OZONE, NITRIC OXIDE, AND AVIAN INFLUENZA:
Preliminary theoretical considerations and possible therapeutic directions

Gerard V. Sunnen, M.D.
© Copyright 2005

Abstract

Influenza is a recurrent global disease with, in pandemic conditions, significant morbidity and lethality. The dynamics of avian influenza are complicated by the fact that its virus is capable of evolving in a variety of animal and human reservoirs. Able to infect all members of the human population in its pandemic phase, influenza presents supremely challenging problems in light of its pathogenic capacity and mutational potential.

Recent advances in immunology have clarified some of the complex mechanisms of antigen-antibody reactions. This paper explores two main gases that, produced at the molecular level by cellular elements of the immune system, perform crucial roles in microorganism inactivation.

The idea that gases are produced in vivo to perform a panoply of essential biological functions has, in the last few years, revolutionized concepts about cellular signaling.

These two physiological gases are nitric oxide and ozone. Suggested is that, in view of the characteristics inherent in avian flu, research into the dynamics of these virucidal agents could assist in the public health response to an influenza plague.

The Avian influenza virus: Virion architecture and molecular biology

The influenza virus belongs to the small family of *Orthomyxoviruses*. *Myxo* refers to the Greek term for mucous and this family's propensity for attachment to the mucoproteins on cell surfaces. In the case of Avian influenza, the target cells are the columnar epithelium of the respiratory tree. The family includes Influenza A, the cause of pandemics, distinguished by its antigenic surface components. Influenza B, a milder disease, does not cause pandemics. Influenza C has a somewhat different genetic structure, infects children and Asian swine, and causes even milder pathology.

The avian influenza virion, 100 to 200 nm in diameter is approximately spherical because of its loose-fitting envelope. Under the electron microscope it appears as an ovoid organism studded with hundreds of spikes, the peplomers. If it were expanded, it would look like a sea urchin.

Within the viral core are eight separate helical single strands of ribonucleoprotein, the software for viral life and replication. This unusual segmented RNA genome encodes the transcription of all viral components, including structural proteins, enzymes, and lipids.

An intricate membrane, the envelope, surrounds the viral genome. Matrix proteins provide internal attachment between the genomic nucleocapsid and its envelope. The Avian influenza envelope has an inner protein (M) shell covered by another shell composed of a double layer of lipids. Approximately 60% of envelope lipids are composed of phospholipids and the rest are cholesterols.

Embedded in the envelope are the roots of the peplomers. Peplomer spikes are essential for viral attachment and penetration into host cells. Peplomers are constructed of carbohydrate and protein components, glycoproteins. Of the several hundred peplomers studding an individual influenza virion, 80% are the triangular-shaped hemaglutinin (HA) glycoproteins, and the rest are the mushroom-shaped neuraminidase (NA) glycoproteins.

HA and NA are vital for avian flu's infectious capacity. With regard to the host, HA and NA are the inimical antigens prodding its immune system's counter-offensiveness. The hemaglutinin HA glycoprotein is able to coalesce the red blood cells of a num-

ber of animal species, hence its name. The neuraminidase (NA) glycoprotein functions as an enzyme, facilitating virus to host cell attachment and viral release from cells. NA has the capacity to destroy a component of the host cell surface, neuraminic acid. The signature proteinic composition of HA and NA determines the virulence of influenza's thrust into host cells.

Since 1971, influenza A viruses have been named according to their HA and NA glycoprotein antigenic compositions. Thus, the influenza H5N1 strain describes the molecular architecture of its peplomers.

Avian influenza: Genetic creativity and infectious transmission

The viral replication cycle follows the pattern seen in numerous mammalian viruses. Virions, once attached to host cell receptors, enter cells by engulfment - the endocytosis process - or by viropexis, which entails a fusion of the viral and the cell envelopes. Once entry into the cell is achieved, virions commence their replication.

The next task of newborn viral nucleocapsids is to exit their incubator cell. During this release process, their viral envelopes are formed. For this to happen, the nucleocapsid fuses with the host cell membrane, itself a lipid bilayer, appropriating its components. The lipid composition of viral membranes thus reflects the lipid composition of the cells through which the particles exit.

