

# Reversing Splenomegalies in Epstein Barr Virus Infected Children: Mechanisms of Toxicity in Viral Diseases

Dana F. Flavin, M.D., M.S., B.S.<sup>1</sup>

## Abstract

*The rapid reversal of splenomegaly in Epstein Barr Virus EBV infected children and young adults was seen in over 50 patients treated with a combination therapy that reduced reactive oxygen species, increased interferon gamma and decreased free nitric oxide in lymphocytes. The patients showed dramatic improvement within 24 hours with successful remission of the disease and complete reversal of splenomegalies. Abbreviations: EBV = Epstein Barr Virus; eNOS= endothelial nitric oxide synthase; iNOS=inducible nitric oxide synthase; NO = Nitric oxide; ROS = reactive oxygen species; XO = Xanthine oxidase*

## Introduction

Viral infections with Epstein Barr Virus (EBV) are often seen in young adults, commonly peaking during puberty. It is often initially misdiagnosed as a flu, later as Chronic Fatigue Syndrome or even depression. Mononucleosis is often responsible for general fatigue and malaise. Though less common in young children, the disease may often run more virulent, particularly in males, causing hepato-splenomegaly with mortality resulting from a rupture of the spleen causing rapid internal bleeding.

Two to three weeks after the onset of fever and malaise symptoms, pharyngitis and often posterior cervical lymph node enlargement or generalized lymphadenopathy set in. Liver function tests are also abnormal in more than 90% of the cases at this stage of the disease.<sup>1</sup>

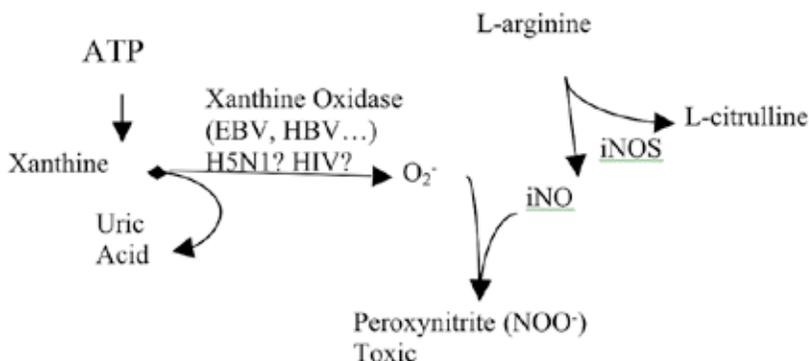
## The Causes of Toxicity in EBV

The basic cause of toxicity in EBV is from the generation of Reactive Oxygen Species (ROS). They are products from the combination of the oxygen radicals from xanthine oxidase<sup>2</sup> (XO), a flavin enzyme, and nitric oxide from the enzyme inducible Nitric Oxide Synthase (iNOS).<sup>3,4</sup> It is known that XO is elevated in the blood of patients with EBV and Hepatitis B 200 times above normal levels.<sup>5</sup> XO is stored in the liver and intestines and released following any inflammatory challenge. It binds onto the glycosaminoglycans on the surface of cells virally infected. The greater the acidity at the surface of the cell, the more receptors exposed. When the adenosine triphosphate (ATP) is broken down intracellularly in virally infected cells, the adenine products hypoxanthine and xanthine act as substrates to XO and generate superoxide radicals. In the presence of free iron, these radicals combine with the gas nitric oxide from iNOS (iNO) to form the toxic product peroxynitrite which can then rapidly convert to a series of further toxic substances.<sup>6</sup> In males, testosterone also increases XO levels which most likely accounts for the increase in toxicity in boys.

iNO alone, however, does not appear to be toxic to virally infected cells without oxygen radicals present; in fact, it can be inhibitory to EBV<sup>7</sup> and other viruses.<sup>8-11</sup> iNO does not have the opportunity to block viral replication when XO is present, but instead combines with the oxygen radicals to generate products toxic to the membrane of the virally infected cells.<sup>12</sup> (Figure 1, p.96).

1. President, Foundation for Collaborative Medicine and Research 24 Midwood Drive, Greenwich, CT 06831  
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Figure 1. Mechanism of toxicity in viral diseases.



