Nutritionally induced oxidative stress: effect on viral disease¹⁻³

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ABSTRACT  It has long been known that the nutritional status of the host can influence both susceptibility to infectious disease and the severity of the disease if contracted. In studies of coxsackievirus infection and selenium deficiency in mice, we found that mice fed a selenium-deficient diet developed myocarditis, but mice fed a diet adequate in selenium did not. Similarly, mice fed a diet deficient in vitamin E developed myocarditis, but mice fed a diet with adequate vitamin E did not. The epidemic of optic and peripheral neuropathy that occurred in Cuba in the early 1990s provides another example of how the nutritional status of the host may affect the impact of a virus. Patients who developed neuropathy had lower blood concentrations of riboflavin, vitamin E, selenium, α- and β-carotenes, and the carotenoid lycopene, which suggests that the disease was associated with an impairment of protective antioxidant pathways. After supplementation of the population with these nutrients, the disease began to subside. The nutritional status of the host can have a profound influence on a virus, so that a normally avirulent virus becomes virulent because of changes in the viral genome. Our studies suggest that outbreaks of disease attributed to a nutritional deficiency may actually result from infection by a virus that has become pathogenic by replicating in a nutritionally deficient host. Am J Clin Nutr 2000;71(suppl):1676S–9S.

KEY WORDS  Oxidative stress, viral disease, selenium, coxsackievirus, myocarditis, antioxidants, mice

INTRODUCTION  The nutritional status of the host has long been known to influence both susceptibility to infectious disease and the severity of the disease if contracted. Many investigations have shown that diets lacking one or more nutrients can exacerbate the consequences of either bacterial or viral infections (1, 2). This increased susceptibility is widely attributed to changes in the immune status of the host. Thus, the current paradigm holds that a nutritional deficiency will decrease the immune response of the host, leading in turn to increased susceptibility to infection. One must consider, however, that the pathogen is replicating in a nutritionally deficient environment (the host), which might also be expected to influence the pathogen. Our work in this area has focused on both changes in the host and changes in the pathogen. We have found that the nutritional status of the host can have a profound influence on a virus, such that a normally avirulent virus acquires virulence as a result of changes in the viral genome.

COXSACKIEVIRUS INFECTION IN SELENIUM-DEFICIENT HOSTS  Our work with coxsackievirus infection and selenium deficiency grew out of a study of Keshan disease, a cardiomyopathy which occurs in regions of China in which the selenium content of the soil is very low (3). Because the food consumed by residents of these regions was grown locally, persons residing in areas with low selenium in the soil became selenium deficient and developed Keshan disease. Supplementation with selenium can prevent Keshan disease, but because its incidence changes seasonally and annually, and because not every person deficient in selenium develops Keshan disease, Chinese scientists concluded that an infectious cofactor was also required. Coxsackieviruses, which are known to infect heart muscle, were suspected, and this virus has indeed been isolated from the blood and tissues of persons with Keshan disease (4, 5). To investigate further the role of infection with coxsackievirus in the development of Keshan disease, we used our well-characterized mouse model of coxsackievirus-induced myocarditis.

Mice were fed a diet either deficient or adequate in selenium for 4 wk, at which time they were inoculated with an avirulent strain of coxsackievirus B3, CVB3/0 (6). This virus replicates in the heart muscle but does not cause myocarditis. Ten days after infection, mice fed the diet deficient in selenium developed myocarditis, which is characterized by inflammation of the myocardium, but mice fed the selenium-adequate diet did not (Figure 1).

Although viral titers were larger in the selenium-deficient mice, the time from infection required to clear the virus from the heart was similar in selenium-deficient and selenium-adequate mice (6). To determine whether the immune response of the selenium-deficient mice was altered, we examined neutralizing antibody titers, antigen, and mitogen responses as well as natural killer cell activity (Table 1). Although neutralizing antibody titers of selenium-deficient and selenium-adequate mice did not differ significantly, both mitogen and antigen responses were significantly lower in selenium-deficient mice (P < 0.001). Natural killer cell activity was not affected by dietary selenium status.

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mice receiving normal nutrition are vulnerable to the virus. Furthermore, once the genomic changes have occurred, even selenium-deficient mice because of changes in the viral genome. avirulent in selenium-adequate mice becomes virulent in selenium-deficient mice subsequent to CVB3/0 infection. We found 6 nucleotide changes in the viral genome of virus recovered from selenium-deficient mice (8). The additional nucleotide change was at nucleotide 2690, from a guanosine in the CVB3/0 input virus to an adenosine in the virus recovered from the knockout mouse. No changes were found in the genome of virus recovered from knockout mice that did not develop pathologic lesions, which provides evidence of a strict association between viral genomic changes and virulence.