Virions are then released into the general blood and lymphatic circulations, ready to infect new cells, other organ systems and, eventually, new hosts. In another scenario, viral particles, by way of their sheer numbers and the over taxation of the cells they invade, may at times provoke cell lysis and death. In an amount of time measured in hours, influenza can flood the body with billions of viral particles.

Transmission of influenza viruses is by droplet, person-to-person contact, and by transfer through fomites (objects).

As is the case in many RNA viruses, *Orthomyxoviridae* mutate at a high rate. Within any one afflicted individual, influenza particles do not show a homogeneous population. Instead, they function as a pool of genetically variant strains known as quasispecies. This is due to the high error frequency of RNA polymerases, the presence of deletion mutants, the high frequency of RNA recombination and point mutations, and the occurrence of defective-interfering RNA (Holland 1993). The net result of these diverse mechanisms is the continuous spawning of novel virions and divergent quasispecies. Some of the genetic creations will find themselves at an advantage in surmounting new host-antibody responses and antiviral drug challenges. They will propagate accordingly, thus expanding their ecological territory. Other genetic configurations, by being too lethal will lead to the demise of their hosts. If we can speak of a viral psychology, an efficient viral survival balance aims somewhere between total defeat by host defenses on one hand and viral suicide through aggressive lethality on the other.

Antigenic drift describes a gradual accumulation of amino acid mutational changes. In the influenza virion, HA and NA antigens slowly change over time. Antigenic shift, on the other hand, represents a dramatic alteration in genetic configuration resulting in the acquisition of completely novel HA and NA antigens.

In a process called reassortment, an individual who harbors concomitantly a human and an avian influenza virus can become an incubator for novel, revolutionary viruses. It is possible, as has happened in past pandemics (Taubenberger 2005), that one of these viral creations becomes doted with the capacity for highly virulent human-to-human transmission.

Avian influenza: The illness

After an incubation of 1 to 5 days, influenza begins with "cold-like" symptoms. "Colds," however, are caused by different viral families such as picornaviruses, rhinoviruses, echoviruses, and coxsackieviruses, and do not escalate into the acute symptomatology of influenza with malaise, fever, headache, myalgia, sore throat, nasopharyngeal congestion, and retro-orbital pain. In the presence of viral pneumonia, there is chest pain and shortness of breath.

The acute symptoms in uncomplicated cases begin to abate in a few days. Recuperation, however, may be slow in some individuals who show lingering malaise. The syndrome is a great stress to the organism. Individuals challenged by heart, liver, pulmonary, endocrine, kidney, immune conditions, or age, are consequently more vulnerable to viral lethality.

Bodily organs injured by influenza are more prone to suprainfections with bacterial species. *Staphylococcus aureus, Streptococcus pneumonia, Klebsiella,* and *Hemophilus influenzae* are commonly implicated in bacterial pneumonia complicating influenza. In

fact, mortality in influenza pandemics has been significantly attributed to bacterial onslaughts on the coattails of the viral invasion. This fact is crucial in public health planning. Indeed, the stockpiling of appropriate anti-bacterial agents is as important as insuring adequate anti-viral supplies.

The importance of physiological gases in antiviral therapy

Current antiviral medications for influenza function via the inhibition of viral components involved in attachment to, or release from, host cells. Oseltamivir (Tamiflu) and zanamivir (Relenza) for example, alter viral neuraminidase peplomers, bridling replicative capacity. Amantadine (Symetrel) modifies M2 proteins, thus interfering with viral nucleic acid release into the host cell.

As effective as such agents may be, it is recognized that viral genetic creativity has the capacity, in time, to circumvent these drug strategies. Viral resistance to amantadine is well documented, and recently, oseltamivir and zanamivir have shown similar fates. Antiviral drug therapies, whether aimed at directly denaturing the virion to compromise its life cycle, or inactivating it through vaccine-mediated immunosuppression are subject to the constant challenge of viral mutational drift and shift.

One complementary line of research aims at understanding the fundamental natural mechanisms of the body's antiviral capacity with a view to enhancing them.

