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Research on Psychoimmunology

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Summary

Several lines of evidence suggest a role for the immune system in the multifactorial pathogenesis of schizophrenia and other psychiatric and neurodegenerative diseases. Later, the role of immune mediators like cytokines became a source of main interest related to the process on inflammation in the CNS. In this article we report the results of our research on cytokines in a different groups of psychiatric patients following their clinical symptomatology and the course of diseases. In particular, we observed a prevalent type 1 cytokine profile in acute multiple sclerosis patients, while IL-10 production predominated in stable multiple sclerosis individuals. The modifications of cytokine profiles observed in schizophrenic patients suggests that clinical improvement is associated with a reduction in the inflammatory-like situation present in those not currently under treatment. Our data on Alzheimer's disease (AD) support the role of the inflammatory process in the pathogenesis of AD and reinforce the hypothesis that the neurodegenerative processes in the AD patients are associated with an abnormal antigen-specific immune response. The activation of immune system mechanisms observed in obsessive compulsive disorders could be due to the combination of endogenous (hormonal alterations associated to the modifications in the hypothalamic-pituitary-adrenal axis) and exogenous (viral or bacterial infections) factors.

Key words: cytokines, schizophrenia, multiple sclerosis, Alzheimer's disease, obsessive-compulsive disorders.

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Introduction

The concept of autoantigenic properties of brain tissue was discussed at the beginning of the 20th Century and Monaenkov later (1963) presented his paper on the 'Factor of individuality in immune processes'.

In the 1980s an interest in immunological research in the field of mental disease appeared in the USA (DeLisi et al. 1984; DeLisi 1987; Ganguli et al. 1987) and by a group of Soviet researchers (Vartanian et al. 1978; Koljaskina et al. 1987).

The development of immunological research in psychiatry has run parallel to that of immunology, reflecting the methodological and theoretical advances made in the latter field.

Three important international meetings focused on viruses, immunology and mental disorder were organized in the years between 1975 and 1991 first in Löwen and then in Montreal through the initiative of Morozov, Lipowsky and Kurstak.

In this field everybody knows the contribution of Professor M. Ackenheil and Professor N. Müller. An International Meeting on Psychoimmunology and Psychopharmacology was chaired by the first author in Milan in 1999.

Psychoimmunology is becoming a very important and fruitful area in schizophrenia where the immunoresponse can also be present in patients with chronic disease, but not clearly in the members in their family (Cazzullo et al. 1998a; Cazzullo 2001)

Later still, the role of immune mediators like cytokines became a source of main interest related to the process on inflammation in the CSM. We devoted a good deal of research to cytokines, especially IL-2, IL-6, IL-12, IL-10 and TNF-a in a different group of psychiatric patients following their clinical symptomatology and the course of their diseases (Cazzullo et al. 1998b).

Basic immunological models

The activation of two different functional compartments is the basis of antigen-specific immune responses.

One is defined as T-helper 1 (TH1), mostly concerned with the activation of cell mediated immunity (CMI); the second is defined as response T-helper 2 (TH2) and is primarily responsible for the stimulation of humoral immunity and the generation of antibodies.

Type 1 cytokines are those that mainly induce CMI. The most important type 1 cytokines are IL-2, IL-12 and g-Interferon (IFN-g). Type 2 cytokines (e.g. IL-4, IL-5, IL-6, IL-10) trigger humoral immunity.

TH1 and TH2 activation, and the secretion of type 1 and type 2 cytokines, are mutually exclusive, as we can realize by the fact that IFN-g blocks the production of TH2 cytokines, while IL-4 and IL-10 suppress TH1 cytokine production.

A disequilibrium in the TH1-TH2 ratio is related to various pathologies such as LES, MS, HIV infection and, possibly, to a number of psychiatric diseases.

• Cytokines

The individual features of various cytokines are as follows.

IL-2 is the principal cytokine responsible for progression of T lymphocytes from G1 to S phase of the cell cycle. IL-2 is produced by CD4+T cells and, in lesser quantities, by CD8+ T cells. IL-2 acts on the same cells that produce it as an autocrine growth factor, and it also acts on nearby T lymphocytes as a paracrine growth factor. IL-2 stimulates the growth of NK cells and enhances their cytolytic function, producing so-called lymphocyne-activated killer (LAK). IL-2 acts on human B cells both as a growth factor and as a stimulus for antibody synthesis.

