Summary Review: Cytokine signature associated with disease severity in ME/CFS 18th August 2017

There has been a lot of talk recently about a new research paper from Stanford University concerning cytokine signatures and disease severity. In this review, we hope to break down some of the main aspects of that study, question some of the science behind it, and explain a little more about what it means for the future of ME/CFS research.

- *Research published in Proceedings of the National Academy of Sciences of the USA (PNAS), 31 July, 2017*
- *Study authors: Jose G. Montoya, Tyson H. Holmes, Jill N. Anderson, Holden T. Maecker, Yael Rosenberg-Hasson, Ian J. Valencia, Lily Chu, Jarred W. Younger, Cristina M. Tato, and Mark M. Davis*
- *This summary review has been written by Charlotte (who has M.E. and holds a degree in biochemistry), who has been volunteering at the ME Association to help produce our recent series of lay reports on key research publications*

Montoya et al. published the paper on the 31st of July and it has since featured in a lot of scientific, as well as mainstream, media outlets. The study was significant and involved measuring the levels of 51 cytokines in 192 patients and 392 healthy controls.

Although the study found little overall difference in the average levels of each cytokine between the two groups, the researchers found an interesting correlation when they divided patients into subcategories based on symptom severity.

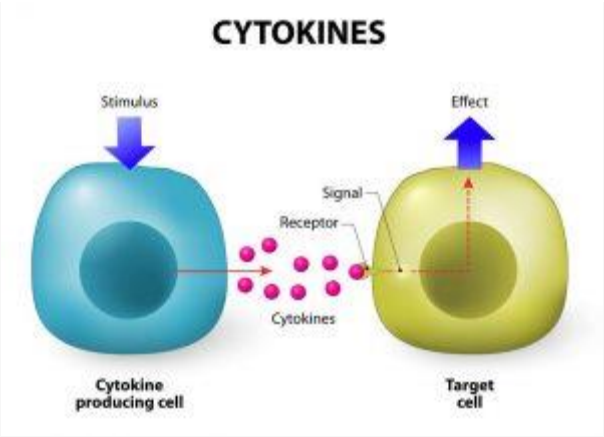
There have been many studies over the years that have suggested an inflammatory role in M.E. However, conventional markers of inflammation commonly used in general practice, such as CRP and ESR (markers your GP would look for) are **not** often elevated in M.E.

⇒ This suggests the profile of inflammation in M.E., you guessed it, is far from straight-forward.

Therefore, these researchers used an extensive panel of cytokines (not your run-of-the-mill markers) – analysed using the latest immune-profiling approach – to see if an abnormal panel of circulating cytokines could be identified.

What are Cytokines?

Cytokines are chemical messengers, or cell signalling molecules, that aid cell to cell communication of immune responses and stimulate the movement of cells towards sites of inflammation, infection and trauma.
There are many different types of cytokines with different names, based on which type of cells produced them, such as lymphokines, monokines and interleukins.
There are both pro- and anti- inflammatory cytokines, and cytokines are responsible for **triggering** fever, inflammation and pain during an infection, and/or can demonstrate immune-system activity etc.



The stars of the show

Only two cytokines were found to be different in patients compared to controls on average; TGF-β (TGF-Beta) was raised and Resistin was lowered.
TGF-β has been mentioned several times over the years in relation to M.E. – a recent review of papers involving cytokines and M.E. found TGF-β was reported as raised in 5 out of 8 studies, making this the 6th study to make this finding.

⇒ An Australian paper published earlier this year found **Activin**, a member of the TGF-β "superfamily", to be raised in M.E. patients, further confirming this to be a common finding.
It seems that we keep confirming what we already know; TGF-β was found to be raised in M.E. patients in a study in 1997, 20 years on and still the same kettle of fish.

The Stanford study, however, used the latest technology, was on a bigger scale, was more accurate and employed a new assay property called 'nonspecific binding'.

What is TGF-β?

Transforming growth factor beta is a cytokine that is primarily immunosuppressive (anti-inflammatory). It has many functions but of most relevance perhaps is that it suppresses inflammatory cytokines and is thought to have neuroprotective properties.

