

Review

Antioxidants and Viral Infections: Host Immune Response and Viral Pathogenicity

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Malnutrition has long been associated with increased susceptibility to infectious disease. The increase in severity from and susceptibility to infectious disease in malnourished hosts is thought to be the result of an impaired immune response. For example, malnutrition could influence the immune response by inducing a less effective ability to manage the challenge of an infectious disease. Work in our laboratory has demonstrated that not only is the host affected by the nutritional deficiency, but the invading pathogen is as well. Using a deficiency in selenium (Se) as a model system, mice deficient in Se were more susceptible to infection with coxsackievirus, as well as with influenza virus. Se-deficient mice develop myocarditis when infected with a normally benign strain of coxsackievirus. They also develop severe pneumonitis when infected with a mild strain of influenza virus. The immune system was altered in the Se-deficient animals, as was the viral pathogen itself. Sequencing of viral isolates recovered from Se-deficient mice demonstrated mutations in the viral genome of both coxsackievirus and influenza virus. These changes in the viral genome are associated with the increased pathogenesis of the virus. The antioxidant selenoenzyme, glutathione peroxidase-1, was found to be critically important, as glutathione peroxidase knockout mice developed myocarditis, similar to the Se-deficient mice, when infected with the benign strain of myocarditis. This work points to the importance of host nutrition in not only optimizing the host immune response, but also in preventing viral mutations which could increase the viral pathogenicity.

Key teaching points:

- A deficiency in selenium and/or a deficiency in selenoenzyme glutathione peroxidase leads to increased susceptibility to coxsackievirus-induced myocarditis.
- A deficiency in selenium leads to increased susceptibility to influenza virus-induced pneumonitis.
- Increased virulence of coxsackievirus and influenza virus in Se-deficient hosts is due to changes in the viral genome.

INTRODUCTION

It has been known for many years that nutritional deficiencies can lead to increased susceptibility to infectious diseases [1,2]. Many viral infections, for example infections with rotavirus, measles, and parainfluenza virus, are much more severe in malnourished hosts as compared with well-nourished hosts. A well-known example is the association of vitamin A deficiency with the development of severe measles infections, leading to a high rate of mortality [3]. Indeed, vitamin A

supplementation is recommended as a treatment for severe measles infection and supplementation with vitamin A is suggested at the time of vaccination for measles infection.

The association of poor host nutritional status with increased susceptibility to infectious disease has long been thought to be related to the host immune response. Thus, a host nutritional deficiency would lead to an impaired immune response. This impairment in immune function would result in increased vulnerability to infectious disease. Both general malnutrition, as well as specific nutritional deficiencies, have been

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reported to be associated with immune dysfunction, including impaired antibody responses, decreased macrophage activity and T cell dysfunction [4,5].

Although the immune response has been demonstrated to be impaired in nutritionally deficient hosts, our laboratory has also shown that the viral pathogen itself may be affected by the nutritional deficiency. Several viruses have been shown to develop increased virulence due to changes in their genomes as a result of replicating in a nutritionally deficient host. The mechanism for the viral genomic changes is not well understood, although it appears to be related to increased oxidative stress in the deficient host. Thus, both the host as well as the pathogen can be influenced by the nutritional status of the host.

KESHAN DISEASE, SELENIUM DEFICIENCY AND COXSACKIEVIRUS

Selenium (Se) is a trace mineral that is an essential component of a number of proteins, including glutathione peroxidase, glutathione reductase and thioredoxin reductase [5]. Se is believed to play an essential role in antioxidant protection due to its incorporation as selenocysteine into several antioxidant enzymes.

A deficiency of Se in China was found to lead to a cardiomyopathy known as Keshan disease [6]. Specific regions in China have Se-deficient soils and thus grains grown in the deficient soil are also deficient in Se. Individuals living in areas with Se-deficient soils who consume locally grown food will develop a deficiency in Se. Keshan disease is a cardiomyopathy characterized by necrotic lesions throughout the myocardium with varying degrees of cellular infiltration and calcification [7]. Supplying people living in Keshan disease endemic areas with selenium can prevent the disease.

However, the deficiency in Se did not appear to entirely explain the epidemiological pattern of the disease. Keshan disease had a seasonal and annual incidence, and not everyone who was Se deficient developed the disease. For these reasons, scientists in China suspected an infectious co-factor was required along with the deficiency in Se for the development of Keshan disease. Using both blood and tissue samples from Keshan disease victims, scientists in China were able to isolate enteroviruses from some of the samples [8]. Coxsackie B viruses were the most commonly identified.

