Summary Review: Natural Killer Cells and Immune Dysfunction as a Biomarker in ME/CFS

Contents

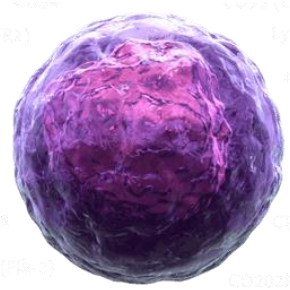
By Charlotte Stephens 16th August 2018.

Introduction

Association of T and NK Cell Phenotype with the Diagnosis of ME/CFS (Rivas et al. 2018)

A relatively large study from a Spanish research group was published recently that found differences in Natural Killer (NK) cell and T cell populations that could contribute to the diagnosis of ME/CFS.

This is by no means the first study to find Natural Killer (NK) cell abnormalities; there have been several, dating back to 1987. In fact, reduced NK cell function seems to be one of the most consistent findings of immune system dysfunction in ME/CFS.



Natural Killer Cell
Source: www.airicerca.org

However, this latest study is different in that it is much larger than previous efforts (149 participants, compared to 28-70 participants in previous studies).

Key highlights from the recent study

- NK and T cell subpopulations were significantly different in the ME/CFS patients compared to healthy controls
- These differences were used to generate a program that was able to correctly identify 70% of the people with ME/CFS from the controls
- The differences found could define a distinct immunological profile that could help in the diagnostic process, as well as contribute to the recognition of the disease and aid in more specific treatments

In this summary review, we hope to provide you with some background knowledge about Natural Killer cells, so that you will be able to better understand how this recent research adds to our understanding of ME/CFS.

NK cell abnormalities may one day help confirm a diagnosis and they also add to the evidence of immune dysfunction. Research evidence also supports the notion of distinct subgroups under the ME/CFS umbrella who may present with potentially different pathologies.

The ins and outs of Natural Killer Cells

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

They make up approximately 10% of our total white blood cells, so we have relatively small amounts, however, they play a very important role (Paust *et al.*, 2011).

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). This is known as cytotoxic activity (Brenu *et al.*, 2013).

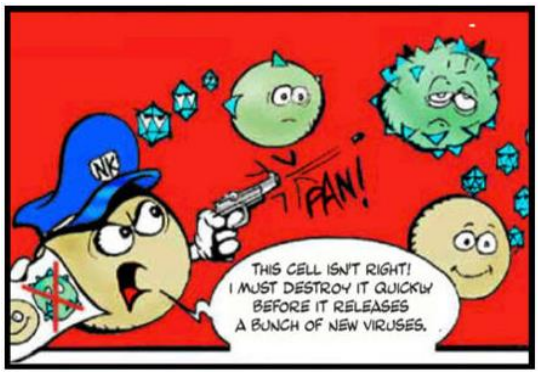
Whilst they are busy containing and attacking the threat, they call in reinforcements (other immune cells) by producing cytokines (chemical messengers) and then await back up, in the form of T cells, which produce specific antibodies against the threat.

How do NK cells recognise "threats"?

All of the cells in our body have little markers on them – labels or tags – that tell immune cells that they are "self" cells and not foreign.

When NK cells read these tags, their cytotoxic (killing) activity is switched off.

Cells which are infected by viruses do not express these "self" tags and so NK cells are able to detect and then destroy them.



Source: virtualmuseum.ca

NK cells also recognise Lipopolysaccharides (LPS), a component of bacterial cells, which also trigger NK cell cytotoxic activity (Brenu *et al.*, 2013).

They also respond to "distress signals" given off by infected cells, in the form of cytokines.

NK cell subtypes

There are several different types of NK cell with slightly different properties, determined by the type of surface markers they express. The following are the main types found to be different in the current study;

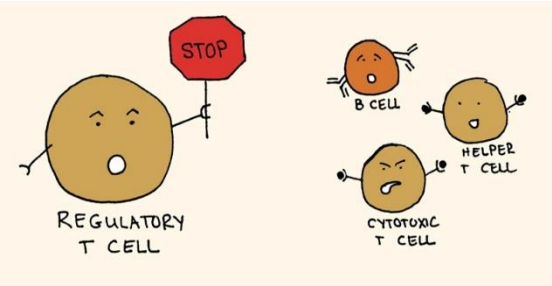
- **NKT-like cells** are NK cells that have developed a type of "memory" for certain types of cells and so can recognise a specific virus, should you be infected with it for a second time. This is similar to the role of T-cells.
- **CD56 bright** subset of NK cells usually make up only about 10% of NK cells and they secrete more cytokines than the other types, particularly IFNy. However, they have very low

cytotoxic activity (killing abilities). They also have a longer life span and can induce T cell proliferation (multiplication) that can lead to autoimmunity and contribute to inflammation.

