MEA Summary Review: MAIT cells are Increased in Severe ME/CFS

By: Charlotte Stephens 15th April 2019

Dr. Jackie Cliff and colleagues from the CureME research team at the London School of Hygiene and Tropical Medicine have just published results from part of a National Institutes of Health (NIH) grant examining immune cell function in ME/CFS.

The team used samples from the UK ME Biobank, including 251 people with ME/CFS – 54 of whom were severely affected – 46 with MS and 107 healthy controls.

They looked at various aspects of immune function, including levels of antibodies against herpes viruses and the number and function of Natural Killer (NK) cells and T-cells.

Members from the ME Biobank Team with Lead Author Dr Jackie Cliff

However, their most significant finding was of increased levels of a subtype of T-cell called MAIT (Mucosal associated inverted T-cell) cells in severe ME/CFS patient samples.

In this review, we go into more detail about the findings from this latest piece of biomedical research, giving possible explanations and discussing what this means for future research.

Key Points

Cliff et al. (2019) Cellular Immune Function in ME/CFS

- The presence of antibodies against six herpes viruses, including Epstein-Barr, was no different between ME/CFS patients and controls.
- No differences were found in NK cell number or function between patients and controls, contrary to previous research findings.
- Some differences were found in the proportions of different subtypes of T-cells in patients with ME/CFS compared to controls.
- Highly increased levels of MAIT cells were found in the severely affected ME/CFS patients.
- The cause and the meaning of these findings is yet to be determined.
- MAIT cells have been implicated in a number of diseases, such as cancer, diabetes and rheumatoid arthritis.

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Introduction

Immune system dysfunction has long been suspected in ME/CFS, with many patients reporting an infection at the onset of their illness, as well as increased frequency of infections during the course of their illness, or in some, being seemingly immune to infections.

Yet, the results of immune studies in ME/CFS have been conflicting and inconclusive.

This is most likely due to the heterogeneous nature of the disease (many different subgroups of patients with different causes under the same diagnosis).

There have been no consistent findings of a persistent or chronic viral infection in ME/CFS.

Many patients report an infection as being the trigger for ME/CFS

Several immune abnormalities have been reported, including altered cytokines levels, abnormal T-cell activation and altered numbers or functioning of NK cells (Brenu *et al*. 2012; Hardcastle *et al.* 2015; Maes *et al*. 2012; Montoya *et al*. 2017; Rivas *et al* 2018; Strayer *et al.* 2015; Theorell *et al.* 2017)

However, many of these findings have been conflicting or have not been reproducible, mainly due to small sample sizes, differences in the characterisation of the patients and the use of different methods of looking at the cells. Because of this, no diagnostic immune biomarker has been identified (VanElzakker *et al*. 2018).

The CureME team wanted to investigate further into immune cell function in ME/CFS, in the hopes of contributing towards a diagnostic profile and also towards treatment avenues.

What did the study involve?

Blood samples and fatigue scale questionnaires were collected from all participants, split into 4 groups; healthy controls, multiple sclerosis (MS) controls, mild-moderate ME/CFS and severe ME/CFS.

The inclusion of an MS control group is useful as they represent another incapacitating illness who act as a good control for the physiological effects of reduced levels of physical activity. The immunological database for MS is also extensively documented and so can provide insight into disease pathways in ME/CFS by comparison.

Lead Author: Dr Jackie Cliff

The research team then used the blood samples to test for:

1. The presence of antibodies (what your body produces to identify and attack specific viruses) against six different Herpes Viruses, including Epstein-Barr Virus (EBV) and Cytomegalovirus (CMV).
2. Number, functioning and types of different white blood cells (immune cells), such as B cells, T cells and NK cells.

What were the main immune cells studied?

