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Different immune patterns in melancholic and non-melancholic major depression

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Abstract The search for immune patterns in major depression has thus far resulted in ambiguous findings, probably because patient samples are psychiatrically heterogeneous. We therefore focused on a detailed classification of subtypes of major depression, comparing patients with melancholic and non-melancholic major depression.

Inpatients suffering from acute major depression were diagnosed and subclassified according to DSM IV criteria. Cell counts were determined by FACS analysis and morphology. Cytokine production (IL-2, IFN-γ, IL-10) upon mitogen stimulation was measured by ELISA in a whole blood assay.

Non-melancholic patients showed increased counts of leukocytes, lymphocytes and NK-cells in the acute stage of disease and after two and four weeks of treatment. Their lymphokine production was unchanged compared to that of healthy controls. Melancholic patients on the other hand demonstrated normal cell counts but a decreased production of IL-2, IFN-γ and IL-10 during the acute stage of disease followed by a normalization with clinical improvement.

Melancholic and non-melancholic patients showed different immune patterns. Classifying melancholic and non-melancholic patients is helpful towards the identification of immune characteristics typical for these diseases.

Key words Major depression · Melancholia · Cytokines · Immunology · Immunocompetent cells

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Introduction

Since the early study of Bartrop et al. (1977) on immune phenomena during bereavement, several studies on the immunology of mood disorders have been undertaken, mostly concerning major depression (MD). Different immune subsystems were studied in a search to establish immune patterns which are typical for depression.

Several studies have investigated the numbers of lymphocytes and their subtypes in MD (Table 1). Most have shown no alterations in total lymphocyte counts (Andreoli et al. 1993, Irwin et al. 1987, Kronfol et al. 1984, Maes et al. 1989 a, 1989 b, 1993), while others reported increased (Müller et al. 1993) or even decreased (Darko et al. 1988 a–c, 1989 a, 1989 b, Kronfol et al. 1989, Marazitti et al. 1992) counts in MD. Such ambiguous findings could not be clarified by investigating lymphocyte subtypes (e. g. Müller et al. 1993, Marazitti et al. 1992).

Even though investigations on natural killer cells (NK-cells) and their activity in MD have provided the most consistent results, considerable discrepancies remain. Several groups demonstrated a decreased NK-cell activity in patients with MD compared to healthy controls (Bauer et al. 1995, Caldwell et al. 1991, Evans et al. 1992, Irwin et al. 1990, Kook et al. 1995, Kronfol et al. 1989, Maes et al. 1992, Reynaert et al. 1995, Schleifer et al. 1996, Urch et al. 1988). However, some have also reported no differences in NK-cell activity (Miller et al. 1991).

NK-cell numbers have been shown to be decreased (Castle et al. 1995, Schleifer et al. 1996), increased (Ravindran et al. 1998, Seidel et al. 1996) or to have remained unchanged (Darko et al. 1988 b, c, Evans et al. 1992, Maes et al. 1994 a, b, Natelson et al. 1998) compared to healthy controls (Table 1).

The proliferative response of lymphocytes after mitogen stimulation was found to be decreased in major depression by most authors (Anesi et al. 1998, Castle et al. 1995, Cosyns et al. 1989, Darko et al. 1988a–c, Hickie et al. 1993, Kanba et al. 1998, Kronfol et al. 1984, 1986,

