Monocyte-Derived Dendritic Cells in Bipolar Disorder

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Background: *Dendritic cells (DC) are key regulators of the immune system, which is compromised in patients with bipolar disorder. We sought to study monocyte-derived DC in bipolar disorder.*

Methods: *Monocytes purified from blood collected from DSM-IV bipolar disorder outpatients (*n *= 53, 12 without lithium treatment) and healthy individuals (*n *= 34) were differentiated into DC via standard granulocyte-macrophpage colony-stimulating factor/interleukin-4 culture (with/without 1, 5, and 10 mmol/L lithium chloride). The DC were analyzed for DC-specific and functional markers and for T-cell stimulatory potency.*

Results: *Monocytes of bipolar patients showed a mild hampering in their differentiation into fully active DC, showing a weak residual expression of the monocyte marker CD14 and a relatively low potency to stimulate autologous T cells. Lithium treatment abolished this mild defect, and monocyte-derived DC of treated bipolar patients showed signs of activation (i.e., an up-regulated potency to stimulate autologous T cells and a higher expression of the DC-specific marker CD1a). This activated phenotype contrasted with the suppressed phenotype of monocyte-derived DC exposed to lithium in vitro (10 mmol/L) during culture.*

Conclusions: *Dendritic cells show mild aberrancies in bipolar disorder that are fully restored to even activation after in vivo lithium treatment.*

Key Words: Bipolar disorder, monocytes, dendritic cells, lithium

We previously reported a raised prevalence of autoimmune thyroiditis, defined as a raised level of thyroperoxidase-antibodies (TPO-Abs), in bipolar patients (Kupka et al 2002). In a consecutive study on the same cohort, not only thyroid autoimmune reactivity but also autoimmune reactivity to pancreatic islets (GAD65-Abs) and to gastric mucosa (H/K-ATPase-Abs) were more prevalent in bipolar disorder (Padmos et al 2004). The raised prevalence of TPO-Abs, GAD65-Abs, and H/K-ATPase-Abs was not associated with the current use of lithium.

To investigate whether a higher prevalence of autoimmunity in bipolar disorder (BD) was associated with a generalized activation of the T-cell system, we extended our studies and measured levels of serum–interleukin-2 receptor (sIL-2R) and numbers of activated T cells in the circulation of BD patients (Breunis et al 2003). The T-cell system was found activated, as evidenced by a higher serum level of sIL-2R and raised numbers of CD3+ CD25+ cells, in both symptomatic and euthymic patients; however, manic patients showed the highest state of T-cell activation. As with the auto-Abs, the T-cell activation was not associated with the use of lithium.

The stimulation of antigen-specific T cells and the production of antibodies, including that of TPO-Abs, GAD65-Abs, and H/K-ATPase-Abs, are under the control of dendritic cells (DC), a specialized group of immune cells. The DC are antigen-presenting cells (APC) par excellence and the only cells capable of stimulating naïve T cells and initiating an effective immune response toward foreign antigens (Banchereau and Steinman 1998; Banchereau et al 2000; Cella et al 1997). Besides that, DC are also involved in preventing autoimmune responses by inducing tolerance to auto-antigens. To exert these functions, DC express major histocompatibility complex (MHC)-class II molecules and the so-called co-stimulatory molecules (CD80, CD86, and CD40) on their cell membrane to be able to strongly stimulate (auto-)antigen-specific T-helper and regulatory cells (Cella et al 1997; Clark et al 2000). One of the main precursors of DC is the blood monocyte (Cella et al 1997; Sallusto and Lanzavecchia 1994).

