CASE STUDY: **Effects of Enzyme Therapy on Vitiligo**

ABSTRACT

Vitiligo is an autoimmune disease that causes skin to lose pigment, resulting in white patches. Autoimmune diseases are thought to develop from the combination of a genetic predisposition, an environmental trigger, and increased intestinal permeability. Gut dysbiosis in particular has been linked to various dermatological and autoimmune disorders due to the impact it has on the immune system. Just as we have a gut-brain axis, we also have a gut-skin axis which represents a bidirectional relationship between the gut microbiome and skin health. There is currently no cure for vitiligo, and treatment success rates using various drugs, creams, light therapy, etc., are inconsistent. We believe this is because conventional treatments for skin conditions such as vitiligo fail to incorporate an internal approach to eradicating this condition. While there is not yet data to suggest that repairing intestinal permeability improves autoimmune skin conditions directly, it stands to reason that targeting the root cause in the gut would lead to symptom improvement, and it has been our clinical experience that proper nutrition and digestive support can help address the root cause of any imbalance to help achieve improved long term results. In line with current concepts relating the microbiome and the immune system, we predict that nutrition and enzyme therapy will promote skin health in someone with vitiligo while simultaneously improving other symptoms related to poor digestion and dysbiosis.

INTRODUCTION

Autoimmune diseases, including skin autoimmune diseases, are on the rise. While the development of a skin manifestation of autoimmune disease is multifactorial, autoimmune skin diseases like psoriasis or vitiligo are the result of an immune response gone awry. Vitiligo occurs when the immune system mistakenly attacks melanocytes, the skin cells that produce melanin, the chemical that gives skin its color. This attack causes the melanocytes to lose their pigment, resulting in patches of lighter or no color in the skin. The exact pathogenesis is unknown, though it was hypothesized that a combination of genetic, immunological, neurogenic, and environmental factors are at play. It is often associated with other autoimmune diseases. Traditionally, vitiligo initially presents on the hands, forearms, feet, and face, often in a perioral or periocular distribution. The disease course tends to spread centrifugally, ultimately involving more of the total body surface area. Complications of vitiligo are social stigmatization, mental stress, eye involvements such as iritis, depigmented skin that is more prone to sunburn, skin cancer, and hearing loss due to loss of cochlear melanocytes. Other complications are related to medications such as skin atrophy after prolonged use of topical steroids.

Treatment of vitiligo is difficult given the often-unpredictable nature of the disease course and the variable responses to interventions. For these reasons, treatment typically requires multiple modalities to elicit a response and is often individualized to meet the needs of each patient. The two major goals of treatment are

to halt active disease and induce repigmentation. Traditionally, topical steroids, oral steroids, calcineurin inhibitors, and phototherapy with narrow-band UVB are considered the first-line therapeutic options. Other therapies include photochemotherapy, lasers, antioxidants, topical Vitamin D analogs, surgical skin grafting systemic medications, and several supplements have been used with varying success. Over the last decade, substantial research and progress has been made in the treatment options for vitiligo. Janus kinase (JAK) inhibitors are an emerging therapy used to treat several dermatologic conditions including vitiligo. Janus kinase (JAK) inhibitors are a type of disease-modifying antirheumatic drug that help treat inflammation and related symptoms by blocking the signaling pathway that causes the body to produce too many cytokines, which can lead to inflammation. This may also help calm the immune system and ease symptoms like joint pain and swelling. However, these drugs come with various side effects due to their immunosuppressive nature.

Inflammation is a natural response from the immune system to tissue damage, bacteria, viruses, and other toxins. It is a protective process that helps the body heal, fight off infection, and maintain homeostasis. Drugs that shut off inflammation and suppress the immune system can be problematic since the immune system relies on inflammation for proper regulation. They also fail to address what is creating the inflammation and driving the symptom, which in this case is vitiligo. Studies have shown that dysregulation of microbiota is common in inflammatory skin conditions such as atopic dermatitis, rosacea, and psoriasis. Some mechanisms that regulate the gut-skin axis include intestinal barrier, inflammatory mediators, metabolites, and the immune system. Diet can have a significant impact on skin health, as the skin is an organ that needs nutrient building blocks from food to build and maintain its structure, function, and resilience, if the food is not digested properly then toxicity, inflammation, and imbalance will begin.

