ELSEVIER

PII: S0308-8146(96)00066-0

Food Chemistry, Vol. 57, No. 1, pp. 47–49, 1996
Published by Elsevier Science Ltd
Printed in Great Britain
0308-8146/96 $15.00+0.00

Viral evolution as driven by host nutritional selective factors: influence of dietary oxidative stress

Orville A. Levander* & Melinda A. Beck

Nutrient Requirements and Functions Laboratory, Beltsville Human Nutrition Research Center, US Department of Agriculture, ARS, Beltsville, Maryland, USA

Frank Porter Graham Child Development Center, University of North Carolina, Chapel Hill, North Carolina, USA

The endemic juvenile cardiomyopathy known as Keshan disease occurs in regions of China with poor selenium nutrition, but a role for an infectious agent was suggested by seasonal changes in disease incidence. Mice fed a selenium-deficient diet suffered more heart damage than normal mice when infected with a myocarditic coxsackievirus B3 (CVB3/20). Increased heart damage was also observed when CVB3/20 was inoculated into vitamin E-deficient mice. Feeding diets deficient in either vitamin E or selenium allowed an amyocarditic coxsackievirus (CVB3/0) to become myocarditic. When CVB3/0 was harvested from deficient mice, passed through HeLa cells and inoculated into normal (non-deficient) mice, it retained its increased cardiovirulence. Virus obtained from the selenium-deficient mice contained six nucleotide changes in the genome compared with the input strain. This is the first report of a nutritional deficiency driving changes in a viral genome. Host nutritional status could have important public health implications for the spread of influenza, hepatitis, polio and perhaps even AIDS. Published by Elsevier Science Ltd

KESHAN DISEASE, SELENIUM AND INFECTION

In 1979, Chinese scientists described an endemic juvenile cardiomyopathy known as Keshan disease that is associated with poor selenium nutrition and could be prevented by improving the selenium status of the population at risk (Keshan Disease Research Group, 1979*a*). Indicators of low selenium intake in the endemic areas included low blood and hair selenium content and low levels of selenium in the foods typically consumed in such regions. The low selenium content of the food supply was attributable to the selenium-poor soils in the Keshan disease zones. Plants do not appear to have a nutritional requirement for selenium and their selenium content tends to reflect whatever amount is available for uptake from the soil.

Widespread selenium supplementation in the deficient areas protected against heart damage so it is clear that selenium deficiency plays a fundamental role in the development of Keshan disease (Keshan Disease Research Group, 1979*b*). However, some features of the disease, such as pronounced seasonal and annual fluctuations in its incidence, could not readily be accommodated solely on the basis of selenium status (Yang *et al.*, 1988). Rather, it was suggested that some infectious agent might be involved and a number of viruses, particularly enteroviruses, were isolated from patients suffering from Keshan disease. One such virus, coxsackievirus B4, was tested for cardiotoxicity in mice fed diets of varying selenium content. Ge *et al.* (1987) found that the cardiopathology induced by the virus was much greater in selenium-deficient mice than in normal mice.

SELENIUM AND VIRAL MYOCARDITIS

More recently, Beck *et al.* (1994*a*) found a similar effect of selenium deprivation in mice infected with CVB3/20, a virulent strain of the coxsackievirus B3. Heart damage appeared more rapidly and was more pronounced in selenium-deficient than in normal mice. Cardiac viral titers were also higher in the deficient mice, thereby providing a likely explanation for the increased heart pathology in these animals.

*To whom correspondence should be addressed.

The mechanism by which the virus causes greater damage in selenium-deficient mice is not clear, but selenium deficiency is known to have direct effects on the cardiomyocytes of several species as well as effects on the host's immune system. For example, myocardial degeneration is a characteristic feature of selenium–vitamin E deficiency in the young of several animals including swine, sheep, cattle and horses (Combs & Combs, 1986). Perhaps the increased heart damage due to viral infection in selenium-deficient mice is merely a function of a generalized decreased metabolic stability of the cardiac muscle in such animals which renders their cardiac tissue more vulnerable to a variety of environmental insults, including attack by an infectious agent. On the other hand, selenium deficiency is known to have a number of deleterious effects on humoral as well as cell-mediated immune functions (Combs & Combs, 1986). Of course, any impairment in immune response could allow the virus to elude host defense systems and replicate more freely, as found in our experiments.

Under our conditions selenium deficiency did not appear to interfere with the ability of the host to mount a satisfactory humoral response since neutralizing antibody production was similar in CVB3/20-infected mice regardless of their selenium status.

Although natural killer (NK) cell activity was slightly depressed in CVB3/20-infected selenium-deficient mice compared with infected adequate mice, the small difference in NK function observed was not thought to be sufficient to account for the great differences noted in cardiopathology. However, appreciable differences in splenocyte function were seen between the deficient and supplemented group. Splenocytes from CVB3/20-infected selenium-deficient mice responded less vigorously to mitogenic (concanavalin A) or antigen-specific (CVB3/20 antigen) stimulation than infected supplemented mice. Whether this defect in lymphocyte proliferation represents the mechanism that allows the CVB3/20 to exert its greater cardiotoxic effect in selenium-deficient mice is a question that can only be answered by additional research.

Beck *et al.* (1994*b*) next turned their attention to the effect of selenium deficiency on the pathogenicity of a benign strain of the coxsackievirus (CVB3/0) that normally causes no heart damage in mice. As expected, in these experiments the hearts of the selenium-supplemented CVB3/0-infected mice appeared perfectly normal and exhibited no evidence of pathology. On the other hand, if the CVB3/0 were given to selenium-deficient mice, the virus caused significant heart damage. Again, the cardiac viral titers were significantly higher in the deficient group when compared to the supplemented group. As in the case of the CVB3/20-infected mice, the CVB3/0-infected mice all developed similar neutralizing antibody titers regardless of their selenium status.

