bacteria (Staphylo - ccocus Aureus American type Culture) was freshly grown before each experiment and allowed to enter an early stationary phase (4 h shaking, at 37°C). Each experimental setup included two controls which were comprised of PBS/G-A and bacteria. The colony-forming units (CFU) were assessed and the log of decrease of colonies was calculated and compared with that of the control counts .

Superoxid Anion Release

This assay was performed as previously reported (Weisbart et al. 1985). The PMNs were suspended in 1 ml of HBSS at a final concentration of 1 x 10^6 cells/ml, with 60 µM of ferricytochrome-C (Sigma), with or without 214 U of

superoxide dismutase (Sigma). To initiate the reaction 0.1µM of fMLP (Sigma) was added, and the rate of superoxide anion release was measured at 550 nm for 5 min, at 37°C, in a UV-260 Shimadzu spectrophotometer. The results of duplicate tests were averaged, and the superoxide anion release calculated using the Massey extinction coefficient of 2.1 x 10^4 M-1 cm-1.

Statistical Analysis

Both the Student's t-test and the Mann-Whitney non-parametric test were used for data analysis of all leukocyte functions.

Results

Superoxide anion generation of schizophrenic patients exceeded significantly that of the healthy control cells i.e. 6.89±0.3 and 5.13±0.55 nmol/min 10^6 cells, respectively (p= 0.015). Chemotaxis ratio of neutrophils from schizophrenic and healthy controls was similar in ratio 2.25±0.2 and 2.36±0.3, respectively (p=0.4). The bactericidal activity of the schizophrenic neutrophils reached the same bactericidal capacity of healthy controls (autologous serum: 0.96+0.1 and 1.02+0.13, respectively; homologous serum: 1.1+0.11 and 0.91+0.12, respectively).

Discussion

In the present study, neutrophil chemotaxis, superoxide anion generation and bactericidal activity were assessed in drug-free schizophrenic patients and healthy controls. The superoxide anion production reached statistically higher levels in schizophrenic patients as compared to healthy controls. Chemotaxis and bactericidal activity, which involve rearrangement of the cytoskeleton to one degree or another (Wolach et al. 1998) were found to be similar to the normal control group. These findings suggest that the cytoskeletal rearrangement is intact in drug-free schizophrenic patients, while the cytoskeleton-free membrane associated-superoxide production is fully overactive. Neutrophil phagocytosis was reported to be reduced in schizophrenic, depressed and manic patients as compared with age and sex matched controls (McAdams & Leonard 1993). Neutrophil phagocytosis was also reduced in all 3 patient groups during the active phase of illness but returned to normal values on recovery. Cosentino et al. (1996) reported that schizophrenic patients on long term medications showed reduced neutrophil

chemotaxis, phagocytosis and oxidative metabolism. The authors concluded that these abnormalities were strongly associated with chronic administration of benzodiazepines and carbamazepine. In our study, in which patients were drug-free, chemotaxis and killing activities were normal; moreover, there was an over-production of superoxide anions by neutrophils. These findings support the assumption that medications could affect neutrophil functions.

In the present study we evaluated the fMLP pathway and found it exacerbated. No parallel overactivation of the bactericidal activity was shown, as only a significant decrease of superoxide production was shown to affect the bactericidal function (Wolach et al. 1982).

The production of superoxide anion by neutrophils is considered a parameter of the phagocytic function (Maes et al. 1992) and is important in the formation of hydrogen peroxide and hydroxyl radical (Fantone & Ward 1982). These reactive oxygen species (ROS) are deleterious to most cellular components, causing lipid peroxidation, protein and carbohydrate oxidation and DNA strand breaks. The brain is exceptionally susceptible to damage by ROS, which may explain the involvement of these metabolites in several neuropsychiatric illnesses, such as Parkinson's, Alzheimer's, Wilson's and Huntington's diseases, as well as schizophrenia (Lohr 1991; Sirota et al. 1999).

This study encourages a further understanding of the pathogenesis of schizophrenia, which may lead to other kinds of treatment, such as free radical scavengers like vitamin E and C (Sirota et al. 1999; Mahadik & Mukherjee 1996).

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Inflammation and Schizophrenia: From basic Research to Treatment Strategies.

Introduction

An inflammatory / immunological pathogenesis has been discussed for a subgroup of schizophrenic patients, since abnormalities in the immune function of schizophrenic patients have been described over the last century (Körschenhausen et al. 1996; Yolken and Torrey 1995). Both, components of the cellular immune system and of the cytokine system came into the focus of interest during the last decades since when modern immunological methods facilitated differentiated investigations of the immune system. The results show that activating cytokines like interleukin-1 (IL-1) and IL-2 in the cerebrospinal fluid are higher in schizophrenic patients compared to controls and high levels of IL-2 in the cerebrospinal fluid is a predictor for the increased probability of a schizophrenic relapse (McAllister et al. 1995).

The upcoming concept to differentiate the T-helper-1 and T-helper-2 $CD4^+$ cells according to their function in the acute immune defense of the cellular immune system (TH-1) and in the humoral immune system more related to chronic diseases (TH-2) allowed a further understanding and interpretation of the immunological findings in schizophrenia.

Moreover, genetic findings also point to an involvement of the immune system. One of the strongest findings of genetic linkage studies in schizophrenia is located on the chromosomal region 6p22-23. In this region several genes are associated with immune function. The HLA-region (6p23.1) is crucially involved in immune function, e.g. during (viral) infection. Additionally, linkage studies revealed a susceptibility locus at chromosome 5 around a region, where the genes for several cytokines are placed. Maybe that a genetic determined dysfunction of these genes contributes to an abnormal immune activation in schizophrenia which is not able to recognize and eliminate common antigenes affecting CNS function.

TH-2 Activation in Schizophrenia

IL-6 is not only a product of macrophage/monocyte activation, but also of the activation of the TH-2 system. Therefore it cannot be differentiated whether a functional increase of the IL-6 system is a product of TH-2 activation or of the monocyte/macrophage line. However, several reports about an activation of different components of the IL-6 system (IL-6, soluble IL-6 receptors, soluble gp130), point to an activation of the TH-2 system in schizophrenia, like other reports on IL-10, IL-4, and IgE.

IL-10 is a cytokine that is produced by TH-2 cells. An increase of IL-10 in schizophrenic patients compared to healthy controls was reported. Another study observed a strong relationship between IL-10 levels and schizophrenic negative symptoms in the cerebrospinal fluid of 62 unmedicated schizophrenics. In medicated schizophrenics, treated with haloperidol, a significant relationship between CSF IL-10 levels and the severity of schizophrenic psychosis - measured by Bunney-Hamburg psychosis rating scale - was found. These findings point out, that IL-10 levels in the CSF are related to the severity of the psychosis, especially to the negative symptoms.

An other characteristic cytokine that is produced by TH-2 cells is IL-4. An increase of IL-4 levels in the CSF of juvenile schizophrenic patients has recently been reported (Mittleman et al. 1997). The production of IgE is also a sign for an activation of the TH-2 immune answer. Increased levels of IgE in schizophrenic patients compared to controls have been observed. Especially the findings in the CSF point out that the probable increase of the TH-2 system in schizophrenia is not only a phenomenon of the peripheral immune system, it also seems to play a role in the CNS immune system. There is a communication between the peripheral immune system and the CNS immune system, where lymphocytes, adhesion

to the vessels and penetration through the endothelium into the brain parenchyma using adhesion molecules and activation of glial cells (*figure 1*).

