The Selenium-Coxsackievirus Connection: Chronicle of a Collaboration

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ABSTRACT This review provides a historical account of a collaboration established between a nutritionist and a virologist to investigate the interrelationship of host nutritional status and viral virulence. The parties to this collaboration consider themselves specialists in the fields of antioxidant nutrition and viral immunology, respectively. The advantages of such talent pooling are discussed (rapid startup, well-focused experimentation, ability to visualize the “big picture”), as are some of the disadvantages (limited common scientific vocabulary, proper apportioning of credit, lack of institutional infrastructure to house such efforts). The common perception that some of the most exciting science occurs when the advancing edges of two disparate disciplines intersect is borne out by this project because host nutrition was shown for the first time to influence the genetic make-up of an invading viral pathogen. Encouragement of joint cooperative ventures should have a high priority as demanded by increasingly difficult scientific problems and as desired by scientists themselves who wish to see their research progress more quickly. J. Nutr. 130: 485S–488S, 2000.

KEY WORDS: • Keshan disease • cardiomyopathy • myocarditis • oxidative stress • vitamin E • viral evolution

People have often asked me “How did you happen to get started working with a . . . a virologist?” Professor Melinda A. Beck initiated our collaboration on the basis of advice she received from her husband, Dr. David J. Thomas. Dr. Thomas is a toxicologist interested in heavy metals and Dave and I had worked together in the past. He suggested that his wife contact me because of her desire to investigate the influence of Se status on the virulence of the coxsackievirus in mice. Coxsackievirus had been isolated from patients stricken with Keshan disease, the Se-responsive cardiomyopathy that afflicts infants, children and women of child-bearing age residing in Se-poor regions of China (Levander and Beck 1997). Furthermore, Chinese scientists had shown that the heart damage caused by coxsackievirus B4 in mice was increased by feeding a Se-deficient diet (Bai et al. 1980).

Thus, on June 2, 1992, Dr. Beck faxed me a letter indicating that she had a mouse model of coxsackievirus infection in her laboratory. In her letter, she asked whether her laboratory could offer any assistance “. . . including using a mouse model of Se deficiency to study coxsackievirus infection.” Not knowing anything about coxsackievirus in particular and very little about viruses in general, but nonetheless aware of a possible link between viral infection and Keshan disease, I indicated my interest to participate in such a joint project.

And so we were on our way. By July 1992, animal protocols were in place and approved and mice were ordered in August. Preliminary results were available in September. On October 13, 1992, Melinda paid a visit to Beltsville so we could meet and discuss our results. She also presented a seminar about our findings. A little more than four months after initial contact, our work was being discussed in a public forum! This time line demonstrates one of the major advantages of collaborative interdisciplinary research, namely, the ability to get things moving quickly by joining two diverse areas of expertise. Without one another’s help and support, it would have been very difficult if not impossible for either one of us alone to accomplish this work so rapidly, and certainly the research was conducted much more efficiently by working in tandem.

Se, vitamin E, and viral myocarditis

Dr. Beck had two different strains of coxsackievirus B3 (CVB3)1 available for testing in her laboratory, one virulent (CVB3/20) and the other avirulent (CVB3/0). That is, in mice fed normal diets, the CVB3/20 strain would cause moderate-to-severe heart damage, whereas the CVB3/0 strain caused no apparent damage. With this model, we showed the following: 1) CVB3/20 caused more severe heart damage in Se-deficient (−Se) mice than in Se-adequate (+Se) mice

1 Presented as part of the History of Nutrition Symposium entitled “Trace Element Nutrition and Human Health” given at the Experimental Biology 99 meeting held April 17–21 in Washington, DC. This symposium was sponsored by the American Society for Nutritional Sciences. The proceedings of this symposium are published as a supplement to The Journal of Nutrition. Guest editors for the symposium publication were Harold H. Sandstead, the University of Texas Medical Branch, Galveston, TX and Leslie M. Klevay, the U.S. Department of Agriculture Agricultural Research Service Grand Forks Human Nutrition Research Center, Grand Forks, ND.

