Experimental autoimmune encephalomyelitis-associated behavioral syndrome as a model of ‘depression due to multiple sclerosis’

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Abstract

Many medical conditions, including inflammatory diseases such as multiple sclerosis (MS), are often accompanied by a high prevalence of depressive episodes. Inflammatory mediators, such as cytokines, were implicated in illness-associated depressive conditions, both in humans and in animals. For example, MS-associated depression (MSD) was attributed to pathophysiological processes such as immune dysregulation and cerebral inflammation. We have recently documented a depressive-like behavioral syndrome in mice with experimental autoimmune encephalomyelitis (EAE), an established model of MS. In the present paper, we discuss the similarities between the EAE-associated behavioral syndrome (EBS) and MSD, in terms of phenomenology, putative mechanisms and responsiveness to anti-depressivetherapy. In particular, we show that: (1) EAE and depression are associated not only with similar behavioral symptomatology, but also with common physiological alterations, including impaired serotonergic neurotransmission, and activation of neuroendocrine (e.g., adrenocortical) and inflammatory cytokine systems; (2) the EBS precedes any neurological deficit during the initial EAE attack, as well as further exacerbations, and remits during recovery and between relapses. Similarly, in many MS patients depression precedes and accompanies the attacks and wanes during remissions; (3) females show increased susceptibility to EBS. Similarly, depression is much more prevalent in women than in men; (4) chronic treatment with the tricyclic anti-depressant imipramine reduced EAE-induced mortality, body-weight loss and behavioral suppression. Similarly, anti-depressant drugs have been used effectively in treating MSD. These findings suggest that the EBS may serve as an animal model for MSD.

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1. Inflammatory diseases are associated with a high prevalence of depression

Studies of depression in randomly selected general medical patients indicate that one fifth of them meet the criteria for major depression, whereas more than one third report some degree
of depression (Hosaka, Aoki, Watanabe, Okuyama, & Kurosawa, 1999; Rodin & Voshart, 1986). Among other diseases, acute and chronic infectious illnesses, such as influenza, gastroenteritis, hepatitis C, and Epstein–Barr virus, are all associated with a range of depressive symptoms, including depressed mood, fatigue, psychomotor retardation, anorexia, as well as attention and memory impairments (Capuron, Lamarque, Dantzer, & Goodall, 1999; Dieperink, Willenbring, & Ho, 2000; Hickie & Lloyd, 1995). Extensive research of depression among HIV patients also revealed an unambiguous comorbidity (e.g., Maj, 1996).

The relationship between infection and depression was also addressed experimentally. Despite the small number of studies, convincing data were collected indicating that induced infections with several viruses (e.g., common cold, influenza, and rubella vaccination), but not others (e.g., cold viruses) produce depressive symptoms (Morag, Yirmiya, Lerer, & Morag, 1998; Smith et al., 1988; Smith, Tyrrell, & Barrow, 1992; Smith, Thomas, Brockman, Kent, & Nicholson, 1993).

Accumulating evidence indicates that the depressive syndrome associated with infectious diseases is produced by inflammatory processes, rather than directly by specific pathogens. Accordingly, depression is highly prevalent in chronic inflammation associated with several types of non-infectious conditions, such as stroke (Robinson, 1997) or autoimmune diseases (Cavallaro, Pozzini, & Thorpe, 1993). Prevalence studies among diabetes mellitus, rheumatoid arthritis, and systemic lupus erythematosus patients documented high numbers of depressive disorder cases (Gavard, Lustman, & Clouse, 1993; Hutchinson, Nehall, & Simeon, 1996; Pincus, Griffith, Pearce, & Isenberg, 1996). Particularly high incidence of depression has been demonstrated in patients with multiple sclerosis (MS) (Foley et al., 1992; Schiffer & Babigian, 1984; Schubert & Foliart, 1993; Whitlock & Siskind, 1980). According to several estimates, the prevalence of MS-associated depression (MSD) is in the range of 37–54% (Schiffer, Caine, Bamford, & Levy, 1983; Joffe, Lippert, Gray, Sawa, & Horvath, 1987; Minden, Orav, & Reich, 1987; Sadovnick et al., 1996; Patten & Metz, 1997).

