Vitamin B12 Regulation of Polyunsaturated Fatty Acid Synthesis

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Abstract

Low-grade, chronic inflammation is associated with a range of diet and age-related disorders, including diabetes, arthritis, and cognitive deficits. Inflammatory cells have the capacity to synthesize complex Polyunsaturated Fatty Acids (PUFAs) called specialized pro-resolution mediators (SPMs) that regulate the extent and duration of inflammatory responses. Humans have a limited capacity to synthesize SMPs, especially as we age, due to decreased expression of the elongase and desaturase enzymes required in their conversion from dietary PUFAs. It was recently shown that vitamin B12, an essential micronutrient, enhances the cognitive benefits of dietary n-3 PUFAs. It is hypothesized that B12 will increase macrophage SPM synthesis and subdue pro-inflammatory cytokine production. Results indicate that vitamin B12 did not significantly regulate or influence gene expression associated with the pro-inflammatory response, but it did seem to enhance the expression of neuroprotective genes.

Introduction

Inflammation is the body's innate response to combat infection, toxins, and a variety of pathologies. Inflammation is required for short-term injuries because it functions to return the body to its homeostatic state, but a state of chronic inflammation is detrimental to health. Chronic systemic inflammation can be associated with increasing age. This low-grade, chronic inflammation can be related to dietary intake of n-6 polyunsaturated fatty acids (PUFA). It has been shown that n-3 PUFAs signal anti-inflammatory pathways, and even more recently suggested, vitamin B12 may enhance the cognitive benefits of dietary n-3 PUFAs. This research study aims to investigate how vitamin B12 regulates polyunsaturated fatty acid synthesis.

Prostaglandins are a class of bioactive molecules that regulate acute and chronic inflammatory responses; they are derived from essential dietary polyunsaturated fatty acids. Omega-3 PUFAs are precursors for bioactive lipids that possess diverse anti-inflammatory effects compared to n-6 PUFAs, which are widely consumed in the American diet and signal pro-inflammatory responses. Humans have a limited capacity to synthesize n-3 fatty acids, especially as we age, due to weak activity of the elongase and desaturase enzymes involved in the conversion of dietary PUFAs; therefore, adequate dietary intake is essential. A prime example of an omega-3 is docosahexaenoic acid (DHA), found naturally in cold-water fish. Its presence is critical for growth and development of the brain while also yielding cognitive benefits in adult brain function. DHA is also a precursor to molecules that regulate the resolve inflammation. Researchers at Stockholm University wanted to know whether diminished DHA synthesis affected macrophage activation (Talamonti, 2017). They investigated the activation status of bone marrow two-derived macrophages, M1 and M2, in mice who were deficient in the enzyme responsible for DHA synthesis in vivo. When comparing the enzyme deficient group

with the control group, both activated M1 and M2 macrophages, but the M1 macrophage from the enzyme deficient group showed increased expression of inflammatory markers and cytokine production. The researchers concluded that a DHA deficiency results in increased pro-inflammatory macrophages, suggesting that adequate consumption of n-3 PUFA is necessary.

Vitamin B12 is an essential micronutrient that acts as a coenzyme in numerous biochemical pathways and is commonly found in multiple animal products. Its role in inflammatory pathways is a relatively new and there is limited available. Researchers in Saudi Arabia sought to find the association between circulating B12 concentration and pro-inflammatory markers like cytokines (Al-Daghri, 2016). Research methods included blood samples from 364 subjects to determine metabolic profiles, mostly concentrating on tumor necrosis factor (TNF-α), which is a classic marker of inflammation in the body. They found a significant inverse correlation between TNF-α and vitamin B12 serum concentration in adults; more specifically, vitamin B12 deficiency resulted in increased TNF-α and this supports pro-inflammatory pathways.

Methods

Four macrophage treatment groups were created: naïve, B12, pro-inflammatory (M1), and M1 plus B12. Groups stimulated to mimic the M1 response were treated with 10 nanograms/mL gamma-interferon (IFN-γ) and 100 nanograms/mL of lipopolysaccharides (LPS). Groups with vitamin B12 were treated with 50 milligrams/ mL of B12. Twenty-four hours after cell stimulation, harvested cells were profiled for gene expression data using single-cell RNA sequencing. This technology evaluated every cell in a sample as completely unique and then groups cells together based on similarities in expression, creating sub-populations. This is

particularly useful for heterogeneous cell populations since it evaluates every cell in a sample as if it were different and then groups them based on similarities in gene expression.

Results

The gene expression data showed that 728 out of nearly 10,000 genes were differentially produced between the samples. The single cell-RNA sequencing produced four distinct populations, which was expected because of the four treatment groups.

