Increased Virulence of Coxsackievirus B3 in Mice Due to Vitamin E or Selenium Deficiency¹,²

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ABSTRACT Nutrition has long been known to affect the ability of the host to respond to infectious disease. Widespread famines are often accompanied by increased morbidity and mortality due to infectious diseases. The currently accepted view of the relationship between nutrition of the host and its susceptibility to infectious disease is one of a direct relationship with host immune status. That is, if the nutritional status of the host is poor—due to either single or multiple nutrient deficiencies—then the functioning of the host immune system is compromised. This impairment of the immune response will lead to an increased susceptibility to infectious disease. Clearly, the immune response has been shown to be weakened by inadequate nutrition in many model systems and in human studies. However, what about the effect of host nutrition on the pathogen itself? Our laboratory has shown, using a mouse model of coxsackievirus-induced myocarditis, that a host deficiency in either selenium or vitamin E leads to a change in viral phenotype, such that an avirulent strain of the virus becomes virulent and a virulent strain becomes more virulent. The change in phenotype was shown to be due to point mutations in the viral genome. Once the mutations occur, the phenotype change is stable and can now be expressed even in mice of normal nutriture. Our results suggest that nutrition can affect not only the host, but the pathogen as well, and demonstrate a new model of relating host nutritional effects to viral pathogenesis. J. Nutr. 127: 966S–970S, 1997.

KEY WORDS: • Keshan disease • cardiomyopathy • enterovirus • viral evolution • myocarditis • mice

The currently accepted model for the effect of nutrition on susceptibility to infectious disease is a unidirectional one that can be represented as follows: Inadequate nutrition → dysfunctional immune response → increased susceptibility to infectious disease. That is, nutritional deficiencies, either in single nutrients or as generalized malnutrition, have deleterious effects on the immune system. For example, a deficiency in zinc leads to decreased natural killer cell and delayed-type hypersensitivity responses (Walsh et al. 1994). General malnutrition leads to reduction in both cellular and humoral immune responses.

Because the immune system is required both for protection from infection and for clearance of infectious agents once infection has occurred, any disruption in immune function would be expected to increase the likelihood of an infectious agent gaining a foothold in the host. For example, rotavirus infection in well-nourished infants results in only mild diarrhea from which the infant recovers. However, rotavirus infection in undernourished children leads to massive diarrhea and high rates of mortality (Guerrant et al. 1983).

Although the unidirectional model for the effect of nutrition on immune function has served well for many years, data from our laboratory suggest it is time to introduce a new model. Specifically, what is the effects of the nutritional state of the host directly on the pathogen? That is, is the pathogen itself responses (Walsh et al. 1994). General malnutrition leads to reduced natural killer cell and delayed-type hypersensitivity responses (Walsh et al. 1994). General malnutrition leads to reduction in both cellular and humoral immune responses.

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Our work with the effect of host nutrition on coxsackievirus-induced myocarditis grew out of observations on the etiology of Keshan disease. Keshan disease, an endemic cardiomyopathy affecting, until recently, thousands of women of child-bearing age and children in China, is a Se-responsive cardiomyopathy (Yang et al. 1988). Individuals living in Keshan disease endemic areas with soils deficient in the trace element Se were found to have cardiac damage. Oral supplementation of the people with Se could completely prevent the disease. However, the seasonal and annual incidence of Keshan disease suggested that an infectious co-factor was required along with the Se deficiency for expression of the disease. Because cox-

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sackieviruses are known etiological agents of viral-induced myocarditis (Shingu 1989) and because scientists in China were able to isolate coxsackieviruses from some of the victims in the development of Keshan disease (Su 1979), it seemed plausible that infection with coxsackievirus along with Se deficiency was required for the development of Keshan disease.

This article will summarize data, both published (Beck et al. 1994a, 1994b, 1994c and 1995) and unpublished, from our studies of a mouse model for coxsackievirus-induced myocarditis. This model closely mimics the human disease and can be used for nutritional studies by varying the diets fed to the animals prior to infection. Using this model, we fed mice a diet deficient in either Se or vitamin E prior to infection with coxsackievirus. We chose to look at vitamin E along with Se because some residents of Keshan disease areas were also of marginal vitamin E status. Both Se and vitamin E act as antioxidants, although by two very different mechanisms.

**DESCRIPTION OF MODEL**

C57H/Hej mice at 3 wk of age were fed either a diet adequate in Se and vitamin E or a diet deficient in either Se or vitamin E for 4 wk. In addition, either menhaden oil or lard was used as the fat source for the vitamin E diets (Beck et al. 1994a and 1994b). Menhaden oil was added as a fat source to increase the rate of depletion of vitamin E stores in the mice. Following the feeding period, mice were inoculated with either 10^7 tissue culture infectious dose (TCID_50) coxsackievirus B3/20 (CVB3/20, myocarditis strain of CVB3) or CVB3/0 (amyocarditic strain of CVB3). Mice were killed at various times after inoculation and examined for presence and severity of heart pathology virus titer and immune function.