Table 1 Review of studies on lymphocytes and their activity in major depression

Publication	Natural killer cell activity	Natural killer cell count	Lymphocyte proliferation	Lymphocyte count
Altshuler et al. 1989			↑	
Andreoli et al. 1992			↔	
Andreoli et al. 1993			↔	↔
Anesi et al. 1994			↓	
Bauer et al. 1995	↓		↔	
Caldwell et al. 1991	↓			
Castle et al. 1995		↓	↓	
Cosyns et al. 1989			↓	
Darko et al. 1988		↔	↓	↓
Darko et al. 1989				↓
Evans et al. 1992	↓	↔		
Hickie et al. 1993			↓	
Irwin et al. 1987				↔
Irwin et al. 1990	↓			
Kauba et al. 1998			↓	
Kok et al. 1995	↓			
Kronfol and House 1984			↓	↔
Kronfol and House 1989			↓	↓
Kronfol et al. 1986			↓	
Kronfol et al. 1989	↓			
Levy et al. 1990			↓	
Maes et al. 1989			↓	↔
Maes et al. 1991			↓	
Maes et al. 1992	↓			
Maes et al. 1993				↔
Maes et al. 1994		↔		
Marazziti et al. 1992				↓
McAdams, Leonard 1993			↓	
Miller et al. 1991	↔			
Müller et al. 1993				↑
Natelson et al. 1998		↔		
Ravindran et al. 1998		↑		
Reynaert et al. 1995	↓			
Schleifer et al. 1996	↓	↓		
Seidel et al. 1996		↑		
Spurell and Creed 1993			↓	
Urch et al. 1988	↓			
Wodarz et al. 1992			↔	

1989, Levy et al. 1991, Maes et al. 1989 a, 1991, McAdams and Leonard 1993, Spurell and Creed 1993). Others found an unchanged (Andreoli et al. 1992, 1993, Bauer et al. 1995, Wodarz et al. 1991) or an increased (Altshuler et al. 1989) lymphocyte proliferative response. Table 1 summarizes the divergent results concerning lymphocytes, NK-cells and the activities of these cell types.

Until now, only a few researchers have investigated the production of lymphokines as markers of T-cell activation in major depression. Some recent studies reported a decreased production of interleukin-2 (IL-2) upon mitogen stimulation in major depression (Anisman et al. 1999, Weizman et al. 1994), while others showed no significant difference concerning IL-2 production in major depression compared to healthy controls (Darko et al. 1988a–c, 1989a–b, Kanba et al. 1998, Natelson et al. 1999, Seidel et al. 1995). The production of interferon gamma (IFN-γ) upon mitogen stimulation was found to be unchanged (Inglot et al. 1994, Natelson et al. 1999) or increased (Maes et al. 1994 b, Seidel et al. 1995) in major depression.

It is clear that the wide range of studies from different research groups has revealed a lack of consistency in the responses of the investigated immune parameters. One obvious reason for this might be that the diagnostic group of MD classified according to DSM III-R and DSM IV criteria is simply too heterogeneous. Few authors have attempted to deal with this problem. Some have investigated patients with melancholic or psychotic features as subtypes of major depression (Hickie et al. 1993, Maes et al. 1989 a, 1992). Another uncommon approach has been to study changes at different stages in the course of the disease (McAdams and Leonard 1993, Seidel et al. 1995, 1996).

Considering the overall situation, we undertook a study that included subjects suffering from MD either with or without melancholic features. The patients were studied in an acute stage of disease and followed-up during clinical improvement after two and four weeks of treatment. We focused on T-helper-cells, NK-cells and related cytokines that could be regarded as functional markers of cellular immunity.

Methods and materials

Patients and controls

Fourty-three patients (15 men, 28 women) who had been hospitalized in the Department of Psychiatry, University of Lübeck School of Medicine, and who were suffering from a major depressive episode (DSM-IV 296.2, 296.3; ICD-10 F32, F33), were enrolled. They were clinically diagnosed by two experienced psychiatrists according to DSM-IV and ICD-10 criteria. Furthermore, the German edition (Wittchen and Semler 1990) of the CIDI (Composite International Diagnostic Interview, Robins et al. 1988) was also applied to diagnose the patients. The mean age at the time of the study was 44.45 years (standard deviation 9.95 years), and the range was between 27 and 62 years. The control group consisted of 43 age- and sex-matched healthy blood donors (mean age 44.45 ± 9.95 years). All subjects gave written informed consent. On admission and follow-ups, patients and controls underwent a medical history and physical examination. They were screened for acute infectious diseases by measuring body temperature, erythrocyte sedimentation rate, c-reactive protein (CRP) and urinary culture. They were free of past physical illnesses (e. g. acute or chronic infections, autoimmune diseases, cancer) and medication that might have influenced immune function (e. g. corticosteroids, lithium, neuroleptics). Patients with serum levels of CRP > 0.6 mg/dl (n=2) were excluded from the study.

