HDFx: A Recently Discovered Biologic and its Potential Use in Prevention and Treatment of Hemorrhagic Fever Viruses and Antibiotic-Resistant Superbugs

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Abstract

Recently, we have reported on the discovery of a new, conserved protein (35-40 kDa) that protects rats, mice, guinea-pigs, and rabbits against lethal hemorrhage, endotoxins, live lethal bacterial and fungal microorganisms, and traumatic injuries when given prophylactally and therapeutically. HDFx was found to stimulate several arms of the innate immune system (e.g. macrophages, NK cells). HDFx was also found to stabilize the microcirculation, prevent rupture and leakage of postcapillary venules, prevent adhesion of platelets to endothelium and loss of platelets, stabilize falls in arterial blood pressure, and prevent stasis and pooling of blood in the postcapillary vessels, as observed by intra-vital high-resolution TV microscopy. HDFx also stimulates phagocytic uptake of foreign particulate matter and bacteria by liver Kupffer cells, splenic macrophages, and circulating macrophages. It also prevents explosive release of cytokines and chemokines from macrophages and lymphocytes in animals subjected to live bacteria, endotoxins, trauma and combined injuries. Surprisingly, HDFx was found to accelerate wound healing and aid the regeneration of tissues. Repeated administration of HDFx, over many months, does not result in either diminished protective activity or detectable organ or tissue pathologies. One of the major consequences of infections and wars/conflicts is loss of the ability to regenerate normal physiologic functions of numerous organs and tissues. A major characteristic of invasion of the body by septic-endotoxic microorganisms and hemorrhagic fever viruses (HFVs) is that these entities eventuate in rupture of the microvessels in the capillary circulation of numerous organs and tissues leading to massive blood and fluid loss, making the body susceptible to superimposed infections and loss of immuno-competence. About 100 million people are infected worldwide, annually, with about 60,000 to 75,000 deaths per year from HFVs. Added to these numbers are the numerous hospital-borne and food-borne infections along with infections resulting from major disasters (hurricanes, tornados, earthquakes, etc.) that cause 75,000 to 100,000 deaths per year in the U.S.A. alone. The ability and uniqueness of HDFx to minimize infections, accelerate wound healing, and promote tissue regeneration should greatly aid treatment and recovery of these victims and be of great value in infections from HFVs and on battlefields.

Keywords: Antiviral drugs; Biothreats; Military medicine; Emerging diseases; Innate immune system; Battlefield injuries

Introduction

In the next two decades, the civilian and military populations of the Western World will, most likely, be faced with an array of biothreats and infectious microorganisms, some emerging from the environment with others generated by bioterrorists, particularly hemorrhagic fever viruses (HFV). Vaccines (to be developed), antibiotics and antiviral drugs all will be useful in protecting against many of these threats but cannot be counted upon in the event of rapid assaults from genetically-altered, mutated, or drug-resistant microbe [1-4].

Thousands of people have died worldwide from Ebola, Middle Eastern Respiratory Virus (MERS virus), SARS virus, and H1N1-mutated flu viruses over the last ten-15 years. Approximately 100 million people are infected worldwide, annually, with about 60,000 to 75,000 deaths per year from various HFVs [5]. Added to this, are the approximately two million cases of hospital-borne (nosocomial) infections with approximately 100,000 deaths per year in the USA alone. These patients, unfortunately, eventuate in a compromised immune system, particularly a loss of many innate immune functions [6-9].

A new disturbing trend in antimicrobial resistance of both gram-negative and gram-positive pathogens and "superbugs" has seriously complicated the treatment of these immuno-compromised subjects [6-11]. Too this major problem must be added the numerous hospitalizations and deaths from contaminated meats, vegetables, seafoods, and dairy products [12-14]. Many of the emerging diseases such as the H1N1 flu, SARS, MERS, dengue fevers, etc, have a very serious hemorrhagic component to them which complicates effective treatment [15-18]. Governmental resources are being overstretched and often remain powerless to combat these assaults on our populations.