The recognition of the comorbidity of depression with physical illnesses is reflected by the relatively new psychiatric entity ‘mood disorder due to a general medical condition’ (DSM-IV, 1994). To meet the criteria of this entity, one’s symptoms should be “the direct physiological consequence of a general medical condition”, rather than a psychological response to stress or disability. Several studies on autoimmune diseases-associated depression, especially on MSD, suggest that the depressive symptoms reflect the action of a basic physiological mechanism, such as immune activation, rather than a mere psychological reaction to the consequences of the disease (e.g., distress due to functional losses): (1) Compared to patients with other neurological diseases, MS patients show higher levels of depressive symptoms at the time of the diagnostic interview, and higher number of depressive episodes since their diagnosis has been made (Schiffer & Babigian, 1984; Whitlock & Siskind, 1980). (2) MSD patients are frequently characterized by the presence of vegetative symptoms and diurnal variations in mood and energy (Whitlock & Siskind, 1980). This quality of depression may suggest an ‘organic’ rather than a ‘reactive’ depression. (3) In many cases the onset of depression precedes the neurological diagnosis (Schiffer & Babigian, 1984). (4) A prospective study of MS depressed patients revealed that immune dysregulation preceded and accompanied the development of depression (Foley et al., 1992). (5) MSD is related to cerebral inflammation. In a recent study (Fassbender et al., 1998), patients were divided into two groups according to two measures of cerebral inflammation, e.g., white blood cells count in the cerebrospinal fluid (CSF) and gadolinium enhancement in magnetic resonance assessment. Cerebral inflammation was associated with higher scores of depression, suggesting a role for inflammatory processes in MSD. In contrast, MSD was not correlated with the degree of the neurological impairment. Together, these findings suggest that at least some of the depressive symptoms that accompany MS are not merely a reaction to the medical condition, but are caused by immune changes that precede and coincide with the appearance of clinical symptoms.

2. Cytokines and other inflammatory mediators may underlie illness-associated depressive symptoms

The complex relationship between depressive symptoms and pro-inflammatory factors has already been reviewed recently elsewhere (Dantzer, Wollman, Vitkovic, & Yirmiya, 1999; Maes, 1995; Yirmiya, 1997; Yirmiya et al., 1999, 2000). In this
paper only the aspects that are relevant for the following discussion will be mentioned. Among the immune activation markers that were found in depressed subjects are high counts of white blood cells and elevated levels of inflammatory cytokines particularly interleukin (IL)-1, IL-6, interferon-γ (IFNγ) and tumor necrosis factor α (TNFα), as well as high levels of positive acute phase proteins (APPs) and low levels of negative APPs (Connor & Leonard, 1998; Maes, 1995). Interestingly, animal models of depression show similar immunoreactivity (Kubera et al., 1996; Song & Leonard, 1995). For example, chronic mild stress-induced anhedonia was associated with several alterations in immune parameters, such as increased IL-1 production. Furthermore, in another model of depression, learned helplessness produced by inescapable shock, intracerebroventricular administration of IL-1 receptor antagonist attenuated the depressive-like response (Maier & Watkins, 1995).

Direct administration of IL-2 or interferons to human patients produces a depressive episode, which resolves immediately following termination of this immunotherapy (Capuron, Ravaud, & Dantzer, 2000; Dieperink et al., 2000; Meyers, 1999). Importantly, this depressive syndrome is attenuated by anti-depressive treatment (Musselman et al., 2001). Consistently, in a within-subject, cross-over double-blind study, we have recently demonstrated that endogenous release of cytokines by endotoxin (lipopolysaccharide) in human volunteers, produced affective symptoms (i.e., depressed mood and anxiety) and memory disturbances. Moreover, cytokine levels in the blood of the subjects were positively and significantly correlated with the depressive symptoms (Reichenberg et al., 2001). Together, these findings indicate that activation of cytokine networks is involved in mediating illness-associated depression, although more studies are needed to establish a clear causal role of cytokines in depression.

