Author's response to reviews

Title: Central pathways causing fatigue in neuro-inflammatory and autoimmune illnesses.

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Author's response to reviews: see over
Reviewer's report

Title: Central pathways causing fatigue in neuro-inflammatory and autoimmune illnesses.

Version: 1 Date: 1 December 2014

Reviewer: Yehuda Shoenfeld

Reviewer's report:

An interesting subject alluding to the immune stimulation responsible for C.F.S.

This article should be supplemented by adjuvant stimulation and CFS like in MMF (Macrophage faciitis syndrome, silicone induced CFS, and vaccines (i.e. aluminium) induced CFS (i.e. the "ASIA" syndrome).

This is now addressed as:

These parameters and elevated number of circulating T cells seen in premenopausal women may be one reason for the powerful prolonged activation of inflammatory pathways and adverse reactions to aluminium adjuvants seen in females following administration of a range of vaccines [383, 384]. The engagement of TLR receptors by aluminium, as well as the activation of the NLP3 inflamazome, could create a state of chronic inflammation and oxidative stress in a person with functional polymorphisms in immune genes as discussed above and hence could be a cause of Autoimmune Inflammatory Syndrome Induced by Adjuvants (ASIA) alternatively known as Schoenfield’s Syndrome [385-387]. The activation of TLR4 by silicon [388] could also explain the connection of this element with the development of ASIA and the chronic activation of TLRs can potentially explain many environmental contributions to the “mosaic of autoimmunity” [389].

Needs an explanation how by chronic stimulation of TLR lead to fatigue.
In the Intro we have added a section how chronic TLR activation may cause fatigue:

This association may be explained by chronically increased levels of pro-inflammatory cytokines and reactive oxygen and nitrogen species (ROS/RNS) produced by the TLR-radical cycle upon stimulation by PAMPs and DAMPs [4]. We have reviewed previously that some pro-inflammatory cytokines, including IL-1β, TNF-α and IL-6, and increased O&NS processes may cause fatigue in some vulnerable individuals [1, 4, 6, 7].

Refer to cytokonic differences found in patients with CFS.

We have added a section on cytokine changes in CFS:

]. We have reviewed previously that patients with CFS and ME show different cytokine profiles, e.g. a Th1-like pattern, with increased levels of IFNγ, IL-2, IL-12 and IL-2 receptor, or a Th2-like pattern, with increased levels of IL-10, IL-4 and IL-5, or combinations thereof [1].

Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

REFEREE 2

Overall this is a very interesting review piece. Nice basic justification for the work and for theoretical immunology as a whole. Very clearly articulated introduction justifying this rather ambitious endeavor. The hypothesis is clearly stated and well justified. My comments may be summarized as follows:

Major Compulsory Revisions:

1. A diagram illustrating the causal links being described in the text would be extremely helpful to the reader in order to help synthesize the significant amount of material. Perhaps one that captures the union of the component mechanisms while also highlighting how these might differ between MS and CFS for example.
We have added three figures, see Fig 1, 2 and 3 which describe the pathways discussed in the text.

2. Because the central theme involves immunity and metabolism it might be worth introducing some thoughts regarding the impact of gender and sex hormones on the nature of these imbalances.

We have added a section on sex- and gender-related effects as this is indeed important:

All disorders reviewed here, except Parkinson’s disorder, are more frequent in women than in men. For example, in patients with rheumatoid arthritis a 4-5 greater incidence is found in women than in men when less than 50 years old, whereas these differences are less pronounced in 60-70 years old individuals. The female predilection is also observed in depression, CFS, MS, Sjogren’s syndrome, and systemic lupus erythematosus [371-375]. In Parkinson’s disorder the male / female incidence rate ratio is 1.6 to 1 [376]. One main difference between Parkinson’s disease and the other disorders discussed here is that the autoimmune component is less pronounced in Parkinson’s disease. An increased incidence rate in women is observed in most autoimmune disorders [371]. Nevertheless, also in Parkinson’s disease autoantibodies are observed and they are associated with specific symptom profiles, including depression [377]. It is argued that these sex-related differences in incidence may be explained by endogenous sex-hormones.