Diversity and similarities of hemorrhagic fever viruses

Viral hemorrhagic fever viruses are a group of "superbugs" that present themselves by a profound loss of homeostasis eventuating in widespread bleeding and circulatory shock. HVFs are induced by a series of RNA zoonotic viruses from four different families; i.e., Arenaviridae, Bunyaviridae, Flaviviridae and Filoviridae [5]. Systemic bleeding seems to occur more with Ebola and Marburg viruses (Filo viruses), Crimean Congo Hemorrhagic Fever (Bunya virus), and the South American hemorrhagic fever virus (Arena viruses), compared to dengue and Yellow Fever (Flavi viruses), Rift Valley Fever (Bunya virus), and Lassa fever (Arena virus)[5-9]. Why the latter is so is not exactly known. However, common to all HFV is that all of them infect vascular endothelial cells, diminish platelet numbers and functions, and result in disseminated intravascular coagulation [5-11]. Clearly, their means for replication are diverse in mechanisms as is often the organs and tissues they attack [5-11].

Although each of the numerous types of hemorrhagic fever viruses (e.g., Ebola, Marburg, Hantavirus, Dengue, Yellow fever, Lassa virus, Rift Valley fever, MERS, etc.) are molecularly different, they all present with several common features in patients such as high fever, bleeding disorders, platelet losses, disseminated intravascular coagulation, swelling caused by edema, low blood pressure (hypotension), bone marrow dysfunctions, and circulatory shock [5-18]. Added to these characteristics are loss of functional macrophages, monocytes, dendritic cells, and natural killer cells (NK cells) [5,6-19]. HFVs also modifies these cells abilities to handle and process antigens and their cytokine-producing functions [5,19-22]. We believe that any drug, vaccine, or adjuvant(s) designed for both prevention and treatment of HFVs must be able to ameliorate or prevent most of these attributes and unique characteristics of HFVs.

Rationale and background for the discovery of a new immuno-stimulant-HDFx

Each year, in the USA, alone, more than 150 million prescriptions are written, 60% of which are for antibiotics. Of these, it has been estimated that 50 million of these costly prescriptions are probably unnecessary [15]. Added to this is the ever-growing and soaring worldwide use of antibiotics in agriculture. How much is this indiscriminate use of antibiotics contributing to the ever-growing resistance of pathogens to antibiotics noted above?

Ever since our laboratories' earliest studies in diverse forms of circulatory shock, more than 50 years ago [13-32], we have been interested in the treatment and mechanisms of septic-endotoxin shock and have designed some vasoactive drugs which manipulate both the microcirculation and the innate immune system [23-28,31-46]. These studies, early-on [24-32], suggested to us as Elie Metchnikoff, the great father of immunology hypothesized, more than 135 years ago [47], that the body might, under stressful circumstances, produce a powerful immuno-stimulant(s) which perforce would act on different arms of the innate immune system and serve to protect the host against major insults. Metchnikoff's very early studies pointed to the important contributions of macrophages and phagocytic leukocytes to natural (innate) resistance against pathogenic bacteria and viruses. Over the past three to four decades, considerable evidence has accrued to support a strong relationship to the functional (physiological) state of macrophages-phagocytes and natural killer (NK) cells to host defense and resistance to pathogens [48-58]. Since many of the characteristics of septic-endotoxin shock have many similarities to those presented by

HVFs, e.g., high fever, bleeding disorders, intravascular coagulation, diminished numbers of circulating platelets, increases in capillary fragility and permeability, diminished numbers of active-functional macrophages, monocytes, and dendritic cells, drops in arterial blood pressure, severe lung pathological alterations, loss of functional NK cells and dysfunctions in handling of dangerous antigens, we have used our septic-endotoxin shock animal models, live gram-negative and gram-positive bacteria, along with combined injury shock models [23-33,35,37,41-46,59-67] to explore the actions and mechanisms of HDFx [68-70].

Our laboratories, over the past several years have discovered a new protein molecule (i.e., HDFx) found in the bodies of rodents, rabbits, guinea-pigs, dogs and subhuman primates which we believe may have the necessary attributes for ameliorating, preventing and combating the deadly effects of HFVs and, potentially, counteract new emerging bacterial and viral disorders [68-70], even those, possibly, like Zika virus. Below, we have summarized the biochemical, physiological, and therapeutic attributes of this remarkable molecule, i.e., HDFx.

What is HDFx and where is it produced in the body?

