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Cell-mediated immunity in patients with chronic fatigue syndrome, healthy control subjects and patients with major depression

A. LLOYD*†, I. HICKIE‡, C. HICKIE‡, J. DWYER† & D. WAKEFIELD†
*The Departments of †Immunology and *Infectious Diseases, Division of Medicine and the ‡Mood Disorders Unit, Division of Psychiatry, The Prince Henry Hospital, Sydney, Australia*

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SUMMARY

The chronic fatigue syndrome (CFS) is characterized by severe persistent fatigue and neuropsychiatric symptoms. It has been proposed that the abnormalities in cell-mediated immunity which have been documented in patients with CFS may be attributable to a clinical depression, prevalent in patients with this disorder. Cell-mediated immune status was evaluated in patients with carefully defined CFS and compared with that of matched subjects with major depression (non-melancholic, non-psychotic) as well as healthy control subjects. Patients with CFS demonstrated impaired lymphocyte responses to phytohaemagglutinin (PHA) stimulation, and reduced or absent delayed-type hypersensitivity (DTH) skin responses when compared either with subjects with major depression or with healthy control subjects ($P < 0.05$ for each analysis). Although depression is common in patients with CFS, the disturbances of cell-mediated immunity in this disorder differ in prevalence and magnitude from those associated with major depression. These observations strengthen the likelihood of a direct relationship between abnormal cell-mediated immunity and the etiology of CFS.

Keywords fatigue depression immunity anergy

INTRODUCTION

The chronic fatigue syndrome (CFS) is a disorder of unknown etiology characterized by extreme fatigue. Minimal physical activity exacerbates the fatigue which is associated with a prolonged recovery period lasting hours to days. This fatigue is associated with an inability to perform tasks that were previously achieved with ease, and is accompanied by a constellation of other symptoms including difficulties with concentration and memory, myalgia, headaches, sleep disturbance and frequently depression. The onset of the disorder often follows an infection or an infection-like illness. The syndrome has been variously named myalgic encephalomyelitis (ME; [1]), post-viral fatigue syndrome [2] and chronic Epstein–Barr virus infection [3].

Recent studies have demonstrated abnormal cell-mediated immunity in patients with CFS [2,4,5], and an immunological basis for the syndrome has been proposed [6]. Research over the last decade has suggested an association between depression and impaired cell-mediated immunity, although the results have not been consistent [7–9]; for review see Hickie *et al.* [10]. Neuropsychiatric symptoms including depression, as well as fatigue, are prominent features of the symptom complex in patients with CFS [11]. It has been postulated, therefore, that the abnormalities in cell-mediated immunity demonstrated in patients with CFS may be attributable to the concurrent psychiatric morbidity, particularly depression [12].

Although evaluation of the psychiatric status of patients with CFS does not support the contention that CFS is simply a depressive equivalent [11], patients with the non-melancholic subtype of major depression remain the most relevant and comparable psychiatric control group. These patients with major depression are similar to patients with CFS, in that they typically have an onset of illness in the third or fourth decade and are predominantly female [13].

Therefore, cell-mediated immunity was examined in patients with CFS, and compared with that of matched patients with major depression as well as matched, healthy control subjects.

Correspondence: Dr Andrew Lloyd, Laboratory of Molecular Immunoregulation, Building 560, Rm 31-19, National Cancer Institute, Frederick, MD, 21702, USA.

PATIENTS AND METHODS

Patients

Twenty patients with CFS who gave a history of marked exercise-aggravated muscle fatigue, of at least 6 months duration, associated with typical constitutional and neuropsychiatric symptoms [14] and in whom physical examination and investigation did not provide an alternative diagnosis were

enrolled. These investigations included: a blood count, differential count and film, renal, liver and thyroid function tests including estimation of thyroid-stimulating hormone, measurement of creatine kinase, serum immunoglobulins, anti-nuclear antibody and rheumatoid factor assays, as well as serological tests for syphilis, hepatitis B and HIV. The patients with CFS were individually matched to two control subjects, one with a major depression, the other a healthy volunteer. The three subject groups were sex-matched, while ages differed by no more than 3 years.

Subjects with major depression were interviewed by a psychiatrist (I.H.) and fulfilled DSM-II-R criteria for major (non-melancholic) depression [15]. All subjects completed the 30-item General Health Questionnaire (GHQ), a screening instrument for psychiatric morbidity [16].

The healthy volunteers were recruited from hospital and laboratory staff as well as members of the local community who had agreed to participate in studies of immunity.

Exclusion criteria

Potential subjects from any group were excluded if they had taken any psychotropic medication other than benzodiazepines, or any other medication known to impair cell-mediated immunity (including topical or systemic corticosteroids) in the 2 weeks before testing. Subjects were also excluded if they had a history of any other physical illness associated with abnormal cell-mediated immunity (e.g. an auto-immune disease or a malignancy) or a recent viral infection (within the last 2 weeks). Healthy subjects were excluded if their score on the GHQ was greater than 5, indicating significant psychiatric morbidity. None of the subjects had undergone previous immunological evaluation.