It is produced by many different cells, including Macrophages, a type of white blood cell that has a central role in the immune system, fighting against foreign bodies, cancerous cells and infections. TGF-β also helps in the regulation of T-cells, another key player in the immune system helping to fight infection. And it is involved in pathways important in the regulation of glucose and energy balance.

However, TGF-β may not be such a "good guy" as it has been shown to have pro-inflammatory roles. Although TGF-β suppresses inflammation on a systemic level (in the body as a whole), it can actually **stimulate** immune and inflammatory responses at the local level – and so it may be important to find out where these high levels of TGF-β are being produced.

TGF-β is involved in the development of cancer, which could help to explain why older patients with M.E. are often predisposed to certain types of cancer.

What is Resistin?

Resistin is a cytokine originally thought to be primarily produced by adipocytes (fat cells) which causes insulin-resistance and is linked to diabetes.

However, it has since been shown to also be released by many immune cells and is now thought to play important regulatory roles in a variety of biological processes; cardiovascular disease, autoimmune disease, cancer and inflammatory bowel disease. It is also known to stimulate other pro-inflammatory cytokines, such as IL-6 and TNFα.

The significant finding

What caused the most excitement, was that 17 cytokines were found to be linked to disease severity, and that 13 of them are classed as pro-inflammatory (See figure 1 – below).

Interestingly, the levels of these cytokines in mild patients were generally *lower* than that of the controls. The patients in the moderate category had levels similar to controls, and then the patients in the severe category had higher levels than the controls – creating an overall upward trend in relation to severity.

⇒ Preliminary results coming from a study at 'younger labs' – home to Dr Jarred Younger – are also finding similar results relating to severity

This upward trend can be known as "dose-dependence", meaning as the number of cytokines in the body increases, the number of symptoms – or the severity of symptoms and the impact that M.E. has on a person's health – increases.

⇒ It is unlikely that cytokines are *causing* the symptoms in M.E., considering the levels are *lower* than healthy controls in milder patients, who still have symptoms.

Instead, it is more likely that M.E. causes the altered cytokine profiles because of another mechanism. Furthermore, although these 17 cytokines showed a clear relationship with severity, they do not distinguish patients from healthy controls and so are unlikely to be used as diagnostic markers.

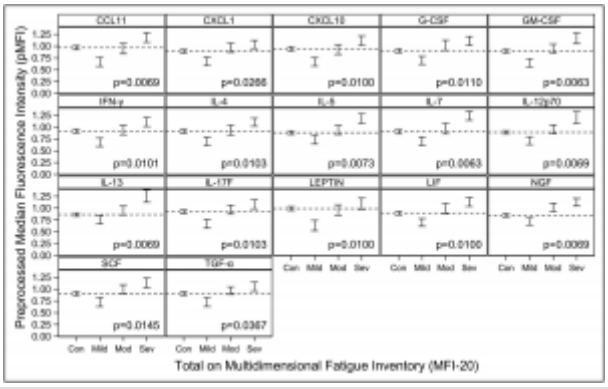


Fig 1. Shows the varying levels of each of the 17 cytokines associated with disease severity. Each line shows the values for controls, mild, moderate and then severe patients in an upward linear trend.

Quick statistics lesson; The "p-value" is showing the statistical significance of each of the trends; the **lower** the number (closer to 0.0001, further away from 1), the more significant the trend. The more significant something is, the more important, or worthy of further attention it is.

The cytokine that's also a hormone…

Leptin was one of the cytokines found to correlate with disease severity. Another study (from Dr Younger) also found fluctuating levels of Leptin to be associated with fatigue severity. Leptin plays a key role in energy homeostasis (stability) and lowered levels of Leptin are also associated with reduced cognition, as well as anxiety and depression.

⇒ Interestingly, Leptin levels are decreased by sleep deprivation, as well as low food input, which could again suggest that this lowered level is a *result* of M.E. and not a cause of it. However, as Leptin was only found to be lowered in Milder patients, this cannot be an explanation for all patients.

Adipokines (cytokines originating mainly from fat cells) have long been implicated as mediators of chronic inflammation and two of these were found to be important in this study; Resistin and Leptin. These adipokines have recently been implicated in neuroinflammation and neurodegenerative diseases and so could help explain the cognitive and neurological symptoms experienced in M.E.