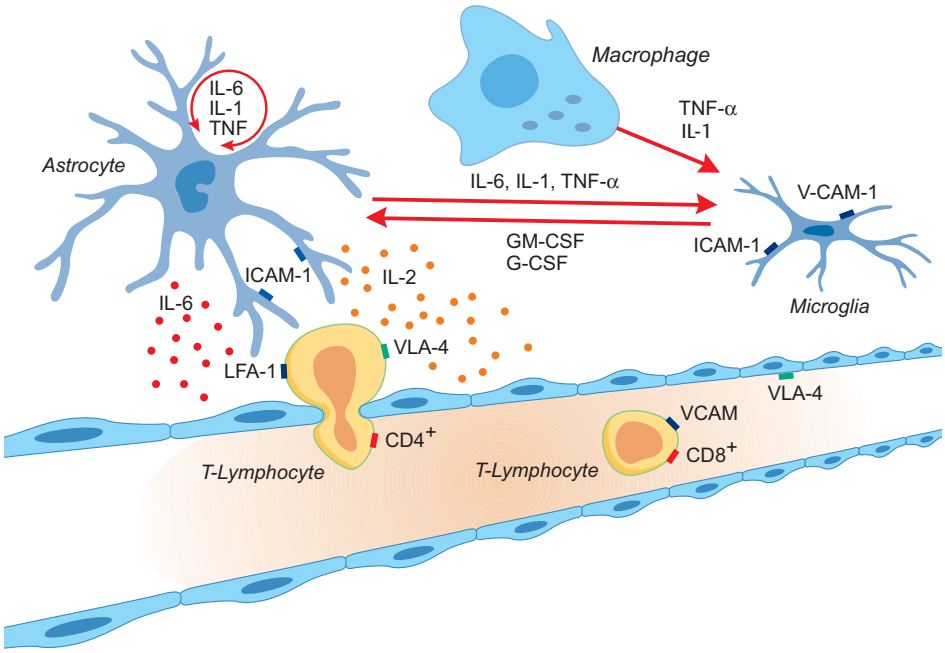

Figure 1.:
Schematic overview on the network of blood lymphocytes, adhesion, glial cells, cytokine production after activation, and the blood-brain-barrier.

Earlier descriptions of elevated $CD3^+$- and $CD4^+$-cells in unmedicated schizophrenics fit as well with the hypothesis of a shift to the TH-2 system with a diminished TH-1 immune response in schizophrenia (Müller et al. 1991; Sperner-Unterweger et al. 1999).
As shown in *figure 2* there is a functional balance between the TH-1 and TH-2 system. It would be expected that an overactivation of the TH-2 system is associated with an underactivation of the TH-1 system. A lot of different findings over decades of years point to a decreased activation of the TH-1 system in schizophrenia.

T-Helper-1 system and schizophrenia

The key characteristics of the TH-1 system are the production of interferon-γ and IL-2. One of the often replicated findings in schizophrenia is the decreased in-vitro production of IL-2. This phenomenon has often been interpreted as the

consequence of an exhaustion of the lymphocytes after overproduction of IL-2, but, however, it might reflect as well the reduced capacity of lymphocytes to produce IL-2. The observation of a decreased production of IL-2 fits well with another finding: The decreased production of interferon-γ (Rothermund et al. 1996). Both findings point to a blunted production of TH-1 related cytokines and to an underactivation of the TH-1 system in schizophrenia. A lack of activation of the TH-1 related cellular immune system has also be postulated by other researchers (Sperner-Unterweger et al. 1999). Own findings of the decreased production of lymphocytes after stimulation with different specific antigens also might reflect the reduced capacity for a TH-1 mediated immune answer in schizophrenia. Especially after stimulation with tuberculin, which provokes a TH-1 mediated immune answer, the reaction was blunted (Müller et al. 1991).

Levels of sICAM-1 in schizophrenia

Recently, decreased levels of the soluble intercellular adhesion molecule 1 (sICAM-1) in the serum of schizophrenic patients have been described (Schwarz et al. 2000). ICAM-1 is a molecule that mediates the adhesion of lymphocytes to other lymphocytes, to endothelial cells and to parenchymatic cells on the one hand, but it also mediates the signal for the activation of the cellular immune system. ICAM-1 is part of the TH-1 immune answer. Therefore, decreased levels of the soluble form of ICAM-1, which is shedded from lymphocytes, seems to represent the state of activation of the TH-1 system. However, reduced sICAM-1 levels have been found not only in the serum, but also in the CSF of schizophrenic patients recently (Schwarz et al., in preparation). The latter finding points out that the blunted activation of the TH-1 system may not be restricted to the peripheral immune system, because CSF parameters reflect more directly the immune pathology of the CNS.

One of the ”classical” epidemiological findings in schizophrenia research is the negative association between schizophrenia and rheumatoid arthritis. This negative association can be interpreted as two sides of the TH-1/TH-2-balance coin – represented by increased

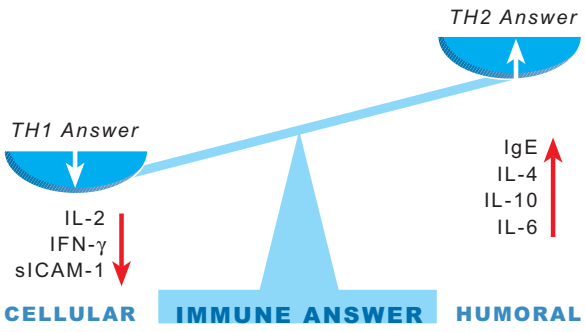

Figure 2.:
Dysbalance of the immune system in schizophrenia. Insufficient activation of the TH-1 answer and relative overactivation of the TH-2 system.

sICAM-1 levels in rheumatoid arthritis and decreased sICAM-1 levels in schizophrenia. SICAM-1 is also a key-molecule that mediates the inflammatory reaction in rheumatoid arthritis, increased sICAM-1 levels are regularly found in rheumatoid arthritis. Rheumatoid arthritis is a disorder that is primarily mediated by the cellular TH-1-related immune system. *Figure 2* shows blunted TH-1 answer of the 'cellular' immune system which is represented by decreased secretion of IFN-γ, IL-2, or sICAM-1, while preferably TH-2 cytokines are secreted.

Therapeutic consequences of these findings

Pharmacological down-regulation of activating cytokines in the CNS using anti-inflammatory therapy may possibly have favorable effects in some schizophrenic patients. This view is supported by the fact that atypical antipsychotics have immunomodulatory properties that may lead to a down-regulation of the immune response in the CNS.

With these findings, it seemed meaningful to study the effects of anti-inflammatory therapy using an add-on design together with a well-proven neuroleptic in schizophrenic patients. We used celecoxib as add-on therapy to risperidone. Celecoxib is a selective cyclooxygenase-2 (COX-2) inhibitor, which enters the CNS well and has few adverse side effects. Moreover, COX-2 inhibition is known to balance TH-1 and TH-2 by activating TH-1 and downregulating the TH-2 immune answer. Risperidone was selected because it is an atypical neuroleptic with high efficacy in the therapy for both positive and negative symptoms of schizo-phrenia, as well as a wealth of experience with risperidone treatment.

As expected, both groups of schizophrenic patients showed significant improvement in the positive and negative syndrome scale (PANSS), and in all subscales, during the five weeks treatment with risperidone. However, the celecoxib add-on therapy group showed a significant group effect in the PANSS total score (Müller et al. 2002). Additional treatment with celecoxib had significant positive effects on the therapeutic action of risperidone with regard to the total schizophrenia psychopathology.

Moreover, the fact that treatment with an immunomodulatory drug shows beneficial effects on the symptomatology of schizophrenia, indicates that immune dysfunction in schizophrenia is not just an epiphenomen, but related to the pathomechanism of the disorder. However, a non-immunological NMDA receptor mediated therapeutic effects of celecoxib has to be taken into account.

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Influences of subtle changes in plasma cytokine levels on brain function

Introduction

The central nervous system (CNS) is involved in host response mechanisms during infection and inflammation: Beside the induction of fever and neuroendocrine activation as, for example, of the stress hormone systems in animals behavioral changes have been observed as well. These alterations in sleep-wake behavior, food intake, motivation, cognition, and other CNS functions are usually called "sickness behavior". It is known from a variety of experiments that in animals inflammatory cytokines such as interleukin (IL)-1, IL-6 or tumor-necrosis-factor (TNF)-α are the most important mediators of these behavioral changes (Yirmiya et al. 2000).

Also in humans changes in immunological functions are accompanied by changes in behavior and emotionality: depressive mood and cognitive impairments have been observed during therapeutic treatment with interferons (Dieperink et al. 2000). However, during treatment with interferons, circulating cytokine levels are strongly increased and the patients experience quite severe flu-like symptoms. It is unclear at present whether much more subtle changes in circulating cytokine levels as they occur during chronic inflammatory or malignant diseases might be causally involved in comorbid depressive disorders (Yirmiya et al. 2000).