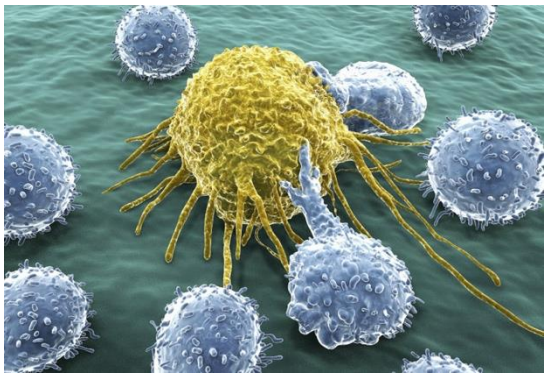
Natural Killer (NK) cells

Natural Killer cells (NK cells) are a type of white blood cell that comprise part of the immune system.

They are the "first response" team, acting like security guards on patrol; circulating around

the body looking for potential threats, such as viruses or cancer cells.

When they spot one of these invader cells, they "attack" by releasing chemical bombs (called perforins and granzymes), which punch holes in the cells, causing them to die (through a process called apoptosis) (Brenu *et al*., 2013).



NK cells (blue) attacking an invader cell (yellow)

Whilst they are busy containing and attacking the threat, they call in reinforcements (other immune cells) by producing chemical messengers (cytokines) and then await back up, in the form of T cells.

Several studies have found that NK cells have reduced cytotoxic activity (killing abilities) in ME/CFS. However, others have found no differences at all.

➢ We produced a detailed review in 2018 on the role of NK cells in ME/CFS

T-cells

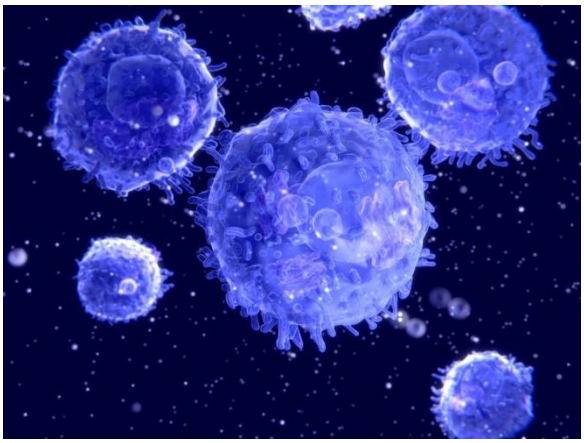
T-cells (named because they are made in the Thymus) are another type of white blood cell that play a very important role in the immune system. There are many different types of T cell and not all of their functions are known, but there are two general categories that they can be divided into; Killer T-cells and Helper T-cells.

Killer T-cells are like soldiers that scan the body for viral-infected or cancerous cells and seek and destroy them. Helper T-cells are like team leaders, they send out chemical messages to recruit and activate other immune cells that ingest germs or produce antibodies against a particular type of infection.

T cells can remember and respond to a specific germ they encountered years ago. T cells are also responsible for the immune responses that lead to allergic reactions and autoimmune diseases.

Problems with T cells have been identified in a number of diseases, such as cancer.

Differences in T cells (including changes in the expression of receptors on their surface and clonal expansion of subsets of T cells) have been previously reported in ME/CFS (Brenu *et al*. 2016; Proal 2017; Maes *et al*., 2015).

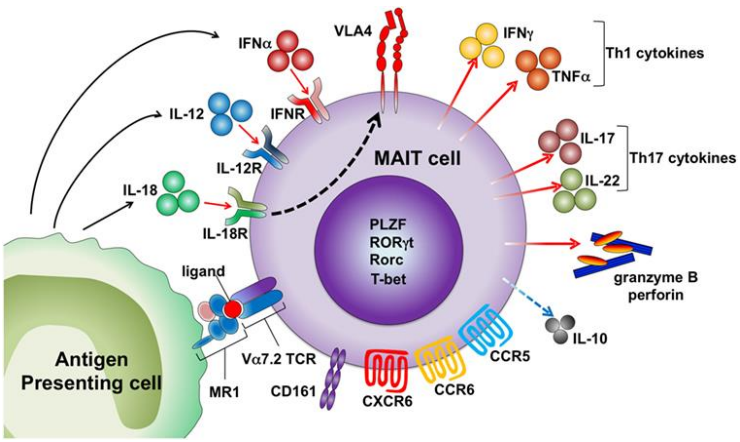


Differences in T cells have been previously reported in ME/CFS

MAIT cells

Mucosal Associated Invariant T-cell (MAIT) cells are an unconventional type of T cell that are relatively new in the research-world, so we are only just beginning to learn more about them.