SELENIUM AND VIRAL EVOLUTION

In order to determine whether the enhanced virulence of the CVB3/0 seen in the deficient mice was due to a genetic change in the amyocarditic virus, viruses were isolated from selenium-deficient and -adequate mice infected with the benign strain. When the virus was isolated from the deficient host and reinoculated into normal mice, it was still found to exhibit significant myocarditis. Thus, passage of the benign virus through a deficient host had altered its ability to cause heart damage in a subsequent normal host. The most straightforward explanation for this result was that passage of the benign strain through a deficient host had changed the genetic make-up of the virus.

Characterization of the nucleotide sequence of the benign CVB3/0 that had been passed through a selenium-deficient mouse (now called CVB3/0Se$^-$ by Beck *et al.*, 1995) revealed that the newly virulent virus had a genome that acquired the base composition of the wild type virulent strain (CVB3/20) at six of seven positions that are known to influence virulence in the coxsackievirus. As far as the authors were aware, this was the first report describing a change in the genetic structure of a virus as influenced by the nutritional status of the host. In the past, whenever nutritionists talked about nutrition–infection interactions, the discussion always focused on effects of host nutritional status on the host, not the pathogen. Yet the results of Beck *et al.* (1995) clearly show that host nutrition can have a profound influence on the pathogen infecting the host as well, even to the point of altering the genetics of the pathogen itself.

The mechanism by which host selenium status altered viral genetics is not established but it should be realized that many of the effects described here due to selenium deficiency are also seen in vitamin E-deficient mice (Beck *et al.*, 1994*c*). Moreover, the effects of vitamin E deficiency on coxsackievirus virulence are further exacerbated by the concurrent feeding of fish oil, a known pro-oxidant stressor. The simplest explanation for all these virulence-enhancing effects due to host nutritional manipulation (selenium deficiency, vitamin E deficiency, fish oil supplementation) is that host oxidative stress is somehow altering viral expression within the host. Additional research will be needed to determine whether host oxidative stress is accomplishing this by directly stimulating the mutation rate of the virus to a more virulent form or by allowing conditions favorable for the selection of an already mutated virulent form to dominate. In either case, the end result is the same: nutritional status has permitted the emergence of a virulent virus.

ROLE OF HOST OXIDATIVE STRESS IN OTHER VIRAL DISEASES

If the results of Beck *et al.* (1995) with the coxsackievirus are applicable to other RNA viruses, these findings could be highly significant from the public health point of view. For example, Pace & Leaf (1995) have recently summarized the information favoring the hypothesis that oxidative stress may be implicated in the progression of HIV disease. Several antioxidants,

including selenium and vitamin E, occur in lower concentrations in HIV patients than in healthy people. Markers of oxidative stress such as serum malondialdehyde and hydroperoxide levels are also elevated in individuals infected with HIV. The pathogenesis of HIV could be exacerbated by host oxidative stress by a number of interrelated mechanisms including stimulation of viral replication, increased inflammatory response or decreased immune proliferative response. Obviously, the numerous investigative leads generated by the novel research of Beck and colleagues should be pursued vigorously.

REFERENCES

Beck, M. A., Kolbeck, P. C., Rohr, L. H., Qing, S., Morris, V. C. & Levander, O. A. (1994*a*). Increased virulence of a human enterovirus (coxsackievirus B3) in selenium-deficient mice. *J. Infect. Dis.*, **170**, 351–357.

Beck, M. A., Kolbeck, P. C., Rohr, L. H., Qing, S., Morris, V. C. & Levander, O. A. (1994*b*). Benign human enterovirus becomes virulent in selenium-deficient mice. *J. Med. Virol.*, **43**, 166–170.

Beck, M. A., Kolbeck, P. C., Rohr, L. H., Qing, S., Morris, V. C. & Levander, O. A. (1994*c*). Vitamin E deficiency intensifies the myocardial injury of coxsackievirus B3 infection in mice. *J. Nutr.*, **124**, 345–358.

Beck, M. A., Shi, Q., Morris, V. C. & Levander, O. A. (1995). Rapid genomic evolution of a non-virulent coxsackievirus B3 in selenium-deficient mice results in selection of identical virulent isolates. *Nature Med.*, **1**, 433–436.

Combs, G. F., Jr & Combs, S. B. (1986). *The Role of Selenium in Nutrition*. Academic Press, Orlando.

Ge, K.-Y., Bai, J., Deng, X.-J., Wang, S.-Q., Xue, A. N. & Su, C.-Q. (1987). The protective effect of selenium against viral myocarditis in mice. In *Selenium in Biology and Medicine, Part B*, eds G. F. Combs, Jr, O. A. Levander, J. E. Spallholz & J. E. Oldfield. Van Nostrand, New York, pp. 761–768.

Keshan Disease Research Group (1979*a*). Epidemiologic studies on the etiologic relationship of selenium and Keshan disease. *Chinese Med. J.*, **92**, 477–482.

Keshan Disease Research Group (1979*b*). Observations on effect of sodium selenite in prevention of Keshan disease. *Chinese Med. J.*, **92**, 471–476.

Pace, G. W. & Leaf, C. D. (1995). The role of oxidative stress in HIV disease. *Free Radical Biol. Med.*, **19**, 523–528.

Yang, G. Q., Ge, K., Chen, J. & Chen, X. (1988). Selenium-related endemic diseases and the daily selenium requirement of humans. *Wld Rev. Nutr. Diet.*, **55**, 98–152.