2 Abbreviations used: apo, apoprotein; ATG, aurothioglucose; ATM, aurothiomalate; CVB3, coxsackievirus B3; DPPD, N,N'-diphenyl-p-phenylenediamine; GPX, glutathione peroxidase; TBARS, thiobarbituric acid reactive substances; TR, thioredoxin reductase.
(Beck et al. 1994c); 2) CVB3/0 caused moderate heart damage in −Se mice but had no apparent effect in +Se mice (Beck et al. 1994a); 3) CVB3/20 caused more severe heart damage in vitamin E–deficient (−VE) mice than in vitamin E–adequate (+VE) mice (Beck et al. 1994b); 4) CVB3/0 caused moderate heart damage in −VE mice but had no apparent effect in +VE mice (Beck et al. 1994b); and 5) feeding fish oil in the −VE diet increased the heart damage caused by either the CVB3/0 or the CVB3/20 strain (Beck et al. 1994b).

The first observation confirmed what the Chinese scientists had already reported, namely, that the cardiotoxicity of a virulent coxsackievirus was increased in Se-deficient mice. The observation that attracted the most interest, however, was 2 above, that is, the fact that a normally benign strain of coxsackievirus B3, CVB3/0, could be converted to cardiovirulence as a result of feeding the host a Se-deficient diet. This was a novel finding with many potentially important implications. What the results seemed to say was that one could be immersed in a sea of benign coxsackievirus without any apparent ill effects until one suffered a decline in Se nutriture to the point that the virus would exhibit its cardiotoxic properties. This seemed a rather unsettling prospect. Nevertheless, the data appeared to fit what one might expect for a regional endemic disease such as Keshan disease. The viral infectious agent could exist silently everywhere in the environment because of its lack of pathogenicity except in those areas in which the inhabitants had poor Se status.

Observations #3 and #4 showed that deficiency of vitamin E could also lead to increases in viral virulence. These results were important because they showed that factors other than Se had to be considered when attempting to predict the effect of a particular diet on the ability of a host to resist viral infection. Of course, we selected vitamin E for testing in the coxsackie/mouse model because of its well-known close metabolic relationship with Se.

Oxidative stress

An appealing hypothesis was the idea that increased oxidative stress in the host due to deficiency of either Se or vitamin E allowed the virus to exert a greater cardiotoxic effect. We soon had other data that supported this concept (observation #5). Feeding fish oil increases the vitamin E requirement of animals because of its large proportion of readily peroxidizable highly unsaturated fatty acids. Feeding a vitamin E–deficient diet containing fish oil (menhaden oil) readily peroxidizable highly unsaturated fatty acids. Feeding a vitamin E–deficient diet containing fish oil (menhaden oil) increased the heart damage caused by either the CVB3/0 or the CVB3/20 strain (Beck et al. 1994b). With this background, it was a logical extension of earlier work to determine whether DPPD would protect against the increased cardiopathology observed in vitamin E–deficient mice infected with CVB3/20. For these studies, mice were fed a vitamin E–deficient casein-based diet containing 4% menhaden oil and 1% stripped corn oil or the same diet supplemented either with 35 mg vitamin E/kg diet or with an equimolar amount of DPPD. After 4 wk of feeding, the mice were inoculated with CVB3/20. Ten days postinfection, heart histopathology scores were lower, indicating less damage in the vitamin E–supplemented group than in the vitamin E–deficient group and were lower still in the DPPD group (Beck 1997 and 1998).

Both of us were now persuaded that oxidative stress was responsible for the increased cardiotoxicity of the coxsackievirus that was observed in our selenium- or vitamin E–deficient mice. No other hypothesis seemed to accommodate all our data. But what was the mechanism whereby the dietary oxidative stress enabled the virus to exert a greater cardiotoxic effect? To me, the most straightforward explanation was that the nutritional deficiencies rendered the host cardiomyocytes more vulnerable to viral attack. Dr. Beck, however, suggested that maybe the heightened oxidative stress in the host cells might in some way have a direct effect on the virus itself. To test this hypothesis, we carried out the “passage experiment.”