- **NKG2C** and **NKCD69**- both are markers showing *activated* NK cells.

(Mandal and Viswanathan, 2015)

T regulatory cells



Source: knowyourscience.blogspot.com

The other type of cells studied in this latest research were T regulatory cells, an immune cell that can regulate or supress other immune cells.

Regulatory T cells actively suppress activation of the immune system and help prevent autoimmune disease – where the body's immune system starts attacking its own cells.

Existing knowledge of NK cells in ME/CFS

There have been over 20 studies published on NK cells in ME/CFS since 1987, with almost all of them concluding that **NK cell cytotoxicity is reduced** in people with ME/CFS (Strayer *et al*., 2015). This means that their killing abilities are not very good.

Caligiuri *et al.* (1987) found that the populations of NK cells in people with ME/CFS were all back to front; the main types of NK cell found in healthy individuals were found in low numbers in people with ME/CFS and the type that are in low numbers in healthy people were very high in people with ME/CFS.

> "When tested for cytotoxicity against a variety of different target cells, patients with CFS consistently demonstrated low levels of killing" and also "most patients remained unable to lyse Epstein-Barr virus-infected B cell targets."
>
> Cligiuri *et al.* 1987.

Other studies have found reduced levels of perforin; the "chemical bomb" used by NK cells to attack other cells (Maher *et al*., 2005).

A much longer, 12-month study in 2012 by Brenu *et al*. also found reduced cytotoxic activity of NK cells in people with ME/CFS that was consistent during the 12-month period.

Studies have also found a correlation between low NK cell activity and symptom severity; the lower the activity, the more severe the condition (Fletcher *et al*., 2010).

Of course, there have also been studies finding **no differences** found in NK cells in people with ME/CFS. This could be due to differences in methods used in different studies or due to the heterogeneous nature of ME/CFS (different causes/presentations and probable subgroups within the same diagnosis).

Blood collection and processing

Interestingly, one of the papers (Theorell et al., 2017) that was unable to find any abnormalities in NK cell populations – in people with ME/CFS and healthy controls – used a method of freezing and then thawing their blood samples before processing.

This was different to other studies (including the current one) which performed the testing and analysis immediately after blood collection, on fresh blood.

This different approach could have contributed to the results. The authors of that paper even commented on this themselves:

> "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 in whole blood or immediately after isolation."
>
> Theorell *et al*., 2017.

What they suggested was that the NK cells themselves function normally but, when in the blood of people with ME/CFS, their function is reduced, perhaps due to the effects of some other factor that is abnormal in the blood of the patients.

The recent Spanish study

Association of T and NK Cell Phenotype with the Diagnosis of ME/CFS (Rivas et al. 2018)

The aim of the study was to further examine differences in T and NK cell subpopulations that had been identified in previous studies in order to assess their use in **improving diagnosis** and characterization of ME/CFS.

The group hoped to overcome a limitation of previous studies of small sample sizes by using a larger cohort of patients. They also looked at any relationships between immune cell types and severity of illness, onset of illness and viral involvement (EBV and HCMV).

Immunophenotyping (identifying subgroups of different types of immune cells) was carried out on the whole blood of 76 patients (fulfilling the revised Canadian Consensus Criteria, as well as an assessment with a medical professional) and 73 healthy controls.

ME/CFS patients were excluded from the study if they had any other medical condition that could explain their symptoms.

Interestingly, controls were excluded if they had a first or second degree relative with ME/CFS. This is not an exclusion seen in many studies (none that I have read, anyway) and reflects that there may be a hereditary component to ME/CFS yet to be identified.

However, oddly, there is no mention of excluding controls who also had another medical condition, which could have potentially affected the results.

Unlike some of the previous studies finding no differences in immune cells, this study analysed fresh (not frozen) whole blood **within 6 hours** of collection.

