NeoGenesis MB-1 with CRISPR Technology Reduces the Effects of the Viruses (Phages) Associated with Acne – A Real World Case Study

gregory maguire[1]

[1]Affiliation not available

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Dr. Greg Maguire, Ph.D. and Steven T. McGee

California Physiological Society and Neogenesis, Inc

Abstract

We present a case of acne successfully treated with a topical spray containing live bacteria. Because acneic skin contains bacteria in the microbiome where a shift toward non-CRISPR bacteria occurs, these bacteria are susceptible to bacteriophage infection and lysogeny. Normalizing the bacterial microbiome to one containing more CRISPR containing bacteria renormalizes the microbiome by killing inflammation-causing bacteriophage infecting the non-CRISPR bacteria.

Viruses are an important part of our health, indeed of a healthy planet. But in the modern world, we are interacting with viruses in ways that we did not evolve to encounter them (Pappaioanou et al, 2009). This includes viruses that directly infect us, such as SARS-CoV-2 that causes Covid-19, where our encroachment on and destruction of animal habitats brings us most of our viral diseases, so called zoonotic diseases. This includes zoonotic viruses other than SARS-CoV-2, for example mumps, rubella, and measles (for which we give the MMR vaccine). But viruses also indirectly affect our health through their infection of the bacteria within us and on our skin (Tetz and Tetz, 2018). Bacteriophages, viruses that infect bacteria, are the most abundant organisms on Earth. Crosstalk between bacteriophages (viruses that infect bacteria) and the immune system occurs in our epithelial tissues, including the skin. Although bacteriophages don't generally infect our own skin cells (Tetz and Tetz, 2018; van Belleghem et al, 2019), the indirect influences of viruses, including the bacteriophages, frequently called phages, on the immune system induces an inflammatory immune response (Bruggemann and Lood, 2013; Sinha and Maurice, 2019). Viral, including Phage, infection may lead to the release of PAMPs (Pathogen-associated molecular patterns), that can translocate through the epithelium and induce proinflammatory responses. The bacteriophages contain genetic material composed of either DNA or RNA (but not both) that encodes viral structural components and synthetic and replication enzymes. Various structural components, including viral DNA, double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), and surface glycoproteins, are recognized as PAMPs by toll receptors (TLR) and other types of receptors, called pattern recognition receptors (PRRs) in our skin cells (McInturff et al, 2005). Among the TLR family members, TLR3, TLR7, TLR8, and TLR9 are involved in the recognition of viral nucleotides, the DNA and RNA inside the viruses. The recognition of viral components by PRRs commonly induces type I IFN (interferon) production that can activate target cells in both an autocrine (affecting cells of the same type) and a paracrine (affecting other types of cells) manner. When this happens in the skin, an inflammatory response results.

In the case of imbalanced phage communities, infection of certain bacterial species by phage, may lead to

an altered microbiota, overgrowth of pathogens, and chronic inflammation. Phages displaying a lysogenic cycle do not kill the host but, rather, become long-term residents as prophage. Thus, the phage genome is passed to the bacterial daughter cells without killing any of the host cells. This is likely to happen in most *P. acnes (* also called *Cutibacterium acnes)*strains (Farrar et al, 2007) because they do not contain a CRISPR system, a system used by some bacteria to kill viruses. And when*P. acnes* proliferate in the acneic conditions of the skin, PAMP release from the associated bacteriophages can induce an inflammatory immune response. Prophage-encoded genes can aid pathogens in their abilities to damage and invade the skin cells and evade the immune system by directly inhibiting phagocytic cells (immune cells that "eat" the pathogens). Prophages integrated into bacterial chromosomes or maintained as plasmids (nanoparticle capsules of the viral DNA or RNA) within bacterial cells account for important genetic differences between strains of the same species, leading to the spread of more virulent forms of *P. acnes* . Genetic sequences carried by prophages have been found to increase the pathogenic potential of their host bacteria, either through the expression of phage-encoded virulence factors or other proteins that assist in immune evasion. Thus, the genetic material that prophages provide to their lysogens, i.e., their host bacterium *P. acnes* , has consequences for how the immune system responds to, or can control, certain members of a microbial community. Therefore, virulent forms of *P. acnes* may be the result of phage infection.