**SERUM GLUTATHIONE PEROXIDASE ACTIVITIES AND VITAMIN E CONCENTRATIONS**

Mice fed Se-deficient diets had decreased glutathione peroxidase activity compared with mice fed diets adequate in Se (Beck et al. 1994c). We used glutathione peroxidase as a biomarker of Se status. Vitamin E-deficient diets led to decreased levels of serum α-tocopherol, with the most severe deficiency seen in mice fed the vitamin E-deficient diet containing menhaden oil as a fat source (Beck et al. 1994a).

**HEART PATHOLOGY OF MICE FED NUTRITIONALLY ADEQUATE OR DEFICIENT DIETS**

As shown in Figure 1, mice infected with CVB3/20 developed more severe heart pathology when fed the diets deficient in either Se or vitamin E as compared with mice fed diets adequate in both Se and vitamin E. The most severe pathology was seen in mice fed the menhaden oil–containing vitamin E–deficient diet. As expected, when mice were infected with the amyocarditic CVB3/0 strain, no pathology was noted in the mice fed the adequate diets. However, cardiac pathology was observed in the mice fed the deficient diets (Fig. 2). Again, the most severe pathology was seen in mice fed the menhaden oil–based vitamin E–deficient diet.

**VIRUS TITERS IN HEARTS OF INFECTED MICE FED NUTRITIONALLY ADEQUATE OR DEFICIENT DIETS**

Because the increase in heart pathology in the Se- or vitamin E–deficient animals may have been related to a change in virus titers in the heart, we examined heart virus titers at various times after infection. Virus titers were elevated in CVB3/20-infected mice fed the Se- or vitamin E–deficient diet.
Histopathology of Se- and vitamin E-adequate recipient mice inoculated with CVB3/0 virus recovered from Se- or vitamin E-adequate or deficient donor mice

<table>
<thead>
<tr>
<th>Diet fed donor mice</th>
<th>Incidence of heart lesions</th>
<th>Pathologic index of heart lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lard: +Se + E</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td>Lard: - Se + E</td>
<td>8/10</td>
<td>2+</td>
</tr>
<tr>
<td>Lard: + Se - E</td>
<td>9/10</td>
<td>2+</td>
</tr>
</tbody>
</table>

1 Recipient mice were killed 10 d after inoculation. Pathologic index was determined as described in Figure 1. (Modified from Beck et al. 1994a and 1994b).

IMMUNE RESPONSE AFTER VIRAL INFECTION IN MICE FED NUTRITIONALLY ADEQUATE AND NUTRITIONALLY DEFICIENT DIETS

To determine whether the immune system was compromised in the mice fed the deficient diets, thus leading to the increased virus titers, we examined several immune indicators. Immune functions studied included spleen cell proliferative responses to mitogens and specific CVB3 antigen, virus-specific neutralizing antibody responses and natural killer (NK) cell activity. Although neutralizing antibody titers were equivalent among diet groups (data not shown), mitogen (Fig. 3) and antigen (Fig. 4) responses were significantly decreased in the animals fed the deficient diets compared with those fed the adequate diets. Natural killer cell activity was not affected, except for mice fed the diets with menhaden oil, regardless of vitamin E status (Beck et al. 1994a). Menhaden oil is known to decrease NK activity (Gauntt et al. 1989), and a similar decrease in NK activity was also found in our study. Therefore, although B cell activity was intact, as measured by the production of neutralizing antibodies, T cell proliferative responses to both mitogen and antigen were decreased in mice fed diets deficient in either Se or vitamin E. The decreased responsiveness of the T cells may have allowed higher cardiac titers to occur in the deficient animals after CVB3 inoculation.

VIRAL PHENOTYPE CHANGE IN NUTRITIONALLY DEPRIVED MICE

There were two possibilities to account for the results that demonstrated that diets deficient in either Se or vitamin E led to increased cardiopathy after CVB3 infection. One possibility was that the condition of the host was critical. That is, the elevated oxidative stress status of the host, induced by a diet deficient in either Se or vitamin E, was critical for the increased damage that occurred after infection. This model predicts that the increased pathology would occur only in mice fed the deficient diets. The other possibility was that the virus phenotype itself had changed. That is, once the phenotype had been altered in the deficient animal, the virus would now maintain that phenotype even in nutritionally adequate animals. To test this hypothesis, we isolated virus from CVB3/0-infected Se- or vitamin E-deficient donor mice and passed the isolated viruses back into nutritionally adequate recipient mice. Ten days after infection with the passaged virus, the mice were killed and the hearts examined for pathology. The nutritionally adequate mice developed illness similar to that in the nutritionally deprived mice (Table 1). That is, CVB3/0 virus obtained from either Se- or vitamin E-deficient mice caused disease in nutritionally adequate mice, even though the parent virus (the original inoculum) could not. Control experiments, in which CVB3/0 was passaged through nutritionally adequate mice, demonstrated that passage alone did not alter the phenotype of the virus. Thus, the phenotype of the virus was altered when the virus replicated in the nutritionally deprived animals. After the phenotype of the virus was altered, it was no longer a requirement for replication to occur in a deficient host for the change in phenotype to be expressed.
proteins. 2A is a nonstructural viral protease.


