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A dynamic course of T cell defects in individuals at risk for mood disorders

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ABSTRACT

Objectives: T cell abnormalities have been repeatedly reported in adult patients with mood disorders, suggesting a role of these cells in the pathogenesis of these disorders. In the present study, we explored the dynamics of circulating T cell subsets over time in a population at high familial risk for developing a mood disorder.

Methods: Children of a parent with bipolar disorder (bipolar offspring, $N = 140$) were assessed at three time-points: adolescence, young adulthood and adulthood. We carried out a detailed fluorescence-activated cell sorting (FACS) analysis to determine various T cell subsets from frozen stored peripheral blood mononuclear cells of bipolar offspring and age- and gender-matched healthy controls at each time-point.

Results: Throughout the period of observation reduced levels of CD3+ and CD3+ CD4+ T cells were observed. In bipolar offspring T_h1, T_h2, T_h17 and natural T regulatory cells (T_{regs}) followed a dynamic course over time with reduced levels of T_{regs} in adolescence and a reduced relative number of T_h1, T_h17 cells in young adulthood. In post hoc analysis T_{regs} were inversely associated with the pro-inflammatory monocyte state determined previously ($r_s = -0.220$, $p = 0.001$). Significant associations between T cell subset abnormalities and psychopathology such as mood disorders were not found.

Conclusions: A subtle partial T cell defect was present in bipolar offspring from adolescence through adulthood. Within this defect the dynamic change of inflammatory and regulatory T cell subsets suggests a high inflammatory state during adolescence, a reduced inflammatory state during young adulthood and a virtually normalized state at adulthood.

1. Introduction

Mood disorders such as major depressive disorder (MDD) and bipolar disorder (BD) are highly prevalent disorders with great societal impact (Gustavsson et al., 2011). Recent attempts to unravel the underlying pathogenesis involve the immune system. Various reports on an imbalanced cytokine profile and mild chronic inflammation (Kunz et al., 2011), to an increased occurrence of auto-immune disease and infection (Benros et al., 2013), and lately the higher activation status of monocytes, macrophages and microglia (Drexhage et al., 2011; Beumer et al., 2012; Barbosa et al., 2014), support the hypothesis for an essential role of the immune system in mood disorder development.

Apart from the cells of the myeloid lineage, cells of the lymphoid lineage, such as T and natural killer (NK) cells, are important players in the immune response. Lately, some excellent reviews on the role of T cells in mood disorders have been published (Miller, 2010; Herbert and Cohen, 1993; Toben and Baune, 2015). T cells are essential for antigen-specific cell-mediated immune responses. There are different subsets of T cells with highly diverse functions which can a.o. be distinguished on the basis of membrane bound

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"cluster of differentiation (CD)" proteins and by the cytokines they produce.

In addition to the CD3 protein complex common to all T cells, T helper cells (T_h) express the CD4 molecule. CD4+ cells interact with and regulate the function of other immune cells, hence their name helper cells. T_h cells develop after antigen stimulation from naïve T cells and are capable of actively secreting cytokines upon (re)-activation Fietta and Delsante, 2009. The production of these small signaling proteins defines the functionality of the T_h cells and the produced cytokines stimulate and/or inhibit various components of the immune reaction. At present three effector subsets of the CD4+ cells are known and called T_h1, T_h2 and T_h17 respectively. T_h1 cells produce IFN-ɣ and stimulate macrophages. T_h2 cells produce interleukin-4 (IL-4) and IL-5 instrumental in the stimulation of B cells. IL-4 also counteracts the effects of IFN-ɣ, and the T_h1/T_h2 cell balance therefore reflects one of the pro/anti-inflammatory balances operative in the immune system. T_h17 cells produce a.o. IL-17, this cytokine stimulates macrophages to pro-inflammation in a fashion similar IFN-ɣ for T_h1 cell.

Apart from the effector T_h there is another subset of CD4+ T cells. This subset develops naturally and spontaneously in the thymus and cells are called natural T regulatory cells (T_{regs}). T_{regs} are $CD3^+CD4^+CD25^{high}$ $FoxP3^+$ cells. This T cell subset has the capacity of dampening inflammatory responses by dampening T_h1, T_h2, T_h17 cells and monocytes/macrophages (Wing and Sakaguchi, 2010). T_{regs} are also important for immune homeostasis and tolerance towards auto-antigens.

There is an extensive literature on CD3+ and CD4+ T cells in mood disorder patients, yet a clear picture has not emerged. The present idea is that in patients with an active mood disorder a pro-inflammatory state prevails with raised numbers of T_h17 cells and reduced numbers of T_{regs}, while in mood disorder patients in remission/recovery normal number of T cells can be found. Yet T cells seems to have an impaired capability for expansion and an increased apoptosis when stimulated with a mitogen (Toben and Baune, 2015).