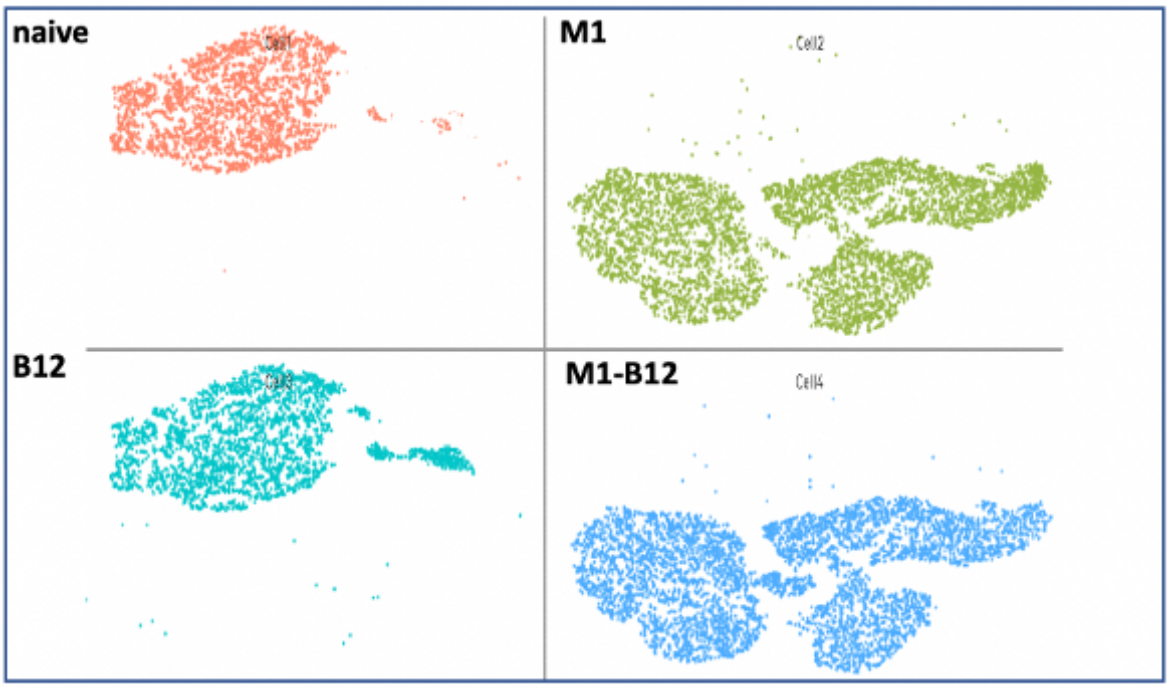


Figure 1: Treatment groups created by single cell RNA-seq

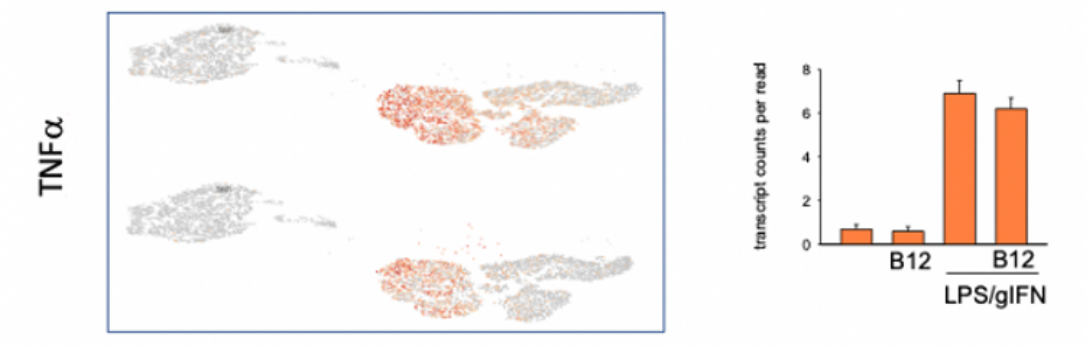
The clusters for pro-inflammatory (M1) and pro-inflammatory with the addition of B12 (M1-B12) did not differ significantly, as they were defined mainly by the absence or presence of vitamin B12. This leads to the assumption that B12 doesn't considerably alter the pro-inflammatory response; if it did, the two clusters would be expected to look different. Figure 1 shows that the two treatment groups look similar. The heat map below shows the differentially expressed genes between treatment groups. The red indicates an increase in gene expression, while the blue indicates a decrease in gene expression.

Figure 2: Heat map of treatment groups

As expected, the genes upregulated during the stimulated pro-inflammatory responses (M1 and M1-B12) were downregulated in the unstimulated cells (naïve and B12) and vice versa.

