Selenium Deficiency and Viral Infection[1]

Melinda A. Beck,*[2] Orville A. Levander† and Jean Handy**

*Departments of Pediatrics and Nutrition and **Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 and †U.S. Department of Agriculture, Agricultural Research Service, Beltsville Human Nutrition Research Center, Beltsville, MD 20705

ABSTRACT The discovery that the juvenile cardiomyopathy known as Keshan disease likely has a dual etiology that involves both a nutritional deficiency of the essential trace mineral selenium (Se) as well as an infection with an enterovirus provided the impetus for additional studies of relationships between nutrition and viral infection. An amyocarditic strain of coxsackievirus B3, CVB3/0, converted to virulence when it was inoculated into Se-deficient mice. This conversion was accompanied by changes in the genetic structure of the virus so that its genome closely resembled that of other known virulent CVB3 strains. Similar alterations in virulence and genomic composition of CVB3/0 could be observed in mice fed normal diets but genetically deprived of the antioxidant selenoenzyme glutathione peroxidase (knockout mice). More recent research has shown that a mild strain of influenza virus, influenza A/Bangkok/1/79, also exhibits increased virulence when given to Se-deficient mice. This increased virulence is accompanied by multiple changes in the viral genome in a segment previously thought to be relatively stable. Epidemic neuropathy in Cuba has features that suggest a combined nutritional/viral etiology. Further research, both basic and applied, is needed to assess properly the possible role of malnutrition in contributing to the emergence of novel viral diseases. J. Nutr. 133: 1463S–1467S, 2003.

KEY WORDS: • *coxsackievirus* • *infection* • *influenza* • *oxidative stress* • *virus mutation*

The role of nutrition in infectious disease has long been associated with changes in the immune response of the nutritionally deficient host. It has been shown in a number of studies that nutritionally deficient humans or animals are more susceptible to a wide variety of infections (1–4). This increase in susceptibility is thought to be the result of an impaired host immune response due to a deficient diet.

However, recent studies have demonstrated that not only is the host immune response affected by the deficient diet, but the viral pathogen itself can also be altered (5). Dietary deficiencies that lead to oxidative stress in the host [e.g., selenium (Se)[3] deficiency] can alter a viral genome such that a normally benign or mildly pathogenic virus becomes highly virulent in the deficient, oxidatively stressed host. Once the viral mutations occur, even hosts with normal nutriture can be affected by the newly pathogenic strain. Here we review our work with animal models of Se deficiency and glutathione peroxidase (GPx)-1 deficiency (knockout model) and viral infection as well as an epidemiological study of a human population in Cuba.

Keshan disease

In the early 1930s a cardiomyopathy termed Keshan disease was first described in China. Necrotic lesions throughout the myocardium (6) that are often associated with inflammatory infiltrates and calcification characterize the disease. Women and children were particularly susceptible to the development of Keshan disease, which had a high mortality rate.

Investigations into the epidemiology of Keshan disease revealed that individuals living in areas with Se-poor soils were susceptible to development of the disease. Individuals living in those areas had low dietary intakes of Se that were reflected in low serum and hair levels of Se. Populations living in areas of China with Se-rich soils did not develop Keshan disease (7).

[1] Published in a supplement to *The Journal of Nutrition*. Presented as part of the 11th meeting of the international organization, "Trace Elements in Man and Animals (TEMA)," in Berkeley, California, June 2–6, 2002. This meeting was supported by grants from the National Institutes of Health and the U.S. Department of Agriculture and by donations from Akzo Nobel Chemicals, Singapore; California Dried Plum Board, California; Cattlemen's Beef Board and National Cattlemen's Beef Association, Colorado; GlaxoSmithKline, New Jersey; International Atomic Energy Agency, Austria; International Copper Association, New York; International Life Sciences Institute Research Foundation, Washington, D.C.; International Zinc Association, Belgium; Mead Johnson Nutritionals, Indiana; Minute Maid Company, Texas; Perrier Vittel Water Institute, France; U.S. Borax, Inc., California; USDA/ARS Western Human Nutrition Research Center, California and Wyeth-Ayerst Global Pharmaceuticals, Pennsylvania. Guest editors for the supplement publication were Janet C. King, USDA/ARS WHNRC and the University of California at Davis; Lindsay H. Allen, University of California at Davis; James R. Coughlin, Coughlin & Associates, Newport Coast, California; K. Michael Hambidge, University of Colorado, Denver; Carl L. Keen, University of California at Davis; Bo L. Lönnerdal, University of California at Davis and Robert B. Rucker, University of California at Davis.