In animals, autoimmunity and infection with a wide range of pathogens, or alternatively, the administration of pro-inflammatory cytokines result in an elevation of body temperature and a cluster of behavioral alterations that were collectively termed ‘sickness behavior’ (Avitsur & Yirmiya, 1999; Dantzer, 2001; Szechtman et al., 1997; Watkins & Maier, 1999). These include anorexia and loss of body weight, reduced motor activity, social disinterest, anhedonic behavior (e.g., reduced interest in pleasant activities such as consumption of sweet solutions, sexual behavior, and intracranial self stimulation), and cognitive impairments (particularly memory dysfunction). As will be discussed below, the symptoms that comprise this syndrome resemble the criteria for a depressive episode. Furthermore, this behavioral syndrome is attenuated by several types of anti-depressant drugs (Castanon, Bluthé, & Dantzer, 2000; Shen, Connor, Nolan, Kelly, & Leonard, 1999; Yirmiya, 1996; Yirmiya et al., 2001).

Together, these findings suggest that inflammatory processes and the production and secretion of cytokines mediate the depressive symptoms that are frequently associated with many physical diseases.

3. EAE is associated with a behavioral depressive-like syndrome

To study the underlying mechanisms of ‘depression due to medical condition’, it is advantageous to develop an animal model of the human condition. This issue was addressed earlier in MRL mice, which are autoimmunity prone and are considered to model the human disease systemic lupus erythematosus (SLE). Autoimmunity was associated with a sickness behavior syndrome and was related to cytokine production (Szechtman, Sakic, & Denburg, 1997). To further explore this association, we used another disease model, experimental autoimmune encephalomyelitis (EAE). EAE is a neuroautoimmune disease, in which CD4+ T helper cells are activated by exogenously administered myelin self-antigen, and induce an inflammatory response within the central nervous system. The inflammation and tissue damage result in neurological deficits, of which the most salient and easy to detect is ascending paralysis. Depending on the kind of myelin self-antigen, the species and the strain used, EAE might present as an acute reversible attack, or chronic relapsing-remitting disease (Gijbels, Engelborghs, & De Dein, 2000). Although not identical in every aspect, EAE is considered an established model of MS (Gijbels et al., 2000; Steinman, 1999).

In our laboratory, EAE was induced by proteolipid protein (PLP139-151) or myelin oligodendrocyte glycoprotein (MOG35-55), common myelin-associated proteins, and several behavioral parameters were assessed along the paralytic attack. We found that EAE mice displayed dramatic reductions in food and sucrose intake, body weight and social exploration during the acute phase of the disease. Importantly, sickness
behavior and muscle weakness were not correlated temporally, indicating that motivational changes, rather than motor dysfunction, account for the behavioral alteration (Pollak et al., 2000).

Phenomenologically, these behavioral alterations resemble depression symptoms. Depression is clinically defined by a set of behavioral and cognitive symptoms (DSM-IV, 1994), some of which can be modeled in animals (Anisman & Merali, 1999; Willner, 1994, 1997; Yirmiya, 1996). Anhedonia, the inability to experience pleasure, is one of the core symptoms of depression, and can be observed in animals when hedonic stimuli render less attractive. Sweet solutions are pleasant to mice, therefore, a reduction in sucrose consumption, as in the case of EAE, may reflect anhedonia (Willner, 1994, 1997). Moreover, the reduction in liquid consumption in EAE mice was specific to sucrose, since water consumption levels remained unchanged, suggesting an alteration in the hedonic value of sucrose (Pollak et al., 2000).