Antibody-antigen reactions have long been central to concepts of primordial defense. Initiated by viral challenges, the immune system, once set in motion, activates its cellular elements and synthesizes antibodies. Activated macrophages and leucocytes engulf virions. Antibodies fasten themselves to viral particles to neutralize them. But how do activated immune cells and antibodies really kill virions?

Recent research has focused on the role of gases in this process. The idea that physiological gases are produced in our bodies, albeit at the molecular level, has been so counterintuitive that it is only recently that this notion has gained gradual conceptual acceptance. The gases principally involved in microorganism inactivation are nitric oxide and ozone.

A crucial physiological gas: Nitric oxide

A gas, with a half-life of only a few seconds, generated in vivo, as an essential component of the immune system, with crucial functions in the nervous and the cardiovascular systems?

In only the last few years, nitric oxide (NO) has received recognition for its multidimensional physiological functions. Indeed, NO has been documented to have a role in vasodilatation, neurotransmitter and immune action, inflammation, angiogenesis (blood vessel growth), smooth muscle relaxation, and apoptosis (programmed cell death).

Nitroglycerin, a century-old medication for angina pectoris has been found to exert its beneficial action on blood vessels via nitric oxide formation. By way of its genesis in the arginine-nitric oxide pathway and its vasodilative action, nitric oxide is implicated in the ongoing modulation of blood pressure, and in erectile function.

In the nervous system, nitric oxide acts as a neurotransmitter. It regulates circadian rhythms, assists in the formation of memory, and influences the release of pituitary hormones.

Nitric oxide is also a bactericide. Cytokine-activated macrophages produce nitric oxide, as one component of defense against bacteria, viruses, and nascent cancer cells. Nitric oxide exerts its antipathogenic functions by disrupting bacterial enzymes, by interfering with bacterial metabolic pathways such as the Krebs cycle, and by disorganizing bacterial genes and mitochondrial function (Snyder 1996).

A critical physiological gas: Ozone

The fact that reactive oxygen species (ROS) are produced by immune system cells during infectious processes has been appreciated for a long time (Babior 2000; Kourie 1998; Valentine 1995). ROS, including the hyroxyl radical, nitric oxide, and hydrogen peroxide, were only thought to be toxic by-products of metabolic redox reactions requiring rapid neutralization by enzyme systems. Ozone had hitherto been seen as a molecule capable of inducing the formation of ROS but certainly not as a molecule specifically produced within the body to fight infection. The crucial role of ozone in the task of staving off invading

microorganisms had not been as fully explained as in the following landmark study.

A greatly under-publicized article with momentous implications (Wentworth 2002, Max 2002) documented that ozone is indeed produced in the body in the context of immune function. Its synthesis is triggered by antigen-antibody reactions, generated by activated neutrophils. At this molecular level, ozone thus becomes a pivotal factor in the neutralization of microorganisms. Additionally, ozone functions as a signaling agent by stimulating production of nuclear factor kappa B, interleukin 6, and tumor necrosis factor a. There is ample evidence for ozone's activation of cytokines (Bocci 2005).

Although the coupling of antibody to antigen – in this discussion, antibody to virus – has been known for its outcome, namely the killing of virions, its fundamental mechanics have largely been a mystery. Recent research sheds light on this vital phenomenon. Cellular elements of the immune system (e.g., neutrophils, macrophages), it is now appreciated, have the capacity to generate singlet oxygen, a single oxygen atom, which in turn reacts with tissue oxygen to produce ozone. Fusing with water, ozone generates hydrogen peroxide. A combination of ozone and hydrogen peroxide, peroxone, is significantly more powerful in its virucidal power than either agent alone.

Ozone, hydrogen peroxide, peroxone, and nitric oxide are powerful oxidants. They interact with the lipids, lipoproteins, proteins, glycoproteins, RNA, and DNA of virions to disrupt their morphology, their functional capacity, and their infectivity.

The detailed intricacies of these reactions are far from clear. Certainly, the fact that gases, at the molecular level, constitute the cornerstone for repelling the relentless challenge of microorganisms and maintaining the harmony of health has opened new conceptual vistas.