Coxsackieviruses, small RNA enteroviruses in the Picornaviridae, are known to infect the heart and can cause myocarditis, or inflammatory heart disease. Using the technique of RT-PCR, several groups have reported coxsackievirus B3 sequences from archived Keshan disease hearts [9]. Thus, it appeared that a deficiency in Se together with a coxsackievirus infection resulted in Keshan disease.

In order to further characterize the relationship between

infection with coxsackievirus and a deficiency in Se, a mouse model was used. Mice are well-established models for coxsackievirus-induced myocarditis and develop a pattern of heart inflammation similar to that found in humans. In addition, well-characterized strains of coxsackievirus, both myocarditic and amyocarditic in mice, are available.

Mice were fed a diet deficient in Se beginning at the time of weaning. After a period of 4 weeks, glutathione peroxidase activity, a marker of Se status, was 1/5 of the activity of glutathione peroxidase from Se-adequate mice. Thus, short term feeding of a Se-deficient diet led to a moderate deficiency in Se.

Se-deficient and Se-adequate mice were infected with a normally amyocarditic strain of coxsackievirus B3 (CVB3/0). At various times post infection, the mice were killed and tissues were removed for study. As expected, the Se-adequate mice did not develop myocarditis when infected with the amyocarditic strain of virus. However, the Se-deficient animals did develop a moderate level of myocarditis [10]. The myocarditis was characterized by inflammatory foci scattered throughout the myocardium.

Heart virus titers revealed that the Se-deficient mice had 10 to 100-fold higher levels of virus in the heart post infection compared with the Se-adequate mice. This result suggested that there was impairment in the immune response, such that the virus was not as controlled in the Se-deficient mice as in the Se-adequate mice. Interestingly, the deficiency in Se did not affect the timing of clearance, as both Se-adequate and Se-deficient mice cleared the virus from the heart by day 14 post infection.

The immune response of the Se-deficient mice was found to be altered. Although the production of neutralizing antibody responses was not affected, the proliferative response of T cells to both mitogen and antigen were decreased. Because inflammation is the hallmark of coxsackievirus-induced myocarditis, expression of mRNA for several inflammatory chemokines was examined [11]. Monocyte chemoattractant protein-1 (MCP-1) was highly expressed at day 10 in the Se-deficient animals as compared with the Se-adequate animals. This increase in MCP-1 mRNA expression may be responsible for the inflammation found in the infected Se-deficient mice.

In addition to alterations in the expression of mRNA for MCP-1, expression of mRNA for γ -interferon (IFN) was greatly decreased in the Se-deficient mice [11]. γ -IFN is important in protecting cells from viral infection, and a decrease in γ -IFN may have been related to the increase in viral titers in the Se-deficient animals.

Thus, it appeared that an altered immune response might have been responsible for the myocarditis that developed in the Se-deficient mice infected with an amyocarditic strain of CVB3. Alternatively, the viral pathogen was also exposed to a Se-deficient environment and might also be affected.

COXSACKIEVIRUS GENOME CHANGES IN SE-DEFICIENT MICE

To determine if host factors alone were responsible for the development of myocarditis in the Se-deficient CVB3/0 infected mice, a passage experiment was performed. Se-adequate and Se-deficient mice were infected with CVB3/0. Seven days later, their hearts were removed and the virus isolated. The virus was renamed to reflect the tissue from which it had been isolated (CVB3/0Se⁺ isolated from Se-adequate animals and CVB3/0Se⁻ isolated from Se-deficient animals). CVB3/0Se⁺ and CVB3/0Se⁻ were passed back into Se-adequate mice. If the induction of myocarditis was due solely to host conditions, then the Se-adequate mice should not develop myocarditis from infection with either CVB3/0Se⁺ or CVB3/0Se⁻. However, Se-adequate mice infected with CVB3/0Se⁻ developed myocarditis, whereas Se-adequate mice infected with CVB3/0Se⁺ did not. These results strongly suggested that the virus that replicated in the Se-deficient mice underwent a genomic change.

To confirm that a change in viral genome had occurred, viruses recovered from Se-adequate and Se-deficient mice were sequenced [12]. The sequence of CVB3/0Se⁺ (recovered from Se-adequate mice) was identical to the original stock virus used to inoculate the mice. However, the sequence of CVB3/0Se⁻ (recovered from Se-deficient mice) had 6 point mutations. Each of the 6 mutations was also found in myocarditic strains of CVB3 virus. Thus, replication of CVB3/0 in a Se-deficient host leads to an alteration in viral genotype, changing a normally avirulent virus into a virulent one due to point mutations in the viral genome.