[2] To whom correspondence should be addressed. E-mail: melinda_beck@unc.edu.

[3] Abbreviations used: CSF, cerebral spinal fluid; CA9, coxsackievirus A9; CVB3, coxsackievirus B3; GPx, glutathione peroxidase; HA, hemagglutinin; IL, interleukin; M, matrix; NA, neuraminidase; Se, selenium.

Supplementation of individuals with Se tablets (as sodium selenite) could completely prevent the development of Keshan disease, which provided evidence that a deficiency in Se was the cause of Keshan disease. However, the complete epidemiological picture of Keshan disease did not wholly fit with Se deficiency as the exclusive cause. For example, the incidence of Keshan disease fluctuated seasonally and annually. If the disease was due entirely to a deficiency in Se, then the time of the year should not have been a factor in the development of the disease. Second, not every Se-deficient individual developed the disease. Taken together, these findings by scientists in China suggested that an infectious cofactor was required along with a deficiency in Se for the development of Keshan disease.

To determine whether there was a role for infection in the development of Keshan disease, scientists in China examined blood and tissue samples from Keshan disease victims for the presence of virus. Enteroviruses and in particular coxsackieviruses were isolated from Keshan disease victims (8). Recently, using RT-PCR techniques, archived heart tissues from Keshan disease victims have also been shown to contain coxsackieviruses (9).

Bai et al. (10) demonstrated that mice fed grains from Keshan disease areas developed a deficiency in Se. When these mice were infected with a strain of coxsackievirus B4 that was isolated from a Keshan disease victim, the mice developed severe heart pathology. Mice that were fed grains from non-Keshan endemic areas developed only mild heart pathology when infected with the virus, which suggests that together with the deficiency in Se, an infection with a coxsackievirus was required for the development of Keshan disease.

Selenium deficiency and coxsackievirus infection

The possible relationship between a deficiency in Se and infection with coxsackievirus was explored further in our laboratory. Weanling mice were fed a diet deficient in Se for 4 wk before intraperitoneal inoculation with an amyocarditic strain of coxsackievirus B3 (CVB3/0). In normal mice, this virus does not induce cardiac inflammation, although it will replicate in heart cells.

Se is an essential component of the antioxidant enzyme GPx (11). There are four isozymes of GPx that are involved in the degradation of hydrogen peroxide and organic peroxides. Serum samples from the mice were tested for the presence of GPx activity as a biomarker for Se status. Mice that were fed a diet deficient in Se had a fivefold decrease in GPx activity as compared with mice fed a diet adequate in Se (12).

At various times postinfection, mice were killed and the heart pathology was examined. As expected, infected mice that were fed a diet adequate in Se did not develop myocarditis. However, infected mice that were fed a diet deficient in Se developed myocarditis (12). In addition, heart viral titers were elevated in the Se-deficient mice as compared with the Se-adequate mice.

To determine whether the Se status affected the immune response of the infected mice, serum neutralizing-antibody titers and spleen cell proliferative responses were determined. Levels of neutralizing antibody in the Se-deficient mice were equivalent to levels found in the Se-adequate mice, although the ability of spleen cells in vitro to proliferate in response to either mitogen or CVB3 antigen was severely impaired in the Se-deficient mice. In addition, mRNA for the chemokine macrophage chemotactic protein-1 and interferon-γ were overexpressed in the Se-deficient mice (13). Thus, it appeared that the deficiency in Se had altered the host immune response to infection with CVB3/0.

