

A systematic review and critical evaluation of the immunology of chronic fatigue syndrome

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Abstract

Objective: Immune dysfunction in patients with chronic fatigue syndrome (CFS) has been widely but inconsistently reported. Traditional reviews of the literature have produced a variety of conclusions. We present the results of the first systematic review of the subject. **Methods:** EMBASE, MEDLINE and PSYCHINFO databases were searched, and leading researchers in the field were contacted. Inclusion criteria were applied, and studies were then divided into groups based on the quality of their methodology. Study results were collated and described. **Results:** Studies ranged

widely in quality. There was an inverse association between study quality and finding low levels of natural killer cells, suggesting that the association may be related to study methodology. On the other hand, reports of abnormalities in T cells and cytokine levels were not related to study quality. **Conclusions:** The conclusions of this systematic review differ from a recent traditional narrative review of the immunology of CFS. No consistent pattern of immunological abnormalities is identified.

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Introduction

Chronic fatigue syndrome (CFS) is characterized by disabling physical and mental fatigue, lasting at least 6 months, without an apparent physical cause [1]. The aetiology of CFS is unclear, but many have suggested a role for infection, and for changes in the immune system. Papers reporting immunological changes in CFS are numerous. However, taken as a whole, the body of literature is inconsistent and, in places, contradictory. Few firm conclusions have been drawn.

What are the reasons for this? Strober has suggested several: using groups of patients with differing primary symptoms and differing duration of illness, failing to control for potential confounding factors and using different laboratory procedures when analysing samples [2].

Several reviews of the immunology of CFS have been published. Buchwald and Komaroff [3] found “evidence of diffuse immunological dysfunction... it has not been shown that immunologic findings explain... the symptomatology of CFS.” Similarly, Wessely et al. [4] concludes that “there is evidence of some abnormality of immune function, but such changes are inconsistent, non-specific and rarely correlate with the clinical condition” and Lloyd and Klimas [5] that “no clear conclusions can be drawn from the data.” Most recently, Patarca-Montero et al. [6] have written that “CFS is associated with immune abnormalities that can potentially account for physio- and psychopathological symptomatology” and also that “assessment of immune status reveals a heterogeneity among CFS patients.”

No systematic review has been completed. The importance of systematic reviews—which can be loosely defined as reviews in which there is a methods section—is established beyond doubt if unbiased conclusions are to be reached [7,8]. Our group has already shown that nonsystematic general reviews in the field of CFS are associated with bias, influenced by professional

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affiliations and country of origin of the authors [9]. The aim of this paper is a systematic review of the immunology of CFS.

Method

EMBASE, MEDLINE and PSYCHINFO databases were searched from 1966 to 2000 using the strategy presented in Fig. 1. Additional checks were made with key investigators and using a personal database of 3000 CFS references maintained by one of the authors in which

- 1 immu\$.mp. [mp=title, abstract, registry number word, mesh subject heading]
- 2 cytokine\$.mp. [mp=title, abstract, registry number word, mesh subject heading]
- 3 1 and 2
- 4 1 or 2
- 5 antibod\$.mp. [mp=title, abstract, registry number word mesh subject heading]
- 6 1 or 4
- 7 interleukin\$.mp. [mp=title, abstract, registry number word, mesh subject heading]
- 8 4 or 7
- 9 antigen\$.mp. [mp=title, abstract, registry number word mesh subject heading]
- 10 8 or 9
- 11 lymphocyte\$.mp. [mp=title, abstract, registry number word, mesh subject heading]
- 12 10 or 11
- 13 HLA.mp. [mp=title, abstract, registry number word, mesh subject heading]
- 14 12 or 13
- 15 MHC.mp. [mp=title, abstract, registry number word, mesh subject heading]

Fig. 1. Search strategy.

• Specific hypothesis (2)	—
• Blinded (2)	—
• Control group	—
▪ Age	—
▪ Sex	—
▪ Inactivity	—
▪ Depression	—
▪ Therapy	—
▪ Diurnal rhythm	—
• Samples treated appropriately (cells fresh, serum frozen)	—
• Functional study	—
• Recognised immunological markers	—
• Immunologist as author	—
Total	(/15)

Fig. 2. Rating proforma.

immunological measures are coded after visual inspection (in contrast to MESH terms). Contact was made with leading researchers in the field to check for missing/unpublished studies.

Certain a priori criteria were set for inclusion in the review: subjects had to have been suffering from medically unexplained, disabling or distressing fatigue as a predominant symptom for longer than 6 months; a sample size of greater than 10 was required; articles had to be written in English. The latter was because of a lack of access to translation facilities. Where it was unclear if two or more papers from the same group represented different samples, authors were asked for clarification.