However, the validity of sucrose consumption as an hedonic measure in EAE should be further established since it might also reflect basic hunger motivation and because in previous research immune activation did not produce alterations in taste reactivity to sucrose (Aubert & Dantzer, 1998; Cross-Mellor, Kent, Ossenkopp, & Kavaliers, 1999). Among other reinforcing stimuli, social environment is less appealing to depressed patients, resulting often in social withdrawal. In medically ill depressed subjects, social disinterest may represent one of the valid criteria for the severity of depression (Clark, von Ammon Cavanaugh, & Gibbons, 1983). In our study, EAE mice showed less interest in social interaction, resembling this depressive symptom. Reduced eating and loss of body weight, which were observed in EAE mice, are also common symptoms of a depressive episode (DSM-IV, 1994). EAE is also accompanied by memory dysfunction (Yehuda, Rabinovitz, Mostofsky, Huberman, & Sredni, 1997), a cognitive impairment that characterizes depressive episodes (Austin et al., 1992).

In addition to the similarity in the behavioral symptoms, EAE and depression are associated with several common physiological alterations.

1. One of the most consistent physiological markers of depression is alterations in monoaminergic systems, such as lowered levels of the 5-HT metabolite 5-HIAA, which is considered to reflect hypoactivity of the serotonergic system (for review see Risch & Nemeroff, 1992). Moreover, cerebrospinal fluid (CSF) 5-HIAA levels predicted the cognitive symptoms of depression (Faustman, Faull, Whiteford, Borchart, & Csernansky, 1990). Reductions in 5-HT levels and damage to bulbospinal serotonergic axons were also documented in EAE (Krenger, Kabiersch, & Honegger, 1989; White, Samathanam, Bowker, & Wessendorf, 1990). Furthermore, EAE-associated increased shock sensitivity was reversed by administration of the 5-HT precursor, 5-HTP (White, White, Barnes, & Albright, 1973).

(2) Another consistent physiological marker of depression is an excessive hypothalamic-pituitary-adrenal (HPA) axis activity, reflected by several findings such as hypercortisolemia (for review see Plotsky, Owens, & Nemeroff, 1998). EAE as well, is associated with high levels of corticosterone. The alterations in glucocorticoid levels correspond to the course of the disease and have a critical role in the recovery of sick animals (del Rey, Klusman, & Besedovsky, 1998; MacPhee, Antoni, & Mason, 1989).

(3) As mentioned above, recent studies strongly suggest that immunological alterations are an important characteristic of depressed patients. Among the more consistent findings are higher levels of IL-1 and positive APPs. In EAE, pro-inflammatory cytokines, such as IL-1, were found to orchestrate the inflammation (Eng, Ghirnikar, & Ling Lee, 1996; Smith Begolka & Miller, 1998), and accordingly high levels are synthesized and secreted during exacerbations. APPs activity in the plasma and sites of inflammation within the CNS is also a characteristic of the acute phase of EAE (Du-Clos, Mold, Paterson, Alroy, & Gewurz, 1981; Hunter, Weston, & Bowern, 1991). Time course studies revealed that elevation of pro-inflammatory cytokines preceded the onset of the neurological symptoms, and that return to normal levels of cytokines preceded their clinical recovery (Kennedy, Torrance, Picha, & Mohler, 1992; Okuda et al., 1995). In fact, the dynamics of cytokine production precisely parallels the EAE-associated behavioral alterations (Pollak et al., 2000).

Taken together, both the behavioral and the physiological alterations that characterize EAE animals suggest that EAE attacks are accompanied by a depressive-like episode.

4. Similarities between MS-associated depression and EAE-associated behavioral syndrome

EAE and MS share many clinical, histological, and pathogenetic similarities, and therefore EAE
is considered by many researchers as an animal model of the human disease (Gijbels et al., 2000; Steinman, 1999). Although there are also several differences between the two conditions, they resemble each other sufficiently to suggest that similar observations may reflect identical mechanisms. Research over the last 20 years elucidated many interesting aspects of MSD. We sought to determine whether these aspects are also part of EBS, to validate the notion that this syndrome models MSD.

(1) Depression does not appear randomly along the course of MS, but tends to occur during attacks or exacerbations and to wane during remissions (for review see Patten & Metz, 1997). To address this point in EAE, a chronic relapsing-remitting form of the disease was induced by immunizing mice with PLP, and reexposing them to...