Eighteen (41.8 %) patients were diagnosed as suffering from a single episode of a major depressive disorder (MD), 12 of whom had MD with moderate severity and 6 of whom had severe MD without psychotic features. Twenty-five patients (58.2 %) were diagnosed as suffering from a recurrent major depressive disorder, 6 with moderate severity and 19 with severe MD without psychotic features. A subgroup of 22 patients was identified which fulfilled the DSM-IV-criteria for major depression with melancholic features (either of the symptoms A and at least three of the symptoms B according to DSM IV). Thirteen patients (30.3 %) were free of antidepressant medication on admission, 23 patients (53.4 %) were on tricyclic antidepressant drugs, and 7 patients (16.3 %) took serotonin reuptake inhibitors (SSRI). Twenty-seven patients included in the study were non-smokers while 16 patients smoked between 4 and 35 cigarettes per day.

Investigations took place on the day after hospital admission (T1) as well as after two (T2) and four (T3) weeks of treatment. The severity of depressive symptoms was rated using the German version of the Hamilton Depression Rating Scale (HDRS) (Baumann 1976, Hamilton 1960).

Leukocytes, natural killer cell (NK-cells), and lymphocyte counts were measured. In addition, the cells' ability to produce cytokines upon mitogen stimulation was investigated.

The following immunological techniques were used:

Flow cytometry analysis

The fraction of NK-cells expressing CD16 and CD56 surface antigens and the fraction of activated T-cells expressing CD25 were determined by flow cytometry. Ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood samples drawn at 8 a. m. were prepared using a Q-Prep Epics immunology workstation (Coulter, Krefeld, Germany). Leukocytes and lymphocytes were counted morphologically.

Whole-blood assay

At 8 a. m., heparinized blood was drawn by venous puncture from patients and controls before it was stored (4 °C) and cultured in a whole-blood assay within 1–2 hours according to a previously described technique (Kirchner et al. 1982). In a 5 ml polystyrol tube (Greiner, Nürtingen, Germany), 100 µl of blood was added to 850 µl of Roswell Park Memorial Institute (RPMI) medium (Biochrom, Berlin, Germany) supplemented with 2 mmol L-glutamine, 100 U/ml penicillin, and 100 µl/ml streptomycin (Gibco, Karlsruhe, Germany). For induction of IL-2, IL-10, and IFN-γ, phytohemagglutinin (PHA) (Borroughs Wellcome, Dartford, Great Britain) was added at a final concentration of 5 µg/ml. The blood suspension was incubated at 37 °C with 95 %air/5 %CO_2 for either 48 hours (for IL-2), 72 hours (for IL-10), or 96 hours (for IFN-γ). The supernatants were recovered and kept frozen at –80 °C.

Determination of cytokines

Cytokine concentrations and the sIL-2R were determined by enzyme-linked immunosorbent assay (ELISA). Recombinant cytokines were used as standards. We used ELISA kits from BioSource International (Camarillo, USA) according to the manufacturer's instructions. The intraassay coefficients of variation were 4.5 % for IFN-γ, 5.7 % for IL-10, 5.8 % for IL-2 and 5.6 % for sIL-2R. The interassay coefficients of variation were 5.7 % for IFN-γ, 5.4 % for IL-10, 7.2 % for IL-2 and 6.7 % for sIL-2R.

We reviewed the data from our previous studies (Seidel et al. 1995, 1996) focusing on the diagnostic classification of the sample. All patients included in those samples met the criteria of major depression according to DSM IV criteria. All but two patients included in the earlier studies suffered from the non-melancholic subtype of major depression (MDNM), while only two patients where identified as suffering from major depression with melancholic features (MDM).

The *cortisol levels* in the serum of patients and controls were determined by the Cortisol Enzymun Test of Boehringer Mannheim (an ELISA/competition test) carried out according to the manufacturer's instructions. Cortisol levels between 27.6 nmol/l (E-3 s of standard 3 a) and 1380 nmol/l can be detected by this test.