Studies were rated by ML on a 15-point scale devised after consultation with an immunologist (MP) and a psychiatrist with special experience in CFS (SW). This is shown in Fig. 2. Methodological quality factors were derived from a general knowledge of the literature on bias (for example, the importance of blinding), added to a specific knowledge of the subject under review.

If a clear a priori hypothesis was stated two points were awarded. A statement in the paper indicating that the investigators were blinded to the experimental groups also

received two points. In neither of these categories were only one point awarded. We felt that providing a clear hypothesis, and blinding as well as evidence that serum and cellular samples were treated appropriately were important markers of a sound scientific method. The presence of an immunologist as a co-author, and the study of well recognized immunological markers using a functional design, all, we felt, were likely to lead to a study which was of a higher technical quality. A point was awarded if one of the authors of a study was clearly described in the paper as an immunologist. A further point was given if the immunological parameters studied were known from the scientific literature to be of direct relevance to the pathogenesis of other immune mediated diseases. We acknowledge that these criteria are arbitrary, but, in the absence of any other scale or instrument, there was no alternative but to develop our own checklist before commencing the review.

Some have suggested that the conflicting literature on the immunology of CFS can be partly explained by the effects of confounders. Hence, studies were awarded one point if a control group was included, and then were more carefully examined for evidence that specific factors had been controlled for. These included simple variables such as the age and sex of subjects. Many prescribed medications have an effect on immune function, hence, a point was awarded if this had been controlled for [10–12]. Depression is common in CFS, and depressed patients have an altered immune response, hence, a point was awarded if studies had either excluded patients with co-morbid depression, or had controlled for its presence [13]. Many immunological variables have been shown to exhibit diurnal variation, and a point was awarded to studies that had controlled for this [14]. Physical inactivity has been shown to have an effect of immune function, and a further point was given if studies made an attempt to control for this [15].

Results

Over 390 studies were located in the original broad literature search. However, when located many of these were only peripherally concerned with CFS, and many others had a sample size of less than 10. Fifty-eight articles met the inclusion criteria. Although one study was principally concerned with Gulf War veterans, subjects were also stated to meet criteria for CFS and hence this was included [22].

Before rating the studies, it was decided to analyse the results in three groups: those studies rated as 10 or above (10 studies), those rated between 5 and 10 (44 studies) and those rated less than 5 (4 studies). After the studies had been rated, it was apparent that a large number were in the middle group. Therefore, this group was split into 2 with those studies rated 8 or 9 (26 studies) and those rated 5, 6 or 7 (18 studies) reviewed separately.

Table 1 summarises the studies that were reviewed.

T cells: quantity and function

T (CD3⁺) cells play a major role in the acquired immune response. There are two major types: cytotoxic (CD8⁺) cells and helper (CD4⁺) cells. Cytotoxic T cells recognize antigen presented on MHC Class I molecules, and are found to be increased in number in many chronic viral infections. Helper T cells produce cytokines in response to antigens presented on MHC Class II molecules on specialised antigen presenting cells. For both types of T cells, antigen exposure causes activation-induced changes, which include increased expression of HLA-DR, CD5, CD11b, CD25, CD28, CD29 and CD38 molecules. Lymphocyte migration is facilitated by adhesion molecules such as CD11a, CD54 and CD58. Chronic exposure to antigen, as in chronic infection, causes CD45RA⁺ (“naive”) cells to change to CD45RO⁺ (“memory”) cells.

Studies rated 10 or more

Eight studies presented data. Four found no difference between CFS subjects and controls in the number of T cells or the presence of activation markers [16–19]. Natelson et al. [20] showed a reduced number of T cells generally and CD8⁺ T cells in particular, in CFS subjects, which they described as “T suppressor cells.” Straus et al. [21] found a reduced percentage of CD4⁺ CD45RA⁺ cells, and increased expression of CD29, CD54 and CD58 markers on CD4⁺ CD45RO⁺ memory cells in CFS patients. Zhang et al. [22] divided his CFS subjects into “gradual” or “sudden” onset (“gradual onset” referred to subjects becoming ill over 1–2 days) and found different changes in each group. Gradual onset subjects had higher numbers of CD8⁺ CD38⁺ cells than controls, i.e., activated cells. Sudden onset subjects had reduced numbers of CD8⁺ CD11b⁺ cells. The precise functional phenotype of these cells is not known. Gulf war veterans with CFS had an increased number of CD4⁺ cells, but showed no difference in markers of activation. In his study of monozygotic twins, which were discordant for CFS, Sabath et al. [23] found that the twins with CFS had an increased number of CD4⁺, CD8⁺, CD45RA⁺ and CD45RO⁺ cells bearing the CD62L activation molecule. There was no difference between twins in the total number of CD3⁺, CD45RA⁺ or CD45RO⁺ cells.