In this experiment, the benign strain of the virus (CVB3/0) was inoculated initially into either Se-supplemented or Se-deficient mice. After 10 d, the hearts of the mice in both groups were excised and virus was isolated from the heart tissue and passed through HeLa cells in culture. Virus was then harvested from the HeLa cells and inoculated into a second series of mice, all fed a normal diet. Virus that had been passed through Se-supplemented mice during the first half of the experiment (CVB3/0Se−2) caused no apparent heart damage when inoculated into the second series of mice. Viruses initially passed through Se-deficient mice (CVB3/0Se−), however, caused significant heart damage when inoculated into a second series of (normal) mice.

The genome

These results suggested that the normally benign virus (CVB3/0) was changed to a virulent form (called CVB3/0Se−) as a result of being passed through a Se-deficient host. Although the results were strongly suggestive, proof of such a change could be obtained only by determining the sequence of the CVB3/0Se− vs. the CVB3/0 viral genomes.
The coxsackievirus genome is ~7500 nucleotides long. Although the CVB3 genome sites that actually determine cardiovirulence remain to be established, Beck et al. (1995) found that the virulent CVB3/0Se− differed from the avirulent CVB3/0 in six of seven positions that were thought to influence cardiovirulence. The newly produced cardiovirus strain, CVB3/0Se−, had a genomic structure identical to that of the virulent strain CVB3/20 except for one nucleotide position. Also worth noting is the fact that the same exact base changes took place when the CVB3/0 was passed through vitamin E–deficient mice (Beck et al. 1996). Because of the diversity in the identity and location of the genomic differences between the benign and virulent CVB3 strains, it was difficult to postulate a biochemical mechanism by which those changes might be taking place.

Mechanistic considerations aside, the discovery by Beck et al. (1995) that the nutritional status of the host could affect the genetics of an invading pathogen has profound implications from the public health point of view. The study of nutrition/infec-tions interactions is not new; the classic monograph in this field was published more than three decades ago by Scrimshaw et al. (1968). In all of these studies and those that followed, however, effects of host diet on the ability of the host to resist infection were always discussed in terms of nutritional effects on the host immune system. The influence of host diet was considered always in terms of effect on the host (Fig. 1). Nothing was said concerning the possible effect of host diet on the pathogen itself. The fact that Beck et al. (1995) provide evidence for the latter constitutes a radical shift in how nutritionists should view the role of diet in determining the outcome of infectious disease. It seems possible that some old observations may have to be reinterpreted in light of these new findings.

As this work was in progress, public interest in emerging viral diseases was intensifying. Several semipopular books (e.g., Preston’s The Hot Zone and Garrett’s The Coming Plague) were published and Hollywood released the hit movie “Outbreak” with Dustin Hoffman and other movies along the same theme. Even the viruses themselves seemed to be cooperating, e.g., sporadic Ebola outbreaks here and there, the continuing burn of the AIDS epidemic world-wide and the unexpected appearance of a lethal hantavirus strain in the southwestern U.S. Dr. Beck and I shared in the excitement of seeing our work progress so well, and we had many telephone conversations speculating about the possible implications of this or that result.

The present

We are continuing to investigate the influence of various types of dietary oxidative stress in the coxsackievirus model. Iron overload is known to exert a prooxidant effect, and evidence is accumulating that viral infection is associated with altered iron metabolism. For example, the progression of HIV infection to more advanced stages is accompanied by increasing body stores of iron, and serum ferritin level was found to be an independent predictor of disease progression (Boelaert et al. 1997). Moreover, iron removal lowers transaminase values during chronic hepatitis C infection and improves symptoms of hepatitis C–associated rheumatic complications (Bonkovsky 1997). Considerable heart damage was observed when we administered the benign coxsackievirus (CVB3/0) to mice that had been fed a vitamin E–deficient diet containing high levels of iron (Levander et al. 1998). The pathology in the infected vitamin E–deficient iron overload group was more severe than that seen in an infected vitamin E–deficient group fed fish oil. Peak cardiac viral titers were highest in the vitamin E–deficient group fed the high iron diet, which is consistent with the highest degree of heart damage in this group. On the other hand, peak cardiac viral titers were the same in the vitamin E–deficient moderate iron and vitamin E–deficient fish oil–fed groups even though the latter suffered much more heart damage than the former. Perhaps the cardiomyocytes from the fish oil–fed mice were more prone to viral damage because tissue peroxidizability as assessed by the formation of hepatic thiobarbituric acid reactive substances was highest in this group (Styblo, M., Beck, M.A., Levander, O.A., unpublished observations).