Statistics

Due to the non-Gaussian distribution of our data, non-parametric tests were applied for statistical evaluation. The Wilcoxon Matched-Pairs Signed-Ranks Test, the Friedman Two-Way Anova Test, the Mann-Whitney-U Test, and the Pearson Correlation Coefficient were used as provided by the SPSS 8.0 program.

Results

The mean score of the Hamilton Depression Rating Scale (HDRS, 21 items version) indicating the severity of depressive symptoms on admission (T1) was 26.82 (standard deviation (SD) 6.92). After two (T2) and four weeks (T3) of treatment the scores were 19.21 ± 7.85 and 17.69 ± 9.73, indicating that treatment induced a significant clinical improvement of the depression (Friedman χ^2=43.80, p < =0.001). The HDRS scores of the subgroups with or without melancholic features are shown in Table 2. At T1, the patients with melancholic features (major depression with melancholic features = MDM) showed a significantly higher HDRS score (Mann-Whitney-U, Z=–2.26, p=0.024) than the patients with non-melancholic major depression (major depression non-melancholic type = MDNM). After 2 and 4 weeks of treatment, no significant differences between the subgroups could be detected.

The leukocyte counts were higher in the total sample of patients suffering from MD (major depression total group: MDT) (T1: 7.0/µl, T2: 7.2/µl, T3: 6.6/µl) compared to the healthy controls (5.7/µl, Friedman χ^2=10.21, p=0.037). In the subgroup of patients with melancholia (MDM), no significant differences could be observed (T1: 6.7/µl, T2: 6.6/µl, T3: 6.4/µl, controls: 5.8/µl; Friedman χ^2=2.47, p=0.48), whereas the group of non-melancholic patients (MDNM) showed significantly increased leukocyte counts (T1: 7.3/µl, T2: 7.7/µl, T3: 6.9/µl; controls: 5.7/µl, Friedman χ^2=8.35, p=0.039).

The absolute numbers of NK-cells were significantly higher in the MDT at all three investigation time points compared to the healthy controls (Friedman χ^2=8.06, p=0.045). The relative counts of NK-cells were significantly higher at intake and after two weeks of treatment (T1: Z=–2.51, p=0.012, T2: Z=–2.94, p=0.003, T3: Z=–1.89, p=0.058). After division of MDT into the two diagnostic subgroups, only the MDNM appeared to show this increase. In the MDM subgroup, no significant difference could be established (Table 3).

The lymphocyte counts were comparable in MDT and healthy controls at all investigation time points. In the MDNM, the absolute lymphocyte counts were significantly higher on admission and after treatment compared to matched healthy controls and the subgroup of patients with melancholic depression (Fig. 1). However, there was no difference in the relative lymphocyte numbers between patients and controls.

Table 2 Score of the Hamilton Depression Rating Scale (HDRS)

	N	T1	T2	T3
Major depression Total group (MDT)	43	Mean 26.82 SD 6.92 Median 27	Mean 19.21* SD 7.85 Median 19	Mean 17.69*° SD 9.73 Median 16
Melancholic depression (MDM)	22	Mean 28.73 SD 7.03 Median 29	Mean 19.33* SD 8.81 Median 22	Mean 19.55* SD 11.15 Median 17
Non-melancholic depression (MDNM)	21	Mean 24.91 SD 6.40 Median 23.5	Mean 19.09* SD 7.03 Median 17.5	Mean 15.74*° SD 7.79 Median 14