IL-6 is synthesized by mononuclear phagocytes, vascular endothelial cells, fibroblasts, and other cells in response to IL-1 and, to a lesser extent, TNF. It is also made by some activated T cells; IL-6 can be detected in the circulation following gram-negative bacterial infection or TNF infusion. This cytokine causes hepatocytes to synthesize several plasma proteins, such as fibrinogen, that contribute to the acute phase response. IL-6 serves as a growth factor for activated B cells late in the sequence of B cell differentiation.

IL-10 is produced by activated macrophages, some lymphocytes, and some non-lymphocytic cell types (e.g. keratinocytes). The two major activities of IL-10 are to inhibit cytokine (i.e., TNF, IL-1, chemokine, and IL-12) production by macrophages, and to inhibit the accessory functions of macrophages in T cell activation. The latter effect is due to reduced expression of class II MHC molecules and reduced expression of co-stimulators, e.g., B7-1 and B7-2. The net effect of these actions is to inhibit both innate and T cell-mediated specific immune inflammation.

TNF-_ is the principal mediator of the response to gram-negative bacteria and may also play a role in innate immune responses to other infectious organisms. The major cellular source of TNF-_ is the LPS-activated mononuclear phagocytes, although antigen-stimulated T cells, activated NK cells and activated mast cells can also secrete this cytokine. TNF-_ is a mediator of both innate and specific immunity and an important link between specific immune responses and acute inflammation. When small amounts of TNF-a are produced, this molecule acts locally as a paracrine and autocrine regulator of leukocytes and endothelial cells: TNF causes vascular endothelial cells to express new adhesion molecules that make the endothelial cell surface become adhesive for leukocytes, monocytes and lymphocytes. TNF stimulates mononuclear phagocytes and other cell types to secrete chemokines that contribute to leukocyte recruitment. This cytokine activates inflammatory leukocytes to kill microbes. At high concentrations TNF-_ is an endogenous pyrogen, acts on mononuclear phagocytes to stimulate secretion of IL-1 and IL-6 into the circulation, and acts on hepatocytes to increase synthesis of certain serum proteins, such as serum amyloid A protein. Long-term systemic administration of TNF__ causes the metabolic alterations of cachexia. Several specific actions of TNF-_ may contribute to its lethal effects at extremely high concentrations: this molecule reduces tissue perfusion by depressing myocardial contractility.

Methods

• Blood sample collection

Whole blood was collected by venepuncture in Vacutainer tubes containing EDTA (Becton Dickinson Co, Rutherford, NJ). Peripheral blood mononuclear cells (PBMC) were separated by centrifugation on lymphocyte separation medium (Organon Teknika Corp, Durham, NC) and washed twice in PBS. The number of viable lymphocytes was determined by trypan blue exclusion and a haemocytometer.

• *In vitro* cytokine production

PBMCs were resuspended at 3x106/ml in RPMI 1640 and were either unstimulated or stimulated with LPS (Sigma, St. Louis, MI)

(10 _g/ml), with a pool of 3 different peptides from the b-amyloid protein as follows: b-A: fragment 25-35 (25 mg/ml); b-B: fragment 1-40 (150 ng/ml); b-C: fragment 1-16 (150 ng/ml) (Sigma, St. Louis, MI); or with influenza virus vaccine (A/Taiwan+A/Shanghai+B/Victoria) (24_g/l; final dilution 1:1000)(Flu) (control antigen) at 37°C in a moist, 7% CO2 atmosphere. Supernatants were harvested after 48 hours for LPS stimulation and after 5 days of culture for the b-amyloid protein peptides and Flu. Production of IL-2 and IL-10 by PBMCs was evaluated with commercial available ELISA kits (ACCUCYTE, Cytimmune Sciences, Inc, College Park, MD). All test kits were used following the procedures suggested by the manufacturer.

• IL-10 genotyping

Genomic DNA was extracted from EDTA-treated peripheral blood (10 ml) using a standard proteinase K and phenol/chloroform method. The DNA concentration and purity were determined by spectrophotometric analysis. A polymerase chain reaction-sequence specific primers (PCR-SSP) methodology was utilised to asses the IL-10 genotypes. The amplification of the sequence in the promoter region of the IL-10 (polymorphic positions –1082, -819, -592) gene were performed using the Cytokine genotyping Tray Method (One Lambda, Canoga Park, CA, USA); the human b-globin gene was amplified as an internal control of genomic DNA preparation. PCR condition were indicated by One Lambda PCR program (OLI-1); the PCR products were then visualised by electrophoresis in 2.5% agarose gel.