To determine whether the change in viral phenotype was due to a change in viral genotype, we sequenced virus recovered from knockout mice and wild-type mice. We found 7 nucleotide changes in the viral genome of virus recovered from knockout mice, 6 of them identical to changes in the genome of virus recovered from the selenium-deficient mice (8). The additional nucleotide change was at nucleotide 2690, from a guanosine in the CVB3/0 input virus to a adenosine in the virus recovered from the knockout mouse. No changes were found in the genome of virus recovered from knockout mice that did not develop pathologic lesions, which provides evidence of a strict association between viral genomic changes and virulence.

These results suggest that the nucleotide changes in the selenium-deficient animals were driven by a decrease in glutathione peroxidase activity. That not all of the knockout animals developed myocarditis, suggests that mechanisms other than a lack of glutathione peroxidase may also be involved in the susceptibility of selenium-deficient mice.

VITAMIN E DEFICIENCY AND COXACKIEVIRUS INFECTION

Could the effect of selenium deficiency on the CVB3 virus be mimicked in other antioxidant nutrient deficiencies? To answer this question, we fed mice a diet deficient in vitamin E, which, like selenium, acts as an antioxidant, but by a different mechanism. Under certain conditions, vitamin E and selenium can spare one another’s activities.
Mice were fed a diet deficient in vitamin E for 4 wk before infection. Menhaden oil, which is rich in n-3 fatty acids, was used in some of the diets to accelerate the vitamin E deficiency because this oil, a peroxidizable fat, is known to increase the rate of vitamin E depletion (9). The other mice receiving a diet deficient in vitamin E were given lard. The mice fed the diets deficient in vitamin E developed myocarditis on infection with the avirulent CVB3/0 virus, but infected mice fed the diet adequate in vitamin E did not (10). The most severe lesions were noted in the vitamin E-deficient group fed menhaden oil. Virus titers were larger in the vitamin E-deficient mice, with viral clearance occurring by the 14th day after infection in all groups (10).

As in the case of selenium-deficient mice, neutralizing antibody titers of deficient and adequate mice did not differ significantly, and both mitogen and antigen responses were smaller in the deficient mice. To determine whether the changes in viral virulence were also due to a phenotype change in the virus, a virulent strain that has been sequenced.

Our results show that a diet deficient in antioxidant nutrients affects not only the host but the viral pathogen as well. We suggest that the current paradigm of nutritional deficiency affecting the host immune system, thereby leading to increased susceptibility to infection, be changed to one in which the nutritional deficiency can affect both the host and the pathogen.

OPTIC AND PERIPHERAL NEUROPATHY IN CUBA

Another example in which the nutritional status of the host may affect a virus is the epidemic of optic and peripheral neuropathy that occurred in Cuba in the early 1990s, affecting > 50 000 people. The illness was associated with dietary limitations and increased physical demands that occurred during food and fuel shortages in Cuba beginning in 1989. Extensive epidemiologic studies carried out with international cooperation (11–15) showed that patients had lower blood concentrations of riboflavin, vitamin E, selenium, α- and β-carotenes, and the carotenoid lycopene, suggesting that the disease was associated with an impairment of protective antioxidant pathways. Smoking was also a risk factor, again thought to be due to injury through oxidative damage. Oral supplementation of the entire population was begun in May of 1993, and the disease began to subside, although cases still occur sporadically.

To rule out an infectious agent, attempts to isolate a virus from cerebrospinal fluid (CSF) of neuropathy patients were made in 1993. Unexpectedly, viruses resembling enteroviruses were isolated from 105 of 125 (84%) CSF specimens (16). Five of these isolates were typical strains of coxsackievirus A9 (CVA9). The other 100 isolates produced a slowly progressive cytopathic effect (CPE) on Vero cells and were designated “light CPE” virus. Antigenically, they were related to both CVA9 and CVB4. In western blot experiments, they were found to lack the capsid proteins typ-

### TABLE 1
Comparison of immune responses of selenium-adequate and selenium-deficient mice infected with CVB3/0

<table>
<thead>
<tr>
<th>Diet</th>
<th>Neutralizing antibody titer</th>
<th>Proliferative response</th>
<th>% specific lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mitogen</td>
<td>Antigen</td>
</tr>
<tr>
<td>Selenium-adequate</td>
<td>153 ± 101&lt;sup&gt;4&lt;/sup&gt;</td>
<td>55 ± 10</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>Selenium-deficient</td>
<td>125 ± 90</td>
<td>5.1 ± 1.3&lt;sup&gt;3&lt;/sup&gt;</td>
<td>9.8 ± 0.9&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Determined as described by Beck et al (6).
<sup>2</sup> As assessed against YAC-1 tumor cells and reported at a 20:1 effector-to-target ratio.
<sup>3</sup> ± SD; n = 20.
<sup>4</sup> X significantly different from selenium-adequate mice, P < 0.001.