This gives the closest representation of what is going on inside the body, and, "…may have allowed for detection of changes that could be lost when using a more processed sample."

They then examined the percentages of a variety of different immune cells (CD4 and CD8 T cells, T regulatory cells and different NK cell subpopulations) and compared the amount between patients and controls.

What did they find?

ME/CFS patients showed significantly **lower** values of T regulatory cells and **higher** NKT-like cells than the healthy controls.

Regarding NK cell phenotypes, NKG2C was significantly **lower** and NKCD69 and NKCD56 bright were significantly **higher** in the patient group (See fig 2 and 3 below).

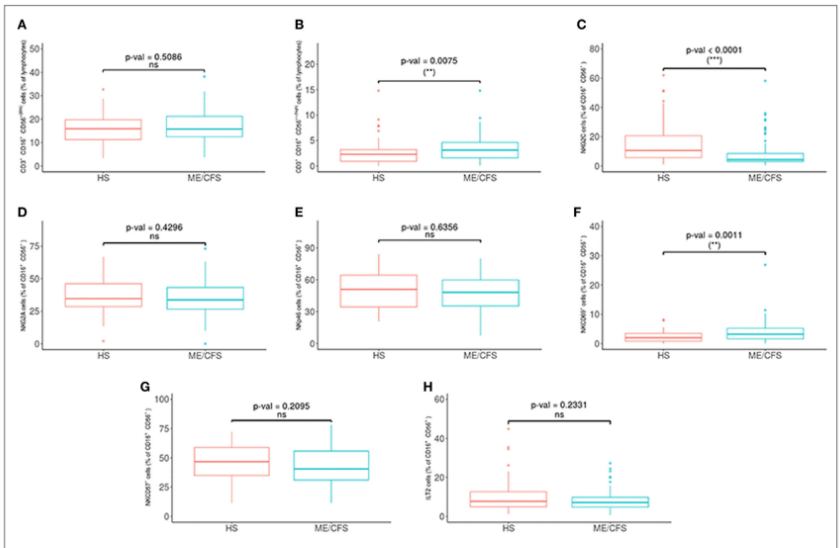


FIGURE 3 | Analysis of NK cell subsets in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) patients (n = 76) and healthy subjects (n = 73). **(A,B)** NK cells as $CD16^{+/-}CD56^{+(dim)}$ and $CD16^{+/-}CD56^{++(high)}$ were obtained in gated $CD45^{+}$ lymphocytes. **(C–H)** Percentages of NKG2C, NKG2A, NKp46, NKCD69, NKCD57, and ILT2 NK cells were obtained after gating for $CD16^{+/-}CD56^{+}$ lymphocytes. Figures show median values (lines), interquartile ranges (boxes), and 10–90 percentile values (bars). p Values obtained by Wilcoxon non-parametric test.

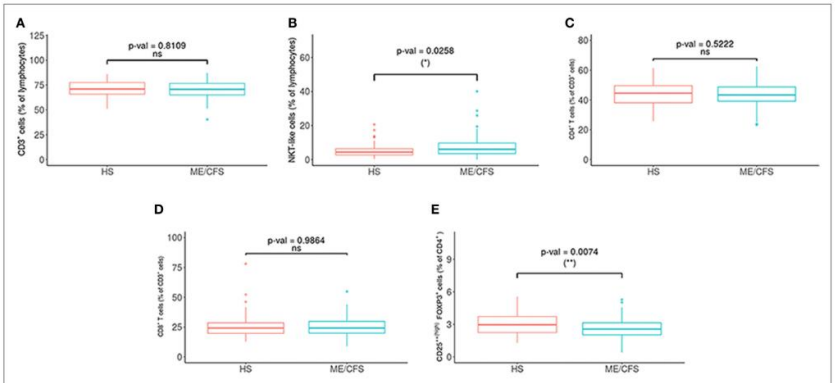


FIGURE 2 | Comparison of lymphocytes subsets in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) patients (n = 76) and healthy subjects (n = 73). **(A)** Percentages of T ($CD3^+$) and **(B)** NKT ($CD3^+CD16^{+/-}CD56^+$) cells were analyzed in gated $CD45^+$ lymphocytes. **(C,D)** Percentages of $CD4^+$ and $CD8^+$ were obtained after gating $CD3^+$ lymphocytes. **(E)** The percentage of T regulatory cells [$CD25^{++(high)}FoxP3^+$] was obtained after gating $CD4^+$ T cells. Figures show median values (lines), interquartile ranges (boxes), and 10–90 percentile values (bars). p Values obtained by Wilcoxon non-parametric test.