Using techniques and isolation procedures, described in our previous studies [68], we have determined, so far, that HDFx is a complicated heat-labile protein molecule (35-40 KDa in size) [68]. It is generated (synthesized) in macrophages and NK cells [68]. Eighty percent of HDFx appears to be derived from macrophages, the other 20% from NK cells [68]. HDFx is stimulated to production in these cell types when the body is under stress of diverse types, e.g., hemorrhage, ischemia, trauma, fungi, infectious microorganisms, systemic body stress (e.g., massive body trauma, centripetal acceleration), among other stressors [68,69]. The more the severity of the insult, the more the macrophages and NK cells appear to respond and be recruited, up to a point of exhaustion, to synthesize and release HDFx into the blood stream [68,69]. Repeated administration of HDFx, over many months, does not result in either diminished protective activity or detectable organ or tissue pathologies [68].

HDFx protects against the systemic effects of blood loss, loss of important blood-formed elements and fluid loss in the capillary microcirculation and stabilizes arterial blood pressure

A major characteristic of invasion of the body by septic-endotoxic microorganisms and HFVs is that these entities eventuate in rupture of the microvessels in the capillary circulation of numerous organs and tissues leading to massive blood and fluid loss, thus making the body susceptible to superimposed infections and loss of immuno-competence [5,17-19,45,48,55,67,71-74].

Such a situation eventuates in loss of white blood cells, loss of monocytes, loss of lymphocytes, loss of macrophages, loss of dendritic cells, loss of circulating platelets, and loss of red blood cells, as well as loss of important plasma proteins (e.g., complement fractions) required for immuno-competence. In addition, the hemorrhagic syndromes induced by HFVs result in profound drops in arterial blood pressure concomitant with decreased cardiac output eventuating in circulatory shock, respiratory failure, cardiac failure and death [16-19].

Using rats, mice, and guinea-pigs, given live and diverse types of lethal bacteria (e.g., *E.coli, S.enteriditis, C. welchii,* etc.*), endotoxins (i.e., E. coli, S. enteriditis, S. typhus),* and/or fungi (e.g., *candidae,*

aspergillus), we found that either pretreatment or post-treatment of the infected animals with the HDFx prevented major blood and fluid loss from the capillary microcirculations in the splanchnic tract, the lung microvasculature, skeletal muscle microvasculature, and the cerebral vasculature [68,69].

The animals that are infected with these diverse microorganisms, when treated with HDFx, either fail to demonstrate significant drops in arterial blood pressure or show an amelioration of the drops in arterial blood pressure.

The result of these diverse, beneficial actions of HDFx results in increased survival and decreased morbidity [68,69]. Whether or not HDFx will produce similar actions in the microcirculations of animals given HFVs remains to be investigated.

HDFx prevents loss of normal vasomotor activity, pooling of blood, and vascular reactivity of microvessels in animals given lethal bacteria: Relationship to microvascular effects of HFVs

Animals and humans challenged with HFVs demonstrate prolonged failure of microcirculatory blood vessels to control and distribute blood flow (or to demonstrate normal vasomotion in the capillary bed) to peripheral and cerebral tissues [16-19]. In an attempt to mimic these phenomena, we injected rats, mice and guinea-pigs with various live bacteria (e.g., *E.coli, S. enteriditis, C. welchii*) or endotoxins and examined the living microvasculatures of mesenteric, skeletal muscle, lung, cutaneous, and cerebral circulations using intra-vital high-resolution TV microscopical techniques which our laboratories have helped to pioneer [36-38,69].

We found that the normal opening and closing of precapillary vessels and sphincters (vasomotion) became, where they exist, eventually non-respondent, thus resulting in inadequate distribution of capillary blood flows coupled to loss of venular tone followed by stasis and rupture of postcapillary venules and intravenular hemolysis with pooling of blood as either the dose of the pathogen was increased or time-elapsed preventing adequate venous return [69].

Both pretreatment and post-treatment of the animals with HDFx either ameliorated or curtailed these detrimental microvascular effects of the deadly pathogens [68,69]. The end result of treatment with HDFx was a dramatic stabilization of the microcirculations when visualized, in situ, under high-power using our TV microscope recording system. Whether injections of HFVs in the presence of HDFx would yield similar beneficial results remains to be investigated.

However, since most of the adverse microvascular effects of HFVs resemble those actions of the gram-negative and gram-positive bacteria in the microcirculations studied, we believe HDFx would most, likely, yield dramatic beneficial results.

It is important to point out, here, that when a subject is infected with lethal bacteria or fungi as we have experimented with, or HFVs, the rupture of postcapillary venules in the microvasculature eventuates in loss of many of the formed elements in blood (e.g., leukocytes, platelets, monocytes, lymphocytes, macrophages, and dendritic cells), thus removing major innate host-defense factors from the body necessary for life functions and survival.