Focus of our research

Our activity has been mainly devoted to the follow topics:

• Multiple sclerosis

Multiple sclerosis (MS) is a chronic neurological disease characterized by multifocal inflammation and damage involving the myelin sheath and the presence of a peculiar psychiatric symptomatology. MS patients present with a variety of clinical patterns, including acute and stable forms. Although the aetiology of MS is still unclear, an immunopathological mechanism, mainly mediated by the activation of cell-mediated immunity (CMI), was suggested long ago (Ferraro and Cazzullo 1948) to be responsible for the destruction of the myelin sheath. Cytokines have been involved in the pathogenesis of MS; thus, increased levels of TNF-a and IFN-g were detected in MS patients with acute disease. Treatment of MS with IFN exacerbates the disease, whereas therapeutic approaches based on the utilization of IFN-a and IFN-b, which are known to be effective in a number of patients, decrease IFN-g production by T lymphocytes, suggesting a direct pathogenic role of this cytokine. We analysed in vitro antigen- and mitogen-stimulated cytokine in multiple sclerosis (MS) patients with either acute (AMS) or stable (SMS) disease and in healthy controls (HC). We also investigated whether immune responses to human endogenous retroviruses (HERV) could be detected in MS and whether these immune responses would be correlated with disease status by analysing cytokine production after stimulation of PBMC with HERV peptides. Results showed that mitogen-stimulated IL-2 and IFN-g was augmented and IL-10 was decreased in AMS compared to both SMS and healthy controls. Whereas the production of the metabolically active IL-12 (p70 heterodimer), was comparable in SMS, AMS and HC, production of the total IL-12 (p70 heterodimer and the p40 chain) were augmented in SMS compared to both AMS and HC. HERV-peptides IL-2 and IFN-g production was more frequent and more potent in AMS compared to both SMS patients and HC. HERV-specific type 2 cytokine production was more frequent and potent in SMS compared to AMS and HC. Thus a prevalent type 1 cytokine profile was seen in AMS patients, while IL-10 production predominated in SMS individuals (Clerici et al. 1999; Clerici et al. 2001; Ferrante et al. 1998).

• Alzheimer's disease

Recent studies of Alzheimer's disease showed that the deposition of amyloid is one of the critical events responsible for the cerebral damage that causes the onset of disease. The amyloid plaques are characterized by the presence of b-amyloid (Ab) stemming from the cleavage of membrane protein. The immune system could play an important role in the neurodegenerative process associated with cerebral plaques. These inflammatory and immune proteins could stimulate the Ab production, facilitate aggregation and increase toxicity of this protein, causing a rise in the severity of AD pathology. The Ab could stimulate the release of inflammatory and immunologically-active molecules from microglial cells and activated astrocytes. However, there are also studies showing a protective role of the immune system in the development of AD. Microglial cells are able to degrade Ab. Recent observations suggest that autoreactive T cells play a role in the peptide elimination. This mechanism seems to be less effective in the AD patients. Thus a specific immune response to Ab could represent a natural defence mechanism in order to prevent the inflammatory processes associated with the amyloidogenic process. The weakening of the immune response and the failure to eliminate toxic metabolites could be the basis of the cerebral chronic inflammatory process in the AD patients.

We have evaluated cytokine production in 50 AD patients and in 30 healthy subjects of comparable age and sex. The peripheral blood mononuclear cells have been stimulated with lipopolysaccharide (LPS) or with a pool of Ab peptides. The results obtained show an alteration in the immune specific response to b-amyloid, an increase in the pro-inflammatory cytokine production, and a reduced IL-10 production in AD patients. It is noteworthy that IL-10 production results in a potent anti-inflammatory response. Analyses on the alleles of the IL-10 gene revealed that the genotype associated with high IL-10 production is extremely infrequent in AD individuals (2% vs. 28%). The presence of low/intermediate-IL-10-producing genotypes (GCC/ATA; ATA/ATA) was associated with an earlier age at disease onset and (ACC/ACC; ACC/ATA) with an accelerated rate of disease progression. These data shed light on the biology of the inflammatory process involved in the pathogenesis of AD by showing that the presence of low-IL-10-allelic isoforms results in a b amyloid-specific impairment of IL-10 production and is associated with the clinical severity of AD. These data thus suggest that the diminished production of this cytokine would facilitate the activation of the chronic inflammatory processes observed in the progression of AD. These data support the role of the inflammatory process in the pathogenesis of AD and reinforce the hypothesis that the neurodegenerative processes in AD patients are associated with an abnormal antigen-specific immune response. These results lend support to the use of anti-inflammatory compounds in the therapy of this disease.

