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Research report

Interleukin-4 is a participant in the regulation of depressive-like behavior

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HIGHLIGHTS

- Deficiency of interleukin-4 leads to an increased depression-like phenotype.
- IL-4$^{-/-}$ mice did not show a higher vulnerability for IFN-α induced depressive-like behavior.
- Unconditioned IL-4$^{-/-}$ mice showed a reduced active avoidance behavior.
- IL-4 plays a critical role in the regulation of depressive-like behavior in a non-inflammatory condition.

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ABSTRACT

Inflammatory immune activation has been frequently associated with the development of major depression. Microglia might serve as an important interface in this immune system-to-brain communication. Interleukin-4, the major Th2 type cytokine, might be protective against depression due to its ability to counter-regulate inflammation and to inhibit serotonin transporter activity. By using an Interferon-α mouse model, we show that a decreased IL-4 responsiveness of microglia was specifically related to the development of depressive-like behavior. IL-4 deficient mice in a BALB/cJ background showed a considerable increase of depressive-like behavior in the forced swim (FST) and tail suspension test (TST) and reduced avoidance behavior in an active avoidance task. Prior conditioning with unescapable foot shocks further decreased avoidance behavior (learned helplessness) but to a similar level as in the wild type strain. IFN-α treatment was not able to further enhance the already increased level of depressive-like behavior in the FST and TST. Thus, IL-4 seems to be a critical participant in the regulation of depressive-like behavior in an untreated baseline condition. Increase of depressive-like behavior during inflammation in wild-type mice might be mediated to some extent by a reduction of IL-4 signaling.

1. Introduction

Accumulating evidence over the past decades has linked the development of major depression to several types of inflammatory conditions and has indicated that immune-brain interactions might be relevant for future treatment strategies. A chronic low-grade inflammation has now increasingly be considered a link between depression and several metabolic comorbidities such as obesity, diabetes, and coronary heart disease, but also ageing, and other more obvious immune-related diseases. Recent meta-analyses have confirmed elevated serum levels of inflammatory parameters (e.g. IL-6, TNF-α, C-reactive protein) in depressed patients [1,2]. Clinical studies have shown antidepressant effects of anti-inflammatory add-on therapies, such as cyclooxygenase (Cox) 2 inhibitors, polyunsaturated fatty acids, minocycline, and anti-TNF-α therapies [3–8]. Prolonged treatment with IFN-α induces major depressive episodes in 20–40% of patients, and even more

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develop mild depressive symptoms with a significant impact on their quality of life [9,10]. IFN-α induces a Th1 shift of the immune response supporting antiviral or antitumor immune responses, and mediates the release of other inflammatory parameters [11–13].

To unravel the mechanisms by which a peripheral inflammation is transmitted to the brain and how it is finally affecting the neuronal network to trigger depressive mood changes will be relevant for antidepressant treatment. It is currently assumed that microglia, which are the resident immune cells in the brain, might function as an important interface in transferring this information. Microglia exist in a ramified state with highly motile processes that come into close contact with neuronal synapses and that perform continuous surveillance of their microenvironment [14–17]. They participate in generating the neuronal network by eliminating dysfunctional synaptic connections [18]. As immune cells, microglia also function as first line responders in inflammatory conditions [19] and acquire the ability to proliferate, migrate, and produce pro- and anti-inflammatory cytokines and neurotoxic factors [20,21]. Microglia can further specialize into distinct functional subsets depending on the specific microenvironment that they are exposed to. In simplified terms, these functions are located along a continuum with the two polarities known as M1 (classically activated) and M2 (alternatively activated) at opposite sites. M1 cells are induced by IFN-γ, TNF-α, or lipopolysaccharide (LPS) and are characterized by a pro-inflammatory phenotype and secretion of neurotoxic substances. IL-4 and IL-13 induce M2 cells that are characterized by an anti-inflammatory phenotype. They secrete neurotrophic factors and support tissue repair [22–24].