⇒ However, the paper comments that it would have been helpful to have **BMI data** on the patients as Leptin levels greatly correspond to this – as they are produced by fat cells – but they did not collect this data and so correlations were not possible.

Unlike the earlier cytokine study from Hornig et al., this study did **not** find cytokine levels to be associated with the duration of illness, only severity.
However, this could have been due to the small number of patients who had the illness for under 3 years (30) compared to those who had it for over 3 years (162) – making uneven sample sizes which could have affected their data.
Therefore, they were unable to rule out the possibility of cytokine levels differing between different fatigue durations.

Theories relating to TGF-β and ME/CFS

– TGF-β – fighting our battles or leading an army against us?

The Stanford research presents two contrasting theories for the finding of raised levels of TGF-β:

> 'The TGF-β elevation in ME/CFS patients may represent down-regulation by these patients' immune systems **against** unremitting inflammation; if so, however, one would expect TGF-β levels to correlate with ME/CFS severity.'

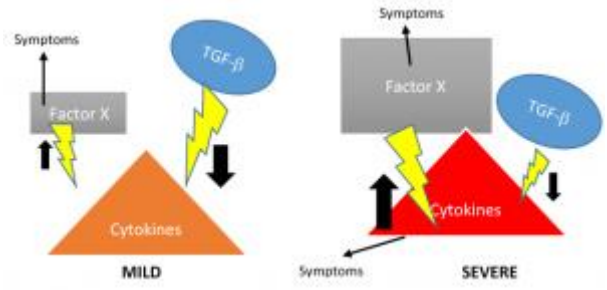
However, TGF-β is also known to be pro-inflammatory, thus a second theory:

> '…elevated levels of TGF-β in ME/CFS patients may actually be **detrimental** and may be a major factor in promoting relentless inflammation.'

So, we're not quite sure yet what TGF-β's role is and whether it's the good guy or not!

It could be that all M.E. patients produce TGF-β to try and ***suppress*** cytokine secretion, which is being produced in response to something else (let's call this "factor X") that also causes its own symptoms. Mild patients are successful in this suppression, whereas in the severe patients, cytokine production is so high (due to more of factor X or a greater immune 'challenge'?) that the production of TGF-β isn't sufficient enough to provide the same degree of suppression, leading to rising levels of inflammation, and adding to symptom severity.

We've tried to show this in the diagram below:



– Lipopolysaccharide

One contender for this "factor X", which has been studied in other papers (here and here), is a molecule called Lipopolysaccharide (LPS). High levels of circulating LPS have been found in M.E. patients, as well as in other infectious and autoimmune diseases.
LPS is an endotoxin released from bacteria during stress or infection and regulates the production of pro-inflammatory cytokines.

Could it be that milder patients are able to adapt to constant stimulation by LPS by down regulating the inflammatory response (possibly through the production of TGF-β), but LPS levels in severe patients are so high that down regulation still isn't enough?

It would be very interesting to measure levels of LPS in different severity subgroups to see if there is a correlation between the amount of LPS and disease severity.

Response to exertion?

Some have suggested that the differences in cytokine profiles between severities may not necessarily be there all the time, they might have in fact been showing a response to exertion.

Although the paper states the blood tests were taken at a baseline, after no physical or emotional stressors, the simple act of travelling to the lab to have blood tests and complete questionnaires may have been enough to put the severe patients above their personal exertion thresholds and trigger post-exertional malaise, whereas this was little exertion for the milder patients and so it did not trigger a response.

⇒ However, it would of course be very tricky to take measurements without ANY exertion for the most severe as it would be impractical (and costly!) to go out to each patient's home.

The critique

Although many are saying this study proves M.E. to be a physiological – and not a psychological or even psychiatric – condition, this is contradictory given that depression gives similar, if not worse, differences in cytokine profiles.

This is not to say that M.E. is a psychological or even a psychiatric illness, but that inflammation alone is probably not the cause and is more likely an **effect** of M.E. or of some other mechanism going on.

– Patient selection

As with all M.E. research, one must question the methods by which they deemed patients to have M.E.

⇒ For instance, in the fatigue questionnaire that was used, some of the M.E. patients answered that they **did not** experience unrefreshing sleep or post-exertional malaise, which many would regard as hallmark symptoms of CFS/ME

Additionally, the way in which the researchers categorised the severities may not have been the most accurate:

⇒ This was done using the "**multifunction fatigue index**" (MFI-20) questionnaire, which some argue may not be the most specific and sensitive test for measuring severity in M.E.
⇒ It is unclear how they decided the boundaries of each category – might there have been overlaps for example, and how would this have impacted the results?