Studies rated 8 and 9

Fifteen studies presented data. In two studies, Lloyd et al. [24,25] reported low levels of T cells in CFS subjects. Hickie et al. [26] reported 30% of his CFS subjects having abnormally low CD8⁺ counts. Peakman et al. [27] noted a correlation between increased fatigue and low CD8⁺ numbers. However, the actual percentage of CD3⁺ cells, CD4⁺ cells, CD8⁺ cells and activated subsets of CD4⁺ cells were normal. The percentage of activated CD8⁺ cells was increased. Klimas et al. [28] reported a reduced percentage of T cells carrying CD8⁺ markers. Eight

Table 1

Ref no., year	Cases				Controls			
	Number	Definition used	Duration of illness	Population used	Number	Population used	Variables matched for ^a	Immunological parameters studied
[16], 1997	26	CDC 1988 ^b	Median 5.3 years	General population	52	General population	A, S, DR	T cells, IFN- γ , TGF- β , IL-1, IL-2, IL-6, B cells, IgG, IgA, IgM, IgE, NK cells
[17], 1998	71	CDC 1994 ^c	> 6 months; < 5 years	CFS clinic	109	General population; out-patients with depression or MS ^d	I, D, DR	T cells, IgG, IgA, IgM, IgE, NK cells
[18], 1990	10	CDC 1988	N/S ^e	N/S	10	Hospital staff	A, S	T cells
[19], 1992	20	CDC 1988	Median 2.6 years	N/S	40	General population; hospital staff	A, D, T, DR	T cell
[20], 1999	30	CDC 1988	> 6 months; < 6 years	N/S	106	General population; out-patients with depression	A, S, D, DR	T cells, IL-1, IL-2, IL-4, IL-6, IL-10, IL-12, B cells, NK cells
[21], 1993	18	CDC 1988	Median 7.4 years	N/S	27	General population; fatigued patients not meeting CDC definition	A, S, I, T, DR	T cells
[22], 1999	111	CDC 1994	N/S	Register of Gulf War veterans	87	General population; Gulf War veterans	A, S, I, D	T cells, IFN- γ , TNF- α , IL-2, IL-10, NK cells
[23], 2000	22	CDC 1994	N/S	Twin Registry	31	Monozygotic twins discordant for CFS	A, S, D, T, DR	T cells, NK cells
[24], 1990	49	CDC 1988	Median 3.9 years	N/S	Results compared against normal values		A	T cells
[25], 1989	100	CDC 1988	Median 3.1 years	N/S	100	Hospital staff	A, S, T, DR	T cells, IgG, IgA, IgM
[26], 1992	33	N/S	Median 4 years	Specialist fatigue clinic	Results compared against normal values		A	T cells
[27], 1997	43	Oxford criteria ^f	Mean 4 years	Specialist fatigue clinic	Results compared against normal values		A	T cells, NK cells
[28], 1990	30	CDC 1988	N/S	N/S	86	N/S	A, S	T cells, NK cells
[29], 1998	44	Oxford criteria	N/S	Specialist fatigue clinic	20	Hospital staff	A, T, DR	T cells, B cells, NK cells
[30], 1991	20	N/S	N/S	GP referrals	35	GP referrals	A, S	T cells, B cells, IgM, NK cells
[31], 1998	57	CDC 1988	N/S	N/S	34	General population; hospital staff	A, S, DR	T cells, B cells
[32], 1991	10	N/S	N/S	N/S	10	N/S	A, S, DR	T cells, TNF- α , TGF- β , IL-1, IL-2, IL-4, IL-6, B cells, NK cells
[33], 1996	76	Oxford criteria	Median 7 years	Specialist CFS clinic	66	Neighbourhood controls	A, S, T, DR	T cells, TNF- α , TGF- β , IL-1, B cells, NK cells
[34], 1999	20	CDC 1988	> 6 months, < 6 years	Specialist CFS clinic	14	General population	A, S, T, DR	T cells, TNF- α , IFN- γ , IL-2, IL-4, IL-10, IL-12, B cells, NK cells