Dr. Allen Smith, collaborating with the author at Beltsville, has initiated a series of investigations into the ability of gold compounds to increase viral virulence. Earlier work had shown that aurothiomalate (ATM) could potentiate the virulence of a number of viruses, including the coxsackievirus (Kabiri et al. 1978). Because aurothioglucose (ATG) is now known to be a potent inhibitor of many selenoenzymes, it seemed reasonable to hypothesize that the virulence-increasing activity of ATM might be due to that property. Thioredoxin reductase (TR) was much more sensitive to inhibition by ATG in vitro than was glutathione peroxidase (GPX) (Hill et al. 1997). We were able to confirm this differential sensitivity of TR vs. GPX to ATG in whole-animal experiments (Smith et al. 1999a). In fact, by careful ATG dose selection, it was possible to inhibit TR activity strongly in a variety of mouse tissues (heart, liver, pancreas and kidney) while still having only a minimal effect on GPX in these organs. These results suggested that TR activity may be more important in determining viral virulence than GPX activity. On the other hand, Dr. Smith showed that both ATM and ATG inhibited TR activity in vivo but that only ATM induced virulence in an avirulent strain of coxsackievirus B3 (CVB3/0) (Smith et al. 1999b). Dr. Paul South, working in the author’s laboratory, found that administration of subacute doses of either mercuric chloride or sodium arsenite caused CVB3/0 to exhibit virulence, and both compounds also had inhibitory effects on TR (South et al. 1999). Dr. Beck tested the effect of CVB3/0 on...
mice that had their gene for GPX-1 disrupted (Beck et al. 1998). A little over half of the GPX-1 "knockout" mice suffered cardiac damage from CVB3/0, whereas there was no damage in the wild-type mice. Additional work is required to clarify the roles of TR vs. GPX in determining an organism's ability to withstand oxidative stress and resist viral infection.

The future

Both Dr. Beck and I are highly pleased that our relatively brief collaboration should have uncovered so many novel and exciting interactions of nutrition and infection. Dr. Beck's use of the GPX-1 knockout mice in her studies shows how these techniques can be applied to further our understanding of such complex interrelationships. The finding that environmental contaminants (mercury and arsenic) can also influence the expression of the benign coxsackievirus indicates the need to take a broad view of these interactions, including not only nutrients and infectious agents but ecological pollutants as well. Other environmental factors (e.g., temperature, radiation or stress) should also perhaps be considered to obtain the complete picture.

Much work remains to be done to establish the scope of nutrition/viral infection interactions. Additional research to determine the effect of oxidative stress on other viral infections is urgently needed. Dr. Smith has started to look at effects of deficiencies of nutrients other than Se or vitamin E on viral infection; this other facet of the interaction also clearly demands more attention. It is hoped that answers to some of these pressing questions can be obtained in the not too distant future.

Epilogue

As scientific problems become more complex, the need to develop interdisciplinary approaches to grapple successfully with these issues becomes more evident (Kahn and Prager 1994). Collaborations present "...formidable challenges, but the benefits...are so great that efforts to remove obstacles must be made" (Macrina 1995). Collaborative research "involving scientists from disparate fields of study can be especially complicated because the parties may not have common vocabularies, compatible working styles, or shared assumptions about the collaboration" (Macrina 1995). In our case, the collaboration was further complicated by the May/September nature of our relationship with Dr. Beck just embarking on her career and the author marking 30+ years in research. The opinion that researchers "...especially untutored ones, proposing research programs that move across disciplinary fences have, and do, put their careers at risk" (Metzger and Zare 1999) is seconded by the comment that because "...independent work is the prevailing measure of scientific identity, junior faculty establishing their own careers need to recognize the importance of balancing collaborative and independent work" (Macrina 1995).

Fortunately, the Beck/Levander collaboration has by and large achieved that balance with each participant receiving a share of the credit. Both parties desire that the collaboration should continue but even if this is not possible, the collaboration will have fulfilled its goals of initiating a new field of endeavor and establishing the career of a young scientist.

LITERATURE CITED


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