Gases, in the 90's, have thus established themselves as a new category of biological modulators, and are in the process of revolutionizing medicine.

Ozone: Physical and physiological properties

The oxygen atom exists in nature in several forms: (1) as a free atomic particle (O), it is highly reactive and unstable (2) Oxygen (O2), its most common and stable form, is colorless as a gas and pale blue as a liquid (3) Ozone (O3), a naturally occurring configuration of three oxygen atoms, has a half-life of about one hour at room temperature, reverting to oxygen. It has a molecular weight of 48, a density one and a half times that of oxygen and contains a large excess of energy in its molecule (O3 é 3/2 O2 + 143 KJ/mole). It has a bond angle of 127º ± 3∞, resonates among several hybrid forms, is distinctly blue as a gas and dark blue as a solid (4) O4 is a very unstable, rare, nonmagnetic pale blue gas which readily breaks down into two molecules of oxygen.

A powerful oxidant, ozone has unique biological properties. Since medicinal ozone is administered by interfacing it with blood, basic research on ozone's biological dynamics have centered upon its effects on blood cellular elements (e.g., erythrocytes, leucocytes, and platelets), and on its serum components (proteins, lipids, lipoproteins, glycolipids, carbohydrates, and electrolytes).

The effects of ozonation on whole blood are extraordinarily complex. The contents of serum, with its multitudes of different proteins, enzymes, immunoglobulins, clotting factors, hormones, vitamins, lipids, carbohydrates, and electrolytes (Dailey 1998), in the face of ozonation, yield a symphony of compounds yet barely inventoried.

Erythrocytes have been extensively studied in relation to ozone administration. Many studies using erythrocyte suspension in physiologic saline (Kourie 1998; Fukunaga 1999) have found hemolysis at relatively low ozone dosages (10 to 30 ug/ml). When ozone is administered in whole blood, however, the dynamics of ozone interaction are altered such that hemolysis is observed at significantly higher doses, implying a buffering action by blood constituents. Moreover, the functionality of erythrocyte enzymes is maintained, suggesting a protective role of antioxidant systems (Cross 1992).

Leucocytes show good resistance to ozone because, unlike viruses, they possess enzymes protecting them from oxidative damage. These enzymes include superoxide dismutase, glutathione, and catalase. A promising area of research centers on cytokine and interferon stimulation in ozone administration and its implication for enhancing immune function (Paulesu 1991; Bocci 2002; Larini 2001).

Ozone: Antipathogenic properties

Recently, there has been renewed interest in the potential of ozone for viral inactivation in vivo. It has long been established that ozone neutralizes viruses in aqueous media and it stands to reason that it would be studied for similar applications in living systems. In vivo ozone applications, however, present far greater challenges. Indeed, the technology of medical ozone administration aims to respect the delicate balance of patient safety on one hand and antimicrobial efficacy on the other.

All viruses are susceptible to ozone's neutralizing action. Viruses, however, differ in their relative susceptibility to destruction by ozone. In one study, poliovirus resistance was 40 times that of coxsackievirus. Relative susceptibility in ascending order was found to be: poliovirus type 2, echovirus type 1, poliovirus type 1, coxsackievirus type B5, echovirus type 5, and coxsackievirus type A9. In pure water, at maximal solubility of ozone and room temperature, echovirus type 29 is inactivated in one minute, poliovirus type 1 in two, type 3 in three, and type 2 in seven minutes (Roy 1982). Analysis of viral components showed damage to polypeptide chains and envelope proteins, which could result in attachment capability compromise, and breakage of the single-stranded RNA, producing replicating dysfunction. Other researchers, in similar experiments, concluded that in ozonation, it is the viral capsid that sustains damage (Riesser 1977).