Our observation of an aberrant T-cell activation and a higher prevalence of thyroid, islet, and gastric-Abs in BD patients led to the hypothesis that DC might also be aberrant in these patients. To our knowledge, studies on the function of DC in bipolar disorder have not been performed. Therefore, we induced purified peripheral blood monocytes of 53 BD patients to differentiate into DC with a 6-day-culture of cells in the presence of the differentiating cytokines granulocyte-macrophpage colony-stimulating factor (GM-CSF) and IL-4. The DC character of the cells was verified by the expression level of the DC-specific type II lectin DC-SIGN, of the DC-specific lipid antigen presenting molecule CD1a, and of CD83 on the cultured cells. The functional activity of the cells was determined by the capability of the generated DC to stimulate T cells to proliferate in mixed lymphocyte reactions (MLR). We also measured the expression levels of MHC-class II, CD80, CD86, CD40, and of molecules involved in APC/T-cell interactions (i.e., of the integrins CD11b, CD18, CD29, CD54). We investigated these functional and phenotypic DC parameters in BD patients with current lithium treatment and without lithium treatment separately, because this drug is known to be immune modulatory (Hornig et al 1998; Rybakowski 2000). Outcomes were compared with those obtained using cells of healthy control subjects ($n = 34$). We also investigated whether in vitro co-culture with lithium influenced the capacity of monocytes to differentiate into DC.

Methods and Materials

Patients and Healthy Control Subjects

Patients. Heparinized blood was obtained from outpatients with DSM-IV bipolar I and bipolar II disorder ($n = 53$) participating in the Stanley Foundation Bipolar Network (SFBN), a multi-center research program described elsewhere in detail

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(Leverich et al 2001; Suppes et al 2001). For these experiments, all patients were recruited from SFBN sites in the Netherlands. The Institutional Review Board of the University Centre Utrecht had approved the study protocol and written informed consent was obtained from all patients. A DSM-IV (American Psychiatric Association 1994) diagnosis of bipolar disorder was made by means of the Structured Clinical Interview for DSM-IV Axis I, Patient Edition (SCID-I/P) Research Version (First et al 1996). A detailed illness history, including past and present medication, was assessed at entry into the Network. Blood for the present study was drawn during one of the monthly follow-up visits, when a research clinician also determined present mood (i.e., euthymic, depressed, [hypo-]manic or cycling) by means of a detailed interview including the Young Mania Rating Scale (Young et al 1978), Inventory of Depressive Symptoms (Rush et al 1986), and Clinical Global Impressions Scale—Bipolar Version (Spearing et al 1997). Patient clinical characteristics are shown in Table 1. Patients were included as "not using lithium" (nonLi-BD) when they were either lithium naive or did not use lithium for at least 12 months. The lithium users had effective lithium treatment for at least 6 months (Li-BD). It must be noted that Li-BD had comparable profiles of other psychotropic medication as the nonLi-BD.

Blood was collected in sodium-heparin tubes (Becton Dickinson [B&D], San Jose, California), because lithium-anticoagulant for the collection of blood has been found to influence the in vitro cytokine production (Riches et al 1992). Blood was drawn in the morning and transported by courier to the laboratory in Rotterdam, lasting an acceptable 8–10 hours before further processing.

Healthy Control Subjects. Blood was collected from healthy laboratory and hospital staff members of the Erasmus MC (The Netherlands) ($n = 34$) on the same days and at the same time points as the patients, with the same procedures. All healthy control subjects (HC) gave written information about medication use and medical history. Informed consent was obtained. The exclusion criteria for this HC group were: any immune disorder, serious medical illness, recent infections, fever, psychiatric disorder, or use of any psychotropic or other medication (apart from anti-conceptive hormonal therapy).