We believe the loss of macrophages and platelets, under these conditions, coupled to the inability to recruit NK cells may be a primary reason for the inability of a host to overcome the very dangerous assaults by HFVs.

Macrophages and NK cells are the major sources of HDFx

Early-on in our studies, we hypothesized that the loss of functional macrophages and NK cells from the circulation might be a major reason for morbidity and mortality when animals and humans are either subjected to systemic lethal insults such as massive hemorrhage(>30% blood loss), trauma, peripheral ischemic events(e.g., bowel ischemia), combined injuries (trauma plus superimposed infections), infection with one or more gram-negative or gram-positive microorganisms, burn injuries, vector-borne viruses(e.g., elephantiasis; tularemia), major high-risk surgical events(e.g., lung surgeries, coronary artery bypass surgeries) particularly in the elderly, and exposure to HFVs and fungi. Most of these situations will perforce result in losses in immuno-competence.

Using various antibodies and chemical agents to either block or deplete the bodies of rats, mice, guinea-pigs, and rabbits of either macrophages and/or NK cells, we found that such animals were very susceptible to mortality when exposed to normally non-lethal encounters using small degrees of blood loss, bowel ischemia, trauma, combined injuries, burns, or non-lethal doses of live bacteria or fungi [68,69]. Depletion of only macrophages caused about 75-80% of the loss of resistance to these insults; the other 20% to loss of the NK cells [68]. Selective depletion of either polymorphonuclear leukocytes or monocytes when challenged with the non-lethal stressors exerted very little effects on mortality [68]. After a great many experiments, over several years and thousands of animals, we were able to isolate a heat-labile protein of about 35-40 KDa, larger than known defensin peptides and much smaller than the larger MW fibronectins and complement products [68]. When highly-purified extracts (free of any endotoxin contamination) of the purified active HDFx was given to rats, mice, guinea-pigs, rabbits, and sub-human primates, over several months, we could not detect any obvious organ pathologies [68,69]. However, use of highly-purified HDFx protected, to a large degree, these animals from a variety of insults, including lethal hemorrhage, lethal body trauma, lethal bowel ischemia, lethal burns, lethal fungus infections, lethal bacterial infections, and combined injuries [68,69]. In addition, HDFx stimulated macrophages to engulf more foreign matter than normal and resulted in a stimulation of NK cells [68]. To our knowledge, a molecule/peptide that possess these characteristics/ qualities has not, as yet, been reported. Although neither we nor anyone else has tested HDFx against HFVs, we suspect that, when tested, HDFx will yield surprising beneficial actions. Additional experiments by our group leads us to believe that HDFx may possess remarkable beneficial actions on platelets, cytokine and chemokine release, and regenerative properties that may account for a great deal of its potential recuperative powers [70].

Roles of platelets, cytokines and chemokines in sepsis and HFV infections: Potential beneficial effects of HDFx

Blood platelets are crucial in primary hemostasis [75]. They circulate continuously in the vascular compartment and serve to help repair injured epithelial and endothelial tissues [75]. Under pathological conditions such as invasion of the host by lethal bacteria, fungi, and HFVs, the needs for platelets often exceed the basal levels.

When the latter takes place, the need for transfusions may become excessive. Unfortunately, as of this writing, there are no substitutes available to replace platelets. Platelets contain diverse granules which contain many cytokines, chemokines, ATP, Ca^{2+}, serotonin, platelet-derived growth factors, etc. Platelets can also act as a source of proteins and glycoproteins [5,19,21,75-77] and can ingest a variety of pathogens and interact with phagocytic cells [19,75-77]. In addition, platelets express important toll-like receptors, an array of direct antimicrobial peptides, and kinocidins [19,75]. Dozens of cytokines and chemokines can potentially be released by platelets as can more than 300 glycoproteins, and untold biological-response modifiers [19,21,22,75]. Although it is thought that most of the explosive release of cytokines and chemokines released in septic-endotoxic shock and invasion by HFVs are derived from monocytes and macrophages [19-22,75-77], evidence is accumulating that platelets may play a very significant role in these responses as well [19-22, 75-77].