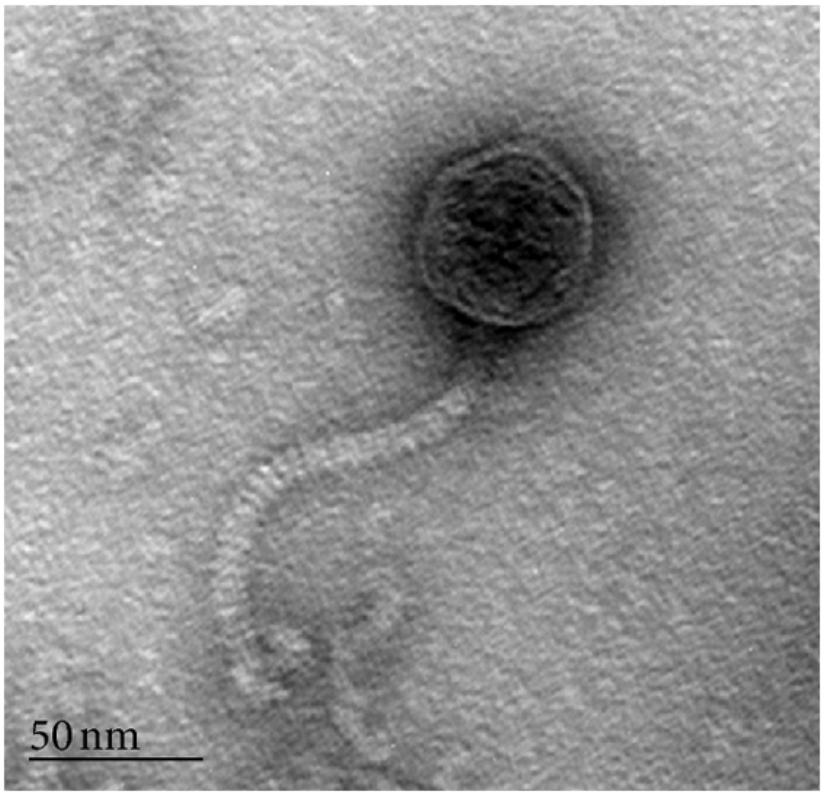

Figure 1. Bacteriophages infecting P. acnes. All bacteriophages infecting P. acnes have been classified as Siphoviruses (dsDNA), due to their long noncontractile tail, and icosahedral heads. (From Bruggemann and Lood,2013).

CRISPR has been in the news lately. Two scientists, Dr. Jennifer Doudna, Ph.D., a professor at the University of California, Berkeley, and Dr. Emmanuelle Marie Charpentier, Ph.D., a professor at the Max Planck Institute in Berlin, were awarded the Nobel Prize in Chemistry 2020 for their discovery of how

CRISPR can be used to more easily and efficiently edit the genetics of living beings, including humans. So powerful is this technology that scientists at Berkeley are teaching physicians at the University of California, San Francisco how to use CRISPR for treating sickle cell disease (SCD), and a clinical trial is underway (Sanders, 2021). SCD is one of the few diseases that have a major genetic component, and therefore genetic modification of the patient can potentially eliminate, or at least, partially remediate the disease. Most disease are a consequence of your exposome (what you're exposed to in life), not your genetics (Rappaport and Smith, 2010; Rappaport, 2016), therefore most disease are preventable by what you do in life. And what we do in life can greatly affect the naturally occurring CRISPR systems in our bodies. CRISPR is used by many of the bacteria in our bodies, and those on our skin. It's a natural way to fight bacteriophage infection, and to maintain balance of our microbiome, including all of the bacteria and viruses that comprise our microbiota. So what is CRISPR? Basically it's a set of genetic messages that the bacteria acquired from bacteriophages, and then those messages are incorporated into the DNA of the bacteria where they are used to detect and destroy DNA from similar bacteriophages during subsequent infections. Mother Nature is clever. The bacteria take something from the bacteriophage and then turn what they acquired from the virus against the virus itself. Thus, CRISPR is used by the bacteria to chop up the DNA or RNA of the bacteriophage virus so that the virus can no longer replicate itself. As such, the viral infection is quelled. Several independent culture-based studies have found that acneic skin is strongly associated with strains from the type I *P. acnes* (McLaughlin et al, 2019); the strains that don't possess a CRISPR system. Thus, the *P. acnes* associated with acneic skin are highly susceptible to bacteriophage infection.