Influences of subtle changes in plasma cytokine levels on brain function

To address the question whether changes in plasma cytokine levels play a role in the regulation of mood and cognition during infectious or inflammatory processes we performed an experimental study with healthy probands in cooperation with the Hebrew University in Jerusalem. As a model for systemic immunological activation 0.8 ng/kg *salmonella abortus equi* endotoxin or placebo was administered to 20 healthy males in a single-blind crossover study. During 12 hours following injection cognitive function, mood and food intake have been repeatedly measured. The subjects did not notice any subjective flu-like symptoms. Nevertheless, a variety of objective changes in the examined variables have been observed, which were similar to the above mentioned “sickness behavior” in animals: endotoxin led to robust increases in the plasma levels of TNF-α and later also of IL-6 and cortisol. Shortly after the injection of endotoxin, when only TNF-α levels were increased, the subjects reported a significantly higher level of anxiety compared to placebo. Later on, when also cortisol and IL-6 levels were increased, a higher level of depressive mood compared to placebo has been reported. As opposed to memory functions, attention or executive function were not impaired during the whole experimental session following the endotoxin injection (Reichenberg et al. 2001).

Finally, the subjects showed less spontaneous food intake shortly after endotoxin injection, when TNF-levels were increased, but ate more when cortisol and IL-6 levels were increased as well (Reichenberg et al., in press). All these behavioral changes were quantitatively correlated with the changes in cytokines and cortisol and cannot be explained by subjective complains of the subjects. Thus, it seems very likely to us that during immunological stimulation with endotoxin changes in mood, cognition and food intake are mediated in humans by changes in plasma cytokine levels and/or by concomittant neuroendocrine activation. Nevertheless, the activation of immune response parameters was more robust than that observed in a variety of chronic diseases (Yirmiya et al. 2000). Thus, it cannot definitely be concluded from the present data whether the depressive symptomatology occuring during such diseases is induced by these immunological changes. Unfortunately, so far there have been no data from experimental studies about changes in cognitive function or mood during such subtle immunological changes. In contrast, some studies have addressed the impact of smaller changes in circulating inflammatory cytokines on another important brain function, the sleep-wake behavior: in animals, changes in inflammatory cytokine levels are known to have robust influences on sleep and to mainly modulate nonREM sleep (Krueger et al. 1995).

In humans, sleep studies have also been performed during experimental endotoxinemia in healthy volunteers (Pollmächer et al. 2000): higher doses of endotoxin as they have been used in the cognition/emotion study primarily disturbe sleep continuity in humans in combination with a neuroendocrine activation (Mullington et al. 2000). Smaller amounts of bacterial endotoxin have an influence on sleep as well without concomittant changes in hormonal secretion: 0.2 ng/kg body weight endotoxin cause a two-fold increase in TNF-α and IL-6 levels compared to placebo. In parallel, nonREM sleep amount and intensity increased in correlation with the changes in TNF-α levels (Mullington et al. 2000). Vice versa, nonREM sleep was transiently suppressed during an experimental reduction of the biological activity of TNF-α by increasing the plasma levels of soluble TNF receptors, the most important endogeneous modulators of the biological activity of TNF-α (Schuld et al. 1999).

In our view all of these studies support the hypothesis that subtle changes in the biological activity, mainly of TNF-α, may be relevant for changes in human brain functions such as sleep, cognition or emotion (Pollmächer et al., in press). It is of particular interest to further elucidate the underlying pathophysiological processes and the brain regions involved.

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Production and dexamethasone-regulation of the cytokine TNF-α in major depression

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Dexamethasone, immune system, neuroendocrine system, major depressive disorder

Production and dexamethasone-regulation of the cytokine TNF-α in major depression

Introduction

Several reports on an altered secretion of TNF-α in major depression have been published during the last years (Levine et al. 1999; Lanquillon et al. 2000; Mikova et al. 2001) after some earlier studies (Bauer et al. 1995; Brambilla et al. 1995): Brambilla and Maggioni (Brambilla et al. 1995) examined elderly depressives and did not find significant changes in the blood levels of this cytokine. Levine et al. (Levine et al. 1999) found no relevant changes of TNF-α levels in the cerebrospinal fluid (CSF) of depressive patients, although they detected a significant correlation between the serum levels of TNF-α and the concentrations of IL-1β in the cerebrospinal fluid (CSF) of depressive patients. Our own data (Lanquillon et al. 2000) and the data of Mikova et al. (Mikova et al. 2001), however, point to a significant and state-dependent increase in the production of TNF-α in this group of patients.

To determine the role of TNF-α in major depression with special regard to the treatment response and to further clarify some of the regulatory influences of an increased HPA-activity on the production of this cytokine, we here describe the production of TNF-α under basal conditions and after incubation with the synthetic glucocorticoid dexamethasone (DEX).

Production and dexamethasone-regulation of the cytokine TNF-α in major depression

Methods

The methods employed have been published previously in detail (Lanquillon et al. 2000). Briefly, 24 depressed inpatients (15 male, 9 female; age: 53.5±15.2 years) with "major depressive disorder, single episode" (296.2) or "major depressive disorder, recurrent" (296.3) according to the DSM-IV diagnostic criteria participated in the study and were matched with 15 controls (9 male, 6 female; age: 52.3±13.5 years). The clinical diagnosis was confirmed by the Structured Clinical Interview for DSM-IV (SCID, German Version). Parameters were assessed before treatment (pre-treatment) and at the end of the study (post-treatment). The psychopathology was quantified by the HAMD (Hamilton Depression Scale; 21-item version) and the MADRS (Montgomery-Asberg Depression Rating Scale). The endpoints of the study were set either at a minimum of 50% reduction of the initial HAMD- and MADRS-scores lasting at least for a one-week period (clinical remission = responder) (n=14) or after 6 weeks with a reduction below 50 % (not sufficiently remitted = non-responder) (n=10).

An adapted commercially available test (Biosource, Ratingen, Germany) was used for the determination of whole blood-derived TNF-α (De Groote et al. 1993). Plates were incubated for 3 h at 37°C for the detection of TNF-α. For further modulation of cytokine production, the steroid compound dexamethasone (DEX; 5, 25 ng/ml) (Sigma, Deisenhofen, Germany) was dissolved in ethanol and added to the plates during the incubation period.

Since modulatory effects of DEX were hardly examinable under basal - unstimulated - conditions by parametrical evaluation, a semi-quantitative procedure was employed: the changes in individual patients and controls were compared to the respective control condition without steroid co-incubation and three groups were established on the basis of the individual reactions: a change between +20% and - 20% was indicated as - 0 -, an increase of more than 20% as - + - and a decrease of more than 20% as - – - .

Results

TNF-α production in major depression under basal conditions
At admission, blood cells of all patients showed significantly higher levels of TNF-α production (56±6.3 pg/5x105 mononuclear cells) than the controls (30.5±5.7 pg/5x105 mononuclear cells) (Kruskal-Wallis One Way Analysis of Variance on Ranks: H = 29.151, df = 7, $p < 0.001$; Mann-Whitney test: * $p = 0.02$)

(Fig. 1A). After treatment, cytokine levels of all patients were significantly decreased (34.5±4.6 pg/5x105 mononuclear cells) (Wilcoxon test: ** p = 0.009) and approached the values of the control group *(Fig. 1A)*.

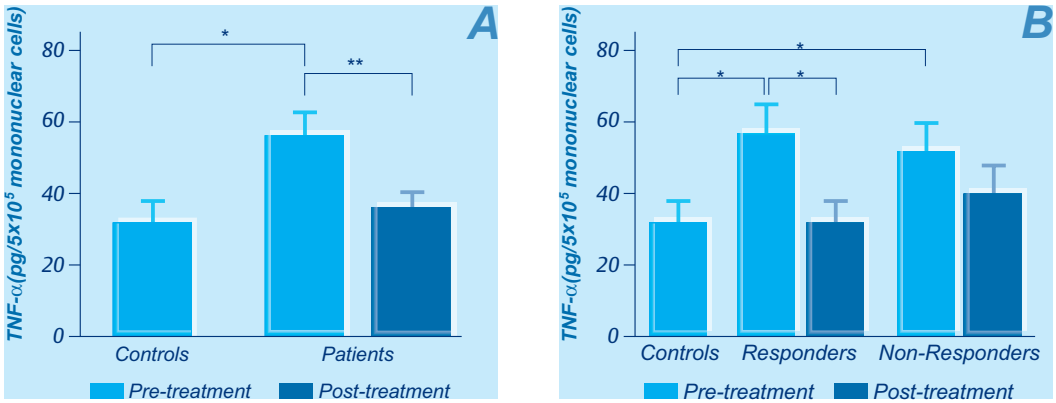


Figure 1:
Produktion of TNF-α in human blood cells with regard to all patients and controls (*A*) (ANOVA; Mann-Whitney test: *p<0.05; for repeated measures: Wilcoxon test: **p<0.01) and with regard to responders and non-responders (*B*) (ANOVA; Dunn's tests: *p<0.05; for repeated measures: Wilcoxon test: *p<0.05 for responders). (Reprinted with permission of Elsevier Science)