[35], 1994	21	CDC 1988	Median 6.2 years	N/S	21	Hospital staff	A, S, DR	T cells, IFN- γ , IL-2, IgA, IgE, NK cells
[36], 1991	18	Oxford criteria	1–20 years	Muscle clinic	18	N/S	A	T cells, IFN- γ
[37], 1991	147	CDC 1988	1–5 years	N/S	50	Hospital staff	A, D	T cells, B cells, NK cells
[38], 1998	18	CDC 1994	1–3 years	N/S	18	General population	A, S, T	T cells, IFN- γ , IL-4
[39], 1990	30	CDC 1988	Mean 4 years	Specialist CFS clinic	Results compared against normal values		A	T cells, B cells, IgG, IgA, IgM, IgE, NK cells
[40], 1993	30	CDC 1988	Median 3 years	Specialist CFS clinic	30	N/S	A, S	T cells, B cells, NK cells
[41], 1994	40	CDC 1988		Specialist CFS clinic	35	Hospital staff; blood donors	A, S	T cells, NK cells
[42], 1990	26	N/S	Median 3.5 years	GP referrals	18	Non fatigued out-patients		T cells, IL-2, NK cells
[43], 1992	35	CDC 1988	>1 year	Infectious diseases clinic	20	Blood donors		T cells, IFN- α , IFN- γ , IL-1, IL-2
[44], 1991	20	CDC 1988	Mean 1.2 years	N/S	20	Hospital staff; patients with AIDS	A, S	T cells, B cells, IgG, IgA, IgM, NK cells
[45], 1997	20	CDC 1988	N/S	N/S	40	Hospital staff	A, S, DR	T cells, NK cells
[46], 1995	70	CDC 1988	N/S	N/S	53	Blood donors	DR	T cells, TNF- α , TNF- β , IL-1, IL-2, IL-4, NK cells
[47], 1994	56	CDC 1988	N/S	N/S	46	Hospital staff		T cells, B cells, NK cells
[48], 1998	27	N/S	N/S	N/S				T cells
[49], 1991	35	CDC 1988	N/S	N/S				T cells, B cells, IgG, IgA, IgM, NK cells
[50], 1997	16	CDC 1988	N/S	Specialist CFS clinic (women only)	16	N/S	A, S, I, T, DR	IL-1
[51], 1994	10	CDC 1988	N/S	Specialist CFS clinic	10	Hospital staff	A, S, D, T	TNF- α , TGF- β , IL-1, IL-6
[52], 1997	93	CDC 1988	N/S	Specialist CFS clinic	176	Blood donors; patients with SLE ^s and MS	A, S, D	TGF- β
[53], 1994	70	CDC 1988	N/S	N/S	53	Blood donors	A, S	TNF- α , TNF- β
[54], 1989	27	CDC 1988	N/S	N/S	25	N/S	A, S	TNF- α , IFN- α , IFN- γ , IL-1, IL-2
[55], 1994	12	CDC 1988	Median 5 years	N/S	13	Hospital staff	A, S, DR	TNF- α , IFN- α , IFN- γ , IL-1
[56], 1996	47	CDC 1988	Median 4.5 years	Specialist CFS clinic	47	Neighbourhood controls	A, S, DR	TNF- α , TGF- β , IL-1, IL-6
[57], 1997	29	CDC 1994	1–5 years	N/S	15	General population		IFN- α
[58], 1999	240	N/S	N/S	N/S	240	Medical out-patients	A	TNF- α
[59], 1991	25	CDC 1988	N/S	N/S	25	Depressed patients; others awaiting an elective myelography or with an aseptic meningitis	DR	TNF- α , IFN- α , IFN- γ , IL-1
[60], 1989	104	CDC 1988	N/S	N/S	22	N/S		IL-2
[61], 1998	18	CDC 1988	N/S	Specialist fatigue clinic	41	Depressed patients; others with “allergies”	A, S	TNF- α , INF- α , IL-1, IL-10
[62], 1989	16	CDC 1988	N/S	N/S	11	N/S		IL-1
[63], 1997	98	CDC 1988	Median 5.4 years	Specialist CFS clinic	105	Blood donors; fatigued patients not meeting CDC definition	A, I	IL-2, IL-6

(continued on next page)

Table 1 (continued)