Lipid-enveloped viruses are sensitive to treatment with ether, organic solvents, and ozone, indicating that disruption or loss of lipids results in impaired or destroyed infectivity. Viruses containing lipid envelopes include the Hepadnaviridae (Hepatitis B) Flaviviridae (Hepatitis C, West Nile virus, yellow fever); *Herpesviridae*, a large family grouping the Simplex, Varicella-Zoster, Cytomegalovirus, and Epstein-Barr viruses; the *Orthomyxoviridae* (Avian influenza); the *Paramyxoviridae* (mumps, measles); the *Coronaviridae* (SARS); the *Rhabdoviridae* (rabies); the Togaviridae (Rubella, encephalitis); the Bunyaviridae (Hantavirus); the Poxviridae (Smallpox); and the *Retroviridae* (HIV), among others. Indeed, once the virion's lipid envelope becomes fragmented, its DNA or RNA core cannot survive.

Viruses that do not have an envelope are called "naked viruses." They are constituted of a nucleic acid core made of DNA or RNA, and a nucleic acid coat, or capsid, made of protein. Some non-enveloped viruses include: *Adenoviridae* (respiratory infections), *Picornaviridae* (poliovirus, coxsackie, echovirus, rhinovirus, hepatitis A), Caliciviridae (hepatitis E, Norwalk gastroenteritis), and *Papillomaviridae*. Ozone, aside from its well-recognized action upon the complex unsaturated lipids of viral envelopes, can also interact with proteins and their constituents, namely amino acids. Indeed, when ozone comes in contact with viral capsid proteins, protein hydroxides and protein hydroperoxides are formed and viral demise ensues.

Viruses, unlike mammalian cells, have no enzymatic protection against oxidative stress.
The enveloped viruses are usually more sensitive to physico-chemical challenges than are naked virions. This has been shown for ozone (Bolton 1982). Although ozone's effects upon unsaturated lipids are one of its best documented biochemical action, ozone is known to interact with proteins, carbohydrates, and nucleic acids. This becomes especially relevant when ozone inactivation of non-enveloped virions is considered.

Ozone: Clinical methodology

Ozone may be utilized for the therapy of a spectrum of clinical conditions (Viebahn 1999, Bocci 2005). Routes of administration are varied and include external, and internal (blood interfacing) methods. In the technique of oxygen/ozone blood administration, an aliquot of blood (50 to 300 ml) is withdrawn from a virally afflicted patient, anticoagulated, interfaced with an ozone/oxygen mixture, and then returned to the patient. This process, called major autohemotherapy (AHT), is repeated serially in a manner consonant with the viral entity under treatment, the clinical course, and the treatment protocol.

Importantly, another more experimental and more intensive technique of ozone administration makes use of the extracorporeal treatment of the entire blood volume using a hollow-fibre oxygenator-ozonizer (Di Paolo 2000; Bocci 2002). This approach is promising because the totality of blood and lymphatic fluids is interfaced with oxygen/ozone mixtures, thus providing integral anti-viral therapy. Research is needed to determine appropriate ozone dosage and treatment duration protocols relative to the viral entity under treatment.

Many studies have reported the safety of ozone administration. As regards efficacy, Wells et al. showed that ozone-treated HIV-spiked Factor VIII maintained its biological capacity and that, concomitantly, an 11-log reduction in detectable virions was achieved. The improvement of liver enzymes in hepatitis C patients after several months of ozone therapy was described (Viebahn 1999; Amato 2000). An 80% hepatitis C viral load reduction in 82 patients using autohemotherapy was reported (Luongo et al., 2000). Reports of many studies in various conditions, albeit in many cases featuring small patient samples and

inadequate controls, may be found in the writings of a number of authors (Bocci 2005; Altman 1995).

Ozone: Possible mechanisms of anti-viral action

The average adult has 5 to 6 liters of blood, accounting for about 7% of body weight. How can any viral load reduction reported via AHT ozone therapy be explained in the face of a technique that treats a relatively small percentage of total blood volume, albeit serially? And how could extracorporeal ozone administration come to the aid of a patient beleaguered by a virulent viral infection?