Investigation of the microvascular beds of the splanchnic tract, skeletal muscle, cutaneous, lung, and cerebral areas of rats and mice, by direct, *in-vivo* high resolution microscopical techniques, in our labs[36-39,78], have demonstrated that administration of several gram-negative bacteria (i.e., *E. coli, S. enteritidis*), endotoxins, and fungi (i.e., *Candida, Aspergillis*))ndicates that these microorganisms eventuate in disseminated intravascular coagulation with adhesion to endothelial surfaces (particularly of the postcapillary venules) and removal of platelets from the intravascular compartments of these tissue sites. Similar effects are thought to take place in the microvasculatures of humans infected with HFVs [19-23,76,77]. Interestingly, treatment of animals with HDFx in our laboratories, attenuated, markedly, these pathological effects on platelets in rats and mice given lethal bacterial and fungal microorganisms. Whether similar, beneficial actions of HDFx on platelet physiology and pathology takes place in humans infected with HFVs remains to be determined.

Effects of septic-endotoxin shock on circulating lymphocytes in animals

Using septic-endotoxic shock (e.g., *E.coli, S.enteriditis*) animal models, we found that lymphocytes also release a variety of cytokines (e.g., TNF-alpha, Interleukins like IL-1, IL-2, IL-6. IL-17, interferons, etc) and chemokines [68] followed by massive apoptosis of the lymphocytes. In our septic shock models, very high serum levels of TNF-alpha usually was a portent of imminent death of the host. However, these explosive releases of both cytokines and chemokines were attenuated, markedly, in the presence of treatment with HDFx. Usually, invasion of a host by HFVs results in high levels of these pro-inflammatory cytokines and chemokines [5,16-22,77]. Whether use of HDFx in invasion of hosts with HFVs will result also in similar beneficial effects is, as yet, not known.

HDFx: A novel biologic immunomodulator accelerates wound healing

A major complication of invasion of a host with either gram-negative or gram-positive bacteria or HFVs, is often loss of a limb or digits due to invasion of healthy tissues by these various microorganisms, often resulting in disfigurement as a consequence of amputation and/or improper tissue healing and or improper healing-regeneration. One of the unique effects and benefits of HDFx is its great ability to regenerate damaged tissues [70]. Using two different experimental animal-wound models(i.e., excision wound model; and incision-wound model) [70], we have reported that HDFx produces greater rates of wound contraction, greater tensile strength, and more rapid healing than control animals [70]. Our data, so far, show that this novel biologic molecule increases hydroxyproline content of granulation tissue coupled with a reduction in superoxide dismutase (SOD) [70]. In addition, we demonstrated that HDFx increases the levels of serum ascorbic acid and stimulates the mononuclear cells of the reticuloendothelial system (RES) [70]. Overall, we believe these new data suggest that HDFx may possess unique regenerative powers. Thus, it is probable that HDFx would result in healing of the difficult, to treat, infections seen in sepsis and infections by HFVs and viruses carried by diverse vectors, possibly like Zika virus.

Potential treatment and prophylaxis with HDFx of infected battlefield-wounded men and women and emerging diseases

One of the major consequences of infections and wars/conflicts is loss of the ability to regenerate normal physiologic functions of numerous organs and tissues in the body. It is often difficult to treat many chronic internal and external wounds and scars (and disfigurement) resulting from assaults by infectious microoganisms such as HFVs. These chronic wounds can become painful and very dangerous, often resulting in sepsis and antibiotic resistance to other microorganisms, vectors, gangrene, and amputation. The debilitated chronically-ill infected patient is, thus, at high risk and particularly susceptible to slow-healing and external, painful wounds.

Overall, we believe our results, so far, with HDFx, demonstrate the potential utility of this recently discovered protein for the warfighter on the battlefield and for victims of major disasters (e.g., earthquakes, plane-train-automobile crashes-pile-ups, severe blood loss after major surgeries, etc.). It is clear from our studies (above) on thousands of experimental animals(of diverse species) that HDFx exhibits a number of powerful, heretofore, unseen attributes, e.g., increases survival rates after hemorrhage, diverse forms of circulatory shock, trauma, diverse infectious microorganisms, and combined injuries, etc. In addition, HDFx elevates arterial blood pressure from very low levels toward normalcy after these diverse lethal insults, restores the pathologies of all microvasculatures investigated, to date, prevents formed elements from pooling in the venous side of the microcirculation in shocked animals and those given lethal doses of bacteria and fungi, stimulates the mononuclear phagocytic system, stimulates several arms of the innate immune system, safeguards viable platelet and lymphocyte functions, and prevents disseminated intravascular coagulation [68-70]. Lastly, HDFx appears to possess a number of powerful attributes which accelerate wound healing.