Because Se is involved in the functioning of enzymes other than GPx, we wanted to determine whether the virulence of CVB3/0 in the Se-deficient mice was due to a deficiency in GPx activity. To establish a relationship between GPx activity and Se deficiency, we used GPx-1–knockout mice. All Se-containing enzymes except GPx-1 are fully functioning in these knockout animals. GPx-1–knockout mice were infected with CVB3/0 and killed at various times postinfection. Myocarditis developed in $>$ 50% of the knockout mice, which suggests that antioxidant protection is important for protection against CVB3-induced myocarditis (14). Interestingly, in contrast to the immune response of the Se-deficient mice, the GPx-1–knockout mice had reduced serum neutralizing-antibody production, although their spleen cells were able to respond to both mitogen and antigen.

Viral genome changes

The ability of the normally amyocarditic CVB3/0 to induce myocarditis in Se-deficient and GPx-1–knockout mice may have been due to effects on the immune system that left the mice vulnerable to the virus. Alternatively, the virus itself may have been altered as a consequence of replicating in an oxidatively stressed host. To determine which of these possibilities is correct, a viral passage experiment was performed.

Se-deficient mice were infected with CVB3/0. At 7 d postinfection, mice were killed and the virus was isolated from their hearts. This virus was then passed back into Se-adequate mice. If the pathogenicity of the virus was due to host factors alone, then the Se-adequate mice should not develop myocarditis. However, the Se-adequate mice did develop myocarditis, which suggests that the virus itself had changed. Virus passed from Se-adequate to Se-adequate mice did not result in pathology, which demonstrates that viral passage alone did not alter the virus (15).

To confirm that the phenotype change of the virus was due to a change in viral genotype, we sequenced virus that was isolated from Se-adequate and Se-deficient mice and compared those sequences to the sequence of the input strain. We found six nucleotide changes between the original CVB3/0 strain and the virus isolated from Se-deficient mice (15): Nt 234: C→T, Nt 788: G→A, Nt 2271: A→T, Nt 2438: G→C, Nt 3324: C→T and Nt 7334: C→T. No changes were found in virus that was isolated from Se-adequate mice.

This is the first report of a specific host nutritional deficiency driving changes in a viral genome and changing a normally avirulent pathogen into a virulent one. Once these changes occur, even hosts with normal nutritional status are vulnerable to the newly virulent virus. The mechanism(s) responsible for the changes is currently under investigation.

Similar to virus that replicated in a Se-deficient host, virus isolated from GPx-1–knockout mice that developed myocarditis also had viral genome changes (14). Virus isolated from GPx-1–knockout hearts that developed myocarditis had the same six mutations as were found in virus isolated from Se-deficient mice. In addition, another nucleotide change occurred: Nt 2690: G→A. The changes in the genome were associated with the increased virulence of the virus, because viruses isolated from GPx-1–knockout mice that did not develop myocarditis were identical to the input strain. Thus, both a deficiency in Se as well as a specific deficiency in GPx-1 activity lead to viral genome changes, which suggests that

increased oxidative stress of the host can affect a viral genome.

Selenium deficiency and influenza virus

Influenza is an RNA virus in the Orthomyxoviridae family with a segmented genome. Each year in the U.S., infection with influenza kills > 20,000 individuals and hospitalizes > 100,000 (16). At highest risk for increased influenza mortality and morbidity are the elderly and individuals with chronic diseases of the lung and heart.

To determine whether a deficiency in Se could have an effect on a viral family other than enteroviruses, mice were fed a diet either deficient or adequate in Se for 4 wk. After the feeding period, the mice were inoculated intranasally with influenza A/Bangkok/1/79 (H3N2), a strain that induces mild pneumonitis in normal mice. At various times postinfection, the mice were killed for study.

At all time points postinfection, mice with a deficiency in Se had much more severe pathology than mice that were fed a diet adequate in Se (17). Interestingly, in addition to being more severe, the lung pathology persisted longer in Se-deficient mice as compared with Se-adequate mice.