Assessment of cell-mediated immunity

All subjects underwent venepuncture between 8 a.m. and 12 midday. T lymphocyte subset analysis was performed using flow cytometry (FACScan, Becton Dickinson, San Jose, CA) by standard methods [17]. The MoAbs used were directed against the CD3, CD4 and CD8 antigens (Ortho Diagnostic Systems, Raritan, NJ) to enumerate the total T lymphocyte population, the inducer and the suppressor/cytotoxic subpopulations respectively. In addition, the HLA-DR$^+$ subpopulation was determined using a commercially available MoAb (Ortho Diagnostic).

Delayed type hypersensitivity (DTH) skin responses were measured by trained operators using a commercially available kit (CMI Multitest, Merieux, France). Results of the DTH skin testing were categorized according to previously determined reference ranges generated by the examination of healthy adult populations [18]. Using this range, a cumulative induration diameter of <10 mm for males and <5 mm for females is designated as an 'hypoergic' response. Cutaneous anergy is indicated by the absence of an induration response to all seven of the test antigens.

Lymphocytes were stimulated with phytohaemagglutinin (PHA) (Phaseolus sp, Burroughs Wellcome, Slough, UK), using a standard method [19]. Quadruplicate cultures were used for each dose of PHA. A dose-response curve for the final concentrations of PHA minus the background activity (at 0 μg/ml) was plotted for each subject (Δct/min). The plateau response (at 0·375 or 0·75 μg/ml) was selected from the response curves to compare the patient and control groups.

Table 1. Peripheral blood lymphocyte counts in patients with CFS, matched subjects with major depression and healthy control subjects

	Absolute number of cells $\times 10^3$/mm^3*				
Subjects	Lymph.	CD3	CD4	CD8	HLA-DR‡
Controls ($n=20$)	2·2 (0·5)	1·5 (0·5)	1·1 (0·4)	0·6 (0·1)	0·36 (0·18)
Depression ($n=20$)	2·2 (0·5)	1·4 (0·4)	1·0 (0·4)	0·5 (0·2)†	0·30 (0·13)
CFS ($n=20$)	2·0 (0·5)	1·3 (0·4)	0·9 (0·3)	0·4 (0·2)†	0·31 (0·18)

* Mean (1 s.d.).
† $P<0·05$ paired t-test *versus* healthy control subjects.
‡ Mononuclear cells displaying Class II MHC antigens.

Statistical analysis

Paired two-tailed t-tests were utilized for comparison of variables between the patients with CFS and their individual control subjects. χ^2 analyses with Yates correction for small numbers where appropriate, were used to compare the DTH skin responses. Analysis of variance for repeated measures (ANOVA) was used to compare the dose-response curves obtained in the PHA assay.

RESULTS

Demographic features and psychiatric status

The 20 patients with CFS included 10 males and 10 females, with a mean age of 36·7 years (s.d. 11·6 years, range 17–60 years) and a median duration of illness of 34 months (range 18 months to 15 years). The mean age of the subjects with major depression was 36·5 years (s.d. 10·9 years) and of the healthy subjects was 37·8 years (s.d. 11·4 years). Twelve of the patients with CFS (60%) and none of the healthy control subjects had scores on the GHQ of 5 or above indicating significant psychiatric morbidity [16]. The mean score on the GHQ in the subjects with CFS was 13 (s.d. 8) in comparison with the patients with major depression who had a mean score of 27 (s.d. 9; $P<0·01$).

Lymphocyte counts

Minor differences in the lymphocyte subset counts were demonstrated only in the CD8 counts, which were lower in the patients with CFS and the subjects with major depression in comparison with matched healthy subjects ($P<0·05$ for each analysis; Table 1). These differences were non-significant if allowance was made for the number of variables tested (5), according to Bonferroni's inequality. There is a similar non-significant trend towards lower CD3 and CD4 lymphocyte counts in patients with CFS (Table 1).

DTH skin responses

Significantly more patients with CFS than subjects with major depression or healthy subjects had abnormal DTH skin responses. Ten of the 20 patients with CFS had abnormal results,