Ref no., year	Cases				Controls			
	Number	Definition used	Duration of illness	Population used	Number	Population used	Variables matched for ^a	Immunological parameters studied
[64], 1995	579	CDC 1988/ Oxford criteria/ Australian criteria ^h	N/S	Specialist CFS clinic	147	Blood donors	A, S	T cells, IgG, IgA, IgM
[65], 1991	58	Oxford criteria	1–20 years	Muscle clinic	37	Hospital staff		IgG, IgM
[66], 1990	78	N/S	Median 4.2 years	N/S	71	General population; hospital staff	A, S, DR	IgG
[67], 1996	46	CDC 1988	Median 7.1 years	Specialist CFS clinic	50	Blood donors	A, S	IgG
[68], 1988	12	N/S	N/S	N/S				IgG, IgA, IgM
[69], 1996	26	CDC 1988	Mean 4.6 years	Specialist fatigue clinic	20	Hospital staff	A, S, DR	NK cells
[70], 1994	10	CDC 1988	N/S	General population	45	General population; fatigued patients not meeting CDC definition	A, S, T, DR	NK cells
[71], 1998	212	CDC 1988	N/S	N/S	30	Hospital staff	A, S	T cells, NK cells
[72], 1994	20	CDC 1988	N/S	N/S	50	N/S	DR	NK cells
[73], 1991	23	N/S	>1 year	N/S	19	N/S		NK cells

^a A: age, S: sex, I: inactivity, D: depression, T: therapy, DR: diurnal rhythm.

^b Holmes G, Kaplan J, Gantz N, et al. Chronic fatigue syndrome: a working case definition. *Ann Intern Med* 1988;108:387–9.

^c Fukuda K, Straus S, Hickie I, Sharpe M, Dobbins J, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. *Ann Intern Med* 1994;121:953–9.

^d Multiple sclerosis.

^e Not stated.

^f Sharpe M, Archard L, Banatvala J, et al. Chronic fatigue syndrome: guidelines for research. *J R Soc Med* 1991;84:118–21.

^g Systemic lupus erythematosus.

^h Lloyd AR, Hickie I, Broughton CR, Spencer O, Wakefield D. Prevalence of chronic fatigue syndrome in an Australian population. *Med J Aust* 1990;153:522–8.

studies reported normal absolute levels [27,29–35]. CD4⁺ counts were low in two studies [25,31] but normal in eight others [27,29,30,32–36]. Markers of activation were raised in three studies; Landay et al. [37] found increased numbers of activated CD8⁺ cells; Lloyd et al. [25] found increased HLA-DR⁺ expression and Peakman showed increased numbers of CD8⁺ CD11b⁺ cells although a large number of other activation markers was normal [27]. Swanink et al. [33] found reduced 11b expression and Hassan et al. [29] found reduced numbers of CD8⁺ CD28⁺ cells in CFS subjects. Klimas et al. [28] showed a reduced percentage of cells expressing CD4⁺CD45RA⁺. Three other studies did not confirm this [27,29,38]. Activation markers were normal in two studies [32,38] and, as with Peakman's paper, studies that showed abnormal levels of one activation marker often showed normal levels of other such markers.

Studies rated 5 to 7

Six of the nine studies in this category found the number of CD8⁺ cells to be normal in CFS patients [39–44]. See et al. [45] found numbers to be lower in CFS subjects. Patarca et al. [46] found an increased percentage of T cells with the CD8⁺ marker. Four studies showed CD4⁺ numbers not to differ between CFS subjects and controls [39–41,45]. Gold et al. [42] found an increased proportion of T cells to be carrying the CD4⁺ marker in CFS patients. In CFS patients, three studies showed raised levels of activation markers [40,41,47]. In particular, both Tirelli's and Barker's papers showed raised HLA-DR⁺ levels on T cells. In contrast, See et al. [45] found lower mean numbers of CD5 and CD11a markers on T cells in CFS patients. Two closely related studies by Tirelli et al. [40,41] found reduced levels of CD4⁺ CD45RA⁺ cells in comparison with normal controls. CD4⁺ CD45RO⁺ numbers were normal. In Peterson's study [39], the number of CD4⁺ CD45RA⁺ cells was normal. All the studies that measured markers of activation found differences between CFS patients and normal controls.

Studies rated less than 5

Wagner showed CFS subjects to have low numbers of CD8⁺ cells but raised levels of activation markers [48]. Hilgers et al. [49] found normal levels of CD8⁺ and CD4⁺ cells.