The immune response was also altered in the Se-deficient mice. Levels of mRNA, which were obtained from lung-draining lymph nodes for proinflammatory chemokines were higher in the Se-deficient mice. The mRNA for monocyte chemotactic protein-1, macrophage inflammatory protein-1α and -1β and RANTES (regulated upon activation, normal T cell expressed and secreted) were all expressed at high levels in the Se-deficient mice (17).

Cytokine mRNA levels were also altered in the Se-deficient mice. Interleukin (IL)-2 and γ-interferon were decreased, whereas IL-4, -5, -10 and -13 were increased, which suggests a skewing toward a T-helper-2-like pattern in the Se-deficient mice as compared with a T-helper-1-like pattern in the Se-adequate mice.

Thus, it appears that a deficiency in Se leads to a more proinflammatory immune response that results in increased pathology in the lungs of the Se-deficient mice. Is the increase in pathology due to a change in the viral genome as we found for coxsackievirus? To answer this question, we passed influenza virus isolated from Se-deficient mice back into Se-adequate mice. These mice developed much more severe pneumonitis as compared with Se-adequate mice infected with virus isolated from Se-adequate mice. To confirm that the viral genome was altered, we sequenced part of the influenza virus genome.

Three segments of the viral RNA, the hemagglutinin (HA), neuraminidase (NA) and the matrix (M) genes, were sequenced from virus isolated from both Se-adequate and -deficient mice. The HA, NA and M proteins have all been associated with virulence of the virus (18). The HA and NA proteins are responsible for viral entry and exit from the infected cell, respectively, and the M protein is associated with viral replication. The M gene codes for both the M1 and the M2 protein.

HA and the NA are viral surface proteins that are exposed to the host's immune response. Mutations in either of these genes are associated with the ability of the virus to escape immune detection by a process known as antigenic drift (19). M1 is an internal protein and thought to be relatively stable among various influenza virus strains. The M2 protein is an ion channel, part of which is exposed on the surface of the virion, and has therefore been found to vary antigenically although to a lesser extent than HA or NA.

Sequencing of the HA, NA and M genes of viruses isolated from either Se-deficient (three separate isolates) or Se-adequate (three separate isolates) mice reveals few changes in HA and NA (20). When compared to the input strain, the isolates from both the Se-adequate and -deficient mice had either zero, one, two or three nucleotide changes. However, the sequence of the M gene of virus isolated from Se-deficient mice was markedly altered when compared with the isolates from Se-adequate mice (20). All three isolates from Se-deficient mice had 29 identical nucleotide changes, six of which led to amino acid changes. In addition, one isolate had an additional five nucleotide changes. One isolate from the Se-adequate mice had one nucleotide change in the M2 protein, and the other two isolates had none.

These results plus our earlier work with coxsackievirus suggest that oxidative stress in a host leads to genome changes of an RNA virus, and that these changes can be associated with increased virulence of the virus. Thus, we propose that RNA viruses in general are highly susceptible to oxidative damage in antioxidant-deficient hosts.

Epidemic neuropathy: Cuba

In addition to animal studies, outbreaks of disease associated with nutritional deficiencies and perhaps a viral component have occurred in human populations. One example is an epidemic of optic and peripheral neuropathy that affected > 50,000 people in Cuba in the early 1990s, which is an incidence of 462 cases per 100,000 people in Cuba's population of 11 million (21).

The epidemic occurred during a period of extreme economic difficulty in Cuba with the loss of Cuba's major trading partners after the dissolution of the Soviet Union. The continuing U.S. trade embargo was tightened in 1992 to exclude trade with subsidiaries of U.S. companies doing business abroad; > 90% of such trade was in food, medicines and medical equipment (22). Because of these factors, Cuban imports were reduced by 1993 to less than one-fourth of their 1989 level. This drastic reduction in importation of food, fuel, raw materials, machinery and spare parts was further exacerbated by a series of storms that seriously reduced the harvest of many food crops. Per capita availability of protein in 1993 was 25% less, and energy 18% less, than in 1989 (23). At the same time, the people faced increased demands for expenditure of physical energy, such as walking or bicycling long distances to work because the unavailability of fuel disrupted public transportation. An analysis for 1992 showed that 76% of per capita caloric consumption was derived from carbohydrates, primarily rice and sugar (23).