The successful initiation of an immune response depends on T cells and macrophages along with the polypeptide factors they produce, called cytokines, which play a key role in communication during normal immunological response as well as infectious, inflammatory, and neoplastic disease states. Bromelain, papain, and amylase have all been demonstrated to induce cytokine production in human peripheral blood mononuclear cells. Treatment with these enzymes leads to the production of TNF-alpha, IL-1 beta, and IL-6 in a time and dose dependent manner. Papain and bromelain have a 10- to 40-fold inducing capacity for TNF production. Macrophage activity is enhanced up to 700% and phagocytosis is also accelerated. Proteolytically active enzymes such as bromelain are known to decrease expression of mRNAs encoding proinflammatory cytokines by human leukocytes in vitro. Significant increases in these proinflammatory cytokines such as granulocyte colony stimulating factor (G-CSF), interferon (IFN)-γ, interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF) are detected in the media from actively inflamed areas in various disease states.

Proteolytic enzymes are safe alternatives to inflammation due to their adaptive ability to listen to the body and upregulate and downregulate the production of various inflammatory markers as needed. Whereas most drug development efforts currently focus on blocking the inflammatory cascade, which most likely is at least in part a secondary rather than a primary event, the anti-inflammatory properties of proteolytic enzymes in conjunction with digestive enzymes and probiotics that ensure optimum digestion and absorption of nutrients and facilitate removal of waste may be a novel therapy for skin conditions such as vitiligo. The purpose of this case study was to determine if Transformation Enzyme Corporation's Professional Protocol™ Protease powder along with digestive enzymes and probiotics could be tolerated and work as a more natural and safer alternative to traditional treatments for a patient with vitiligo and other autoimmune conditions.

CASE STUDY

Here we have a 30-year-old male whose chief complaint is managing his vitiligo with secondary issues being hormonal imbalances, GI issues, fatigue, brain fog, and chronic pain and inflammation. The patient was

diagnosed with vitiligo as a teenager and has tried both allopathic and holistic approaches to eradicate the pigmentation issues with little success. The patient was not new to enzyme therapy and had already been on Transformation's autoimmune protocol along with other nutraceuticals he had seen his peers use in various vitiligo support groups. He did see some symptom improvement but was still not where he needed to be.

Due to the relationship between gut and skin and the role inflammation plays in autoimmune conditions such as vitiligo, we wanted to see how he would respond to a more therapeutic dose of Transformation's Professional Protocol™ Protease. We hypothesized that a higher dose of the protease blend would yield significant clinical improvement due to its ability to modulate the inflammatory cascade and immune system. His protocol was adjusted to include the Protease powder and continue with digestive, GI lining, and microbiome support in the auto immune protocol. The objective is to observe symptom improvement by supporting the gut/skin axis, decreasing GI inflammation and eradicating underlying gut infections.

9/18/2023: The patient completed a comprehensive symptom survey questionnaire.

9/20/2023: Dosing set at 5 grams Protease Powder mixed with liquid three times daily away from food, which the patient took at 5am, 2pm, and 10pm. Continue 16/8 fast with window opening at 12pm and closing at 8pm. Powder to be increased gradually as tolerated until therapeutic dose of 30 grams is met. The patient was also instructed to continue taking Transformation's Professional Protocol™ Digest with each meal and snack, Transformation's Professional Protocol™ Probiotic at bedtime, and other supplements he was on prior to the case study.

9/26/2023: The patient added a fourth 5gram dose at 10am, bringing the total to 20 grams daily, with mealtimes around 12pm and 7-8pm.

10/2/2023: To get a picture of the patient's overall metabolic health and underlying problems such as bacterial overgrowth and how metabolites from these microorganisms can interfere with cellular function, a stool sample was collected for a GI-MAP™ analysis from Diagnostic Solutions Laboratory.