Summary

Data on T cell quantity and function presents a complicated picture. Four of the eight highest rated studies demonstrated no differences between CFS subjects and controls [16–19]. The four others showed a reduced number of T cells in CFS [20], a reduced percentage of CD4⁺ CD45RA⁺ memory cells and increased levels of activation molecules on CD4⁺ CD45RO⁺ cells [21], increased CD62L activation marker on CD4⁺, CD8⁺,

CD45RA⁺ and CD45RO⁺ cells [23], and increased CD8⁺ CD38⁺ cells in Zhang et al.'s "gradual onset" subjects [22]. Overall, these results suggest a trend towards increases in T cell activation. Fifteen other studies showed no change in T cell numbers [27,29–35,39–44,49], six demonstrated low levels in fatigued subjects [24–26,28,45,48] and one found the opposite [46]. As regards activation molecules, in the group of studies rated eight or nine, two found no differences [32,38], three found increased levels in CFS subjects [25,27,37] and two reduced levels [29,33]. Four out of five studies in the five- to seven-point group showed differences between CFS subjects and controls. Three found increased levels [40,41,47], and the other reduced levels in CFS patients [45]. Three other studies suggested reduced levels of memory cells in CFS subjects [28,40,41].

Cytokine levels

Cytokines are soluble proteins, which act as a signal between the cells of the immune system and other cells of the body, including other immune cells. They are produced by T cells and other immune cells in response to an immunological insult. Briefly, of the many cytokines known, IL-1, IL-2, IL-4, IL-6, IL-12, TNF and IFN- γ are proinflammatory. IL-10 and TGF inhibit the proliferation of immune cells. Some have suggested that it is the effect of cytokines on various organs, which could be an important mechanism in producing the symptoms of CFS. Possible causes of reduced cytokine production are a mild immunodeficiency or hyper-differentiated T cells which may be hypo-responsive to stimulation.

Measuring serum cytokine levels is difficult as the sensitivity of assays varies, the half-life of cytokines is short and decay may occur during storage. Some studies have measured in-vitro cytokine production by stimulated lymphocytes.

Studies rated 10 or more

Of the four studies in this category, two compared serum cytokine levels in CFS subjects and normal controls. Both showed no differences across a range of cytokines including IL-1, IL-2, IL-4, IL-6, IL-10, IL-12, TNF- α , TNF- β and IFN- γ [16,20]. Mawle et al. [16] also showed no differences after lymphocytes were stimulated in-vitro with phytohaemagglutinin (PHA). Cannon looked at in-vitro production of cytokines over the course of the menstrual cycle and found that the production of IL-1 β changed in CFS subjects but not in controls [50]. Zhang et al. [22] showed that Gulf War veterans with CFS had increased serum levels of IL-2, IL-10, TNF- α and IFN- γ when compared with veterans not suffering from the condition.

Studies rated 8 and 9

In the 16 studies in this group, an increase in the serum level of TGF- β was the most noted abnormality. Two studies reported this, in each case using the same bioassay

for TGF- β activity [32,51]. Bennett et al. [52] found similar levels in CFS patients and controls. Surprisingly, in Chao's study [32], CFS patients had lower levels of TGF- β than controls when lymphocytes were stimulated by lipopolysaccharide (LPS). This was not the case when they were stimulated with PHA. Raised levels of TNF- α and TNF- β were reported in CFS subjects by Patarca et al. [53]. In contrast, seven studies found no such increase [32–34,51,54–56]. Swanink et al. [33] showed lower levels of TNF- α production from the lymphocytes of CFS patients stimulated by LPS than controls. Rasmussen et al. [35] demonstrated raised levels of IFN- γ from CFS patient's lymphocytes stimulated by PHA and LPS. In contrast, Visser et al. [38] showed reduced levels of IFN- γ after the LPS stimulation. Three studies did not show any difference between serum levels of IFN- γ when CFS subjects were compared with normal controls [34,54,55]. Vojdani et al. [57] found increased levels of IFN- α in CFS subjects. Data on IL-1 β levels was equally contradictory; Chao et al. [32] showed raised levels of IL-1 β when lymphocytes were stimulated by LPS; Swanink et al. [33] demonstrated the reverse and four studies showed no abnormal IL-1 β serum levels [51,54–56]. Four of the 13 papers recorded no differences across a wide range of cytokines [34,54–56].

Studies rated 5 to 7

Among the nine studies reporting data, Patarca et al. [46] and Moss et al. [58] both showed raised levels of TNF. Lloyd et al. [59] found levels to be normal. Levels of IFN- α and IFN- γ were recorded as normal in two papers [43,59]. Interestingly, Lloyd also measured IFN- α levels in the cerebrospinal fluid of CFS patients. CFS patients had higher levels than patients awaiting an elective myelography, but lower levels than patients diagnosed with aseptic meningitis [59]. Cheney et al. [60] found IL-2 levels to be raised in CFS subjects, but Gold et al. [42] found patients with CFS to produce less IL-2 when their cells were stimulated with PHA. One other study found IL-2 levels not to differ when compared with controls [43]. Other abnormal findings presented in single studies: decreased IL-10 levels in CFS patients [61] and raised levels of IL-1 α [43] and IL-1 β [46] in subjects with CFS. In contrast, two out of nine studies found no abnormal cytokine levels [62,63].