By using an IFN-α mouse model, we previously showed that microglial M1 activation only occurred in mice that responded to peripheral IFN-α treatment with the development of depressive-like behavior [25]. Inflammatory signals then may act on the neuronal network by neurotoxic activities or might directly influence mood regulation e.g. via the serotonin system. A number of inflammatory cytokines such as IL-1β, TNF-α, IFN-α, and IFN-γ are able to up-regulate the serotonin transporter activity and to reduce the availability of extracellular serotonin [26–28]. So far, only one cytokine, namely IL-4, has been described that is able to inhibit serotonin transporter activity [29] and thus is acting via the same mechanism as currently used antidepressant medications (SSRI). IL-4 reduced serotonin uptake in a dose dependent manner of human immortalized B cells, which was preferentially seen in cells homozygous for the long high-activity allele in the promoter region of the serotonin transporter [29]. In the current manuscript, we focus on the role of IL-4 for the development of depressive-like behavior. We show that mice responding with depressive-like behavior after IFN-α treatment were specifically characterized by a down-regulation of the IL-4Rα on microglial cells. IL-4$^{-/-}$ knock out mice display inherent depressive-like behavior in the forced swim (FST) and tail suspension test (TST) and reduced avoidance behavior in an active avoidance task. In the absence of IL-4, IFN-α is not able to further increase the already enhanced level of depressive-like behavior.

2. Material and methods

2.1. Animals

BALB/c mice were purchased from Charles River Germany (Sulzfeld, Germany), BALB/cJ from Charles River UK (Kent, UK), and BALB/c-*Il4*^{tm2Nnt}/J (IL-4$^{-/-}$) from Jackson Laboratories (Maine, USA). All three strains were maintained in community cages in the central animal facility of the Ruhr University Bochum under standard housing conditions. The mice were kept under a reversed 12:12 h dark/light cycle. Animal care and experimental procedures were performed according to institutional guidelines and were approved by the local authorities.

2.2. IFN-α treatment

Murine IFN-α (PBL Interferon Source, #12100-1, endotoxin tested) was dissolved in phosphate-buffered saline (PBS; VWR, Leuven, Belgium). The BALB/c and IL4$^{-/-}$ males were eight weeks old at the beginning of the experiments. Daily intraperitoneal (i.p.) injections of 0.2 ml were administered at the concentration of 60,000 U/kg/day for 14 days, while control mice received 0.2 ml of PBS.

2.3. Behavioral tests

All behavioral tests were performed during the dark phase. In the first set of experiments, male BALB/c mice were tested in the FST before and after IFN-α/PBS administration. The FST was performed the day before starting the treatment and again on day 14 of treatment. In the second set of experiments, untreated BALB/c, BALB/cJ, and IL-4 deficient BALB/cJ mice were tested in the light/dark test (LD), TST and FST. All mice started with the less stressful LD, which was followed by the TST on the same day with a break of 4 h between the two tests for each animal. The FST was performed on the following day. The day after, IL-4$^{-/-}$ mice were treated with either IFN-α or PBS over two weeks. On day 14, mice underwent again the LD and TST, which was followed by the FST on day 15 of treatment.

2.3.1. Forced swim test (FST)

The experimental setup was in accordance with a modified version of the Porsolt swim test. The mice were placed in a vertical cylinder filled with 20 cm deep water at 23 ± 2 °C. The test was run for 6 min. The time spent in an immobile floating condition indicates behavioral despair/depressive-like behavior. An investigator who was blinded to the treatment groups analyzed immobility time manually.

2.3.2. Tail suspension test (TST)

The test was performed in a box-like enclosure with a hook on the ceiling, by which the mice were suspended by the tail using adhesive Scotch tape so that they could not reach the floor. Movements were recorded for 6 min. The time spent in an immobile posture is regarded to measure behavioral despair/depressive-like behavior. Immobility time was analyzed manually.

2.3.3. Light/dark test (LD)

The mice were placed in a shuttle box (Multi Conditioning System, TSE Systems, Germany), which was divided into a dark and a lighted compartment of equal size. The mice could move between the two compartments. The test was run for 10 min. The cumulated time that mice spent in the dark compartment is automatically measured and saved by the TSE system and is regarded to measure anxious behavior.

2.4. Learned helplessness

The test was carried out in a shuttle box (Multi Conditioning System, TSE Systems, Germany), which was divided into two chambers with a lockable door. During two test sessions on two consecutive days (conditioning phase) mice got randomized foot shocks (0.1 mA, 1 – 3 s; time between shocks 5 – 15 s) over 52 min while the door was closed and they could not escape. On day three, all animals (conditioned/unconditioned) were tested in an active avoidance and escape paradigm in which they underwent 30 trials. Each trial started with a 5 s light stimulus followed by a foot