GLUTATHIONE PEROXIDASE AND COXSACKIEVIRUS

Why would a deficiency in Se lead to a change in viral genotype? One possibility is the association of Se with antioxidant enzymes. In particular, glutathione peroxidase, of which there are 4 isozymes, is a major component of the cellular antioxidant system. A deficiency in Se leads to a decrease in glutathione peroxidase activity. To determine if the decrease in glutathione peroxidase activity was associated with the increase in susceptibility to CVB3/0 induced myocarditis of Se-deficient mice, glutathione peroxidase-1 knockout mice were utilized.

Glutathione peroxidase-1 (GPX-1) knockout mice develop normally and do not have compensatory increases in other antioxidant enzymes under normal conditions [13]. However, GPX-1 knockout mice are at a higher risk for mortality when exposed to the pro-oxidant compound paraquat [14]. When infected with CVB3/0, a little over half of the GPX-1 knockout mice develop myocarditis, whereas CVB3/0 infection of wild-type mice does not induce myocarditis [15]. These results suggested that the increased susceptibility of Se-deficient mice

to develop myocarditis when infected with CVB3/0 was associated with a decrease in the activity of GPX-1.

The immune response of the GPX-1 KO mice was also altered in response to infection with CVB3/0. Neutralizing antibody levels were greatly decreased in the GPX-1 KO mice as compared with wildtype mice, although T cell proliferative responses to both mitogen and antigen were not affected. These results are in contrast to the results from the CVB3/0 infected Se-deficient mice, in which T cell proliferative responses were greatly inhibited and no changes in the production of neutralizing antibody were noted.

Cardiac viral titers were equivalent between CVB3/0 infected GPX-1 KO mice and wildtype mice. This was in contrast to Se-deficient mice, which developed higher cardiac titers when compared with Se-adequate mice. As was found for Se-adequate and Se-deficient mice, both wildtype and GPX-1 KO mice cleared virus from the heart at an identical rate.

Sequencing of virus recovered from CVB3/0 infected GPX-1 KO that developed myocarditis revealed seven nucleotide changes when compared with the stock virus [15]. Virus recovered from the infected wildtype mice had no genome changes when compared with stock virus. Six of the seven nucleotide changes found in virus recovered from GPX-1 KO mice were identical to the changes found in Se-deficient mice. Of particular importance, genomic changes were found only in virus recovered from CVB3/0 infected GPX-1 KO mice that developed pathology. The sequence of the CVB3/0 virus isolated from infected GPX-1 KO mice that did not develop cardiac pathology was identical to the stock virus. Thus, changes in the viral genome were responsible for the development of myocarditis in the GPX-1 KO mice.

INFLUENZA VIRUS AND SE-DEFICIENCY

Clearly, replication of coxsackievirus in a Se-deficient host leads to changes in the viral genome. Once these genomic changes occur, even mice with normal nutrition are susceptible to the newly pathogenic strain of virus. To determine if viruses other than coxsackievirus were also susceptible to changes in the viral genome due to replication in a Se-deficient host, infection with influenza virus was studied.

Influenza virus is a segmented RNA virus in the Orthomyxoviridae family. These viruses are responsible for a great deal of morbidity and mortality each year. Older adults and those with chronic diseases of the lung and/or heart are at the highest risk of dying from an influenza virus infection. Influenza viruses have a propensity to alter their surface proteins in order to escape early detection by the immune system of an infected host [16]. One such process is known as antigenic drift and is responsible for new strains of influenza that arise each year and infect their newly susceptible hosts due to small

changes in the hemagglutinin (HA) and neurominidase (NA) proteins, both of which are exposed on the viral surface. The HA and NA are recognized by the host antibody response, and changes in these two proteins can lead to the ability of the virus to escape immune detection. In addition, large-scale changes in the influenza virus surface proteins due to reassortment of viral RNA segments between influenza strains are also possible. Known as genetic shift, these large changes in the viral genome are responsible for worldwide pandemics that have occurred several times throughout history.

To determine if a deficiency in Se would affect the pathogenicity of an influenza virus, Se-deficient and Se-adequate mice were infected with a mild strain of influenza, influenza A/Bangkok/1/29. At various times post infection, the mice were killed and the tissues harvested. Se-deficient mice developed much more severe lung inflammation post influenza infection when compared with the infected Se-adequate mice [17]. The infiltrate in the lungs of the infected mice consisted predominantly of macrophages, CD4+ and CD8+ T cells. Se-deficient mice had decreased percentages of CD8+ cells infiltrating the lungs, compared with Se-adequate mice.