Summary

No clear differences were demonstrated between CFS subjects and normal controls as regards cytokine levels. In the studies rated as 10 points or higher, the two papers that looked at a wide range of cytokines did not reveal any significant differences [16,20]. In the other studies, including Zhang's study of Gulf War veterans, there were more abnormal results. Raised levels of TGF- β levels were demonstrated in two papers [32,51], but not in two others [16,52]. Other abnormalities could rarely be replicated in more than one paper.

B cells: quantity and function

B cells produce immunoglobulins in response to infection following cognate interaction with helper T cells.

Studies rated 10 or more

Only two studies included information on B cells, and neither showed a difference in B cells numbers between CFS subjects and controls [16,20].

Studies rated 8 and 9

Seven studies looked at B cell quantity and function in CFS [29–34,37]. All showed no difference from normal controls.

Studies rated 5 to 7

Tirelli et al. [40] found an increased number of B cells in CFS subjects. Three other studies did not find this [39,44,47].

Studies rated less than 5

No differences were found by Hilgers et al. [49] in B cells numbers between CFS patients and controls.

Summary

Across all three groups, no differences in B cell quantity and function were shown.

Immunoglobulins

Immunoglobulins are produced by B cells. They bind to foreign antigens either to neutralize their effect, or to facilitate their uptake by phagocytes (opsonisation). They also trigger the "classic" complement cascade leading to the destruction of infected cells. There are different types of immunoglobulins: initially after infection IgM is secreted, IgA is present in mucous membranes, IgG facilitates opsonisation and fixes complement, and IgE is released in parasite infections and is involved in allergic reactions. Changes in IgG₁ and IgG₃ concentrations are considered to reflect CD8⁺ cell responses, and changes in IgG₂ and IgG₄ to reflect CD4⁺ responses.

Studies rated 10 or more

Two studies found no difference in immunoglobulin levels in CFS patients when compared with normal controls [16,17].

Studies rated 8 and 9

Four studies presented data. Lloyd et al. [25] found total IgG levels to be normal, although IgG₁ levels were low in CFS subjects. Bates et al. [64] found IgG levels to be higher than in controls. Two studies found normal IgM levels in CFS patients [25,65]. In contrast, Bates et al. [64] showed low levels. Similarly with IgA concentrations, two studies found normal levels [25,64], but Rasmussen et al. [35] showed low levels when compared with controls. Rasmussen also found low levels of IgE in CFS subjects.

Studies rated 5 to 7

Of the four studies in this category, Peterson et al. [39] showed reduced total IgG levels, and, in particular, low IgG₁ levels. Wakefield et al. [66], studying children with CFS, found total IgG levels to be normal, but reduced levels of IgG₁, IgG₂ and IgG₃. In adults, Bennett et al. [67] found increased levels of IgG₁. Total IgG levels were not measured. Gupta et al. [44] did not show any difference between CFS patients and normal controls in IgM, IgG or IgA levels. Peterson et al. [39] found normal levels of IgA and IgE.

Studies rated less than 5

Levels of IgG, IgA and IgM were normal in Hilgers et al.'s study [49]. Komaroff looked at a highly selected group of CFS patients with a past history of hyper or hypogammaglobulinaemia. Three out of the 12 patients had altered levels of immunoglobulins. There was no consistent pattern, and serum levels changed with repeated testing [68].

Summary

No differences in the level of immunoglobulins were found in the highest rated studies. In others, IgM levels tended to be normal. However, IgG levels and, in particular, IgG₁ levels tended to be low in CFS subjects [25,39,66].

NK cells: quantity and function

NK cells are lymphocytes, which lack a T or B cell phenotype. They carry either CD16 or CD56 markers and have nonspecific antiviral and antitumour activity. The number and activation status of NK cells increases in response to infection. However, it may vary over the course of the illness. Within population variation in the number of NK cells is large, and measurement is further complicated by a subpopulation of CD3 T cells carrying CD56 markers.