The viral culling effects of ozone in infected blood may recruit a variety of mechanisms. Research is needed to ascribe relative importance to these, and possibly other mechanisms of ozone's anti-viral action:

1. The denaturation of virions through direct contact with ozone. Ozone, via this mechanism, disrupts viral lipids, lipoproteins, and glycolipids. The presence of numerous double chemical bonds in these molecules makes them vulnerable to the oxidizing action of electron-hungry ozone. By readily donating one of its oxygen atoms, ozone reconfigures the bonds of viral lipid envelopes, fatally disrupting viral architecture. Deprived of an envelope, virions cannot sustain nor replicate themselves.

2. Ozone proper, and the peroxide, hydroxyl radical, and peroxone compounds it creates, may alter the viral structures necessary for attachment to host cells. Peplomers, the glycoproteins protuberances gluing virions to host cell receptors are likely sites of ozone action. Even minimal alteration in peplomer integrity through glycoprotein peroxidation could impair attachment to host cellular membranes foiling viral attachment and penetration.

3. Introduction of ozone into the serum portion of whole blood induces the formation of lipid and protein peroxides. While these peroxides are not toxic to the host in quantities produced by the protocols of ozone therapy, they nevertheless possess oxidizing properties of their own which persist in the bloodstream for several hours. Peroxides created by ozone administration show long-term antiviral effects that may serve to further reduce viral load.

4. The immunological effects of ozone have been documented (Bocci 1992; Paulesu 1991). Cytokines, proteins manufactured by several types of immune system cells, regulate the functions of other cells. Mostly released by leucocytes, cytokines are important in mobilizing immune reactivity. Ozone-induced release of cytokines may constitute an avenue for the reduction of circulating virions via the activation of immune cells.

5. Ozone action on viral particles in infected blood yield several possible outcomes. One outcome is the modification of virions so that they remain structurally grossly intact yet sufficiently dysfunctional as to be nonpathogenic. This attenuation of viral particle functionality through slight modifications of the viral envelope, and possibly the viral genome itself, not only modifies pathogenicity but also allows the host to diversify its immune response. The creation of dysfunctional viruses by ozone may offer unique therapeutic possibilities. In view of the fact that so many mutational variants exist in any one afflicted individual, the creation of an antigenic spectrum of crippled, fragmented, and attenuated virions could provide for a unique host-specific stimulation of the immune system, thus permitting the creation of what may be seen as a host-specific autovaccine.

6. Finally, a very exciting avenue of research suggests that the fundamental virucidal properties of antibodies are predicated upon their ability to catalyse singlet oxygen which, in its reaction with tissue oxygen produces ozone (Marx 2002; Wentworth 2002). A key element in the viral-inactivating capacity of antibodies may thus reside in the formation of ozone integral to antigen-antibody reactions. Exogenously administered ozone may, in this model, amplify the efficacy of antigen-antibody dynamics.

Physiological gases: Implications for research and therapy for influenza

The importance of discovering how physiological gases exert their virucidal action resides in finding methods of enhancing their effects. Therapeutic strategies for increasing the production of gaseous oxidants at the antibody-virion interface could assist in countering a number of viral pathologies, including influenza.

One such approach could aim at activating the immune system's production of singlet oxygen. This could be done in a variety of ways once the metabolic steps for singlet oxygen production are discovered.

Another method makes use of administrating oxidative gases to bodily fluids, the logical choice being blood, as performed in

ozone autohemotherapy and in extracorporeal ozone therapy. A question perennially presents itself with these methods: Do exogenously applied oxidative gases injure the cellular elements in blood such as erythrocytes, leucocytes, and platelets, and its molecular constituents?

Ozone has a decades-long history of practices involving its interfacing with blood for a variety of clinical situations. At appropriate doses and protocols, ozone/oxygen treatment produces no untoward effects in blood. Its patient safety is well established.

There are no references to introducing nitric oxide in the circulation. The reason is that nitric oxide, in even minute amounts, has propensities to induce cellular damage, even though, at the molecular level, it is essential to life.

Avian influenza, like most human viral pathogens, constantly seeks to find breaches in the immunological defenses in its target population, aiming to strike an optimal balance between its dynamic propagation and its lethality.