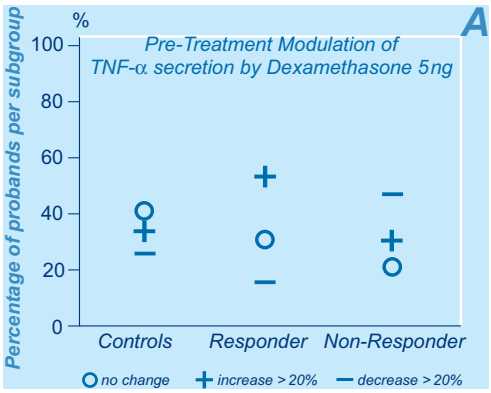
If the group of patients was splitted into a responder and a non-responder subgroup *(Fig. 1B)*, similar changes were detectable: TNF-α levels were significantly increased in both subgroups at admission (Responders: 57.2±9.7 pg/5x10^5 mononuclear cells; non-responders: 54.7±7.3 pg/5x10^5 mononuclear cells), if compared to the control group (for comparison between subgroups: Kruskal-Wallis One Way Analysis of Variance on Ranks: H = 9.73, df = 2, p = 0.008; Dunn's tests: * p < 0.05) *(Fig. 1B)*. However, at the post-treatment time point, only the TNF-α levels of the responder subgroup were significantly reduced compared to the pre-treatment production of the cytokine (Responders: 29.8±5.5 pg/5x105 mononuclear cells; Wilcoxon test: p = 0.041). The non-responder subgroup also showed a decrease in the levels of the cytokine, although to a lesser and not significant degree (41.1±7.8 pg/5x10^5 mononuclear cells; Wilcoxon test: p = 0.11) *(Fig. 1B)*.

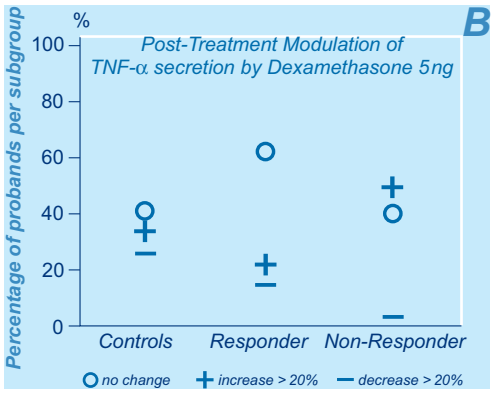
Effects of dexamethasone on TNF-α production under basal conditions in major depression

For the examination of the effects of DEX on the production of TNF-α, a semi-quantiative and statistically non-parametric approach was used, which was based on the percentages of probands/patients with either no or a pronounced up- and down- (more or less 20%) reaction. An incubation with DEX at a concentration of 5 ng/ml did not lead to significant changes in the percentage of probands/patients with such reactions, neither at the pre-treatment time point *(Fig. 2A)* (Pearson Chi-square = 4.2; df = 4; p = 0.38), nor at the post-treatment time point *(Fig. 2B)* (Pearson Chi-square = 3; df = 4; p = 0.55).

Interestingly, the higher concentration of 25 ng/ml DEX did induce significant changes in the distribution of the proband/patient's subgroups with the different extents of a reaction: No (n = 0) proband of the control group showed a more than 20% increase of TNF-α production after DEX treatment, 33% showed a decrease and 67% did not exhibit any changes in the reaction. This was significantly different at the pre-treatment time point for the responder (+ = 62%; - = 23%; 0 = 15%)(Pearson Chi-square = 13.8; df = 2; p = 0.001) and for the non-responder subgroup (+ = 40%; - = 40%; 0 = 20%)(Pearson Chi-square = 8.8; df = 2; p = 0.012), respectively *(Fig. 2C)*.

At the post-treatment time point, only the non-responder subgroup showed significant differences (+ = 40%; - = 30%; 0 = 30%) versus the control group (+ = 0%; - = 40%; 0 = 60%) Pearson Chi-square = 7.6; df = 4, p = 0.023) *(Fig. 2D)*. The responder subgroup (+ = 23%; - = 31%; 0 = 46%) did not show significant differences to either the control group (Pearson Chi-square = 4; df = 2; p = 0.14), or to the non-responder subgroup (Pearson Chi-square = 0.8; df = 2; p = 0.63) *(Fig. 2D)* at this time point.





Production and dexamethasone-regulation of the cytokine TNF-α in major depression

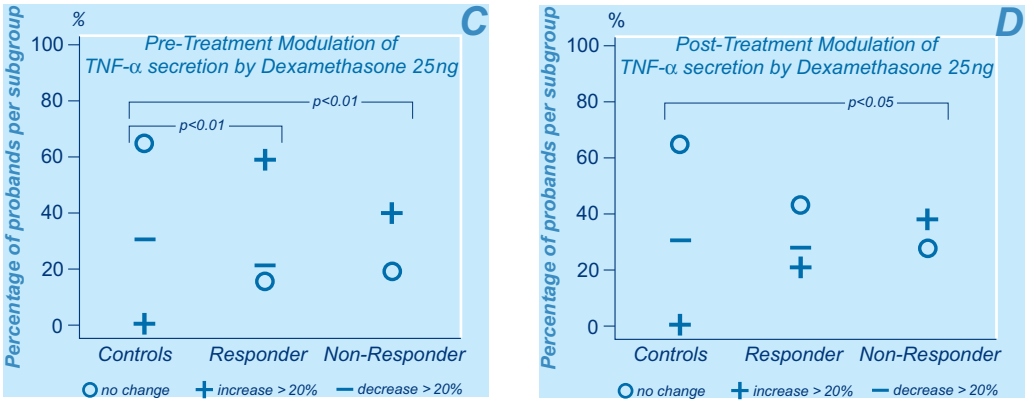


Figure 2:
Modulation of the production of TNF-α in human blood cells by Dexamethasone (5 ng/ml [*A*, *B*] and 25 ng/ml [*C*, *D*]) and pre- [*A*, *C*] and post-treatment [*B*, *D*]). Statistical evaluation was achieved by Pearson Chi-square tests. (A change between + and - 20% was indicated as - 0 -, an increase of more than 20% as - + - and a decrease of more than 20% as - – -).

Discussion

Several studies point to a role of the cytokines IL-1β and IL-6 in major depression and in the activation of the HPA-axis found in this disorder (Maes 1995; Kronfol et al. 2000). Moreover, these cytokines are able to directly induce behavioral changes such as anorexia, weight loss, sleep disturbances, motor retardation, anergia and loss of interest (Dantzer et al. 1999; Dantzer 2001). Beside IL-1β and IL-6, TNF-α is one of the major proinflammatory mediators of the immune system, which is most likely also involved in the behavioral changes in depression (Danzer et al. 1999; Dantzer 2001; Bluthe et al. 2000; Yirmiya et al. 2000). Such changes correspond to the so-called "sickness behaviour" which has been demonstrated for this cytokine in animal (Bluthe et al. 2000) and in human models (Dantzer 2001; Yirmiya et al. 2000).

Our data (Lanquillon et al. 2000) which have been recently confirmed by Mikova et al. (Mikova et al. 2001) suggest a significant relationship between the depressive symptomatology and increased TNF-α levels. Moreover, Levine et al. (Levine et al. 1999) showed that serum TNF-α levels were positively correlated to IL-1β levels in the CSF of patients with major depression, suggesting a central origin of an increased secretion of IL-1β, which may be related or contribute to increased peripheral levels of TNF-α (Bluthe et al. 2000; Butler et al. 1989).

With regard to the underlying increase in TNF-α levels in major depression (Lanquillon et al. 2000; Mikova et al. 2001), our here presented data suggest that in major depression glucocorticoids such as DEX may exert a state-dependent increased drive on TNF-α levels under basal conditions and in low concentrations. Other results show that peripheral TNF-α is able to contribute or directly induce an activation of the HPA-axis of the neuroendocrine system (for review see: (Mulla et al. 1999)). Taken together, these data suggest that a "feed-forward" stimulation of the "immunological circuit" of HPA-axis activation might occur in major depression. A functional interaction between TNF-α and this part of the neuroendocrine system has been demonstrated in the rodent (Butler et al. 1989). Moreover, an inducing action of DEX on TNF-α levels was also detectable in alveolar mononuclear cells of the rat after immune activation by intravenous LPS (Renz et al. 1992). It has to be noted that this effect was specific for TNF-α, since IL-1α and IL-6 levels were expectedly reduced under these conditions by the DEX treatment.