NeoGenesis MB-1 uses a blend of naturally occurring nitrifying bacteria found in the soil, salt water, and fresh water that contain the CRISPR system, and hence are capable of remediating and preventing bacteriophage infection, and the resulting immune induced inflammation that those viruses may be causing. In the modern world our normal living conditions are upset, meaning the set of chemicals and bacteria with which we evolved are no longer those with which we have contact in the modern world. Many of the CRISPR containing bacteria normally found in our guts (Soto-Perez et al, 2019) and on our skin (Toyomane et al, 2021) may no longer be present. Recent studies suggests that bacteriophage may not be as specific to what they infect as originally thought, and may actually aid bacteria in their infections of humans (Putra and Lyrawati, 2020). Phage may even play an important role in the initiation of Parkinson 's disease (Tetz et al, 2018). Indeed, recent studies have found the induction of immunity by the over population of bacteriophage can worsen autoimmune disease, a chronic inflammatory condition (Gogokhia et al, 2019). And in acneic skin, the type I strains of *P. acnes* associated with acne appear more susceptible to phage infections compared to those from the type II phylogroup (Webster and Cummins, 1978). Therefore the bacteriophage infection of proliferating type 1 strains of *P. acnes* may be an important inducer of immune inflammation.

MB-1 from NeoGenesis returns a proprietary set of bacteria to the skin that contains the CRISPR system. Thus, one of the ways in which MB-1 helps to reduce inflammation in acneic skin likely arises from the CRISPR system reducing bacteriophage infection of the bacteria on the skin, including the virulent type 1 strains of *P.acnes* , and thereby reducing the more virulent strains of *P. acnes* and their inflammation-inducing PAMPs, found in acneic skin. As shown in Figure 2, topical MB-1, b.i.d. to the affected areas of skin, the redness and inflammation (A) was greatly reduced in just 57 days (B).

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Figure 2. Twice daily application of MB-1 topically to the affected areas of the skin greatly reduces redness

and inflammation associated with acneic skin.

MB-1 is a new, natural means to treat inflamed skin that employs CRISPR-containing bacteria found in the environment in which humans evolved; namely in our uncontaminated and untreated fresh water, salt water, and soil. The CRISPR technology found in MB-1 reduces the overabundance of bacteriophage on the skin, and helps to rebalance the*P. acnes* microbiome to one with fewer infected and inflammation-causing virulent strains within the Type 1 class. MB-1 also contains nitrifying bacteria that lead to the production of nitric oxide to relax blood vessels and oxygenate the skin, thus decreasing the anaerobic environment of the skin where virulent strains of *P. Acnes* proliferate. Interspecies interactions and antagonism between *P. acnes* and other bacteria have been demonstrated through the production of antimicrobials and fermentation products (Christensen et al, 2016). Thus, the nitrifying bacteria used in MB-1 may compete with the *P. acnes* to reduce the number of these bacterial strains in acneic skin, and along with CRISPR in the nitrifying bacteria help to quell the acneic inflammation associated with bacteriophage induced inflammation. Further, by disabling the bacteriophage virions shed from lysed *P. acnes* bacteria (DePaepe et al, 2014) as a result *P. acnes* bacteriophages having a broad infection range of other bacterium types (Marinelli et al, 2012), the CRISPR enabled bacteria may further stop the spread of bacteriophage replication and the related inflammation induced by these bacteriophage.

Written Informed Consent

The authors have obtained written informed consent from the subject presented in our paper.

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