The earliest neuropathy cases occurred among men of working age, most of whom were smokers, in the western tobacco-producing province of Pinar del Río. Late in 1991, physicians there began to report an unusual number of patients with progressive loss of visual acuity in both eyes that developed over days to weeks. Nearly half of these patients also had symptoms of sensory peripheral neuropathy such as tingling and burning sensations in the feet and hands. Typically, patients reported easy fatigability and weight loss before onset of the visual symptoms.

Because the illness resembled "tobacco-alcohol amblyopia," which is also known as "nutritional amblyopia" and is related to B-group vitamin deficiency (24), the patients were treated with high doses of parenteral B-complex vitamins plus folate and vitamins A and E. Most patients responded well, especially when treated soon after onset of their illness (23).

New cases continued to appear, however, and by December 1992, 472 patients had been identified in six provinces scattered throughout the island. The number of reported cases then rose exponentially and reached 4,461 by March and 45,584 by June 1993. There were no fatal cases, but some patients had residual visual impairment particularly if their symptoms were far advanced before treatment was started.

Both sexes were affected, but almost exclusively between the ages of 25 and 64 y of age; the illness was extremely rare in children, pregnant women and the elderly. The age distribution may have been related to the priority given to these groups under the food-rationing system (24). Pérez et al. (23) present data that show higher per capita consumption of energy, protein, fats and some vitamins in the youngest and oldest age groups of the population.

A standard case definition was developed in March 1993 (25). By then it was evident that the illness could manifest as optic neuropathy, peripheral sensory neuropathy or a mixture of the two. Four separate case-control studies demonstrated that the illness was associated with various measures of the decreased frequency, quality and quantity of food intake. All four studies demonstrated that smoking was associated with illness in a dose-related manner (23,25–27). Extensive epidemiologic and laboratory investigation revealed no association with exposure to any of a long list of toxins that may affect the optic nerve (24).

Pérez et al. (23) summarized data from several laboratories that support the existence of oxidative stress in the Cuban population during the epidemic. Serum levels of malonyldialdehyde were above normal limits in both patients and controls. Patients had significantly lower levels of sulfhydryl compounds than did control subjects with normal serum albumin levels in both groups. Serum Se was significantly lower ($P < 0.001$) in patients than in controls in two studies, although both values were within accepted limits of normal. Serum zinc levels were in the low-normal range but did not differ between patients and controls. Both patients and controls had levels of glutathione reductase in erythrocytes that were significantly below historic Cuban normal values. A laboratory assay showed a significantly higher frequency of sister-chromatid exchange as evidence of oxidative damage to DNA in patients than in controls.

Viral isolation attempts from cerebrospinal fluid (CSF) of neuropathy patients, which were undertaken to rule out an infectious agent, unexpectedly yielded viruses resembling enteroviruses from 105 of 125 (84%) CSF specimens cultured (28). Five of these isolates were typical strains of coxsackievirus A9 (CA9) that were identified by standard techniques of neutralization and immunoblotting. The other 100 isolates had some antigenic similarity to CA9 but differed from it biologically and serologically; they produced a slowly progressive "light" cytopathic effect on Vero cells, and in plaque assays the plaques were pinpoint and slow to develop. Similar viruses were isolated from two of 30 control CSF specimens obtained from people who required lumbar puncture for anesthesia or other purposes ($P < 0.01$ compared with patients).

Light viruses were reisolated from the CSF of 24 of 25 patients who were recultured after 21–30 d. One patient who remained ill was recultured after 1 y, and virus was again recovered from the CSF. One patient's CSF yielded CA9 in the initial virus culture and a light virus 1 mo later (28).