In summary, theses data support an emerging role of the cytokine TNF-α and its neuroendocrine and behavioural effects in major depression and perhaps also in diseases such as schizophrenia, multiple sclerosis and rheumatoid arthritis (Mikova et al. 2001; Kronfol et al. 2000; Straub et al. 2001).

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Induction of TNF-alpha and its soluble receptors by certain psychotropic drugs: What for!?

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Induction of TNF-alpha and its soluble receptors by certain psychotropic drugs: What for!?

Introduction

During the past 10 years we and our colleagues at the Max-Planck-Institute of Psychiatry have shown that certain psychotropic drugs induce increased plasma levels of TNF-alpha, its soluble receptors sTNFR-p55 as well as sTNFR-p75 and of some other cytokines in psychiatric patients treated regularly with the respective drugs. The atypical antipsychotic clozapine significantly increases plasma levels of TNF-alpha and its soluble receptors as early as by the end of the first week of treatment (Pollmaecher et al. 1996). This occurs in almost every patient treated with clozapine independently of any of the known adverse effects such as fever, blood cell dyscrasia or hypersalivation. In addition, plasma levels of sIL-2R are increased, as well, whereas those of IL-6 and IL-1ra do not show any significant changes during these first 6 weeks of treatment with no adverse effects occurring (Pollmaecher et al. 1996; Pollmaecher et al. 1995). If these results are compared to those of patients on combined therapy with clozapine and fluvoxamine, manipulating the hepatic metabolism of clozapine, still the circulating levels of TNF-alpha and its soluble receptors are increased (Hinze-Selch et al. 2000a).

In another study, we investigated whether or not this increase in plasma levels is due to increased secretion by peripheral blood mononuclear cells (PBMC)

(Hinze-Selch et al. 1998). In a complex in vitro design spontaneous and clozapine-in-vitro- stimulated secretion of TNF-alpha was investigated in patients before and during the first 6 weeks of treatment. In this in vitro paradigm, we disproved this hypothesis. However, in vivo treatment with clozapine has an effect on PBMC that seem to be sensitized to clozapine in the course of in vivo treatment.

In order to test whether or not these effects on cytokine secretion are specific to clozapine, we investigated plasma levels of TNF-alpha and its soluble receptors before and during the first 4 or 6 weeks on monotherapy with further psychotropic substances. Whereas haloperidol (Pollmaecher et al. 1997) does not significantly affect these plasma levels, the atypical antipsychotic olanzapine does (Schuld et al. 2000); it increases circulating levels of sTNFR-p55 and sTNFR-p75 but not those of TNF-alpha itself. With respect to antidepessant drugs, mirtazapine increases plasma levels of TNF-alpha as well as of its soluble receptors, and amitriptyline increases plasma levels of sTNFR-p75 only, whereas paroxetine and venlafaxine do not affect these plasma levels at all (Hinze-Selch et al. 2000b; Kraus et al. in press). Thus, the effect on circulating levels of TNF-alpha and its soluble receptors is neither specific to a certain compound nor random. Moreover, because the effect on this cytokine and its soluble receptors is not restricted to the occurrence of adverse effects, it might be associated with particular therapeutic effects of the respective compounds.

The TNF cytokine system plays a role in the immune response (for review see Hinze-Selch and Pollmaecher 2001), in sleep regulation (for review see Pollmaecher et al. 2000), in body weight regulation together with leptin (for review see Hinze-Selch and Pollmaecher 2001), and in cell death, apoptosis and differentiation (for review see Hinze-Selch and Pollmaecher 2001). Thus, this cytokine system that is proven to be present in the CNS (for review see Hinze-Selch and Pollmaecher 2001) has several functions that are relevant for psychotropic treatment. Table 1 describes the effect on the plasma levels of TNF-alpha, its soluble receptors and leptin, and therapeutic actions of the compounds listed. We suggest that psychotropic drugs that affect circulating levels of these cytokines and soluble receptors are likely to exert sedating effects and modulate body weight regulation. Thus, the induction of cytokines and soluble cytokine receptors might be involved in therapeutic and adverse effects and, therefore, be an additional field to be considered when using and designing new compounds. However, the present results are not sufficient yet to establish any causal relationships in this network.

Induction of TNF-alpha and its soluble receptors by certain psychotropic drugs: What for!?

	Sedation and Weight gain	Leptin	TNF-alpha	STNFR-p55	STNFR-p75
Paroxetine[1]	o	o	o	o	o
Venlafaxine[2]	o	o	o	o	o
Haloperidol[3]	o	o	o	o	o
Olanzapine[4,5]	+	+	o	+	+
Clozapine[5,6,7]	+	+	+	+	+
Amitriptyline[1]	+	o	o	o	+
Mirtazapine[2]	+	o	+	+	+

Table 1:
Effect of various psychotropic medication on cytokine plasma levels and on sedation and weight gain in psychiatric patients
1) Hinze-Selch et al. 2000b, 2) Kraus et al. in press, 3) Pollmaecher et al. 1997, 4) Schuld et al. 2000, 5) Kraus et al. 1999, 6) Pollmaecher et al. 1996, 7) Hinze-Selch et al. 1997

These effects are independent of the question whether or not schizophrenia and major depression are associated with disturbances in these cytokine systems. There is a broad body of literature in this field but only very few studies on the TNF-system (e.g. Kaminska et al. 2001, Naudin et al 1997, Schattner et al. 1996, Theodoropoulou et al 2001; Mikova et al. 2001, Leonard 2001). We showed in a large scale cross sectional study that psychotropic medication has a significant effect on plasma levels of TNF-alpha and its soluble receptors whereas the diagnosis of major depression only displayed a weak effect on plasma levels of TNFR-p55 only (Haack et al. 1999). In terms of the large body of literature on in vitro secretion by PBMC, we have reviewed the published results on schizophrenia and could not support any relevant association between certain cytokines or soluble cytokine receptors and this diagnosis (Hinze-Selch and Pollmaecher 2001). However, again medication and factors such as smoking appeared to be relevant for the results and were not sufficiently controlled for yet.

In conclusion, we suggest that certain psychotropic drugs exert significant effects on TNF-alpha and on the shedding of sTNFR-p55 as well as sTNFR-p75 and thereby modulate this multifunctional cytokine system. This is involved in the desired and adverse effects of the respective compounds. However, further research including more basic research is needed to disprove or support this hypothesis that suggests a new insight in the treatment and pathophysiology of major psychiatric disorders.

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Amantadine and Borna disease virus (BDV) infection in affective disorders

Abstract

Borna disease virus (BDV) is known as pathogenic in certain animal species. Symptoms in infected animals range from subclinical manifestations to fatal neurologic disorders with neurobehavioral and/or emotional disturbances. Psychiatric diseases were considered to be potentially associated with human BDV infections, since BDV-antibodies were detected in humans, and human strains of BDV were isolated from patients with recurrent mood disorders. Amantadine is known to have direct effects on different neurotransmitter systems. In addition, immune modulating as well as antiviral efficacy has been described. However, amantadines antidepressive efficacy was highlighted by the discovery of its antiviral effect on BDV. This report focuses on a viro-psycho-immunological disease-model with regard to human BDV-infection and the use of amantadine in the treatment of BDV-infected patients with affective disorders.

Introduction

Borna Disease Virus (BDV) infections are widespread in animal species. This neurotropic, negative and single-stranded enveloped RNA virus spreads via axo

nal and transsynaptic pathways quite specifically into olfactoric and limbic structures. The symptoms in BDV-infected animals range form unapparent and subtle clinical manifestations to fatal neurological disorders. The severe and fulminant course of the infection, which is often accompanied by neurobehavioral and "emotional" disturbances, occurs sporadically and, at least in experimentally infected animals (rats), is thought to be mediated by immunopathology (Ludwig & Bode 2000 for review). Increases in serum-BDV antibodies have also been detected in neuropsychiatric patients. In addition, viral antigen, viral RNS and BDV-specific circulating immune complexes (CICs) have been observed in acutely ill major depressive patients (Bode et al. 2001), leading to the conclusion that BDV may be causally related to psychiatric disorders, in particular to affective disorders.