They are found mainly in the liver, lungs and gut. They are highly abundant in the liver, making up 50% of the total T cells there, whereas MAIT cells usually account for around 1-10% of T cells in the blood. It is thought that MAIT cells may play a role in the immunological balance of the liver (Schubert *et al*. 2017).



MAIT cell responding to cytokines and producing cytokines.

MAIT cells target and attack cells infected with particular types of bacteria. They also respond to and produce pro-inflammatory cytokines (chemical messengers), which have been found to be raised in ME/CFS (Montoya *et al.,* 2017).

When someone has an infection, levels of MAIT cells in the blood will *decrease*. This is because they migrate to the infected tissue, so higher levels will then be found at the specific tissue that is infected, such as the lungs (Murugesan *et al.* 2017).

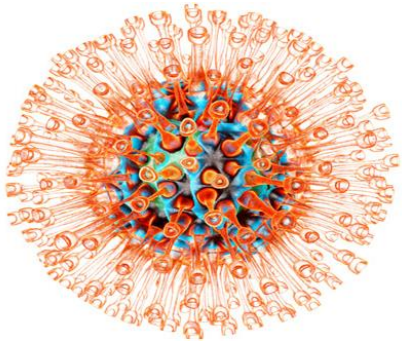
Abnormalities in MAIT cells have recently been implicated in many non-infectious diseases, such as cancer, diabetes, colitis and autoimmune diseases. For example, in Rheumatoid Arthritis, high levels of MAIT cells have been found located at the site of tissue inflammation, suggesting that they contribute to joint inflammation in rheumatoid arthritis (Chiba *et al*. 2012).

Results of the study

1. Herpes viruses

There were *no significant differences* in the levels of antibodies in the blood for the six Herpes Viruses tested for between the groups.

This means that, although people with ME/CFS do have antibodies against Herpesviruses, they do not have them in any higher numbers than seen in people with MS or healthy controls.



Herpes Virus cell

The researchers were looking to see if infection with any of the six herpes viruses tested for (Epstein-Barr Virus (EBV), Cytomegalovirus (CMV), Herpes simplex virus 1 and 2 (HSV1/HSV2), Human herpesvirus (HHV6) and Varicella-zoster virus (VZV)) were any more common in ME/CFS than in controls, and found that they are not.

This agrees with other studies that also found no differences in the presence of antibodies against herpes viruses in ME/CFS compared to controls (Rivas *et al*. 2018).

This is to be expected, given how common Herpes viruses are – it's been reported that up to 98% of the population are infected with herpes viruses, and so the presence of antibodies against them is not likely to distinguish ME/CFS from controls (Olsson *et al*. 2017 Rivas *et al*. 2018; Wald and Corey 2007).

However, antibody testing is not very specific; it only shows that you may have been infected with the virus at some point in your life, it does not show if the virus is still in you – in an active state or lying dormant.

Herpes viruses are known to lay dormant, sometimes in the nervous system, and are opportunistic, meaning they 'attack' or become active again when the bodies defence systems are weak, for example in periods of high stress or when we are feeling 'run down'.

So, just because the results here were no different doesn't mean to say that there is no herpesvirus involvement in ME/CFS pathology – they could still play a part in triggering and perpetuating the illness.

For example, Epstein-Barr Virus DNA sequences have been detected in B cells (a type of immune cell) in ME/CFS patients, that could reveal viral activity *even though the antibody profile was similar to healthy controls* (Scheibenbogen *et al.*, 2014).