Table 1. Clinical Characteristics of Bipolar Patients and Healthy Control Subjects at the Time of Drawing Blood

	Bipolar Patients	Healthy Control Subjects
Group size		
Total	53	34
1st Series	14	11
2nd Series	15	9
3rd Series	8	8
4th Series	16	6
Age (years)[a]	45 (22–62)	38 (23–62)
Men	25 (47%)	12 (35%)
Women	28 (53%)	22 (65%)
Psychotropic medication		
Carbamazepin	35 (66%)	
Valproate	16 (30%)	
Thyrax	10 (19%)	
Antidepressives	14 (26%)	
Antipsychotics	9 (17%)	
Benzodiazepins	18 (34%)	
Duration of treatment (years)[a]	14 (1–36)	
Li users	41 (77%)	
Li titer in serum (mmol/L)[b]	.78 ± .17	
Cumulative period of Li treatment	76 (6–203)	
Patients never used Li (naïve)	6 (11%)	
Patients without current Li but with Li treatment in past	6 (11%)	
Period without Li (months)[a]	43 (12–114)	
Mood		
Euthymic	31 (58%)	
Depressed	12 (23%)	
Manic	8 (15%)	
Cycling	2 (4%)	
Duration of illness (years)[a]	21 (3–42)	
Hematological parameters		
Hb[b,c]	8.7 ± .8	
Leucocytes[b,d]	6.9 ± 1.4	
ESR[b,e]	5.8 ± 2.8	

Li, lithium; Hb, hemoglobin; ESR, erythrocyte sedimentation rate.
[a]Mean (range).
[b]Mean ± SD.
[c]Reference values: 7.4–9.6 mmol/L.
[d]Reference values: 4.0–10.0 × 10^9/L.
[e]Erythrocyte sedimentation, reference values < 10 mm/hour.

Generation of DC

Peripheral blood mononuclear cells (PBMC; i.e., the lymphocytes and monocytes) were prepared from the heparinized blood via Ficoll (Pharmacia, Uppsala, Sweden) gradient centrifugation, as has been described in detail before (Canning et al 2001). The PBMC were thereafter re-suspended in RPMI 1640 with 25mmol/L HEPES and L-glutamine (Bio Whittaker Europe, Verviers, Belgium), additionally containing 10% inactive fetal calf serum (FCS) (FCSi; Bio Whittaker), penicillin (100 units/mL)/streptomycin (100 μg/mL; P/S; Bio Whittaker), and extra ultra-glutamine (UG; 2 mmol/L; Bio Whittaker) (RPMI+). Samples were frozen in 10% dimethylsulfoxide and stored in liquid nitrogen. This enabled us to test patient and control monocytes in the same series of experiments at appropriate times. The frozen PBMC were quickly thawed and washed twice in RPMI+ (trypan blue exclusion: > 85% viable cells). To separate monocytes from lymphocytes, Percoll (density 1.063 g/mL; Pharmacia) density gradient centrifugation (for 40 min, 400 g) was used. Monocytes were cultured in four series (see Results) at a concentration of .5 × 10^6 cells/mL on 24-well culture plates (Falcon; B&D) under plastic-adherent conditions in RPMI+ in the presence of GM-CSF 800 units/.5 × 10^6 cells/mL (series 1 + 2: Pepro Tech, Rockyhill, New Jersey; series 3 + 4: Biosource, London, England) and IL-4 1000 units/.5 × 10^6 cells/mL (series 1 + 2: Biosource; series 3 + 4: Pepro Tech). In series 2–4, we added lithium chloride (LiCl) to the culture plates (1, 5, and 10 mmol/L). Cells were thereafter incubated at 37°C, 5% CO_2, and 95% humidity. On day three, the culture fluid was refreshed with complete RPMI+, containing cytokines (and, where appropriate, LiCl). After six days, DC were collected by re-suspending and washing the wells thoroughly with cold phosphate buffered saline (PBS) pH 7.4 (Bio Whittaker) containing 3 mmol/L ethylene diamine tetra-acetic acid (Sigma-Aldrich, Steinheim, Switzerland). This culture method is well established (Sallusto and Lanzavecchia 1994).

T-Cell Isolation

The pellet of the Percoll gradient was washed twice with PBS (Bio Whittaker Europe) containing .1% bovine serum albumine (Bayer, Kankakee, Illinois) (PBS/.1%BSA), and the cells were incubated with anti-CD3 microbeads (20 μl/10^7 cells: Miltenyi