A number of studies have furnished evidence of abnormal immune functions in mentally ill patients (e.g. Besedowsky & del Rey 1996). In addition, stress has been shown to decrease immune responses to viral infections (Lederman et al. 1997). On the basis of these findings it was hypothesized that human BDV-infection represents a co-factor in the development or course of certain psychiatric diseases (Dietrich et al. 1998): stress may cause immunosuppression and thus induce activation of persisting BDV in the limbic system. The resulting neuropathological changes might influence the serotonergic or dopaminergic neurotransmitter systems. In addition, a specific affinity of BDV structural elements for aspartate and glutamate receptors in the hippocampal formation might directly induce an imbalance of these transmitter system interactions, causing affective and behavioural disturbances.

Methods

Basing on such a hypothesis an antiviral therapy against BDV should lead to a reduction of clinical symptoms and BDV-activity in infected patients. Amantadine represents such a compound (Bode et al. 1997). Therefore, we investigate the clinical effect of amantadine on BDV-infected chronically depressive patients (n=25, 17 women, mean age 49.9±14.3 years) with retrospective evaluation in an open trial. Patients suffered from major depression (MD, n=11), bipolar disorder with major depressive episode (n=12, 6 bipolar I and 6 bipolar II patients) or dysthymia (n=2), all diagnoses established following DSM-IV-criteria. Two patients had an additional Axis II diagnosis of a personality disorder. All patients reported therapy-resistance (non-response to two antidepressants of different chemical subgroups, given in adequate dosages over at least six weeks) in their history. Further details may be taken from Dietrich et al. (2000).

Amantadine and Borna disease virus (BDV) infection in affective disorders

Results

It appeared that amantadine may have a pronounced and/or rapid antidepressive efficacy in the depressive episodes of these BDV-infected patients. The overall response rate, regarded as reduction of HAM-D-score >50%, change from severe to mild depression with OPCRIT (McGuffin et al 1993) and change to no depression, of the amantadine treatment in these 25 patients was 68%, but 80%, having excluded patients with dysthymia and Axis II diagnosis of personality disorder. In addition, bipolar I patients improved faster and did not show any (hypo-)mania following depression. BDV-infected patients with MD superimposed to dysthymia or personality disorders did not seem to respond to this therapy (see Table 1). BDV-infection was measured in blood samples investigated basically according to immunofluorescence (IF) for detecting BDV antibodies (Bode et al 1992) and enzyme immuno assay (EIA) for BDV antigens in peripheral blood mononuclear cells (PBMC) (Bode et al. 1996).

Diagnose	mean age	number of patients	Treatment Response ++	+	(+)	o	Response Rate	after (x) weeks
BP I	43.0 ± 14.3	6	5			1	83%	(2.0)
BP II	46.4 ± 6.8	5	2	2		1	80%	(3.3)
MD	54.6±16.6	10	4	4		2	80%	(3.3)
BP II/MD + Personality Disorders; Dysthymia	53.3 ± 8.7	4			2	2	0%	(---)
	49.9 ± 14.3	25	11	6	2	6		(2.9 ± 1.5)
			68% (responders)		32% (non-responders)			

++ No depression (HAM-D; OPCRIT-diagnostic questionnaire) after treatment
+ Reduction of HAM-D score > 50%/change from severe to mild in OPCRIT
(+) Mild to moderate improvement (HAM-D-reduction < 50%/reduction of one diagnostic step, e.g. from moderate to mild depression in OPCRIT
o No relevant change. „++“ and „+“ was regarded as treatment response

Table 1: Treatment response to the therapy with amantadine in BDV-infected depressive patients (n=25) diagnosed as having either major depression (MD) or bipolar disorder (DSM-IV-criteria). Change of depressive symptoms was documented by the Hamilton rating scale for depression (HAM-D) or with an operationalized diagnostic criteria system (OPCRIT, version 3.31):

++ No depression (HAM-D; OPCRIT-diagnostic questionnaire) after treatment
+ Reduction of HAM-D score > 50% / change from severe to mild in OPCRIT
(+) Mild to moderate improvement (HAM-D-reduction < 50% / reduction of one *diagnostic step*, e.g. from *moderate* to mild depression in OPCRIT
0 No relevant change. „++“ and „+“ was regarded as treatment response.

The virological evaluation of BDV suggested that the strong improvement of clinical symptoms corresponded with a decrease of acute infection markers, whereas a lack of clinical improvement coincided with an increase or maintenance of acute infection. Similar evidence was found in a small group of patients with obsessive-compulsive disorder and secondary depression (Dietrich et al. 2001).

Discussion

The clinical efficacy of amantadine in our trial appeared to be comparable to the efficacy of standard antidepressants in these patients. The therapeutic effect was observed in mostly therapy-resistant patients suggesting this efficacy to be at least partially related to amandadines antiviral effect on BDV. The hypothesis of BDV representing an etiopathogenetic co-factor in subtypes of affective disorders was, furthermore, supported by the following finfings: The majority of responders ended up with antigen-negative blood tests, whereas more than 80% of the non-responders were still positive for acute markers. The bipolar I patients who were shown to have a high prevalence for BDV (Bode et al. 2001), showed a quick improvement without development of (hypo-)mania. However, amantadines neuropharmacological efficacy (Huber et al. 1999) may have been responsible for this beneficial clinical response. Furthermore, indirect immunological factors could have been involved as well. Nethertheless, these good clinical results and the high prevalence of BDV in bipolar and recurrent depressive patients suggest a certain etiopathogenetic link of BDV and affective disorders.

Further studies appear to be necessary to substantiate these preliminary findings: e.g. placebo-controlled and double-blind studies with special emphasis on immunological, molecular, and antiviral aspects but also on potentially prophylactic and antimanic effects of the amantadine treatment.

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Cerebrospinal fluid filtration in therapyresistant Borna disease virus-related psychosis: therapeutic effect, findings

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Cerebrospinal fluid filtration in therapy resistant Borna disease virus-related psychosis: therapeutic effect, findings

Introduction

Borna disease virus is a frequent cause of meningoencephalitis in horses and sheep in middle Europe, and a broad variety of species can be infected by BDV (Rott and Becht 1995). Symptomatology and course of disease is variable depending from route of infection, virus strain, genetic and immune factors of the host (Herzog et al. 1997). Serum antibodies against BDV were found in humans with depressive disorders and later in a variety of psychiatric disorders and in normal persons. However, findings differ considerably from 100% - 0% between studies, and in blinded ring studies especially PCR methods were not valid whereas serological methods rather reliable (Richt et al. 1997; Herzog et al. 1997; Nübling et al. 1998); reviews: Richt and Rott 2001; Carbone 2001; Schwemmle 2001; Bechter 2001). In natural BD in horses serological methods (IFA, immunoblot) allow in vivo diagnosis of BDV infection, respectively BDV encephalitis with CSF investigation (Herzog et al. 1994). Human (meningo-) encephalitis is sensitively diagnosed in acute phases by PCR, in subacute or chronic phases by demonstrating increased agent specific immunoglobuline G within CSF spaces (Reiber 1998). BDV specific immunoglobuline G we found increased in CSFs of 20% BDV seropositive patients during acute schizophrenic or affective psychosis (Bechter et al. 1995), suggesting mild immune pathologic BDV encephalitis underlying

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respective psychoses (Bechter 1998). In Guillain-Barré syndrome, an inflammatory immune pathological neurological disorder, CSF filtration is an effective treatment (Wollinsky et al. 2001). We introduced CSF filtration in therapy resistant BDV related psychoses.

Patients and methods

According to Helsinki Declaration we yet experimentally treated 4 BDV seropositive patients with therapy resistant psychosis (1 patient paranoid schizophrenia ICD-10:F20.09, 3 patients major (melancholic) depression (ICD-10:32.x). In short: a spinal catheter was introduced into the lumbar subarachnoid spaces, connected to a PALL-CSF-1E-filter and system. Over 5 days consecutively about 300 ml CSF were filtrated daily under sterile conditions.

Therapy resistance was defined. Psychopathology (BPRS, PD-S, HAMD, MADRS, SCL-90-R) and test performance (ZVT, Stroop, d2-test, reaction time, continuous performance) were assessed over months before and after filtration (over about one year totally).

BDV infection was investigated by serological (indirect immunofluorescence, immunoblot) and molecular methods (nested-RT-PCR, RT-PCR) in blood and CSF; within CSFs cells, proteins, oligoclonal bands, glucose; QYNAD by HPLC and neurophysiological methods (compare Brinkmeier et al 2000). T cell repertoire of lymphocytes in CSF and blood was analysed as described (Oleszak et al 1995).

