Host Nutritional Status and Its Effect on a Viral Pathogen

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The nutritional status of the host has long been associated with both severity and susceptibility to infectious disease. The accepted model system proposes that inadequate nutrition impairs the functioning of the immune system, thus resulting in increased susceptibility to infection. However, current work suggests that not only can the nutritional status of the host affect the immune response, but it can also affect the viral pathogen. In a mouse model, a benign strain of coxsackievirus B3 became virulent and caused myocarditis in selenium- and vitamin E-deficient mice. This change in pathogenicity was due to mutations in the viral genome, which changed an avirulent virus into a virulent one. Once these mutations occurred, even mice with normal nutriture developed disease from the mutated virus. These results suggest that the oxidative stress status of the host can have a profound influence on a viral pathogen.

Studies of the interaction between infectious disease and host nutritional status have generally shown that poor host nutrition leads to increased pathogenicity of the infecting agent. The increase in either susceptibility to or severity of infectious disease in the nutritionally deficient animal has generally been considered to be host related. That is, poor host nutritional status leads to a decrease in host protective factors against disease (weight loss, mucosal damage, lowered immunity). Indeed, a number of nutritional deficiencies lead to lowered immunity and can lead to increased severity, duration, and susceptibility to infectious disease. However, work in our laboratory suggests that an alternative pathway may be responsible for the increase in virus-induced pathogenicity in malnourished hosts. We found that the nutritional status of the host can have a profound effect on the viral pathogen itself. Specifically, we found that a decrease in dietary nutritional antioxidants can change a normally avirulent B3 coxsackievirus into a virulent virus by inducing changes in the viral genome. Once changes in the viral genome have occurred, even a host with adequate nutritional status is susceptible to the newly acquired virulent properties of the pathogen.

Here we review the work that led to our description of a model system in which the oxidative stress status of the host is an important consideration during a viral infection. We hope these results provide a framework for other investigators, both nutritionists and virologists, to collaborate on a variety of other nutrition-infectious disease models.

Keshan Disease

In China in the early 1930s, there was an outbreak of a cardiomyopathy characterized by necrotic lesions throughout the myocardium. This disease was found only in regions of China in which people were deficient in selenium. Named Keshan disease after the province in which it was first described, the disease could be wholly prevented by providing selenium supplementation to the selenium-deficient population [1]. However, the deficiency in selenium alone was not the sole cause of the disease. Scientists in China noted that Keshan disease had a seasonal and annual incidence that was more characteristic of an infectious disease than one caused by a nutrient deficiency. Screening of tissues from Keshan disease victims revealed the presence of a number of viruses, including coxsackieviruses [2]. Coxsackieviruses, and in particular coxsackievirus B3, infect heart muscle and cause myocarditis. In some cases, the myocarditis develops into dilated cardiomyopathy, a serious disease that leads to an accelerated course and death. Coxsackieviruses are small RNA viruses in the Picornaviridae family.

In order to study how a deficiency in selenium influenced a coxsackievirus infection, we fed mice a diet either deficient or adequate in selenium. After 4 weeks on the diets, the mice were infected with either a myocarditic strain of coxsackievirus, CVB3/20, or an amyocarditic strain, CVB3/0. Both strains will replicate to equivalent titers in the mouse heart, yet only CVB3/20 will induce myocarditis. Furthermore, of about 7400 nucleotides (nt), there are only 7 nt differences between CVB3/20 and CVB3/0. At various times after infection, we sacrificed the mice and removed their hearts for study.

We found that selenium-deficient mice that were infected with CVB3/20, the myocarditic strain, developed much more severe pathology than CVB3/20-infected selenium-adequate mice [3]. Virus titers in the heart and liver were also elevated in the selenium-deficient mice. Of note, selenium-deficient mice in-
fected with the normally benign strain, CVB3/0, developed myocardi-tis, whereas infected selenium-adequate mice did not [4]. Although this response was interesting, it was not wholly unexpected. Other studies have demonstrated that a deficiency in selenium leads to increased susceptibility to viral infection, which was related to impairment in the host immune response. Indeed, in our model, the spleen cell proliferative response to both mitogen and specific viral antigen were decreased in the selenium-deficient mice when compared with that of the selenium-adequate mice.