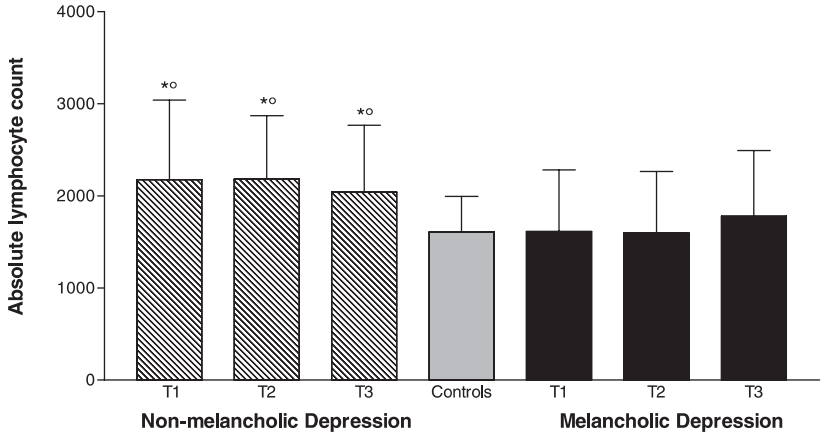
* significantly different from T1 (p < =0.001)
° significantly different from T2 (p < =0.05)
T1 admission; *T2* after 2 weeks; *T3* after 4 weeks; *SD* Standard Deviation

Table 3 Natural killer cell (NK-cells) counts in major depression (MD)

	NK-cells number T1	NK-cells number T2	NK-cells number T3	NK-cells Number controls	NK-cells % T1	NK-cells % T2	NK-cells % T3	NK-cells % controls
MDT	537.9* ± 384.2	530.4* ± 365.4	552.2* ± 388.9	360.8 ± 201.5	27.9* ± 11.2	28.0* ± 11.8	28.1 ± 12.9	22.6 ± 11.2
MDM	469.9 ± 310.6	472.7 ± 328.0	509.9 ± 399.1	382.6 ± 226.8	29.2 ± 12.1	28.9 ± 13.3	28.0 ± 12.4	23.6 ± 12.2
MDNM	629.4* ± 458.7	573.7* ± 404.0	587.1* ± 408.6	325.8 ± 179.1	26.6* ± 10.7	26.4* ± 10.1	27.3* ± 14.0	20.6 ± 9.6

* significantly different from healthy control ($p < =0.05$)

Fig. 1 Lymphocytes in major depression.

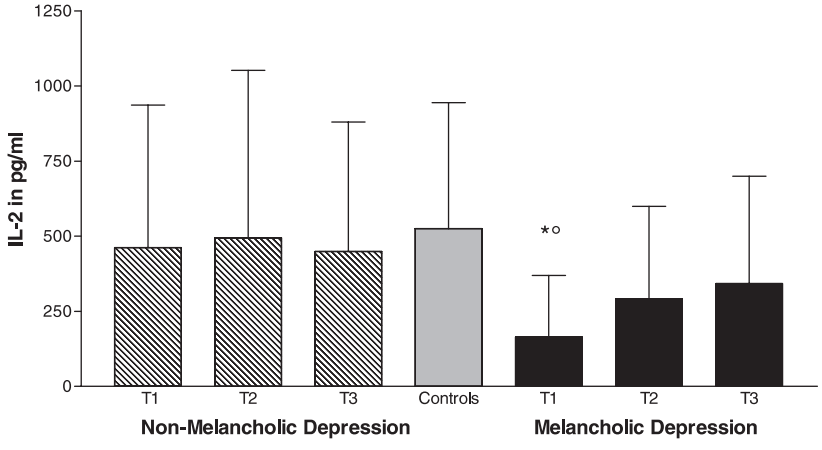


* significantly different from healthy controls ($p \leq 0.05$)
° significantly different from Melancholics at corresponding point of investigation ($p \leq 0.05$)

The production of the lymphokine interleukin-2 (IL-2) upon mitogen stimulation as a marker of cellular immunity (mean: 420.6 ± –480.3 pg/ml) was significantly decreased in patients suffering from major depression compared to healthy controls (Wilcoxon Z=–2.37, p=0.018) on admission. After two (mean: 426.6 ± 421.6 pg/ml) and four (mean: 441.3 ± 432.9 pg/ml) weeks of treatment, there was no significant difference concerning IL-2 production between patients and healthy controls (mean: 525.2 ± 499.8 pg/ml). Patients with MDM produced significantly less IL-2 than patients with MDNM upon admission (Fig. 2, Mann-Whitney-U Z=–2.60, p=0.009) and there was no difference between patients with MDNM and healthy controls. There were no differences in the amounts of CD25+ cells and the soluble IL-2 receptor between the depressed patients and healthy controls or among the subgroups (data not shown).