Reactive Oxygen Species:  $O_2^-$ , Peroxynitrite,  $H_2O_2$ ,  $OH^-$

The radical induced toxicity to the membrane of virally infected cells also increases the release of toxic cytokines including Prostaglandin E2, Tumor Necrosis Factor, bradykinins, and complement, resulting in a myriad of toxic substances being produced from the initial ROS insult.<sup>13</sup>

### The Cause of Splenomegaly in EBV

The decrease of lymphocyte movement in EBV is caused by the generation of nitric oxide (NO) from another nitric oxide synthase enzyme, endothelial nitric oxide synthase (eNOS). The properties of eNO are different from the properties of iNO product in that eNOS nitric oxide (eNO) is a slow-released, longer active gas that slows down the movement and activity of lymphocytes. This latter activity is responsible for the splenomegaly in EBV. iNO, on the other hand, is short lived, rapidly generated, and rapidly dissipates locally.<sup>14</sup> For this reason and most likely a fine structural difference in the gas at the biophysical level, iNO rapidly combines with superoxide radicals and creates the toxic product peroxynitrite. eNO, on the other hand, neutralizes the oxygen radicals

and has been shown to decrease oxygen radical toxicity as in cancer cells, reperfusion injury and many other situations.

In addition to the eNO, another substance is released in EBV, BCRF1, a cytokine-like compound, similar to the cytokine IL-10. Like IL-10, BCRF1 suppresses IFN gamma and the Th-1 antiviral and anticancer lymphocytes. Since IFN gamma is the strongest anti EBV interferon, the virus actually keeps the host from defending itself.

### Inhibitors of EBV Toxicity and Replication

In order to inhibit the viral replication in EBV it is important to:

- Block NF Kappa b to prevent viral replication.
- Decrease XO activity to decrease ROS.
- Increase Interferon gamma IFN gamma<sup>15</sup> to increase viral defense.

NF Kappa b can be inhibited by antioxidants. In these patients, vitamin E as mixed tocopherols and vitamin C as a natural compound were used for this purpose. Many other antioxidants may be helpful here but we chose to use the classic antioxidants for our purpose since they are so extensively researched.

Xanthine oxidase activity was decreased by the flavonoids in *succus liquoriteae* (licorice root). Most flavonoids decrease the XO activity often reversing it to xanthine dehydrogenase.<sup>16</sup> Flavonoid containing substances include milk thistle (silymarin), garlic, red and black berries.

Glycyrrhnic acid in licorice root increases IFN gamma release and therefore adds an additional antiviral effect in EBV.<sup>17-19</sup> For hundreds of years licorice root has been known and used for its medicinal properties. Research over the past few years indicates that both flavonoids and glycyrrhnic acid have a synergistic effect to decrease viral toxicity at the same time increase interferon gamma.

**Inhibitors of Splenomegaly**

The eNO-caused lymphocyte immobility is reversed by adding N-acetylcysteine (NAC) or methionine which binds NO and alters its function resulting in a rapid increase in lymphocyte movement which therefore results in a complete reversal of the splenomegaly in a matter of hours.<sup>20</sup> (Figure 2, p. 98)

**Additional Supportive Therapy**

Because of the severity of the Epstein Barr Virus disease in many of the young patients, when they are ill for over three weeks with constant fevers, malaise and extreme splenomegaly, and weakened antiviral defenses, it has been

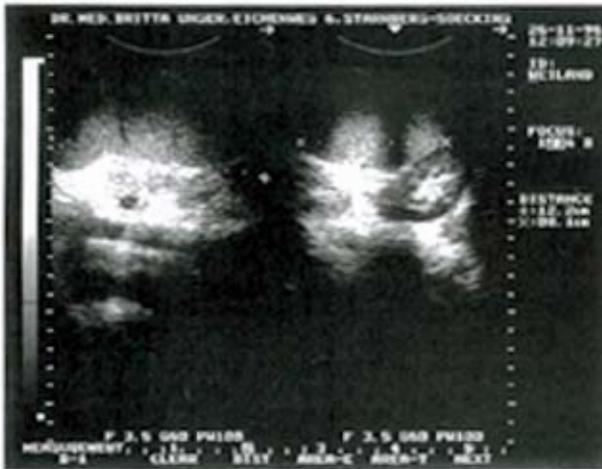
Table 1. Anti EBV hepatosplenomegaly mechanisms.

| Substance  | Mechanism  |
|--|--|
| Licorice Root<br>14.8 ml b.i.d or                                      | Increases Interferon gamma<br>helps decrease XO activity flavonoids <sup>23</sup><br>500 mg glycyrrhnicic acid t.i.d.    |
| N-acetylcysteine<br>500 – 750 mg t.i.d. or<br>methionine 500 mg b.i.d. | Binds Nitric oxide increasing lymphocyte<br>movements and reversing splenomegaly<br>Increases Th-1 lymphocytes antiviral |
| Zinc<br>40 – 60 mg/day   | Increases the efficacy of interferon 10 fold   |
| Vitamin E<br>Mixed Tocopherols<br>800 IU/day                           | Decreases NF Kappa b activity to decrease<br>viral replication. <sup>24,25</sup><br>Protects the lipid membrane.         |
| Vitamin C<br>1 gm t.i.d.   | Decreases NF Kappa b activity<br>and protects the hydrophilic membrane   |
| Sodium selenite<br>200 mcg children<br>500 mcg adults                  | Antiviral activity<br>Helps reduce peroxide products via GSH px.   |

Figure 2. Spleen size before and after therapy.