However, we reasoned that in addition to host changes that the virus itself may be affected by host conditions. In order to answer this question, we designed a virus passage experiment (figure 1). The benign CVB3/0 virus was inoculated into either selenium-deficient or selenium-adequate mice. After 7 days, the mice were killed and virus was recovered from the hearts. Isolated viruses, renamed CVB3/0Se⁺ (isolated from selenium-adequate mice) or CVB3/0Se⁻ (isolated from selenium-deficient mice) were then inoculated individually into selenium-adequate mice. Thus, if the virulence of the virus was dependent on host factors, the selenium-adequate mice should not develop myocarditis following infection with the normally benign virus. However, the result obtained was not consistent with a change in host response. Selenium-adequate mice infected with CVB3/0Se⁻ developed myocarditis, whereas selenium-adequate mice infected with CVB3/0Se⁺ did not. This result demonstrated that the virus itself had changed as a consequence of replicating in a selenium-deficient animal.

In order to confirm that the pathogenic phenotype of the normally benign virus was altered due to a change in viral genome, we sequenced both the CVB3/0Se⁺ and CVB3/0Se⁻ viruses. We found that the genome of CVB3/0Se⁺ had indeed changed: 6 point mutations were found in the CVB3/0Se⁺ genome when compared with the input virus, CVB3/0 [5]. No changes occurred in the CVB3/0Se⁻ genome. These 6 nt changes were also found in the virulent virus, CVB3/20. Thus, 6 of 7 nt in the virulent CVB3/20 were also found in the CVB3/0Se⁻ virus.

Our work was the first report of a specific host nutritional deficiency altering the genome of a viral pathogen, changing it from an avirulent virus to a virulent one. Once these genomic changes occur, even hosts with normal nutritional status are at risk for developing myocarditis after infection.

**Oxidative Stress and Viral Changes**

What is the mechanism by which the genomic changes occur in the avirulent virus that replicates in a selenium-deficient animal? One of the functions of selenium is as an essential cofactor for the antioxidant, glutathione peroxidase. Therefore, we reasoned that a deficiency in other antioxidant nutrients might have the same effect. To test this hypothesis, mice were fed a diet deficient in vitamin E, with or without the addition of fish oil. Vitamin E, a lipid-soluble vitamin, functions as an antioxidant by free radical scavenging. Fish oil was added to one of the diets in order to further exacerbate the oxidative stress potential of the vitamin E-deficient diet by accelerating the vitamin E deficiency. After 4 weeks on the diet, mice were infected with the amyo-carditic virus.

As with the selenium-deficient diet, mice fed the vitamin E-deficient diet developed myocarditis when infected with the normally benign strain of CVB3 [6]. The mice fed the vitamin E diet with fish oil had the most severe cardiac pathology. Sequencing of virus obtained from the vitamin E-deficient mice yielded the same nt changes as those found in the virus recovered from selenium-deficient mice. The common mechanism appeared to be one of increased oxidative stress in the host due

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**Figure 1.** Diagram depicting virus passage experiment and outcome. Mice were fed either a selenium (Se)-adequate or Se-deficient diet before inoculation with amyo-carditic coxsackievirus strain CVB3/0. Seven days after infection, virus was isolated from the mice (renamed CVB3/0Se⁺ or CVB3/0Se⁻) and passed back into Se-adequate mice. Only the CVB3/0Se⁻ virus induced myocarditis in Se-adequate mice.
a nutritional deficiency in antioxidant protection, which led to genomic changes in a normally avirulent virus.

To further investigate the hypothesis that oxidative stress of the host was the underlying driving mechanism for the viral genomic changes, we made use of glutathione peroxidase 1 knockout mice (GPX-1 KO). If the action of the selenium deficiency was due to a decrease in antioxidant protection due to a decrease in the glutathione peroxidase activity, then the GPX-1 KO mice should respond similarly. Indeed, we found that over half of the GPX-1 KO mice developed myocarditis when infected with the avirulent strain CVB3/0 [7]. However, none of the wild type mice developed myocarditis. Sequencing of the virus isolated from the GPX-1 KO mice revealed 7 nt changes, 6 of which were identical to changes found in virus from selenium-deficient animals. Furthermore, the genomic changes were associated with pathologic changes in the heart. Virus recovered from GPX-1 KO mice that did not develop pathology did not exhibit genomic changes. This experiment provided further evidence that the mechanism for the viral genome changes was related to the oxidative stress status of the host.