What does this mean?

Many of these findings suggest an **autoimmune involvement** to ME/CFS.

T regulatory cells are involved in **preventing** autoimmunity, and levels of these were found to be **low** in ME/CFS. T regulatory cells have also been found to be lower in autoimmune conditions such as Rheumatoid arthritis and systemic lupus erythematosus (Barreto, *et al*. 2009; Kawashiri *et al.,* 2011)

In fact, this was one of the suggested reasoning's behind the possible treatment of ME/CFS with Rituximab.

Increased levels of NKCD69 have also been found in infectious and autoimmune pathologies (Rodriguez-Munoz *et al*., 2016).

In a study with rheumatoid arthritis induced in mice, CD69 played a key role in the autoimmune pathology and inflammation by increasing **TGF-beta**, a cytokine that has consistently been found to be increased in ME/CFS patients (Zhang *et al*., 2011; Blundell *et al.*, 2015).

The CD56 bright NK cells produce high levels of a particular cytokine called Interferon gamma (IFNγ). Increased IFNγ expression is associated with a number of autoinflammatory and autoimmune diseases, including systemic lupus.

Interferon gamma (IFNγ) also has the ability to inhibit viral replication directly and elevated levels of IFNγ have been associated with high levels of EBV (Epstein Barr Virus) reactivation (Cardenas-Mondragon *et al.*, 2017)**.**

How does this help in diagnostics?

The researchers used the results that were most different between ME/CFS patients and healthy controls to create a program that would try to identify if someone had ME/CFS based on these immune cell findings.

Using this program, they were able to **correctly classify** individuals as either ME/CFS patients or healthy controls in **70% of cases** (which translates to about 3 in 4 patients).

Further Investigations

The research team also examined the relationship between the immune cell phenotypes and **severity of fatigue**, using questionnaires. However, they **did not** observe a significant correlation between any of the cell phenotypes and the degree of severity.

The ME/CFS patients were then split into two subgroups; those whose illness was triggered by an infection and those that were not. The phenotypes of the two groups were compared and a **significant difference** was observed in two different **NK subtypes**.

Could this finding potentially represent a diagnostic tool to be used to differentiate between two subpopulations of ME/CFS patients? Ones with possibly different underlying disease mechanisms, and perhaps different treatment routes?

Disease onset groups

Increased levels of NKT-like cells were found in the group of patients that had described **an infection before the onset** of the disease. These populations have a role in the regulation of the immune response through their cytokines.

Higher levels of NK CD56 bright cells were found in the **"no infection" onset group**, which the researchers hypothesised could be due to exposure to raised levels of catecholamines (adrenal hormones such as **adrenaline**) as a result of chronic activation of the hypothalamic–pituitary–adrenal axis (the central stress response system), as described by Loebel *et al.* (2016).

EBV (Epstein-Barr Virus)

Levels of **antibodies** against EBV (responsible for many illnesses and suggested as a trigger for ME/CFS, such as glandular fever) and HCMV (Human Cytomegalovirus Virus) were determined and compared between the patients and healthy individuals.

No difference was found between the patients and healthy individuals with regard to the prevalence of positive antibodies. This means that the people with ME/CFS **did not** have more or less EBV antibodies than the healthy controls (see table 3).

TABLE 3 | Prevalence of positive and negative IgG anti-Epstein–Barr virus (EBV) VCA and CMVH serology, in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) patients and healthy subjects.

		ME/CFS (n = 76)	HS (n = 73)
EBV	POS	72 (94.7%)	68 (93.15%)
	NEG	4 (5.2%)	5 (6.8%)
CMV	POS	53 (69.7%)	50 (68.4%)
	NEG	23 (30.2%)	23 (31.5%)

This is interesting as viruses like EBV are often hypothesised as causes of ME/CFS. These result show that it isn't necessarily the virus, but rather the immune system's **response** to the virus that may be the problem.

Even more interesting, nearly 95% of all the participants in the study (including healthy controls) had antibodies against EBV, suggesting nearly all of them had been infected by it at some point in their lives.