Spleen size before (14.6 x 9.4 x 5.2 cm)



Spleen size after (12.2 x 8.4 x 4.2 cm)

determined that two additional supportive substances, zinc and selenium, are best included to insure the safety and efficacy of the treatment.

According to the literature, zinc increases the efficacy of interferon ten-fold which

our patients results confirmed.<sup>21</sup> Selenium was given in the form of sodium selenite for its antiviral effect<sup>22</sup> as well as being a valuable part of the enzyme glutathione peroxidase, responsible for eliminating toxic peroxide products in the cell. (Table 1, p. 97)

### Discussion

Mononucleosis, an Epstein Barr Viral EBV infection, is treatable and rapidly reversible. By understanding the basic pathology and molecular biology of viral diseases we are now able to inhibit the toxicity of the disease while simultaneously increasing the immune defense to stop the viral replication.

Nitric oxide is a gas that can be protective or destructive depending on its environment.<sup>26,27</sup> iNO is toxic when associated with oxygen radicals from oxidase enzyme XO. In inflammatory diseases other than viral, NADPH oxidase, monoaminoxidase and other ROS generating enzymes also play a role.<sup>28</sup> In viral diseases, however, the radical generation is predominantly caused by XO. This enzyme is not only found in leucocytes and lymphocytes but is carried through the blood like a hormone or cytokine, active in another part of the body from whence it originated. This was an astounding discovery which not only has helped us to cure EBV in 24 hours in most cases, but may very possibly be a major mechanism of toxicity in the Asian bird flu H5N1, HIV, and many other viral and inflammatory diseases. The effect of eNO on lymphocytes mobility discovered by Eisenstein also enabled us to correctly surmise, as born out by testing, that through binding eNO we might be able to reverse splenomegalies.

It was not until the work of Chisari at Scripps was published that we saw how important the increase in IFN gamma was in our treatment.<sup>29</sup> Although our colleague Jan Anderson in Sweden used IFN gamma with prednisone in a cerebral involvement of EBV, he informed us the patient did not improve until he removed the prednisone and the T-cells increased.<sup>30,31</sup> We devised a much better solution by increasing the Th-1 cells with NAC by binding NO and<sup>32</sup> removing the inhibition on the motility of the lymphocytes.

In some cases it was necessary to use acetaminophen to lower fevers, although children respond to the biochemical changes from nutrients much faster than adults, due to their increased metabolism.

The next step in our research will be to investigate whether or not XO is elevated in HIV and H5N1 Asian bird flu and develop further treatments for these diseases. Should the mechanism indeed be the same as what we have found in so many other viral diseases we should be able to develop many new treatments to increase the immune system while simultaneously decreasing the toxicity in these diseases. In viral infections, the redox relationship determines the prognosis and morbidity of the disease. Literature searched showed similar correlations in the mechanism involved in viral toxicity and treatments for the following viruses: Cocksackie virus,<sup>33</sup> Epstein-Barr virus,<sup>34,35</sup> Hepatitis,<sup>36,37</sup> HIV,<sup>38,39,40</sup> Influenza virus,<sup>41</sup> Japanese Encephalitis virus,<sup>42</sup> Murray Valley Encephalitis virus,<sup>43</sup> Vaccina and Vesicular stomatitis virus,<sup>44</sup> and Varicella-zoster virus,<sup>45</sup> giving credence to the probability that the viral toxicities are the same in all of these diseases.

### Summary

Until this study, EBV infection, (mononucleosis), was a disease whose only treatments were bed rest and acetaminophen. In children it has been known to be lethal when splenomegaly lead to a ruptured spleen. The only therapy for splenomegaly in the past was a splenectomy leaving the children and young adults susceptible to future septic bacterial infections. We have been able to reduce the viral infection load including reversing splenomegaly within hours with our treatment from research on the disease's pathology. We were able to block specific biochemical targets to enhance the mobility of the lymphocytes out of the

spleen as well as drastically decreasing the toxicity and the viral replication itself. The pharmacology of the individual nutrients elucidates the intricate interaction in this therapy and explains the dramatic improvement and successful reversal of all symptoms in this disease for a rapid return 24-48 hours to optimal health.

It is most probable that the mechanism of toxicity in most, if not all viral diseases, including HIV and H5N1, is the same as in EBV and HBV with the symptoms varying according to where the viruses are located. With a better understanding of the mechanisms involved in the pathology of these diseases, we shall be able to apply this in the future to develop further successful therapies for viral diseases.

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