Among all patients with major depression (MDT), there was no difference concerning the production of interferon-gamma (IFN-γ) compared to healthy controls. However, melancholic patients (MDM) showed a significantly lower production (mean: 1789.6 ± 2053.6 pg/ml) than their matched healthy controls (3819.8 ± 4423.8 pg/ml, Wilcoxon Z=–2.06, p=0.039) upon admission (T1). Upon follow-up, no differences could be shown. Fig. 3 shows the production of IFN-γ at all the investigation time points. Concerning IFN-γ production, patients with MDNM differed significantly from patients with MDM (Mann-Whitney-U Z=–2.26, p=0.024).

Fig. 2 Production of IL-2 upon mitogen stimulation in major depression.



* significantly different from matched healthy controls ($p \leq 0.05$)
° significantly different from non-melancholic MD at corresponding point of investigation ($p \leq 0.01$)

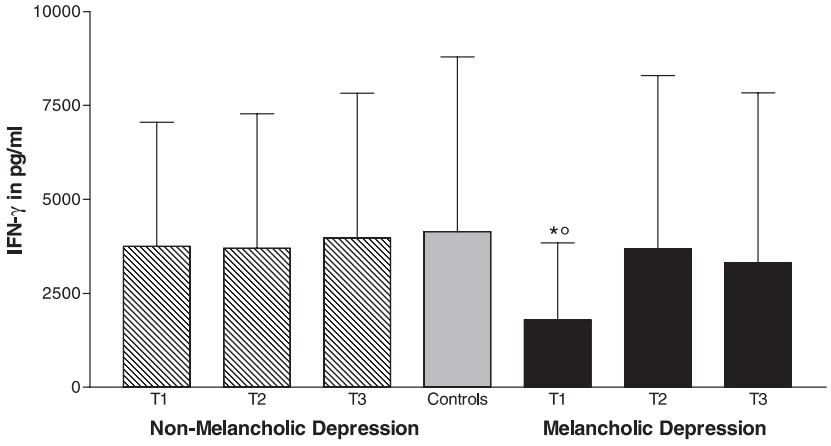


Fig. 3 Production of IFN-γ upon mitogen stimulation in major depression.

* significantly different from matched healthy controls ($p \leq 0.05$)
° significantly different from patients with non-melancholic Depression at at corresponding point of investigation ($p \leq 0.05$)

The production of IL-10 (a negative immunoregulatory cytokine) was decreased in patients with melancholic depression upon enrollment (T1: 155.2 ± 143.5 pg/ml; controls 234.6 ± 138.7 pg/ml; Wilcoxon Z=–2.32, p=0.02), and approached normal values after two (206.5 ± 155.5 pg/ml) and four weeks of treatment (211.2 ± 167.3 pg/ml). The IL-10 production in patients with MDNM (T1: 271.6 ± 205.6 pg/ml; T2: 233.8 ± 180.9 pg/ml; T3: 266.4 ± 210.4 pg/ml) did not significantly differ from healthy controls (232.0 ± 158.2 pg/ml).

No differences concerning cell counts or cytokine production between medicated and unmedicated patients were detected. There was no correlation between immune parameters and HDRS scores. No sex-related differences were observed. Patients suffering from recurrent depressive episodes did not differ from patients experiencing the first depressive episode concerning the studied immune parameters. Smokers and non-smokers did not differ concerning cytokine production and cell numbers.

Cortisol levels did not differ between patients with major depression (MDT) and healthy controls (T1: 555.7 nmol/l, SD 222.9 nmol/l, T2: 503.1 nmol/l, SD 205.9 nmol/l, T3: 533.1 nmol/l, SD 202.7 nmol/l, healthy controls: 463.6 nmol/l, SD 157.9 nmol/l, Friedman χ^2=3.49, p=0.32). In melancholic patients (MDM) the cortisol levels were slightly increased compared to matched healthy controls but did not reach the level of statistical significance (T1: 616.6 nmol/l, SD 214.3 nmol/l, T2: 524.0 nmol/l, SD 177.6 nmol/l, T3: 574.8 nmol/l, SD 221.2 nmol/l, controls: 506.8 nmol/l, SD 151.4 nmol/l, Friedman χ^2=4.62, p=0.20). Cortisol levels and the investigated immune parameters were not significantly correlated at any time point.