It would have been useful to know how many patients there were in each category of severity and the extent to which this also might have affected the results.

⇒ For example, if there were only a small number in one category of severity, the results could be seen as a misrepresentation of cytokine levels – as it could skew the average in one direction or the other.

– Controls

Unlike other studies, this study was very rigorous in its controls; matching them very accurately by age, gender and race and correcting for these things in their data analysis. This is good scientific practice and would have helped greatly in increasing the accuracy of the results.

⇒ However, all the patients used in the study were **aged around 50**, which may have had an influence on the results and so it would have been useful to see the same cytokine profiles in a group of younger patients as this may not be a good representation of the M.E. population as a whole.

– Cross-sectional design

The study is cross-sectional in design, meaning it is only looking at a snapshot of cytokine levels at one moment in time. What we need are more **longitudinal studies**, building up a picture of fluctuating levels over a long period. More studies are also needed that examine cytokine levels "before and after" exertion.

Clark et al. recently carried out a study in the UK looking at cytokine levels before and after exertion.

They concluded there to be **no differences** in expression and that cytokine levels in general (with the exception of our old friend TGF-β) to be no different to that of controls.
However, the Clark study has been **criticised** for its poor execution and scientific errors, such as dividing the samples into batches that were looked at years apart by different lab technicians!
It would have been far more useful if this study had not been published, but had been repeated once the problems had been identified and corrected – enabling more accurate conclusions to be reached.
N.B. We are hoping to bring you a more thorough review of this research as soon as we can.

Conclusion: where do we go from here?

The paper concludes:

> **'Findings in this study provide further evidence that ME/CFS likely involves a systemic inflammatory process' and 'support the suitability of exploring immunomodulation as a primary or adjuvant therapy.'**

Although this study does not provide a diagnostic test for M.E., and it is doubtful that cytokine markers ever will, it has highlighted the importance of looking at different severities of M.E. – as different subgroups who present differing disease profiles – which may respond differently to treatment.

The research also provides further evidence for immune and inflammatory involvement in M.E. and supports the suitability of treatment using immunomodulatory therapies, such as that being investigated in Norway using Rituximab.

As usual, the findings are not straightforward and could leave scientists wondering what they all mean. They are significant and exciting, of course, albeit puzzling and adding to the mystery of a case that no one seems currently able to crack.

⇒ It is very likely that inflammation is just one of a multitude of contributing factors; a small piece of the puzzle that is slowly being pieced together, one break-through study at a time.

The Stanford Symposium

In a recent conference at Stanford, Dr Mark Davis, one of the lead researchers on the paper and a top immunologist, said:

> **"The increased cytokine levels that correlate with disease severity indicate a strong inflammatory component to M.E."**

He then went on to present his theory of an autoimmune component to ME, where our T cells (important players in the immune system that help to identify and destroy foreign cells) are actually attacking cells in our own tissues.

Many studies have found increased numbers of circulating cytotoxic CD8+ T cells (White blood cells that kill infected or damaged cells) bearing activation antigens; suggesting the body is actively fighting something.

- Interestingly, TGF-b and Leptin (Two of the cytokines found to be different in M.E.) have both been shown to have regulatory roles in T cells, further supporting this T cell theory.

In MS (multiple sclerosis), there are T cells activated which attack the myelin sheath (a protective layer around nerve cells) and Davis proposes a similar mechanism may be happening in M.E., but targeting a different cell type.

- Dr Davis then spoke of some research currently underway to try to identify which tissues these T cells are targeting, which sounds very promising!

At the very least, the wide press-release and positive media attention this study has received, along with the fact that it's published in a highly respectable journal, is very encouraging and provides hope for more funding and more biomedical research.

The Stanford study has certainly made its mark in the scientific world, grabbing people's attention, generating more interest in the disease among researchers and making the voices of M.E. sufferers that bit louder; things are looking up!

If you have found this information useful, then please donate, whatever you can afford, to help us continue with our work and make the UK a better place for people with ME/CFS.

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