Estrogen, progesterone and testosterone play important immunomodulatory roles and influence the quantity and pattern of cytokine secretion by antigen presentation cells and T lymphocytes and immunoglobulin production by B cells. Sex hormones also regulate the Th1/Th2 balance of the immune system, the production of regulatory T cells and the functionality of granulocytes and natural killer cells [378, 379]. An interested reader is referred to an excellent review by [380] for a detailed consideration of the mechanistic effects of sex hormones on individual classes of immune cells. In the light of the discussion above it also seems noteworthy that estrogen is neuroprotective in many animal models of neuroimmune and neurodegenerative disorders essentially by downregulating the expression of neuroinflammatory genes in glial cells such as those coding for elements of the complement system, proinflammatory cytokines and TLRs [381]. Thus, excessive oestrogens but less androgens may
favor activation of B cells, a Th2-like response and increased numbers of autoimmune cells and thus autoimmune responses [371]. Nevertheless, the precise effects of sex-or gender-related factors on the increased incidence of autoimmune-related disorders has remained elusive. Future research should delineate not only sex but also gender-related effects according the gendered innovations approach [382].

3. The authors perform an impressive review of these processes in MS, PD, CFS, depression, SLE, etc... It is not entirely clear however why these specific illnesses were selected. Fatigue is a fairly ubiquitous and non-specific symptom. Are these illnesses examples along a spectrum of imbalance involving various degrees of mitochondrial dysfunction, inflammation, OS and NOS? It would be helpful to perhaps explain this at greater length and cast the choice of these illnesses in the context of the underlying processes being discussed. Once again a descriptive diagram would be very helpful.

In the Introduction we now discuss that:

These specific disorders were selected as examples along a spectrum of imbalance involving various degrees of activation of immune-inflammatory and O&NS pathways, and mitochondrial and brain metabolic dysfunctions in systemic auto-immune, immune-inflammatory and neurodegenerative disorders.

We also added a new figure displaying the pathways being discussed:

Figure 1 shows the underlying processes and pathways associated with secondary fatigue, which we will discuss in the following sections.

4. Additional discussion describing the avenues of cross talk between brain and peripheral immunity would be helpful, for example, peripheral immune feedback to the brain via the vagus nerve. This would further reinforce the authors’ arguments and describe them in the context of an anatomical/physiological scaffold.
3.8. Cross-talk peripheral and CNS inflammation

There is now copious evidence that chronic or intermittent inflammation, as observed in the abovementioned systemic disorders, can worsen or trigger neuroinflammatory or neurodegenerative diseases via the induction of “primed microglia” [8][12]. Briefly, prolonged or intermittent peripheral inflammation and immune activation acts to prime microglia which thereafter become exquisitely sensitive to future inflammatory stimuli [8]. Once microglia have achieved this sensitized status, subsequent peripheral inflammation and proinflammatory cytokines production mediated by a number of insults (e.g. biotoxin exposure or pathogen invasion) provokes an exaggerated response from microglia and the production of excessive concentrations of neurotoxic molecules, such as NO, peroxinitrite, prostaglandins, COX2 and cytokines [6][7]. The secretion of these neurotoxins and alarmins leads to the activation of astrocytes and the combined activation of these glial cells provokes dysregulation of brain homeostasis, development of chronic neuroinflammation, and profound neurotoxicity. Both humoral and neuroendocrine routes mediate proinflammatory signalling to the brain. The neural route operates via the dorsal motor nucleus of the afferent vagus nerve [6]. The humoral route is facilitated by circulating proinflammatory cytokines that communicate their presence to the brain via direct and indirect routes. Such pathways involve engagement with specific transporters in the blood brain barrier (BBB), the activation of endothelial cells and macrophages, creating a mirror pattern of production on the adluminal side of the BBB, and passive diffusion into areas of the brain lacking a functional BBB (e.g. circumventricular organs) and thereafter into the glial limitans [1]. The cumulative effects of pro-inflammatory cytokines and activated astrocytes cause disruption of the BBB allowing abnormally high numbers of activated T cells and B-cells to circulate between the peripheral immune system and the brain, acting as more channels of communication between the peripheral and central immune system [13]. It should be noted that cytokines are able to diffuse from the CNS into the bloodstream as well as vice versa [13]. Finally, the presence of pro-inflammatory cytokines in the brain activates the hypothalamus instigating the cholinergic anti-inflammatory pathway designed to terminate the immune response [1][6]. These processes are depicted in Figure 2.
5. In the discussion, the inclusion of comments and examples describing current and upcoming therapeutic approaches targeting these interlinked illness components would be very interesting for the reader. For example, have antioxidative compounds shown benefit in any of these illnesses?