Fig. 1. EAE-associated sickness behavior. Female SJL/J mice (n = 8) were injected with lymph nodes cells activated in vivo and in vitro against proteolipid protein (PLP) as described elsewhere (Pollak et al., 2000). Several days following adoptive transfer of cells, mice presented a transient paretic attack. Ten weeks following the initial immunization mice were re-inoculated with 100μg PLP emulsified in complete Freund adjuvant, and 2 weeks later developed a relapsing-remitting disease. The neurological impairment was scored on a scale of 0–6, with 0 meaning no neurological signs, 1 meaning tail weakness, up to 6, which signifies the death of the animal. Body weight, food, and sucrose intake and social interest, reflected by the time spent in sniffing a con-specific juvenile in the home cage, were measured daily. EAE initial attack, as well as subsequent exacerbations, were accompanied by a reduction in body weight (A), consumption of food (B), sucrose solution (C), and social exploration (D).
the same antigen 10 weeks following the acute phase. As shown in Figs. 1A–D, EAE-associated reductions in food intake, body weight, sucrose drinking and social exploration were observed only during the initial acute phase and subsequent relapses, but not between successive relapses. Hence, both EBS and MSD accompany exacerbations and not intermittent periods.

(2) In many MS cases depression precedes the onset of the neurological symptoms and the diagnosis of the disease (Schiffer & Babigian, 1984; Sullivan, Weinschenker, Mikail, & Edgley, 1995). For example, a retrospective inquiry of MS patients' medical records revealed that in one sixth of MSD cases the diagnosis of depression preceded that of MS (Schiffer & Babigian, 1984). To examine the temporal relationship between the neurological and depressive symptoms in EAE, we monitored the behavior of EAE mice during the days preceding the onset of the motor dysfunction. We found that the reductions in the behavioral parameters preceded the onset of the neurological symptoms by 1–2 days (Pollak et al., 2000). As shown in Figs. 1B–D, the decrease in food and sucrose consumption, and the suppression in social exploration preceded the onset of detectable paresis in the initial attack as well as in subsequent relapses.

The findings in humans can be interpreted in two ways. Dysphoric emotional state might be involved in the immunopathological process and consequently may contribute to the pathogenesis of MS. Alternatively, the depressive symptoms may reflect the pathological process and should be regarded as an early symptom of MS. The finding that EAE, which is deliberately induced, is also preceded by a depressive-like episode suggests that the behavioral alterations are early manifestations of the disease.

(3) Depression is twice more prevalent in women than in men (DSM-IV, 1994). The results of studies on gender differences in MSD are inconsistent. A higher risk of depression in female MS patients was recently reported (Schiffer, Weitkamp, Wineman, & Guttmersen, 1988). In this study, affective symptoms were assessed in a sample of 56 MS patients reporting that 67.5% of the female patients suffered from depression or another affective disturbance, compared to 25% of the males. However, two earlier studies did not

![Fig. 2. Gender differences in EBS. EAE was induced in female and male SJL/J mice as described in Fig. 1. Behavioral parameters were measured daily. Clinical score was similar in both genders (A). The decrease in body weight (B) and sucrose intake (D) began earlier, but sustained for a shorter period in EAE males, reflected by a significant interaction between group and day of disease ($F(6, 54) = 2.87$ and $2.75, p < .05$, respectively). The reduction in food intake was greater in magnitude in EAE female mice (C), reflected by a significant group effect ($F(1, 9) = 9.99, p < .05$). The decrease in social exploration was slightly lower in magnitude and shorter in males (E), without reaching statistical significance. Data represent means ± standard errors.](image-url)
find gender differences in MSD (Minden et al., 1987; Schiffer et al., 1983).

To address this point in the animal model, EAE was induced in male and female SJL/J mice using adoptive transfer of lymph node cells, sensitized to PLP. Motor deficits, body weight, food, and sucrose intake, as well as social exploration, were evaluated daily. No differences between the genders were found in motor score (Fig. 2A). In contrast, the reduction in body weight, and the consumption of food and sucrose solution began earlier in males compared to females, but were significantly less prominent or shorter (Figs. 2B–D). Males also showed a small attenuation in the severity of social exploration reduction (Fig. 2E), which did not reach statistical significance. These findings indicate that gender differences are manifested in some, but not all, components of EBS. Thus, future studies should examine the hypothesis that female MS patients are more susceptible to specific MSD symptoms.