Results

4 BDV seropositive patients improved under and after CSF filtration considerably, usually from the 2nd or 3rd day of filtration, especially cognitive and negative symptoms previously therapy resistant. Test performance improved overall from 10 to 50% percent rank before filtration to 40-90% after filtration. 2 patients relapsed some weeks after 1st filtration series and were again filtrated. Positive therapeutic effect was repeated in both cases. Considerable dose reduction of neuroleptic or antidepressant medication was possible after filtration. Catamnesis 1-3 ½ years. Within the CSFs we detected QYNAD (or a similar molecule) increased (3-5fold), reduced during filtration to normal. T cell repertoire of CSF lymphocytes was monoclonally expanded, whereas in blood polyclonal (= normal) (Oleszak et al 2001).

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Changes of systemic immunity in Alzheimer's Disease

Rationale

Immunological mechanisms may play an important role in the etiology of Alzheimer's Disease (AD). Inside the plaques activated microglia cells, acute phase reactants and the proinflammatory interleukin 6 (IL 6) were found indicating an inflammatory response in the brain of AD patients (Mc Geer et al. 1995; Hüll et al. 1996). For study of systemic immunity in AD we focused our research on T-lymphocyte subsets in peripheral blood, on in vivo concentrations of cytokines in serum and CSF, and on cytokine production in lymphocyte cultures.

Patients and Methods

T-Lymphocyte subsets in peripheral blood: 17 patients with probable AD (NINCDS-ADRDA criteria, 9 female and 8 male, 64-88 years old, median 75 years) were investigated. Controls were 13 healthy volunteers (7 female and 6 male, 58-84 years old, median 65 years). Surface antigens of lymphocytes in peripheral blood were analyzed by FACS. In vivo concentrations of cytokines and soluble receptors in serum and CSF: 20 patients with probable AD (16 female and 4 male, 60-88 years old, median 76 years) were investigated. Controls were

21 patients with other neurological disorders, with exception of organic brain syndrome, and with normal CSF status (7 female and 14 male, 59-82 years old, median 68 years). Serum and CSF were analyzed for concentrations of cytokines by ELISA. In vitro production of cytokines in mitogen stimulated lymphocyte cultures: 19 patients with probable AD (12 female and 7 male, 56-90 years old, median 73 years) were investigated. Controls were 23 healthy volunteers (16 female and 7 male, 62-84 years old, median 69 years). Mitogen stimulated lymphocytes were cultured in whole blood samples with medium following the Luebeck protocol (Kirchner et al. 1982). Cytokine concentrations in the supernatant were measured by ELISA.

Results and Discussion

The results of this study are compiled in Table 1, Table 2, and Figure 1. In AD patients a decrease of CD8 + cells in peripheral blood, a decrease of IL 6, s-IL 6R, and TNFα in CSF, and a decrease in the production of IL 6, γ INF, and TNFα in lymphocyte cultures was observed. All results point to a suppression of systemic immunity in AD. It is known from other studies that proinflammatory cytokines (IL 1, IL 6) accumulate in the brain of AD patients (Hüll et al. 1996; Rempel et al. 2001). These cytokines may trigger a systemic antiinflammatory response without preceding systemic inflammation (Woiciechowsky et al. 1999). This is probably done by stimulation of the HPA stress axis by cytokines which in turn enhances sympathetic nervous system activity. Additionally, cortisol secretion is increased possibly leading to a suppression of the systemic immune system, too. Therefore, in AD there is both a condition of acute inflammation in the brain and a suppression of systemic immunity.

Changes of systemic immunity in Alzheimer`s Disease

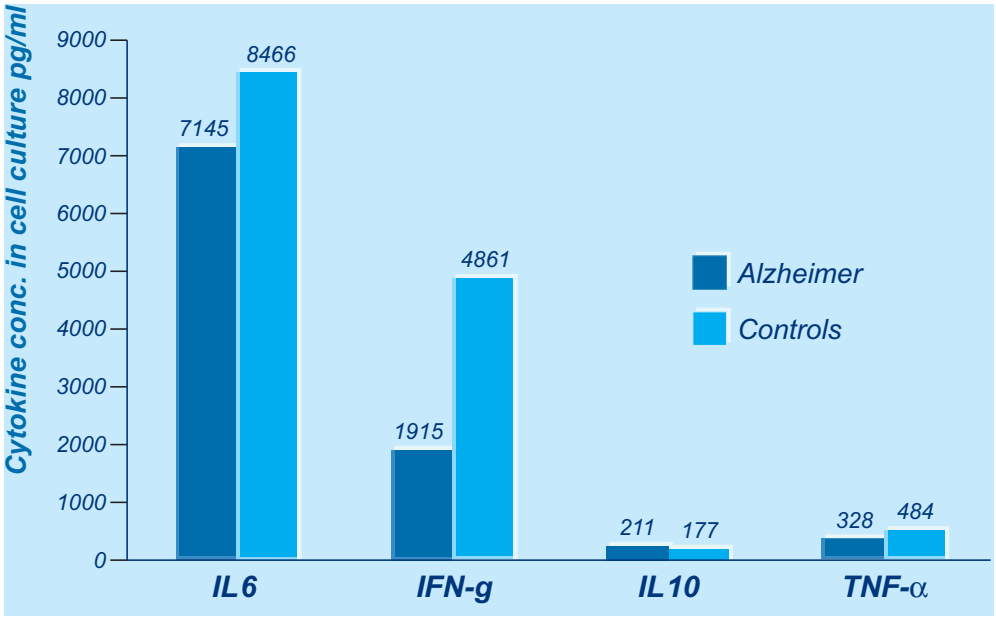

Figure 1:
Cytokine conc. in cell culture pg/ml

	AD-PATIENTS		CONTROLS	
	Median (Range)	Mean (SD)	Median (Range)	Mean (SD)
CD 3-cells	64.47 (45.22-79.17)	63.35 (9.99)	64.55 (43.1-78.17)	65.15 (8.30)
CD 19-cells	9.92 (1.43-16.33)	9.59 (3.68)	8.38 (4.09-17.83)	9.85 (4.14)
CD 4-cells	44.51 (23.0-63.41)	45.87 (8.99)	43.24 (30.04-50.45)	43.73 (5.41)
CD 8-cells*	13.13* (6.17-30.36)	15.34 (7.16)	20.8 (11.17-32.89)	22.26 (6.85)
CD 4/CD 8 ratio**	3.68** (1.35-8.02)	3.58 (1.62)	2.16 (1.0-3.82)	2.18 (0.78)
CD 16+56 cells	15.91 (2.99-40.28)	17.47 (10.15)	18.09 (7.34-30.17)	18.24 (7.60)

*Abbreviations: *p = 0.010; **p = 0.007*

Table 1:
Relative Percentage of Lymphocyte Subsets and B-Lymphocytes

Changes of systemic immunity in Alzheimer`s Disease

	Serum AD	Serum Controls	CSF AD	CSF-Controls
IL 1β	0	0	19.6 (8.9)	23.3 (8.8)
IL 2	0	0	0	0
s IL 2-R	421 (159)	447 (172)	47.6 (8.3)	55.6 (13.6)
IL 6	4.6 (10.7)	16.1 (13.9)	4.6 (2.1)*	10.6 (17.6)
s IL 6-R	21026 (8403)	24080 (6230)	574 (173)*	766 (192)
TNF-α	0	0	14.0 (1.6)*	19.3 (1.8)
s TNFα-RI	1527 (544)	1942 (1217)	681 (151)	667 (257)

Concentrations are given as means and Standard Deviations in parentheses in pg/ml
*Abbreviations: AD Alzheimer's Disease; *p<0.05*

Table 2:
Cytokine Concentrations in Serum and CSF

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Conditioning of immune functions

Introduction

Behaviorally conditioned immunomodulation has been described as the most impressive demonstration of brain to immune system interaction. Conditioned immunomodulation provides a model for investigation of the afferent and efferent communication pathway between the CNS and immune system. Moreover, the discovery that immune conditioning is able to elicit physiologically relevant immune alterations suggests that this model may be of clinical relevance.