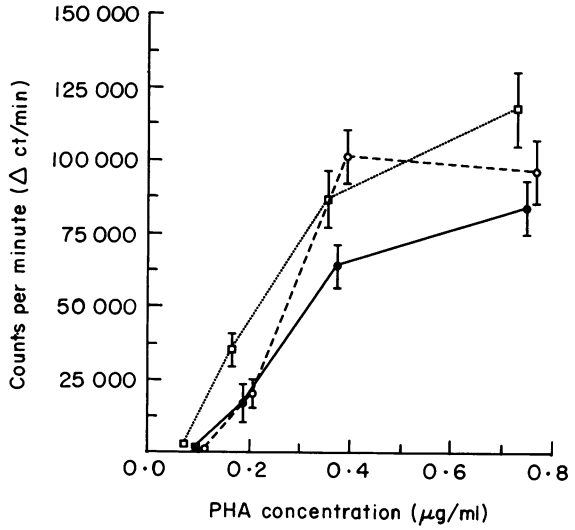

Fig. 1. Phytohaemagglutinin (PHA)-induced lymphocyte stimulation in patients with CFS (●), subjects with major depression (□), and healthy control subjects (○). Each point represents group mean ± s.e.m. ($n = 20$) of Δct/min values at various concentrations of PHA.

four patients (20%) had cutaneous anergy and six (30%) demonstrated hypoergic responses, in comparison with two subjects with major depression and two healthy control subjects (10%) who had hypoergic DTH responses ($\chi^2 = 5{\cdot}83$, $P = 0{\cdot}02$). Cutaneous anergy was only seen in the CFS group.

Lymphocyte proliferation in vitro

The proliferation of lymphocytes in response to PHA from patients with CFS (Fig. 1) was significantly reduced in comparison with that seen in healthy subjects ($F = 4{\cdot}47$, $P = 0{\cdot}005$; ANOVA), and subjects with major depression ($F = 2{\cdot}49$, $P = 0{\cdot}06$). The plateau response in this assay was lower in the patients with CFS than in the subjects with major depression ($P < 0{\cdot}05$, *t*-test), who demonstrated the highest mean plateau response. The dose-response curve for the patients with CFS was shifted to the right in comparison with the healthy control subjects and the subjects with major depression.

DISCUSSION

This study has demonstrated abnormalities in cell-mediated immunity in patients with CFS when compared with healthy subjects and with subjects with major depression. No significant difference was detected in the measurements of cell-mediated immunity between the healthy subjects and those with major depression. Therefore, the evidence of disordered cell-mediated immunity found in the closely matched patients with CFS should not be attributed to concurrent depression.

Although the link between depression and immunological disturbance is commonly cited, the studies on which this association is based were uniformly small in size, inappropriately included medicated subjects, and demonstrated significant differences only in the pattern of lymphocyte responses to mitogen stimulation (for review see [10]). The evidence for the association has recently been cast into doubt by the report of Schleifer *et al.* [9] who performed the largest study of this kind, and found no substantial differences in measures of humoral or cell-mediated immunity between 91 unmedicated patients with major depression and matched control subjects. More recently, we have demonstrated that abnormalities in standard measures of cell-mediated immunity, including cutaneous anergy, may be present but are restricted to severely affected, hospitalized patients with the melancholic subtype of depression [35]. Psychiatric morbidity in patients with CFS has not been found to resemble that seen in patients with the melancholic subtype of major depression [11].

Chronic fatigue syndrome is commonly reported to follow acute viral infection [2,3,14]. In only a minority of cases of CFS in the reported literature has the initial infectious agent been documented, with a range of common viral infections implicated as apparent precipitants for CFS, including varicella [20], Epstein–Barr virus [21], rubella [2], mumps and enteroviruses [22]. The pattern of abnormalities in cell-mediated immunity documented in patients with CFS in this and other studies [2,4,5] is also seen in association with acute viral infection, including T cell lymphopenia, cutaneous anergy and impaired lymphocyte proliferation in response to mitogen [23,24]. It is possible therefore that a disordered immune response associated with these precipitating infections fails to clear viral antigen, or alternatively a persistent and inappropriate immune response even in the absence of antigen may give rise to the symptoms of CFS [6]. In addition, deficits in the number and function of natural killer (NK) cells have been reported in patients with CFS [5,25,26], thus raising the possibility that this important initial immune defense against viral infection may also be disturbed.

Chronic and excessive production of cytokines such as interferon-alpha (IFN-α) have been proposed as a pathophysiological mechanism for the symptoms of CFS [6]. T cell lymphopenia [27], impaired lymphocyte responsiveness to mitogen [28] and cutaneous anergy [29] may be produced by administration of IFN-α. Therapy with IFN-α has been associated with a syndrome of adverse effects marked by fatigue and neuropsychiatric symptoms suggestive of CFS [30].

Both psychiatric morbidity and abnormal cell-mediated immunity are prevalent in patients with CFS, suggesting either that both may *result* from a disorder of the CNS, or that an immunological disorder outside the CNS may directly affect cerebral function, potentially via the interaction of cytokines with specific receptors within the brain [31]. Studies of the pathophysiological basis of 'fatigue', the cardinal symptom in patients with CFS, have demonstrated that muscle performance under controlled laboratory conditions (including optimal motivation) is essentially normal [32,33] but that perceived exertion may be abnormal [34]. It is likely therefore that this symptom also, and perhaps the disorder itself, is generated and maintained by an immunopathological process within the CNS.

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