GI-MAP™ baseline findings

The Gastrointestinal Microbial Assay Plus (GI-MAP™) is an innovative clinical tool that measures gastrointestinal microbiota DNA from a single stool sample with state of the art, quantitative polymerase chain reaction *(qPCR or real-time PCR)* technology. The GI-MAP™ was designed to detect microbes that may be disturbing normal microbial balance or contributing to illness as well as indicators of digestion, absorption, inflammation, and immune function. Initial findings from the patient's GI-MAP™ showed a High positive for *H. pylori*. *H. pylori* is generally associated with the gastrointestinal system, but it can also infect the skin, liver, and heart. Some studies have shown that GI-MAP™ can trigger the immune system, which may be linked to skin diseases like vitiligo and psoriasis vulgaris. *H. pylori* is also correlated with other symptoms the subject was experiencing such as sleep issues and anxiety. This could also explain findings of low magnesium on the OMX™ report due to suppression of stomach acid which is needed for proper absorption of this crucial mineral. There was significant overgrowth of opportunistic bacteria that further painted the picture of low stomach acid and improper digestion. Initial stool analysis also showed high proportions of histamine producing and mast cell activating bacteria within the GI. Immune function was evaluated and showed low levels of secretory IgA (SIgA) which can make it difficult to fight off invaders that enter the gut lining. This can lead to inflammation and the breakdown of tight junctions that keep toxins and undigested food out of the bloodstream. These toxins and substances can then trigger immune and inflammatory reactions throughout the body, causing discomfort and pain. Digestive markers were within range, which was not surprising considering he had been on digestive enzymes, however results did show zonulin to be

high, indicating intestinal permeability. This was also not surprising considering the amount of LPS being produced. One of the most studied bacterial surface molecules lipopolysaccharide (LPS), is produced by most gram-negative bacteria. These gram-negative bacterial toxins can cause endotoxemia, possibly leading to the activation of various neuroinflammatory responses morgenella and psuedomonus and bacterodes and enterobacter are all LPS producers that trigger zonulin.

H. PYLORI & VIRULENCE FACTORS	Baseline results	
Helicobacter pylori	2.25e3	High ↑
Virulence Factor, virB	Positive	
COMMENSAL BACTERIA		
Enterococcus spp.	2.52e8 H	
BACTERIAL PHYLA		
Bacteroidetes	8.18e12 H	
Firmicutes	7.60e11 H	
DYSBIOTIC & OVERGROWTH BACTER		
Enterococcus faecalis	1.37e4	High ↑
Enterococcus faecium	3.43e5	High ↑
Morganella spp.	2.48e7	High ↑
Pseudomonas spp.	2.38e9	High ↑
Pseudomonas aeruginosa	1.14e6	High ↑
Staphylococcus aureus	1.42e3	High ↑
Streptococcus spp.	6.80e3	High ↑
IMMUNE RESPONSE		
Secretory IgA	337 L	
ADD-ON TESTS		
Zonulin	188.8 H	

10/4/2023: Patient increased Protease powder dosage to 25 grams daily.

10/7/2023: Patient increased Protease powder dosage to the goal of 30 grams daily with no issues.

11/7/2023: Protocol modified to include Transformation's Professional Protocol™ Protease IFC for shoulder pain and 2 caps 3 times daily of Transformation's GastroZyme formulas for the elevated zonulin, an indicator of intestinal permeability which will also drive inflammation.

11/8/2023: The patient completed a second comprehensive symptom survey questionnaire.

3/5/2024: A stool sample was collected for a follow-up GI-MAP™ analysis.

GI-MAP™ follow-up findings

The chart below shows a side by side comparison of the subjects initial GI map to the GI map results post protocol. We would like to note that while we did see positive changes in microbial balance and digestive health, the powder protease protocol was not followed properly for the first three months. Had the powder been consumed properly, the results would have been more remarkable. The follow-up test did indicate that things were titrating up as evidence by improved *H. pylori* score and significant improvement in overgrowth of opportunistic microorganisms. SecIGA scores also improved significantly, indicating protease was positively influencing his immune function and resiliency. Digestive markers remained stable while the patient's GI inflammation in intestinal permeability scores

worsened. This is most likely due to the pathogenic infection and foodborne illness he contracted as evidenced by the *E. coli* markers observed in this sample. *E. coli* causes massive inflammation and zonulin shifts. Comparing the total number of out-of-range scores from both tests, it is significant to note the ratio of those initial areas which had resolved (56%) and improved (11%) compared with new areas that had fallen out of range on the follow-up tests due to the foodborne illness (33%).