Classical conditioning, a 'learning of a physiological reflex', was initially described by the Russian Physiologist Ivan Pavlov in dogs. In this classical example, a neutral stimulus, such as a tone (conditioned stimulus/CS), was paired with a physiologically relevant unconditioned stimulus (e.g. food). The unconditioned stimulus (UCS) produced a physiological response (unconditioned response, e.g. salivation). After repetitive CS-UCS pairings re-exposure to the tone (CS) alone, led to a conditioned response (CR, e.g. salivation). The initial work of Ader and Cohen (1975, 1982) on conditioning of immune functions became the corner stone of modern psychoneuroimmunology research. In the early 70s, Ader and Cohen

used the paradigm of conditioned taste aversion (CTA), a variant of classical conditioning (Garcia & Hankins 1977). The consumption of a novel taste (usually a saccharin-solution) is paired with a substance (UCS) that causes gastrointestinal complaints (e.g. cyclophosphamide or lithium chloride). During re-presentation of the CS alone, the animal will avoid drinking the solution due to the association with the unpleasant consequences of the drug. One side effect of cyclophosphamide, which Ader was using as the UCS, led to the coincidental discovery of conditioned immune response. The conditioned animals showed a higher mortality rate than control animals. Since cyclophosphamide also exerts immunsuppressive effects, Ader and Cohen postulated, and subsequently proved, that these animals experienced not only a conditioning of taste aversion, but also a conditioned immunosuppressive effect. In the last 25 years a wealth of animal studies have demonstrated the conditionability of immunosuppression as well as of immune enhancement. Generally, it is possible to alter both humoral and cellular immune functions via behavioral conditioning procedures.

Our Group has developed a conditioning model in the rat using the potent immunosuppressant cyclosporin A (CsA) as the unconditioned stimulus (Exton et al. 1998; 2002). Cyclosporin A is widely used in transplantation medicine, due to its inhibitory effects on IL-2 and other signaling proteins of T lymphocytes (Kahan 1989). Therefore, the drug reduces the T-cells response and prevents the rejection of a transplanted organ. In our model, male DA rats were conditioned on three separate days, receiving 0.2% saccharin solution (CS) followed by an intraperitoneal application of CsA (UCS) (20mg/kg) (figure 1). In the afternoon a saline injection was administered paired with water. The sham conditioned control animals received the CsA together with water, while the saccharin was given paired with saline injections. Two additional controls groups, one CsA-treated group to control for the drug effect and an untreated group, were included. Following the third conditioning trial, animals were re-presented each with the saccharin (figure 1) and sacrificed after the third day for immunologocial assays. Since CsA inhibits IL-2 production, a cytokine necessary for T lymphocytes proliferation (klonale expansion), the proliferative response and cytokine production of lymphocytes from the spleen in response to a mitogen (Concavalin A; ConA) was analyzed in vitro. The conditioned rats showed a significant reduction in splenocyte proliferation and IL-2 production comparable to the immunosuppression in the CsA-treated controls.

The mechanisms of conditioned immunomodulation are not fully understood. However, there is evidence suggesting the endocrine mediators, such as opioids and catecholamines, are involved in the conditioned immune response (Exton et al. 2000). The central nervous system may communicate with the immune system via direct neural innervation of the lymphoid organs and the release of neurotransmitter affecting lymphocyte functions. In the spleen, noradrenaline is the primary transmitter released, and functionally operates as a neurotransmitter between the nerve and the lymphocyte. Since we observed conditioned suppression of lymphocyte proliferation in the spleen, but not in the mesenteric lymphe nodes, we denervated the spleen two weeks prior to conditioning. Surgical denervation of the spleen significantly reduced the splenic noradrenaline and completely blocked the conditioned reduction in splenocyte proliferation (Exton et al. 1998).

To test in vivo relevance of the our conditioning model, we used a transplantation model in the rat. In this model a conditioned DA rat received after the third re-exposition a heart from a Lewis rat according to standard procedures. Saccharin was given every day until graft rejection. Compared to the sham conditioned and control animals, the conditioned animals not only showed an prolongation of graft survival time, but also displayed a greater latency to rejection (figure 2A). In sum, in the conditioned animals, saccharin application alone increased the survival time of the transplants to a level that was comparable to the drug effect in the CsA-treated animals. Furthermore, it was demonstrated that the combination of conditioning and a subtherapeutic dose of CsA given during re-exposition even heightened the mean survival time in the conditioned group, with 20% of the manimals developing tolerance against the heart allograft (figure 2B). These results demonstrate that the combination of CS-re-exposition and the subtherapeutic drug application acted synergistically. In sum, the conditioned immunosuppression in animals provides a model to examine the survival time of organ transplants. These data also demonstrate that behaviorally conditioned immunosuppression is biologically relevant.

The capability of brain pharmacology has been most impressively demonstrated in rodents by behaviorally conditioned immune alterations. Specifically, the endogenous pharmacology of the brain may potentially be used as a supplement to traditional drug delivery. Together, these findings may have therapeutic implications for immune modulating drugs by implementing behavioral conditioning paradigms as supplementary therapy to traditional drug regimes. Therefore, future studies need to address the question of whether this model is robust enough to function in a daily clinical setting.

Conditioning of immune functions

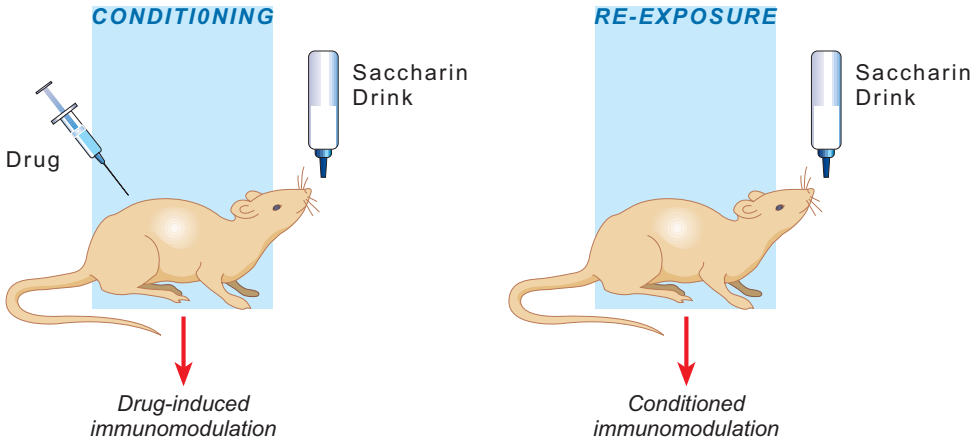

Figure 1:
Conditioning of immune functions
In the conditioning phase animals received a novel stimulus (saccharin) paired by an injection of an immunopharmacological drug. After repetitive pairings, the animals were reexposed to the saccharin drink alone. The conditioned effect induces an immune response similar to the effect of the drug (Exton et al. 2002) Figure 2: Heart transplants survival following conditioning

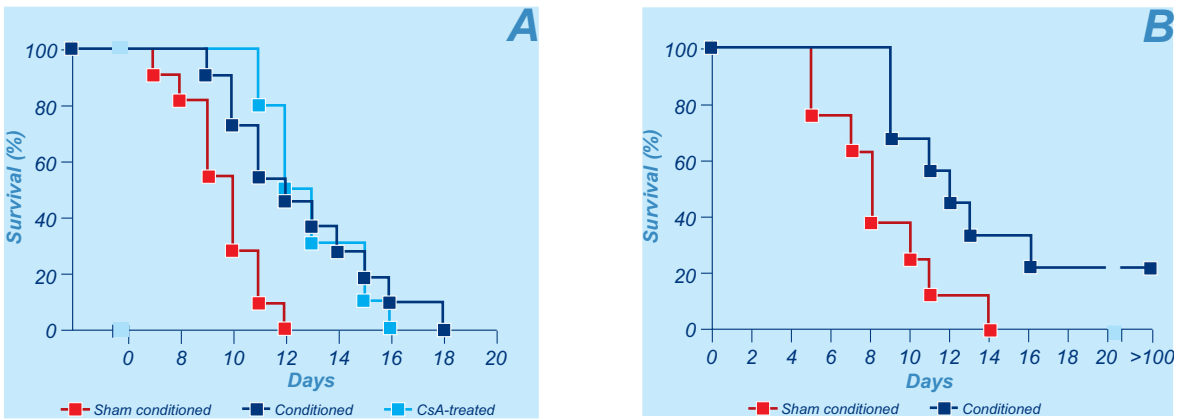

Figure 2:
Heart transplants survival following conditioning
(A) Conditioned animals showed a prolonged survival of transplanted organs.
(B) Treatment with a subtherapeutic dose of CsA significantly potentiated the immunosuppression in the conditioned animals, but in the sham conditioned group (Exton et al. 2002).

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