Examination of draining lymph nodes for mRNA for a variety of cytokines and chemokines revealed that infected Se-deficient mice had an increase in the production of pro-inflammatory cytokines and chemokines. In addition, the type of cytokine production was TH2-like (pro-inflammatory) as opposed to the more TH1-like pattern found in infected Se-adequate animals.

Sequencing of the mRNA segments that code for viral surface proteins (HA-hemagglutinin and NA-neurominidase) revealed little difference between virus recovered from Se-deficient vs. Se-adequate animals. However, the mRNA that codes for the matrix protein revealed 29 nucleotide changes, of which six led to amino acid changes. Virus recovered from Se-adequate animals had two nucleotide changes, of which one led to an amino acid change [18].

This finding was unexpected, as the matrix protein is relatively stable and exhibits little change among influenza virus strains. One possible explanation is that the matrix protein is internal, and thus not subjected to immune pressure from the host antibody response. In contrast, changes in the HA and NA proteins are responsible for the ability of the virus to escape detection by a previously exposed host. The HA and NA are exposed on the surface of the virion and are therefore exposed to immune pressure.

To date, we have sequenced only three of the eight RNA segments of the influenza virus. Although we found many more changes in the matrix protein of the virus recovered from Se-deficient mice as compared with virus recovered from Se-adequate mice, we do not know if other changes are present in the influenza virus genome. Further investigation is currently underway.

CONCLUSIONS

Studies utilizing both Se-deficient mice as well as GPX-1 knockout mice demonstrate both Se and GPX-1 activity as providing a unique role in preventing enhanced virulence of both coxsackievirus as well as influenza virus. As diagrammed in Fig. 1, in addition to having an effect on the immune system of the host, a deficiency in Se and/or a deficiency in glutathione peroxidase activity can lead to enhanced virulence of a viral pathogen due to genetic changes in the viral pathogen itself. Once these changes have occurred, even hosts with normal Se status and glutathione peroxidase activity are susceptible to its newly virulent properties.

This work suggests a new way of examining the effect of host nutritional deficiencies on increased susceptibility to viral infection. Although it is clear that nutritional deficiencies can have a profound effect on host immunity, as shown by us as well as many others, it is also clear that the viral pathogen itself is susceptible to the deficiency.

What is the mechanism that allows the viral pathogen to mutate in the deficient host? One possibility is a selection mechanism. Both coxsackievirus and influenza virus are RNA viruses which have a high mutation rate due to a lack of proofreading enzymes during replication. Thus, mutant viruses will be generated each time the virus replicates and sequencing of the virus reveals only the consensus or dominant sequence. A host deficiency in Se leads to alterations in the immune response of the infected host, which in turn could allow the selection of a new viral variant with more pathogenic properties. A second possibility may involve increased oxidative stress that occurs in the Se-deficient mice due to a lack of the antioxidant glutathione peroxidase. The increased oxidative stress status of the host may cause direct damage to the viral RNA itself, resulting in new mutations that lead to enhanced

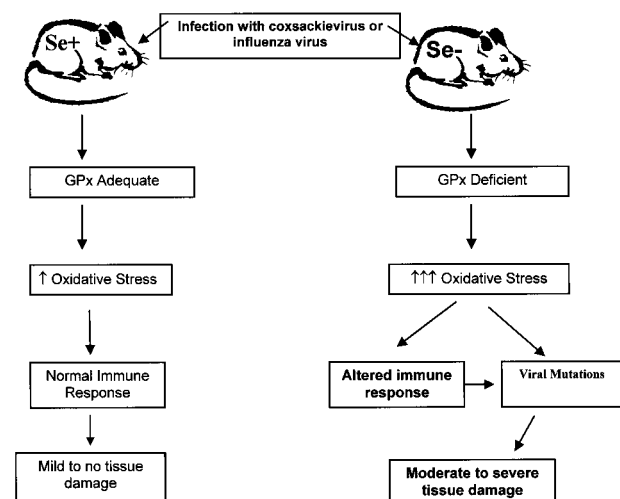


Fig. 1. Diagram depicting the influence of a lack of selenium or glutathione peroxidase activity on the development of pathogenesis post viral infection.

pathogenesis. Both of these possibilities are currently under investigation.

In summary, the nutritional status of the host is an important variable when considering viral pathogenesis. With the current interest in emerging infectious diseases, it would be important to consider the host nutritional status as a driving force for viral mutations.

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