Discussion

Neuroinflammation in age-related diseases, like Parkinson's and Alzheimer's disease, is primarily mediated through elevated pro-inflammatory responses and accumulation of fibrils, or misfolded proteins that form plaques in the brain. Pro-inflammatory cytokines, such as TNF-α, are highly expressed in the regions of neurofibrillary tangles (NFT) in Alzheimer's patients. In age-related pathologies, there is a decline in PUFA synthesis (Joffre, 2020). PUFAs are essential in maintaining the myelin sheath and neuronal health, and reduction in synthesis could be linked to age and a chronic pro-inflammatory response. TNF-α is the hallmark inflammatory cytokine and was expected to increase in the M1 stimulated cells. The addition of B12 did not significantly alter TNF-α expression.



While vitamin B12 did not significantly alter the entire inflammatory response, it did upregulate some neuroprotective genes. An example of an upregulated neuroprotective gene is Elov5. It is a regulator for lipid synthesis, making it a gatekeeper for making PUFAs (Robichaud, 2018). Figure 3 shows that during the M1 response, macrophages tend to lessen the expression of Elov5.

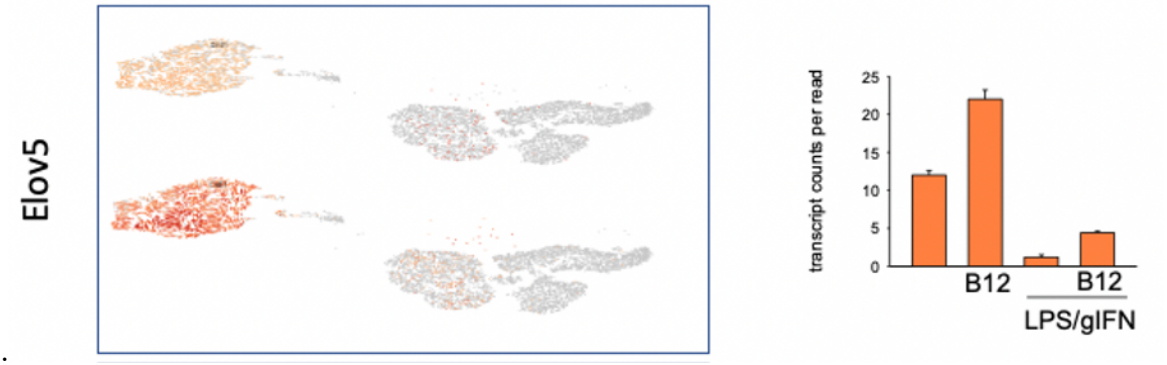


Figure 4: Expression of Elov5

As depicted in Figure 3, the addition of vitamin B12 may increase the expression of Elov5. Another neuroprotective gene that was altered by the addition of B12 is Trem2. This gene stimulates macrophages to clear the misfolded proteins in the brain and has been associated with Alzheimer's disease (Yang, 2020). Based on Figure 4, it is downregulated during the pro-inflammatory response.

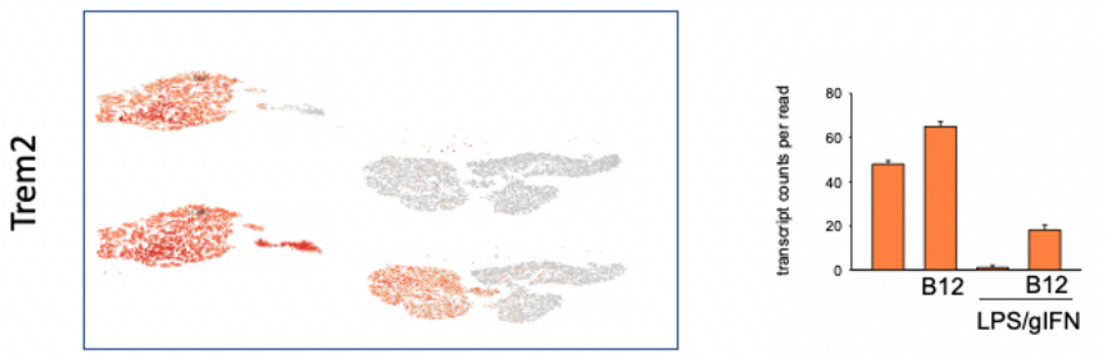


Figure 5: Expression of Trem2

Downregulation means that the misfolded proteins are not being cleared, leading to neuroinflammation. With the addition of B12 to the pro-inflammatory response, it looks as if B12 may upregulate Trem2 expression. This indicates that a subset of macrophages may be more sensitive to B12 than others.

Conclusion

Vitamin B12 did not significantly regulate or influence gene expression associated with the pro-inflammatory response, but it did seem to enhance the expression of neuroprotective genes. This means that B12 differentially regulates macrophage responses involved with neuroinflammation but does not alter the entire pro-inflammatory response. The findings indicate that adequate vitamin B12 intake is crucial in elderly populations, especially those at risk for chronic inflammatory disorders like Alzheimer's, Parkinson's, and arthritis.

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