In addition, a recent study found that a subset of ME/CFS patients exhibit increased expression of 'Epstein-Barr virus induced gene 2 (EBI2)' mRNA in immune cells and that these patients appear to have a more severe disease phenotype (Kerr, 2019).

> "The possibility remains that herpes viruses may be important in ME/CFS pathogenesis, that virus reactivation may trigger a worsening of symptoms, and that measurement of antibody titres to alternative viral antigens might provide a more relevant measure"
>
> Cliff et al. (2019) Cellular Immune Function in ME/CFS

2. Immune cells

The proportions of white blood cells that were B cells, T cells or NK cells *did not differ* significantly between the groups. Meaning, people with ME/CFS have 'normal' levels/amounts of B cells, T cells and NK cells.

However, this does not tell us anything about whether they are functioning correctly or if they have any gene mutations etc.

2a. NK cells

Further investigation into NK cells specifically revealed *no significant differences* in the different subtypes or level of functioning of NK cells between patients and controls.

This is contrary to several previous studies, which found altered levels of NK cell subtypes, as well as *reduced* NK cell function in ME/CFS. In fact, reduced NK cell activity is the most consistently reported finding in ME/CFS (Eaton *et al.* 2017).

"Based on previous reports of abnormal NK cell function in ME/CFS, this was an unexpected finding, although is consistent with a small number of previous reports of normal NK cell proportion and function."

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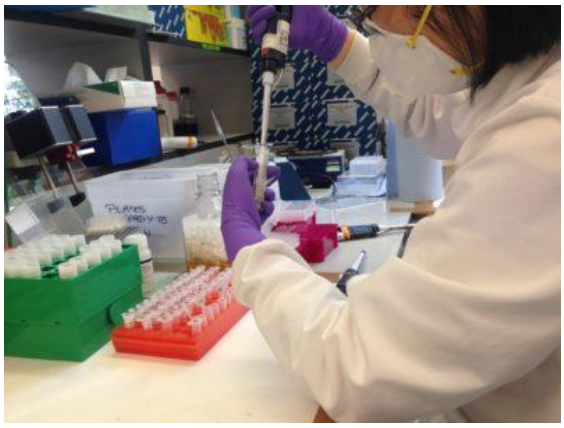
- We produced a detailed review in 2018 on the role of NK cells in ME/CFS

Differences in research methods

The conflicting findings of NK cell function in ME/CFS may be due to different research teams using different methods of looking at the cells.

This current study used isolated immune cells (separated from the blood – away from the plasma, serum and red blood cells) that had been frozen and thawed before analysis.

Whereas, previous studies that had found differences in NK cell function tended to use fresh, whole blood (not separated) samples (Rivas *et al*. 2018).



The current study used cells that had been frozen then thawed before analysis.

This is important, as it may mean that it's not the NK cells themselves that are 'faulty', but 'something' in the blood serum/plasma of people with ME/CFS that may be influencing NK cell function.

This mysterious factor in the blood has also been highlighted in studies by Prof Ron Davis, Dr Karl Morten and Dr Øystein Fluge (Fleming, 2018; Fluge, 2016; Johnson, 2017).

- Read an update on Dr Karl Morten's work at Oxford University, funded by the ME Association Ramsay Research Fund.

> "Timing of functional assays vis-à-vis PBMC isolation may explain differences, with an effect of soluble factors, such as cytokines, catecholamines, and hormones, **waning with time** from cell isolation from whole blood."
>
> "Such a discrepancy would therefore suggest that NK cells from ME/CFS patients in general are intrinsically **normal** but might be **responding to an abnormal external milieu**, rendering them hypofunctional (reduced functioning) **in whole blood or immediately after isolation**."
>
> Theorell et al., 2017

The Biobank researchers also commented on the fact that they used a long culture time, which may have also affected the results: *"In our system, we cultured PBMC for 18 hours to allow time for direct and indirect cell activation: it is possible that differences in degranulation or cytokine production may have been observed with a shorter stimulation period."*

Lastly, Rivas *et al*. (2018) found that the abnormalities observed in NK cells in ME/CFS were different between patients who reported an infection at the onset of their disease and those that didn't. It was not noted in this study whether the ME/CFS patients reported an infection that triggered their illness or not and this may be an important factor to take into account in future studies.