Studies rated 10 or more

Zhang et al. [22], looking at Gulf War veterans, showed a reduced percentage of NK cells in veterans with CFS. Another five studies found no differences between controls and CFS patients [16,17,20,23,69]. Mawle et al. [16] and Sabath et al. [23] showed no difference in the functional ability of NK cells, and See and Tilles [69] and Natelson et al. [17,20] found no difference in the number, or percentage of NK cells, in CFS subjects.

Studies rated 8 and 9

Of the 12 studies in this group, 7 showed no differences in the quantity or function of NK cells in CFS subjects [29,30,32–35,37]. Reduced activity was shown in three papers [70–72]. Klimas et al. [28] found reduced activity of NK cells when assessed by their ability to kill K562 tumour cells, but the number of cells was increased. Peakman et al. [27] found an increased percentage of lymphocytes to be NK cells in CFS subjects.

Table 2

Percentage of studies reporting any statistically significant difference between samples taken from CFS patients and controls

Immunological markers	Rated studies			
	>10	8–9	5–7	<5
T cells	50 (4/8)	60 (9/15)	66 (6/9)	50 (1/2)
Cytokines	50 (2/4)	54 (7/13)	78 (7/9)	–
B cells	0 (0/2)	0 (0/7)	25 (1/4)	0 (0/1)
Immunoglobulins	0 (0/2)	75 (3/4)	75 (3/4)	50 (1/2)
NK cells	17 (1/6)	42 (5/12)	86 (6/7)	100 (1/1)

(–) indicates no studies included in group.

Studies rated 5 to 7

One of the seven studies in this group showed increased numbers of NK cells in CFS patients [41]. An earlier Tirelli et al.'s [40] paper showed reduced CD56/CD57 markers in CFS patients. Gupta et al. [44] also showed this. Patarca et al. [46] and Barker et al. [47] showed reduced NK activity in CFS patients in contrast with Gold et al. [42] who demonstrated increased activity. Peterson et al. [39] found no abnormalities of NK cell structure or function.

Studies rated less than 5

Morrison found increased numbers of NK cells in CFS patients [73].

Summary

Apart from Zhang et al.'s study [22], none of the highest rated studies showed differences in NK cell quantity and function [16,17,20,23,69]. In contrast, six of seven studies in the five- to seven-point group showed differences, although in variable directions [40–42,44,46,47].

The number of studies that showed differences between CFS patients and controls was recorded in each rating group. The results are shown in Table 2.

To detect possible publication bias, these papers were further analysed by year of publication. Positive results were collated by the year of publication of the study. No clear pattern emerged, with papers showing differences between CFS subjects and controls being published throughout the 1980s and 1990s.

Discussion

By using a variety of sources, this literature review has sought to be comprehensive. The inclusion criteria served to limit the review to papers considering subjects with CFS conforming to standard operational definitions. Analysing studies in four groups had certain disadvantages notably that papers using different methodological approaches, and with different strengths and weaknesses, were considered as having a similar importance. However, the sheer number of papers limited the ability to present every finding individually.

We hoped that grouping papers by a priori quality scores would help to clarify the literature. We hypothesized either that the studies showing certain abnormalities, or alternatively finding no differences, would cluster in the higher quality papers. In general, the results support the latter hypothesis—for example, in the NK cell findings, it was clear that the better the study the less likely it was to find differences. This is important because many reviewers have noted, correctly, the large number of studies that do report low NK cells, and some have reached the conclusion that this is indeed a replicated, and hence valid, finding. However, consideration of the relative methodological quality suggests otherwise, with a clear trend for an inverse association between methodological quality and the probability of finding low NK cells. We suggest that any association between CFS and low NK cells is definitely not proven and may be erroneous. Our conclusions therefore differ somewhat from those reached by Patarca-Montero et al. [6] in their traditional review format.

Turning to other variables no clear pattern emerges. For T cell markers, there is no clear relationship between quality and findings—with positive results fairly evenly grouped according to quality. Hence, the introduction of quality criteria has not resolved the issue either way and genuine uncertainty remains.

Given the complexity and size of the CFS literature, this review demonstrates the importance of adhering to the principles of systematic reviews. Given that the CFS literature now contains papers with results to support virtually any conclusion about the nature of the immunological abnormalities, a traditional review without a priori definitions and quality ratings, may be prone to bias. Although this paper is concerned with immunological abnormalities, the same conclusion could be made for most of the CFS literature.

As regards the aetiology of CFS, our results do not rule out the possibility that the syndrome is caused, at least in part, by immunological dysfunction. In particular, changes in T cell number, function and activation markers were seen in some of the highest rated studies. However, the highest rated studies generally failed to show abnormal NK cell numbers or function, or abnormal cytokine levels.

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