Hyperimmune rabbit sera prepared against the light strains neutralized CA9 and coxsackievirus B4 but not vice versa. Immunoblotting experiments demonstrated that the capsid proteins, which contain the major epitopes for neutralization of enteroviruses, were not present in their native form in the light viruses. Hyperimmune rabbit sera produced against these strains recognized capsid proteins of CA9, but in their homologous strains they revealed only a band of higher molecular weight, which was interpreted as possibly an uncleaved precursor form of these proteins. Conversely, antisera produced against CA9 recognized the CA9 capsid proteins but failed to react at all with proteins of the light virus (28).

We suggest that given the prevalence and apparent persistence of coxsackieviruses in the CSF of patients with epidemic neuropathy, it is important to further investigate the role of these viruses and their possible interactions with other factors. Ongoing studies in our laboratory include the sequencing of viral isolates from neuropathy patients for comparison with typical CA9 isolates.

RNA viruses make up the vast majority of all viruses. RNA viruses are continually evolving due to their lack of proofreading enzymes. The propensity for RNA viruses to mutate is considered to confer a survival advantage evolutionarily; that is, viruses that can rapidly mutate to meet changing environmental situations (e.g., immune pressure) can better adapt to new conditions.

The emergence of new viral diseases or the increase in infection from known viruses is often attributed to such things as global warming, destruction of the rain forest, agricultural practices, etc. However, the influence of host nutrition on the evolutionary process of RNA viruses is rarely considered. Our research demonstrates that inoculation of certain strains of coxsackievirus or influenza virus into Se-deficient mice leads to the production of more virulent strains of virus. The epidemic of optic and peripheral neuropathy in Cuba suggests the possibility of a virus mutating in an oxidatively stressed host and thus presenting with new pathogenic characteristics. Therefore, we propose that nutritionally induced oxidative stress can have a profound impact on RNA mutation rates and that host nutritional status should be considered when studying infectious diseases.

LITERATURE CITED

1. Bendich, A. (1996) Antioxidant vitamins and human immune responses. Vitam. Horm. 52: 35–62.
2. Harbige, L. S. (1996) Nutrition and immunity with emphasis on infection and autoimmune disease. Nutr. Health 10: 285–312.
3. Scrimshaw, N. S. (1975) Nutrition and infection. Prog. Food Nutr. Sci. 1: 393–420.
4. Semba, R. D. (1994) Vitamin A, immunity and infection. Clin. Infect. Dis. 19: 489–499.
5. Beck, M. A. & Levander, O. A. (1998) Dietary oxidative stress and the potentiation of viral infection. Annu. Rev. Nutr. 18: 93–116.
6. Gu, B. Q. (1983) Pathology of Keshan disease. A comprehensive review. Chin. Med. J. 96: 251–261.
7. Keshan Disease Research Group (1979) Epidemiologic studies on the etiologic relationship of selenium and Keshan disease. Chin. Med. J. 92: 477–482.
8. Su, C., Gong, C., Li, J., Chen, L., Zhou, D. & Jin, Q. (1979) Preliminary results of viral etiology of Keshan disease. Chin. Med. J. 59: 466–472.
9. Li, Y., Yang, Y. & Chen, H. (1995) Detection of enteroviral RNA in paraffin-embedded myocardial tissue from patients with Keshan disease by nested PCR. Chung Hua I Hsueh Tsa Chih 75: 344–345.
10. Bai, J., Wu, S., Ge, K. Y., Deng, X. & Su, C. (1980) The combined effect of selenium deficiency and viral infection on the myocardium of mice. Acta Acad. Med. Sin. 2: 29–31.
11. Stadtman, T. C. (2000) Selenium biochemistry. Mammalian selenoenzymes. Ann. N. Y. Acad. Sci. 899: 399–402.
12. Beck, M. A., Kolbeck, P. C., Rohr, L. H., Shi, Q., Morris, V. C. & Levander, O. A. (1994) Benign human enterovirus becomes virulent in selenium-deficient mice. J. Med. Virol. 43: 166–170.
13. Beck, M. A. & Matthews, C. C. (2000) Micronutrients and host resistance to viral infection. Proc. Nutr. Soc. 59: 1–5.
14. Beck, M. A., Esworthy, R. S., Ho, Y. S. & Chu, F. F. (1998) Glutathione peroxidase protects mice from viral-induced myocarditis. FASEB J. 12: 1143–1149.