Lastly, there is another important cell of the lymphoid lineage, which has extensively been studied in mood disorder patients, the NK cell. NK cells are non-T cells, thus CD3 negative, but positive for the CD marker CD56.

The Dutch Bipolar Offspring (DBO) Study is a longitudinal study following children of a bipolar parent from adolescence into adulthood. These offspring are at increased familial risk for mood disorder development. This study provides a unique setting for the exploration of T cell and NK cell numbers in individuals at high risk for mood disorder development. We prospectively followed this cohort for 12 years and evaluated the participants and their parents during adolescence (mean age 16 years), young adulthood (mean age 21 years) and adulthood (mean age 28 years). At adolescence 27% of the offspring fulfilled criteria of a lifetime mood disorder. At adulthood (12-year follow-up) the lifetime mood disorder prevalence has increased to 54% (Bergink et al., 2013). In a recent study, Mesman et al. (2014), studied in the DBO cohort the expression of a set of inflammatory and activation genes in the circulating monocytes of the offspring and described an over-expression of these genes in monocytes particularly at adolescence, declining towards adulthood. Findings were not related to psychopathology.

For this study, we determined the T cell composition by FACS analysis and we assessed the percentages of CD3+(T cell), CD4+(T_h cells), CD8+(T cytotoxic cells), $CD3^+CD4^+CD25^{high}$ $FoxP3^+$ (T_{regs}), T_h1 (CD4+ IFN-γ+) cells, T_h2 (CD4+ IL-4+) cells, T_h17 (CD4+ IL-17+) cells, CD3-CD56+(NK) cells and CD14+(monocytes) in peripheral blood. We focused in particular on the CD3+ T cells and CD3+ CD4+ T_h cells, and correlated the percentages of T_{regs} to the previously described (Grosse et al., 2015) inflammatory activation state of the circulating monocytes, since we recently published defects of these T cell populations in MDD and since it is known that T_{regs} control monocyte-induced inflammation (Huang et al., 2011), also in MDD (Grosse et al., 2015). We also investigated potential correlations between the percentages of the various T cell populations and the NK cells found at adolescence to the psychiatric outcome later.

2. Methods and materials

2.1. Ethical procedure

The Medical Ethical Review Committee of the University Medical Center Utrecht (UMCU) approved the study. Written informed consent was obtained from all subjects and their parents (before age 18 years) after a complete description of the study was given.

2.2. Participants

2.2.1. Bipolar offspring

The data presented is obtained from the Dutch Bipolar Offspring (DBO) study. The study design and recruitment procedure of this study have been described in detail by Wals et al. (2001). In brief, 140 children (mean age 16 years, range: 12–21 years) from 86 families with one parent diagnosed with BPD (74% BPD type I and 26% BPD type II) were recruited in the years 1997–1999 and followed for 12 years (follow-up rate: 77%). Subjects were psychiatrically assessed at four time-points: at baseline, and at one-year (adolescence), at five year (young adulthood) and 12-year (adulthood) follow-up. A detailed presentation on the demographic characteristics and psychopathology at the latest assessment has been described elsewhere (Mesman et al., 2013). Various T lymphocyte subpopulations from frozen stored peripheral blood mononuclear cells (PBMCs) were measured at adolescence, young adulthood and adulthood in DBO. At adolescence material of 25 subjects was available and assessed. At young adulthood material of 93 subjects and at adulthood material of 102 subjects was available for reassessment.

2.2.2. Healthy controls

Healthy controls (HC) were recruited cross-sectional at the various stages of the study. During the adolescent phase, participants were recruited from high schools ($N = 25$). The recruitment took place between 2001 and 2002. During the young adulthood phase, recruited HC were laboratory/medical staff, and control groups used in other studies ($N = 48$), recruited mainly between 2001 and 2005. HC in the adulthood phase were recruited from universities ($N = 50$) between 2010 and 2011. Exclusion criteria for healthy controls were medication use (except oral contraceptives), (self-reported) psychiatric disorders and immune and/or endocrine disease in both the subject and their first-degree family members. Both bipolar offspring and healthy controls were excluded from analyses in cases of clinical evidence of acute (severe) infections 14 days prior to blood withdrawal, usage of medication with explicit effect on individual's immune state within 24 h of blood withdrawal, or pregnancy. Demographics for both bipolar offspring and healthy controls are presented in Table 1.

2.3. Immune assessment

Blood drawings took place between 8.00 AM and noon; no fasting advice was given. For the serum preparation clotting-, and for immune cell preparation sodium heparin tubes were used. After direct transportation to the Rotterdam Erasmus MC (within 4 h), serum was stored at -80 degrees while the sodium heparin tubes were used for preparation peripheral blood mononuclear cells