Potential Mechanisms for the Genomic Changes

What is the mechanism for the genomic changes? Several possibilities exist. One is that the oxidative stress status of the host allowed for selection of a normally occurring variant. RNA viruses, like coxsackievirus, exist as a quasispecies [8]. That is, the nt sequence of any given RNA virus represents the consensus sequence or average base composition. This is due in part to the inability of RNA viral polymerases to repair mistakes made during replication [9]. This high rate of mutation is thought to confer a survival advantage to the viruses, allowing a rapid response to an environmental change. Sequences other than the consensus sequence are also represented within the quasispecies, but to a much lesser degree. Thus, the oxidative stress status of the host may shift the consensus sequence to a more virulent one, selecting out a new consensus sequence with an altered phenotype. This rapid selection of a new consensus sequence may be due in part to a decrease in the host’s immune function. In the deficient animals, T cell activity was impaired and accompanied by an increase in virus titers. This increase in virus titer may have allowed for the more virulent genotype to outcompete the avirulent genotype, thus resulting in a new consensus sequence.

An alternative explanation is direct oxidative damage to the viral RNA. The increased oxidative stress status of the host due to a nutritional deficiency in antioxidant protection may lead to an increase in free radicals that could damage viral RNA, resulting in mutations. This model suggests that the immune system either plays no role in the mutations or plays a role by adding to the oxidative stress. The myocarditis is characterized by an influx of inflammatory cells, and the inflammatory process itself contributes to oxidative stress. Both of these hypotheses are not mutually exclusive. We are currently using cell culture techniques to determine if the genome changes can be driven in oxidatively stressed cells independently of an impaired immune system.

Epidemic of Optic and Peripheral Neuropathy in Cuba

Are there other examples of nutritional status of the host affecting a viral genome? One possibility concerns an epidemic that occurred in Cuba in the early 1990s [10]. The disease was characterized by severe decreases in visual acuity, often progressing to near blindness over the course of several months. Defects in color vision or narrowing of the visual field occurred as well, with centrocecal scotomata and visible changes in the nerve fiber layer accompanying these changes. In some patients, a sensory peripheral neuropathy, with burning and tingling of the hands and feet, occurred alone or in combination with the optic form of the illness.

Epidemiologic studies suggested the disease was associated with an unbalanced diet, specifically deficiencies in lycopene, selenium, vitamin E, and the B group vitamins [11]. Smoking of cigars was also a risk factor for development of the disease. However, nutritional deficiencies did not appear to be solely responsible for the disease. A coxsackie-like virus was isolated from the cerebrospinal fluid of patients (85%). Virus was isolated from only a small number of cerebrospinal samples (4%) from nonneuropathy patients [12].

In collaboration with scientists in Cuba, our laboratory is studying virus isolates from neuropathy patients. The Cuban isolates are serologically related to both CVB4 and CVA9. They are slow growing in culture, which is atypical for a coxsackievirus. Western blot analysis revealed abnormal formation of the capsid proteins, with 1 long capsid polyprotein being formed, rather than 4 cleavage proteins.

In the year preceding the Cuban epidemic, an epidemic of coxsackievirus A9 meningitis was experienced. We hypothesize that the CVA9 virus replicated in a population that was deficient in antioxidant protection. Replication under conditions of oxidative stress led to the emergence of a new strain of CVA9, with altered pathogenesis. Sequencing of the Cuban variant virus is currently in progress.

Conclusions

Our work clearly demonstrates that host nutritional factors can have a profound effect on a viral pathogen, specifically by altering its genome, resulting in the expression of a new pathogenic phenotype. This work has important implications for the role of nutrition and infection and suggests that host nutritional status has influence beyond the host itself such that the genome of the virus may be altered.
References