• Schizophrenia

Alterations in the immune system are found in patients affected by psychiatric disorders and in particular in schizophrenic subjects. Several observations underline that the immune system is involved in these pathologies (Cazzullo 2001). The use of IL-2 in schizophrenic patients can provoke the appearance of symptoms related to the disease, and the severity of the symptoms is correlated with the amount of IL-2 used. In schizophrenic patients there is an increase of serum concentration of the soluble IL-2 receptor, and the concentration of this cytokine is elevated in the CSF of non-treated patients. Additionally, IL-10 production is decreased in paranoid patients. Probably the immune system plays a role in this pathology.

In order to analyze this hypothesis and evaluate if patients with different diagnostic subset (paranoid patients vs. non-paranoid patients) have a different immune response, in our first study, we analyzed cytokine production in 37 patients with chronic schizophrenia and in 40 healthy subjects (Cazzullo et al. 1998b). Results showed that chronic schizophrenia is associated with an impairment of soluble antigens-stimulated proliferative responses, and with alterations in the production of cytokines (IL-2 and IL-10). Peripheral lymphocytes of patients belonging to the paranoid subset produce less IL-10 than cells of patients without a diagnosis of paranoia. Moreover, type 1 cytokine production (IL-2 and IFN-g) is significantly increased in non-treated schizophrenic patients compared with healthy controls. We have also observed a positive correlation between the PANSS total score and IFN-g production. These data suggest a possible role for the activation of immune response in the generation of psychotic symptoms.

Then we investigated the production of IL-2, IL-4, IL-10 and IFN-g in 12 drug-free and in 12 drug-naive schizophrenic patients and in 33 healthy controls (Cazzullo et al. 2001). We analysed the modifications of these cytokines during a 3-month period of treatment with risperidone (Cazzullo et al. 2002). The use of risperidone was associated with augmented IL-10 (a suppressor of type 1 cytokines) and decreased IFN-g production. This modification suggests that clinical improvement is associated with a reduction in the inflammatory-like situation present in schizophrenic patients not currently treated.

• Obsessive-compulsive disorders

Based on the immunological alterations that we have observed in schizophrenic patients, we have hypothesised that an impairment of the immunological response could also be present in patients affected by obsessing-compulsive disorders (OCD). Previous studies have demonstrated the presence of high IgG serum concentrations in these patients. Moreover there is an increase of the IgG levels in the CSF of these OCD individuals. Previous studies in which cytokines have been analyzed in OCD patients showed that the plasma concentration of TNF-a plasma is decreased; no differences were detected in the concentration of other cytokines including IL-1b, IL-2 and IL-3 production.

We have analysed type 1 (IL-2, IL-12 and IFN-g) and type 2 (IL-10) cytokine production and the expression of a panel of phenotypical markers in peripheral blood mononuclear of 20 OCD and 12 healthy controls. In particular, eight untreated OCD individuals (NT-OCD) and 12 patients undergoing therapy (T-OCD) have been enrolled in the study. Nine of the analysed patients had psychotic symptoms (P-OCD); these symptoms were absent in the remaining 11 individuals (NP-OCD). The results obtained have demonstrated a significant increase in the absolute number of Natural Killer cells in OCD patients compared to healthy controls ($p < 0.05$). Moreover, the production of IL-2, IFN-g and IL-

10 was significantly increased in P-OCD compared to NT-OCD and T-OCD patients.

The increase of NK cells could indicate the presence of an immune activation in OCD patients. This activation could be due to the combination of the endogenous factors (hormonal alterations associated with the modifications in the hypothalamic-pituitary-adrenal axis) and exogenous factors (viral or bacterial infections).

Conclusions

We would like to recall the intrinsic value of immunological research, especially where it is closely related to the clinical features of various psychiatric diseases.

An open problem is that of the relationship with psychopharmacological agents and the possibility to introduce some immunological mediators.

The second item is in opposition to our law against the use any drugs not specifically indicated for a definite disease.

The future line of research should address any close connection between immunological events and clinical symptomatology, devoting particular attention to patients of a young age.

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