	Baseline results		Followup results	
H. PYLORI & VIRULENCE FACTORS				
Helicobacter pylori	**2.25e3**	**High ↑**	**1.32e3**	**High ↑**
Virulence Factor, virB	**Positive**		Negative	
COMMENSAL BACTERIA				
Enterococcus spp.	2.52e8 H		1.63e7	
BACTERIAL PHYLA				
Bacteroidetes	8.18e12 H		2.53e12	
Firmicutes	7.60e11 H		2.45e11	
DYSBIOTIC & OVERGROWTH BACTER				
Enterococcus faecalis	**1.37e4**	**High ↑**	3.36e3	
Enterococcus faecium	**3.43e5**	**High ↑**	2.71e3	
Morganella spp.	**2.48e7**	**High ↑**	<dl	
Pseudomonas spp.	**2.38e9**	**High ↑**	<dl	
Pseudomonas aeruginosa	**1.14e6**	**High ↑**	<dl	
Staphylococcus aureus	**1.42e3**	**High ↑**	**2.72e4**	**High ↑**
Streptococcus spp.	**6.80e3**	**High ↑**	**3.94e3**	**High ↑**
IMMUNE RESPONSE				
Secretory IgA	337 L		1160	
ADD-ON TESTS				
Zonulin	188.8 H		270.9 H	
BACTERIAL PATHOGENS				
Enterohemorrhagic E. coli	<dl		**8.92e5**	**High ↑**
E. coli O157	<dl		<dl	
Enteroinvasive *E. coli/Shigella*	<dl		<dl	
Enterotoxigenic *E. coli* LT/ST	<dl		<dl	
Shiga-like Toxin *E. coli* stx1	<dl		**4.77e5**	**High ↑**
Shiga-like Toxin *E. coli* stx2	<dl		<dl	

PROTOCOL RATIONALE

The role of supplemental enzymes in alleviating autoimmune disorders involves clearing persistent immune complexes, controlling inflammation, enhancing blood flow, and removing necrotic tissues. These benefits of enzyme therapy in the case of vitiligo are multifold, and the types of Transformation™ dietary supplements used in this case study fall into four categories: digestive enzymes, proteases, digestive support, and probiotics.

Digestive enzymes with meals help ensure proper digestion and bioavailability of all nutrients supporting tissue repair and a strong immune system. Professional Protocol™ **Digest** is a comprehensive and therapeutic enzyme blend high in lipase, protease, and carbohydrases to ensure complete digestion of all foods.

A protease formula between meals will help promote optimal circulation and removal of toxins. Proteases also help manage inflammation and support the repair of damaged tissues or removal of dead tissue. Professional Protocol™ **Protease** is a comprehensive and therapeutic blend of acid / alkaline / neutral proteases and

peptidases that targets complete protein digestion when taken with meals. Taken at bedtime or away from meals, Protease supplies a comprehensive and therapeutic blend of proteolytic enzymes for systemic benefits.

For additional enzyme support, Professional Protocol™ **Protease IFC** is unique formulation of highly active proteolytic enzymes and antioxidants is designed to help regulate inflammation anywhere on or in the body. This product is ideal for muscle aches, pains, injuries, and stiff joints as well as to promote cardiac health and is applicable for any other inflammatory conditions.

Research has shown that chances of intestinal permeability increase with vitiligo. **GastroZyme** is an herbal formula with marshmallow root, papaya, prickly ash, and gotu kola plus enzymes to heal and repair the mucosal lining of the GI tract, respiratory system, and urinary tract for additional gut lining support.

A probiotic supplement further supports digestion and the immune system while maintaining a healthy gut environment by enhancing the ecological balance of friendly bacteria. Professional Protocol™ **Probiotic** is a comprehensive probiotic formulation that includes six different probiotic sources totaling more than 5 billion colony forming units (cfu) per capsule. Chosen for their safety and efficacy, these GI stable and heat stable microorganisms mirror the ratios found in a healthy GI tract.

NOTE: the original case study was set to last 3 months. During the final interview with subject, it was discovered he had not followed the proper instructions for mixing the protease powder. Enzymes are hydrolytic and activate once they are mixed with a liquid. Unfortunately, the subject misunderstood and was pre-mixing his protease powder each morning and drinking his pre-mixed bottles throughout the day. This unfortunately impacts the clinical efficacy of the protocol and explains why we did not see the drastic changes we were expecting. For this reason, we extended the study for one additional month taking the protease powder correctly to monitor symptom changes.