(4) Anti-depressant drugs can be used effectively in treating MSD, similarly to their effects on other types of depression. This conclusion is based on three clinical trials, which demonstrated the efficacy of tricyclic (desipramine), serotonin selective reuptake inhibitor (sertraline), and monoamine oxidase A inhibitor (moclobemide) anti-depressives to alleviate MSD symptoms (Barak, Ur, & Achiron, 1999; Schiffer & Wineman, 1990; Scott, Nussbaum, McConnell, & Brill, 1995).

In a preliminary study, we assessed the effects of chronic treatment with an anti-depressant drug on EBS. Female C57BL mice were administered daily with imipramine from the day of immunization with MOG. Two control groups were employed, one treated daily with saline and the other was not handled. All mice developed hyperacute EAE (haEAE), characterized by early onset, brain hemorrhages and high mortality rate. Differences among the groups were observed in survival rate and body weight loss. Survival rate was significantly higher in the imipramine compared to both saline and non-handled groups (Table 1). This finding is consistent with the results of a recent study, reporting that treatment with lofepramine, an imipramine derivative, was associated with a reduction in the number of brain lesions in MS patients (Puri et al., 2001). In our study, imipramine also attenuated haEAE-associated decrease in body weight. Data represent means ± standard errors.

Table 1
Effect of chronic imipramine on hyperacute-EAE-associated mortality

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of survival cases</th>
<th>No. of mortality cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-handled</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Saline</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Imipramine</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
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On day 0 and 7, female C57BL mice were immunized with 300μg myelin oligodendrocyte glycoprotein (MOG) emulsified in complete Freund adjuvant. In addition, mice were injected with 500μg pertussis toxin on days 0 and 3. Beginning on day 0, mice were either non-handled or injected daily with saline or 10mg/kg imipramine. Clinical score and behavioral alterations were evaluated daily. Statistical analysis revealed significantly unequal frequencies of mortality cases among the groups ($\chi^2(2) = 7.29, p < .05$), reflecting the attenuating effect of imipramine on EAE-associated mortality.

Fig. 3. Effect of imipramine on hyperacute-EAE-associated loss of body weight. EAE was induced as described in Table 1. ANOVA revealed a significant group by day of disease interaction ($F_{(11, 77)} = 2.1, p < .05$), reflecting the attenuating effect of imipramine on EAE-associated decrease in body weight. Data represent means ± standard errors.

5. Concluding remarks

The validation of an animal model consists of three components: face, construct, and predictive validity. Face validity refers to the phenomenological similarity of the animal condition to the
human condition it is supposed to model. Construct validity means that a theoretical rationale underlying the model will be consistent with the current knowledge of the human condition. Predictive validity demands that manipulations known to influence the human condition will have a similar effect on the model.

EBS can achieve these three kinds of validity. The EAE-associated sickness behavior symptoms resemble the depressive symptoms. Moreover, several behavioral characteristics of MSD (e.g., temporal relationship with the neurological symptoms) are found also in EBS (face validity). Depression is theorized to result from alterations in monoamines, HPA-axis and inflammatory processes, all of which were documented in EBS (construct validity). Finally, depression in general, and MSD in particular, respond to anti-depressive treatment. EBS should respond to the same treatment to achieve predictive validity. Our preliminary findings provide initial evidence for predictive validity, but more comprehensive studies should be conducted to determine the sensitivity and specificity of the response to anti-depressants.

The presence of animal models of illness-associated depression (SLE and MS) should permit and encourage further research to reveal the physiological and behavioral mechanisms underlying this pathological condition, and to help distinguish adaptive and maladaptive aspects of this syndrome. A better understanding of these mechanisms may result in the development of novel therapies for MSD in particular, and ‘depression due to medical condition’ in general.

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References