However, these antibodies against EBV are not very specific; they only show 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 laying dormant.

Scheibenbogen *et al.* (2014) had detected EBV DNA sequences in B cells in ME/CFS patients, that could reveal viral activity even though the antibody profile would be similar to healthy controls.

Discussion

Reduced cytotoxic activity of NK cells would suggest lowered immunity and therefore an increased susceptibility to infections.

Yet it seems from anecdotal evidence that people with ME/CFS are either highly susceptible to new infections and/or have recurring infections, or they hardly ever contract infections.

It would therefore be interesting to see if there are any differences in NK cell populations between these two subtypes of people within the ME/CFS community.

Of note, only 41% of the patients in this study reported that their illness was triggered by infection. It would be interesting to see these results on only patients who had been triggered by infection, to see if the results would be more or less significant.

Low NK cell activity is associated with an increased susceptibility to Herpes Viruses (e.g. EBV). Could this lowered NK cell function found in people with ME/CFS actually be why they were susceptible to getting the infection that triggered ME/CFS in the first place?

On the other hand, the NK cell differences may not be a causative factor in ME/CFS, but rather a consequence of having it.

NK cell studies have looked at the association with severity of illness, but not the length. This study had an average length of illness of 17 years, which is pretty long.

It would be interesting to see if these differences in NK cells change over the course of the illness and it may give more of an idea of whether the NK cell phenotype is what makes people more susceptible to getting ME/CFS or whether it is a consequence of having the disease.

Although the computer program test was promising in being able to correctly diagnose 3 in 4 patients, this may not be viable in a GP setting as they were using freshly collected blood. In reality, blood samples would be frozen and not processed within 6 hours, as in this study.

Potential causes of lowered NK cell function:

1. Psychological and physical stress – the products of which (cortisol) can inhibit NK cell function (Sieber *et al*. 1992; Witek-Janusek *et al.* 2008),
2. Gut dysbiosis – affects the immune system (probiotics have been shown to improve NK cell function (Gill *et al.* 2001; Chiang *et al*. 2000; Takeda *et al*, 2006)),
3. B12 deficiency (Tamura *et al.* 1999),
4. Cancer, viral infections, MS, Rheumatoid arthritis and Systemic Lupus.

Possible ways to enhance NK cell function:

1. Reduce stress,
2. Glutathione – this is needed to produce the chemicals that NK cells release, so low levels of glutathione can lead to reduced NK cell function (Millman, 2008). Oxidative stress and low glutathione levels have been found in ME/CFS and are suggested as causal factors (Shungu *et al*., 2012),
3. Supplements – Curcumin, Magnesium, Probiotics, B12, Ginseng, Echinacea, Chlorella, CoQ10 (Ravaglia *et al.,* 2000; Kwak *et al.,* 2012; Currier *et al*. 2001; Partearroyo *et al*., 2013; Chaigne-Delalande *et al.* 2013; Fiala, 2015).

Conclusion

From this latest research, and the many other studies on the subject, it seems clear that is strong evidence of reduced NK cell function in ME/CFS. However, research is **yet to identify the cause**, or indeed treatment for these abnormalities.

These findings could eventually help in the diagnosis in ME/CFS. However, differences in the methods used in these NK cell studies have made it difficult to compare results between them or to use NK cell function as a consistent biomarker (Brenu *et al*, 2013).

Additionally, the heterogeneous nature of this disease means that it may not be a suitable biomarker for everyone currently diagnosed with ME/CFS.

It is important to note that the NK cell abnormalities found in this study are **a reduction in function**, not a reduction in the overall numbers of NK cells; patients have enough of them, they just aren't doing their job properly! It is not something that can be routinely tested for by your GP.

These immune cell findings further add to the evidence of immune dysfunction and possible autoimmune involvement in the pathology of ME/CFS.

The authors concluded:

> "The observed differences in some of the subpopulations of T and NK cells between patients and healthy controls could define a distinct immunological profile that can help in the diagnostic process of ME/CFS patients, contribute to the recognition of the disease and to the search of more specific treatments.
>
> "However, more studies are needed to corroborate these findings and to contribute to establish a consensus in diagnosis."
>
> Association of T and NK Cell Phenotype with the Diagnosis of ME/CFS (2018)

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