Table 4 Comparison of immune patterns in subtypes of Major Depression

	Non-melancholic depression	Melancholic depression
Leukocyte count	↑	↔
Lymphocyte count	↑	↔
Natural killer cell count	↑	↔
Production of IL-2	↔	↓
Production of IFN-γ	↔	↓
Production of IL-10	↔	↓

Discussion

Many studies on immune functions in patients with major depression have been undertaken before now. Despite this effort, only a few characteristic immune alterations such as reduced NK-cell activity have emerged so far. It needed to be asked whether a more detailed psychiatric characterization of the patients would be helpful towards identifying immune alterations typical for subgroups of depression. Melancholic depression is not simply a more severe form of non-melancholic depression. It represents a distinct clinical subtype with an episodic rather than a chronic course of disease. Melancholia is characterized by a loss of pleasure in all, or almost all activities. There is a lack of response to usually pleasurable stimuli and the depressed mood is experienced as a feeling distinct from that experienced after the death of a loved one. There may be a feeling of excessive or inappropriate guilt. In melancholia, depression is regularly worse in the morning with early morning awakening. Patients may suffer from marked psychomotor retardation or agitation and significant weight loss. From its clinical presentation and the results of research, melancholic depression has often been considered as the most "biological" form of depression. In this study a thorough clinical classification of subgroups of major depression focusing on melancholic features was therefore undertaken.

The increased absolute and relative numbers of NK-cells in the whole group of major depressed patients is consistent with the results of other studies on NK-cells in MD (Ravindran et al. 1998, Seidel et al. 1996). Upon differentiation into subgroups it became clear that it was only the MDNM patients who accounted for this increase. Melancholics showed normal NK-cell counts (as reported by Darko et al. 1988a–c, Evans et al. 1992, Maes et al. 1994 a, Natelson et al. 1998, for MD patients). Most

previous studies have not differentiated between melancholic and non-melancholic subjects. Only Maes et al. (1994a) reported no differences between patients with minor depression, MDNM and MDM. Natelson et al. (1998, 1999) differentiated between patients with dysthymia and MD. The varying make-up of patient samples in the previous studies (concerning melancholic and non-melancholic patients) might explain the divergent findings. Support for this idea comes from one of our earlier studies (Seidel et al. 1996) in which increased NK-cell numbers (as seen here in MDNM patients) were seen, since in that study all but two patients suffered from non-melancholic depression.

Lymphocytosis was observed in MDNM while melancholic patients showed no difference in lymphocyte counts compared to healthy controls. In most published studies, no alterations in lymphocyte numbers were observed (Andreoli et al. 1993, Irwin et al. 1987, Kronfol et al. 1984, Maes et al. 1993). Others reported increased (Müller et al. 1993) and decreased (Darko et al. 1988a–c, 1989a–b, Kronfol et al. 1989, Marazitti et al. 1992) numbers of lymphocytes in major depression. Different percentages of melancholic patients included in the samples of the reported studies may have been responsible for the discrepant results. On the other hand, Maes et al. (1989a) found no differences in lymphocyte counts between patients with minor depression, MDNM and MDM. However, in that study the subgroups consisted of only 10 to 14 patients.