Association between CMV antibodies and NK cell function

Interestingly, the team also looked at the association between levels of antibodies against Cytomegalovirus (CMV) and the functioning of NK cells and found there to be a significant correlation.

They found that those with antibodies against CMV (in all groups – the controls, MS and ME/CFS) had more abnormalities in their NK cells compared to those that didn't have antibodies against CMV.

Therefore, they proposed that the presence of CMV antibodies needs to be assessed and taken into consideration when studying NK cells in ME/CFS as this has an impact on NK cell number and function and could have potentially affected the results of previous studies.

> "We have also confirmed that CMV seropositivity has a major impact on the phenotype and function of NK cells and T cells, underlining the paramount importance of assessing CMV and other herpes virus infection status in studies of human immune status."
>
> Cliff et al. (2019) Cellular Immune Function in ME/CFS

2b. T cells

The overall number and level of functioning of T cells was found to be *no different* between ME/CFS patients and controls.

On a more detailed look into T-cell subtypes, a few differences were found in the types of different T cells in ME/CFS patients, particularly the 'CD8+' subset, although it is not certain what this means yet.

> "The functional role and consequence of change within these different CD8+ T cell subpopulations remains to be determined."
>
> Cliff et al. (2019) Cellular Immune Function in ME/CFS

Previous studies have also reported differences in the CD8+ subset of T cells in ME/CFS (Brenu *et al.* 2016; Maes *et al.* 2015).

MAIT cells

The most significant finding from this study was in a particular subtype of T cell, called Mucosal Associated Invariant T-cell (MAIT). Levels of these were found to be **highly increased in severe** ME/CFS. This is the first study to report this finding.

The cause or the meaning of this finding is yet to be determined. Though the researchers noted that it is possible that the increase in MAIT cells could be driven by changes in the gut microbiome of people with ME/CFS, which should be investigated further.

Alternatively, they suggested that it could be related to *"other, as yet uncharacterised, changes in the immune system."*

"We observed highly significant differences between the groups in the proportion of T cells that are MAIT cells and the proportion of MAIT cells that express CD8."

"…analysis revealed that this was due to increased proportions of MAIT cells ($P < 0.001$), and particularly of CD8+ MAIT cells, in people with severe ME/CFS compared to healthy controls."

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In most diseases, including during an infection, circulating levels of MAIT cells are found to be ***reduced***, not raised (Xiao and Cai, 2017). However, MAIT cell findings have been conflicting in several diseases due to differing research methods, as well as the influence of age and medications.

Raised levels of MAIT cells have been found in those who take corticosteroid medication (Xiao and Cai, 2017).

Therefore, it could be hypothesised that the increased levels of MAIT cells seen in ME/CFS are due to exposure to raised levels of cortisol (a stress hormone produced by the adrenals) as a result of chronic activation of the hypothalamic–pituitary– adrenal axis (the central stress response system), as described by Loebel *et al.* (2016).

MAIT cells are activated by and also produce pro-inflammatory cytokines (chemical messengers), which have been found to be raised in the blood of people with ME/CFS (Montoya *et al*., 2017).

Interestingly, levels of MAIT cells in healthy individuals have been reported to increase following exercise (Hanson *et al.* 2019), suggesting that people with ME/CFS exhibit a post-exercise state when at rest.

This increase in MAIT cells after exercise could be due to the fact that cytokine levels rise after exercise and so the MAIT cells may be responding to the cytokines (Suzuki, 2018; Terink *et al*., 2018).