For millennia, diseases and non-combat injuries have resulted in the vast majority of lost combat days on the battlefield. "During the Mexican (1845-1848) and Spanish-American (1898) wars disease-related deaths outnumbered battlefield deaths by seven to one" [79]. During recent wars in Vietnam (1969-1975), Iraq (2001-present), and Afghanistan(2001-present), American and UN-coalition forces have continued to demonstrate a mounting number of diverse diseases in battlefield troops which have taken great tolls in morbidity and mortality despite improved transportation, treatment, hygiene, and new advances in management [79,80]. Many of the infections produced by a variety of microorganisms, parasites and vectors such as pneumonia, tuberculosis, Q fever, brucellosis, leishmaniasis, and malaria have become antibiotic- and drug-resistant and exhibit

superimposed infections with *Acinetobacter* species [79-82]. In addition, many of the warfighters exhibit gastroenteritis, prolonged diarrhea, and drug-resistant respiratory infections. Inadequate treatment often results in loss of body parts, amputations, and disfigurements. Many of these men and women on return to the USA, and their native countries, require prolonged hospitalization and treatment, often at great cost without restoration to normalcy. The ability and uniqueness of HDFx to accelerate wound healing and promote tissue regeneration should greatly aid treatment and recovery of our warfighters.

It is our belief that prophylaxis of service men and women with HDFx would result in marked attenuation of morbidity and mortality in battlefield troops. Obviously, this suggestion remains to be tested under controlled conditions.

Conclusions and future thoughts regarding HDFx

In summary, our findings demonstrate that we have discovered a naturally-occurring biologic protein which possesses very unique characteristics and qualities not seen in any protein, heretofore, to our knowledge. HDFx ameliorates morbidity and mortality induced by a variety of agencies which result in circulatory shock. HDFx protects, to varying degrees, diverse animal species against severe blood loss, trauma, combined injuries and the effects from diverse gram-negative and gram-positive bacteria, as well as some forms of fungi. In addition, and not to be minimized, is HDFx's ability to accelerate wound healing and regeneration of tissues. HDFx prevents explosive release of cytokines and chemokines from macrophages and lymphocytes in animals given lethal bacteria and endotoxins. In addition, HDFx prevents/ameliorates disseminated intravascular coagulation and loss of circulating platelets in animals given lethal bacteria and endotoxins. HDFx also prevents the profound drops in arterial blood pressure seen in animals subjected to diverse forms of circulatory shock, bacteria, endotoxins, and some fungi. It also preserves macrophages and NK cells when animals are assaulted by diverse infectious microorganisms.

In view of the unique circulatory, antibacterial, antifungal, and microvascular actions of HDFx, we believe it would be judicious to examine its potential protective physiological, biochemical, and immunological effects in human subjects infected with diverse viruses that cause hemorrhagic fevers. It would be propitious to consider using HDFx as a prophylactic treatment for men and women warfighters. We believe that the widespread stimulation of the innate immune system, determined to date, suggests that HDFx would deter invasion of the body by AIDS and Hepatitis viruses. While development of curative treatments or an effective vaccine remains a grim possibility for HIV, measures of success could be gained by dramatic stimulation of the host defense system, as typified by HDFx. Currently, no agent, or drug, or combination of drugs is capable of achieving the latter. However, HDFx seems to be such a promising candidate. Indeed, the possibility of a "vaccination-like effect becomes a potential dynamic objective of our research.

A major objective of our group is to secure adequate funding to elucidate the complete, complex molecular structure of HDFx and then via genetic engineering to produce large quantities of HDFx for further testing in both animals and human subjects under diverse pathophysiological conditions, including infections produced by HFVs and infections seen on the battlefields.

In view of the enormous refugee problem in The Middle East, and the desire (and need) to resettle huge numbers of unvented, potential disease-carrying people, many of whom have contagious diseases, it would be propitious to administer HDFx to thousands of men, women, and children in the host countries for protection against potential multiple assaults on immune systems by unknown bacteria, viruses, and other microorganisms in order to prevent major outbreaks, leading to potential plagues.

Acknowledgements

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