TH1-cytokine production (as a marker of cellular immunity) was decreased in melancholic depression while patients suffering from non-melancholic depression showed a normal production of IL-2 and IFN-γ. Some recent studies also reported a decreased production of interleukin-2 (IL-2) upon mitogen stimulation in major depression without further diagnostic subclassification (Anisman et al. 1999, Weizman et al. 1994), while others found no difference concerning IL-2 production in major depression compared to healthy controls (Darko et al. 1988a–c, 1989a–b, Kanba et al. 1998, Natelson et al. 1999). Seidel et al. (1995) reported an unchanged IL-2 production in a sample of patients with MDNM. Maes et al. found that lymphocyte proliferation is decreased in melancholia compared to MDNM or minor depression (Maes et al. 1989b, 1991). Our results agree with those of Hickie et al. (1993) who reported a decreased cell-mediated immunity in melancholic patients while patients suffering from major depression without melancholic features did not show a decrease. Clearly, there is a relevant difference between MDNM and MDM concerning the production of TH1-cytokines and lymphocyte proliferation. The degrees of change in IL-2 and IFN-γ production (50% decrease) in melancholic patients is comparable to the changes in cytokine production in patients suffering from autoimmune diseases (multiple sclerosis, Graves' disease, rheumatoid arthritis) or viral infections (Cathely et al. 1986, Eisenstein et al. 1994, Faxvaag et al. 1995, Jokinen et al. 1993, Wandinger et al. 1997).

TH1 lymphokine production in melancholic depression was decreased only upon enrollment. After two or four weeks treatment, differences disappeared. With clinical improvement and/ or psychiatric treatment, the lymphokine production became normalized. This contrasts with the findings of McAdams and Leonard (1993) who showed that the decreased mitogen stimulative response in a small sample of eight patients suffering from major depression remained decreased after recovery. Differences in immunological techniques (T-cell replication versus cytokine production) and patient samples (n=8 versus n=43, lower severity of depression) can probably account for these differences.

IL-2 and IFN-γ are inflammatory cytokines while IL-10 is a negative immunoregulatory cytokine. IL-10 is secreted by regulatory T-cells and suppresses the release of IFN-γ (Groux and Powrie 1999). Since two proinflammatory cytokines were decreased in the acute stage of melancholic depression we wondered whether an upregulated antiinflammatory cytokine (like IL-10) would be responsible for the lower proinflammatory cytokine production. However, the decreased production of IL-10 upon admission makes it unlikely that the decreased production of the cytokines IL-2 and IFN-γ is due to the antiinflammatory action of IL-10.

Even though plasma cortisol levels were not significantly increased in the study groups the influence of cortisol on the immune system should be taken into consideration. Cortisol is known to decrease the production of IL-2 and IFN-γ by inhibition of the function of NFκB, a transcription factor important for cellular activation and cytokine production in the immune response. It also promotes the death by apoptosis of leukocytes and lymphocytes. On the other hand, cortisol is known to inhibit inflammatory cell migration from the blood stream to sites of inflammation. This leads to higher leukocyte counts in the blood. The group of melancholic patients showed slightly (but not significantly) higher cortisol levels and significantly lower productions of IL-2 and IFN-γ at T1 but normal leukocyte counts. Further investigations are needed to elucidate the role of corticosteroids regarding the immunological changes observed in melancholic depression.

The high scores in the HDRS upon admission demonstrate the severity of depression in the studied subjects. A significant drop after two and four weeks of treatment shows the extent of the clinical improvement. However, many patients still showed significant psychopathology after four weeks of treatment. In future studies, it might be useful to study the same subjects after complete recovery to help determine whether these immune patterns are state or trait markers.

In summary, patients with non-melancholic MD demonstrate increased absolute numbers of leukocytes, NK-cells and lymphocytes upon admission and after two and four weeks of treatment. Concerning relative cell counts, only NK-cells were increased. Cytokine production was normal in this group of patients at all investigation time points.

Patients suffering from MD with melancholic features exhibit a different immune pattern, i. e. normal leukocyte, lymphocyte and NK-cell counts. Production of the cytokines IL-2, IFN-γ and IL-10 on the other hand is decreased during the acute stage of disease and normalizes with treatment and clinical improvement. These differences cannot be explained by the use of antidepressant medication alone, by age, sex or smoking habits. The results of many previously published studies support the idea that patients suffering from melancholic depression show a different immune pattern compared to non-melancholic patients. Some previous findings cannot be explained by this model, usually because of a lack of clinical information on the patient samples. Therefore, further studies focusing on patients with melancholic depression are required to support this hypothesis. These studies should include detailed clinical information and larger patient samples to facilitate subgrouping.

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