In healthy people, levels of MAIT cells have been reported to increase following exercise.

Stimulated MAIT cells increase TNFα expression after exercise, and TNFα has been found to be raised in ME/CFS (Moss *et al* 1999; Maes *et al.* 2012).

MAIT cells continue to be implicated in more and more non-infectious diseases, such as cancer, diabetes and autoimmune diseases (Xiao and Cai, 2017).

In the future, MAIT cells could represent targets for immunotherapy. However, we need a better understanding of their role in disease first.

The study of MAIT cells is still a relatively new field of research and so it is not clear what their exact role is yet, or their role in contributing towards the pathology of various diseases (Chiba *et al*. 2018).

Next steps/ Future research

This study was the first to find increased levels of MAIT cells in severe ME/CFS and the research team noted that their findings 'warrant further investigation'.

The findings need to be reproduced and confirmed, to make sure that this is a finding in all severe ME/CFS patients, not just the ones studied in this cohort. We also need to identify what might be causing the raised levels and whether they are contributing to the pathology of ME/CFS.

> "In future studies, the functional phenotype of these CD8+ MAIT cells in PWME could be ascertained, to determine if they may be contributing to disease pathology."
>
> Cliff et al. (2019) Cellular Immune Function in ME/CFS

The researchers also highlighted that the gut microbiome (types of bacteria in the gut) in ME/CFS should be investigated further, as a dysfunctional gut microbiome could be related to their findings and is something that has been previously highlighted in ME/CFS (Giloteaux L *et al*. 2016; Nagy-Szakal *et al.* 2017; Newberry *et al*. 2018).

Although their results cannot be used as a diagnostic biomarker alone, they could help to contribute towards a diagnostic profile along with other factors found by other studies.

"Eventually a biomarker signature might be developed for diagnosis of ME/CFS: in this context, the proportion of MAIT cells in peripheral blood had modest discriminatory capacity alone but might contribute to a combined factor signature."

"In this regard, testing of candidate biomarker signatures in large, independent, longitudinal validation cohorts will be vital, especially considering historically mixed reports of biological phenotype in ME/CFS."

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The Biobank group already have some other immune studies in ME/CFS underway, including a long-term study looking at changes in immune cells over time and in correlation with symptom fluctuation:

"Primarily, we are investigating the function of the MAIT cells, as the current publication is only about the proportions of this cell type in blood – so we are looking to see if they are cytotoxic and also whether they are producing cytokines."

"We are also conducting longitudinal studies, to see how cell proportions change with time and how these relate to clinical symptom changes: this for the MAIT cells and also the CD8+ T cell subsets."

"We are also conducting transcriptomic analyses to understand further any biological pathways which are affected in ME/CFS."

Lead Author, Dr Jackie Cliff, ME Biobank.

Conclusion

To summarise, no differences were found in the number of antibodies against herpesviruses in the blood between ME/CFS patients and controls, although this doesn't mean that herpes viruses are not involved.

There were also no differences found in the number of B cells or NK cells between ME/CFS patients and controls.

However, with the findings of changes in the subtypes of T cells in ME/CFS and increased MAIT cells in severe patients, the results of this study demonstrate immune system involvement in ME/CFS.

This is the first study to report the finding of highly increased numbers of MAIT cells in ME/CFS, which warrants further investigation to determine the cause and whether it is contributing to the disease pathology of ME/CFS.

This study leaves us with many questions, which will hopefully be answered by the ME Biobank team and the research they are continuing to produce.

"These abnormalities demonstrate that an altered immunological state does exist in ME/CFS, particularly in severely affected people. This may simply reflect ongoing or recent infection or may indicate future increased susceptibility to infection."

"Longitudinal studies of ME/CFS patients are needed to help to determine cause and effect and thus any potential benefits of immuno-modulatory treatments for ME/CFS."

Cliff et al. (2019) Cellular Immune Function in ME/CFS

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