### TABLE 2
Nucleotide changes that occurred in the genome of CVB3/0, which was isolated from selenium-deficient mouse<sup>6</sup>

<table>
<thead>
<tr>
<th>Viral strain</th>
<th>Strain phenotype</th>
<th>Genomic nucleotide number (5/-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>234</td>
</tr>
<tr>
<td>CVB3/20&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Myocarditic</td>
<td>T</td>
</tr>
<tr>
<td>CVB3/0&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Amyocarditic</td>
<td>C</td>
</tr>
<tr>
<td>CVB3/0Se&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Amyocarditic</td>
<td>C</td>
</tr>
<tr>
<td>CVB3/0Se&lt;sup&gt;5&lt;/sup&gt;-&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Myocarditic</td>
<td>T</td>
</tr>
</tbody>
</table>

<sup>1</sup> Adapted from Beck et al (6).
<sup>2</sup> A virulent strain that has been sequenced.
<sup>3</sup> Sequenced before inoculating either selenium-adequate or selenium-deficient mice.
<sup>4</sup> Isolated from the heart of a selenium-adequate mouse inoculated with CVB3/0.
<sup>5</sup> Isolated from the heart of a selenium-deficient mouse inoculated with CVB3/0. Three other isolates from selenium-deficient mice had the identical sequence.
ical of enteroviruses, which contain the major epitopes for neutralization. Light CPE virus persisted in the CSF of some patients for 1–12 mo. The CSF of one patient yielded CVA9 on the first culture attempt and a virus of the “variant” type from a second culture 1 mo later. Just before the epidemic, CVA9 was circulating in the population. Was the neuropathy due to emergence of a new strain of CVA9? Was it the result of replication of the virus in an oxidatively stressed host with nutritional deficiencies?

To determine the genotypic differences between the virus isolated from the Cuban patients and CVA9, we partially sequenced one Cuban isolate (44/93 IPK) and compared its sequence with the published sequence of CVA9 (Beck and Handy, unpublished observations, 1998). The most striking difference between the 2 viruses was the active site of the 2A protease, which performs the primary cleavage of the structural protein precursor from the rest of the polypeptide chain. This cleavage must occur to yield capsid proteins, which form the surface of the virus (17). The picornavirus 2A protease is structurally and functionally similar to cellular serine proteases, which characteristically fold to form a catalytic triad of histidine, aspartic acid, and serine. In the picornaviruses, however, the catalytic site contains cysteine instead of serine, and the enzyme is inhibited by compounds known to inhibit thiol proteases.

The Cuban isolate, 44/93 IPK, resembles other enteroviruses in that it contains the 3 amino acids of the 2A catalytic triad: His 21, Asp 39, and Cys 110. However, 44/93 IPK, unlike CVA9 or any of the other known enteroviruses, has a mutation that introduces another cysteine 4 residues away from the active site, at position 25. The CVA9 strains studied by Chang et al (18) all have histidine or arginine at this locus; coxsackievirus B strains have histidine, arginine, or serine. The introduction of another cysteine so close to the essential cysteine of the catalytic site suggests that dimerization may occur to form cystine and thereby inactivate the enzyme, especially under oxidizing conditions. 44/93 IPK has 2 other amino acid substitutions within 5 positions of the 2A catalytic site, neither of which occurs in any of the CVA9 or CB strains studied: lysine for threonine at position 26 and isoleucine for valine at position 17. Impairment of the function of the 2A protease would prevent appropriate cleavage of the structural region from the rest of the polypeptide and would interfere with the subsequent processing of polyprotein to form the viral capsid proteins. This may explain the apparent absence of the normal capsid proteins in the western blot experiments (16) and the appearance instead of a high-molecular-weight protein in the western blot experiments (16) and the appearance instead of a high-molecular-weight protein postulated to be a capsid protein precursor.

In contrast with the protease 2A, the other major enterovirus protease (3C) of 44/93 IPK has only 6 amino acid substitutions among its 183 positions when compared with CVA9, and none is near the catalytic site. This variation is consistent with that reported for this enzyme among enteroviruses (17).

Thus, we hypothesize that the virus isolated from Cubans with epidemic neuropathy may be a CVA9 virus that mutated as a result of replication in an oxidatively stressed host. These mutations then led to a change in viral phenotype, altering the pathogenicity of the virus. Further study is required to understand the relation between the altered virus and the neuropathic response.

CONCLUSIONS

New viruses continue to appear through the evolution of existing viruses, and this is particularly true for RNA viruses, which have high mutation rates and lack proofreading capability. Several factors have been invoked to explain the emergence of new viruses and the reemergence of known viruses, including global warming, changes in industrial or agricultural processes, and worldwide travel. Little has been said, however, about the impact of the nutritional status of the host. We believe that the nutritional status of the host should be considered in the context of infectious disease, not just from the viewpoint of the host but also from the viewpoint of the infecting agent. Outbreaks of disease attributed to a nutritional deficiency may actually be the result of infection by a virus whose pathogenicity has changed as a result of replicating in a nutritionally deficient host. Clearly, more interdisciplinary research, bringing together both nutritionists and infectious disease specialists, is needed.

REFERENCES