In the Discussion, we have added a section on possible effects of antioxidative compounds:

Multi-targeting these interlinked dysfunctions may show benefit in these diseases. For example, a number of antioxidant compounds have demonstrated efficacy in modifying pathways leading to chronic inflammation, oxidative stress and immune dysregulation at relatively high doses for a long duration [7]. N-acetyl-cysteine is an example of a multi-target therapeutic approach having the capacity to decrease the levels of ROS/RNS, increase the levels of cellular antioxidants, such as reduced glutathione, and normalize the production of proinflammatory cytokines and immune cell functions [397]. This supplement has demonstrated the capacity to improve fatigue and disease activity in SLE, CFS and major and bipolar depression [7, 398]. ω3 PUFAs and zinc are also very effective antioxidants and anti-inflammatory compounds and supplementation has produced clinical benefit in patients diagnosed with depression and chronic fatigue syndrome [7, 399, 400]. ω3 PUFAs show also a clinical efficacy in SLE and rheumatoid arthritis [398, 401, 402]. Curcumin, another nutraceutical with anti-inflammatory and antioxidative effects, is useful in the treatment of depression and rheumatoid arthritis [403, 404]. Coenzyme Q10 is another powerful antioxidant and anti-inflammatory compound which also has positive effects on mitochondrial function and which displays disease modifying effects in Parkinson’s disease and produced clinical benefit in patients with a diagnosis of CFS [56]. Other approaches aimed at upregulating antioxidant defences include methylfolate and dimethyl fumarate, with the latter displaying disease modifying properties in MS [140]. Methylfolate produces a similar quantum of benefit in MDD to antidepressants and can often be effective in treatment resistant depression [140].
6. As these mechanisms are extensively inter-linked, it might be good to remind the reader as a cautionary note that without a solid prospective timeline and known biochemistry, it remains difficult to distinguish causation from association. A discussion of hypothesized triggers might be helpful if such literature exists.

In the revision, this is now discussed as:

As these mechanisms are extensively inter-related, it should be underscored that without a solid prospective timeline and known systems biomedicine, it has remained difficult to distinguish causation from association. Therefore, future research should delineate a) the overwhelmingly complex and dynamic interactions between these different pathways and the intracellular networks that modulate them; and b) the multifactorial triggers that cause secondary fatigue by activating the networks / pathways in those disorders, including viral and bacterial infections, bacterial translocation, psychosocial stressors, exposure to adjuvants, nicotine dependence, sex- and gender-related factors, etc. Towards this end, a systems biomedicine approach is essential to delineate the genetic and molecular signature of fatigue in those disorders and the non-linear interactions between the many pathways, networks, and trigger and genetic factors that underpin secondary fatigue.

Minor Essential Revisions.

7. The authors mention elevated Th17 and Th1 activation in MS and refer to rituximab in support of this statement. Rituximab primarily targets B cells. This passage should be developed more to help the reader understand the mechanisms involved (section 3.1.2).

We now have discussed Rituximab as follows:

Sex effects may also determine responsivity to drug therapy as for example in MS. Thus, postmenopausal women are poorer responders to Rituximab than males of the same age [390, 391]. This might seem a little counter intuitive from the frame of reference that Rituximab exerts its effects mainly on the B cell population and that B cell levels do not appear to differ in postmenopausal women and age equivalent males to any significant extent [392]. However Rituximab also exerts modulatory effects on the T cell compartment [393]. Numerous
researchers have reported that the clinical benefits seen following the use of Rituximab in Rheumatoid Arthritis and other autoimmune conditions are associated with the antibody’s capacity to increase the expression of FOXP3 [394], suppress the expression of retanoic acid-like orphan receptors ultimately suppressing the production of Th17 T cells and IL-17 [395] and reducing the expression of cytokines by Th1, Th2 and Th17 T cells [396]. It is possible that the Th2 shift in the immune system seen in postmenopausal women negates the benefits of Rituximab on a Th1 / Th17 biased immune system [392]. The positive benefits of Rituximab and Natalizumab on MS [84, 85] is probably most easily explained by the modulatory effects of Rituximab, and likely Natalizumab, on the T cell compartment as well as their well documented effects on B cell depletion.

8. The authors should be careful to not confuse the use of CSF (cerebral spinal fluid) with CFS (chronic fatigue syndrome) and vice versa in the text.

OK, corrected

9. Please be aware of typographical errors in the occurrence of TNFa and IL-1b.

OK, corrected

Discretionary Revisions

10. Section 3.1.3: it is difficult to believe that the association between fatigue and changes in brain imagery is “…is clear cut and such a relationship is now an established phenomena…” when all previous studies were unfruitful and evidence has only recently been published in the author’s suggested reference 109 (2013).

This is addressed in the revision as:
While the relationship between self reported fatigue and neuroimaging changes is still a matter of considerable debate, the positive association between changes in brain activity and objective measures of cognitive fatigue is generally accepted [110, 111].