15. 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. Nat. Med. 1: 433–436.

16. Centers for Disease Control and Prevention (2000) Surveillance for influenza—United States, 1994–95, 1995–96 and 1996–97 seasons. Morbidity and Mortality Weekly Report 49: 13–28.

17. Beck, M. A., Nelson, H. K., Shi, Q., Van Dael, P., Schiffrin, E. J., Blum, S., Barclay, D. & Levander, O. A. (2001) Selenium deficiency increases the pathology of an influenza virus infection. FASEB J. 15: 1481–1483.

18. Ward, A. C. (1997) Virulence of influenza A virus for mouse lung. Virus Genes 14: 187–194.

19. Murphy, B. R. & Webster, R. G. (1996) Orthomyxoviruses. In: Fields Virology, 3rd. ed. (Fields, B. N., Knipe, D. M., Howley, P. M., Chanock, R. M., Melnick, J. L., Monath, T. P., Roizman, R. & Straus, S. E., eds.), pp. 1397–1445. Lippincott-Raven, Philadelphia, PA.

20. Nelson, H. K., Shi, Q., Van Dael, P., Schiffrin, E. J., Blum, S., Barclay, D., Levander, O. A. & Beck, M. A. (2001) Host nutritional status as a driving force for influenza virus mutations. FASEB J. 15: 1846–1848.

21. Centers for Disease Control and Prevention (1994) Epidemic neuropathy—Cuba, 1991–1994. Morbidity and Mortality Weekly Report 43: 183, 189–192.

22. Kuntz, D. (1994) The politics of suffering: the impact of the U.S. embargo on the health of the Cuban people. Report of a fact-finding trip to Cuba, June 6–11, 1993. Int. J. Health Serv. 24: 161–179.

23. Pérez Cristiá, R. & Fleites Mestre, P. (1995) Analisis y discusion de la hipotesis toxico-nutricional como posible causa de la neuropatia epidemica. In: Neuropatia Epidemica en Cuba, 1992–1994 (Rojas, F., ed.), pp. 117–158. Editorial Ciencias Medicas, Havana, Cuba.

24. Román, G. C. (1994) An epidemic in Cuba of optic neuropathy, sensorineural deafness, peripheral sensory neuropathy and dorsolateral myeloneuropathy. J. Neurol. Sci. 127: 11–28.

25. Más Bermejo, P., del Puerto Quintana, C., Barceló Pérez, C., Molina Esquivel, E. & Cañas Pérez, R. (1995) Estudio de casos y controles de la neuropatía óptica epidémica de Cuba, 1993. Bol. Oficina Sanit. Panam. 118: 115–126.

26. Cuban Neuropathy Field Investigation Team (1995) Epidemic optic neuropathy in Cuba—clinical characterization and risk factors. N. Engl. J. Med. 333: 1176–1182.

27. Gay, J., Porrata, C. & Hernández, M. (1994) Factores dietéticos de la neuropatía epidémica en la Isla de la Juventud. Cuba Bol. Oficina Sanit. Panam. 117: 389–399.

28. Más, P., Pelegrino, J. L., Guzmán, M. G., Comellas, M. M., Resik, S., Alvarez, M., Rodríguez, R., Muné, M., Capó, V., Balmaseda, A., Rodríguez, L., Rodríguez, M. P., Handy, J. & Kourí, G. (1997) Viral isolation from cases of epidemic neuropathy in Cuba. Arch. Pathol. Lab. Med. 121: 825–833.