DISCUSSION

The primary goal of our clinical interventions was to improve skin pigmentation and halt the spread of additional spots using Transformation's foundational approach and megadose protease. However, as the root cause of vitiligo is inflammation and other symptoms related to poor gut health, the objective was not just to mitigate vitiligo symptoms but also other symptoms that were associated with the underlying cause of imbalance. While lab testing can provide important objective data, symptom improvement is an easy and cost-effective way to evaluate efficacy of a treatment plan. We used a comprehensive symptom survey questionnaire where the patient marks each symptom as 'Always', 'Sometimes', or 'Never'. Upon completion of the study, the patient completed a final questionnaire on 4/1/2024. The symptoms had shown to have improved significantly overall. Compared with the initial questionnaire, the following symptoms had resolved:

- Afternoon fatigue
- Decrease in physical stamina
- General fatigue, tired, sluggish most of day
- Wake up tired even after 6+ hours of sleep
- Poor memory, forgetful, mental sluggishness
- Muscle soreness, stiffness, achy joints
- Increase in fat distribution, abdomen and hips
- Feeling that bowels do not empty completely
- Sense of fullness during and after meals
- Increased thirst and appetite
- Crave sweets during the day
- Crave salt
- Heartburn
- Night sweats

While not completely gone, significant improvement was observed in the following symptoms:

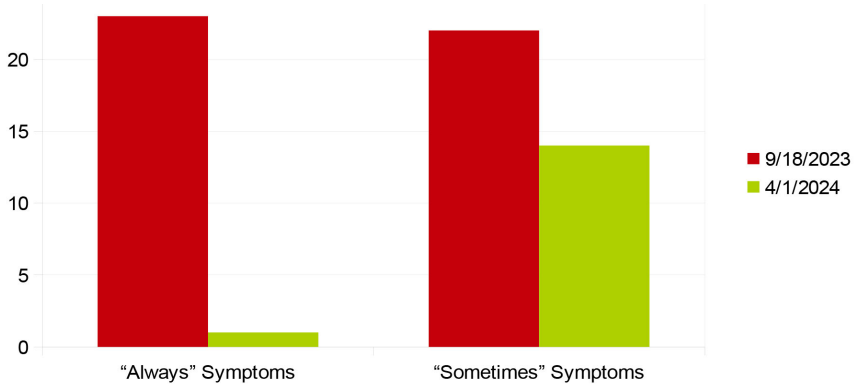
- Nervousness or anxious
- Cannot fall asleep, insomnia

- Cannot stay asleep
- Inability to concentrate or stay focused
- Excessive belching, burping, or bloating
- Pass large amount of foul-smelling gas
- Stools are foul smelling
- Increasedsexdrive

And the following symptoms had resolved from 'Sometimes' to 'Never':

- Difficulty gaining weight
- Difficulty losing weight
- Headaches
- Migraines
- Fatigue after meal
- Some foods cause sinus congestion/ headaches
- Eating sweets does not relieve sugar cravings
- Irritable, lightheaded, or shaky if meals missed
- Leg nervousness at night, restless leg
- Pain inside of legs or heels
- Stomach pain, burning or aching 1-4 hours after eating
- Mucous-like, greasy, or poorly formed stools
- Undigested foods found in stools
- Unexplained itchy skin
- Use of antacids

In total, 100% of the initial 'Always' scores saw improvement and 86% of the 'Sometimes' scores saw improvement.



CONCLUSION

Treatment of vitiligo is difficult given that the disease course is often unpredictable and has variable responses to treatment. Vitiligo has been correlated with an abnormal gut microbiota. This case presentation supports previous study findings of an altered gut microbiome being linked to vitiligo. Although total repigmentation was not accomplished, depigmented patches were much better and spread of patches was also suppressed. There were encouraging indications of improvement in mental state, hormone function, pain, and energy. The analysis and summary of the underlying association in co-occurrence of vitiligo and dysbiosis may amplify the appropriateness of enzyme therapy as a therapeutic option for this disease. Clinicians should pay attention to the use of enzyme therapy for addressing the gut skin/axis and bringing the body back into balance.

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