A universal strategy in mastering viral infections is the culling of pathogenic organisms to the point where they no longer represent a replicative threat. Concomitantly, the actuation of host immune memory serves to repel future infective attempts made by the virus. In the case of influenza, however, the virus continuously creates novel quasispecies, some of which may be so new, and so virulent, that immune memory becomes superfluous. This is the scenario for an influenza pandemic.

Because of its acuteness, avian influenza requires proactive viral culling as soon as the first symptoms arrive. With billions of influenza viral particles generated daily – a reproductive phenomenon commonly observed in the viremic episodes of enveloped viruses – it is apparent that anti-viral therapy needs to be administered in an emergency mode.

As regards an influenza antiviral strategy using physiological gas therapy with ozone/oxygen mixtures, it is suggested that research thrusts explore the following:

The effectiveness of autohemotherapy. Needed are studies on ozone's anti- influenza virions both in vitro and in vivo. This would make possible the determination of optimal inactivation parameters relative to ozone dosage and duration and frequency of blood treatments. Intensive oxygen/ozone treatment of blood aliquots in the acute phases of the influenza infection could reduce morbidity and mortality via any one of the six possible mechanisms of ozone anti-viral action enumerated above.

The effectiveness of extracorporeal treatment of the total blood volume with ozone/oxygen. If ozone administration to blood exerts a therapeutic influence by boosting antigen-antibody oxidative dynamics, it stands to reason that a therapeutic advantage could derive from this method. A limiting factor in internal ozone administration has been the total allowable ozone dosage that, according to some authors, should not exceed 6 milligrams per treatment (Viebahn 1994). The German and Austrian societies for ozone therapy, on the other hand, suggest a dose of 3 mg. Accordingly, respecting these parameters, ozone-mediated enhancement of antigen-antibody reactions in bodily fluids may only require minuscule ozone dosages over long treatment times. Extracorporeal oxygen/ozone treatment could thus provide for the safe and effective culling of virions in the acute viremic phase of influenza, at a time when it is most urgently needed.

Summary and conclusions

Avian influenza is an acute respiratory infection caused by viruses capable of reproducing in various animal reservoirs. Its zoonotic properties promote genetic reassortment in hosts harboring different viral quasispecies. This perennial cross species incubation may be able, as it has in the past, to create highly virulent viral mutants capable of human-to-human propagation.

Described are two physiological gases, produced in vivo, that perform crucial functions in the body's defense against pathogenic microorganisms. Ozone and nitric oxide are central components to the immune system's capacity to mobilize cellular and antibody responses against pathogenic microorganisms. Both gases, most importantly, perform pivotal roles in the core mechanism by which antibodies inactivate noxious viral antigens.

This paper outlines six possible mechanisms by which ozone may exert its antiviral actions. Viruses cannot surmount the oxidative force of the ozone molecule. Consequently, unlike the antiviral agents available today, ozone will show effectiveness across the entire genotype and quasispecie spectrum of influenza.

In conclusion, it is suggested that ozone/oxygen treatment, which has been demonstrated to be innocuous to humans and ani-

mals in contemporary treatment protocols, be granted research consideration for influenza. It may then be found therapeutically useful not only in influenza, but also in future epidemics caused by novel viruses that, unfortunately, are certain to emerge.

The acute phase of avian influenza infection is marked by a rapid progression of symptoms in the context of a fulminant viremia. The emergency culling of the sudden effusion of billions of circulations virions could conceivably recruit the assistance of a physiological gas known to be safe in doses prescribed: Ozone. In summary, two research directions are proposed, each seeking to test this hypothesis, namely:

Determining the effectiveness and optimal administration of emergency ozone autohemotheray in influenza.
Establishing the emergency therapeutic capability of total blood volume viral decontamination via extracorporeal oxygen/ozone administration.

BIBLIOGRAPHY

Ackey D, Walton TE. Liquid-phase study of ozone inactivation of Venezuelan Equine Encephalomyelitis virus. Appl Environ Microbiol 1985; 50: 882-886

Altman N. Oxygen Healing Therapies. Healing Arts Press, VT, 1995

Armstrong. Infectious Diseases, First Ed. Mosby, Philadelphia, 2000

Babior BM. Phagocytes and oxidative stress. Am J Med 2000; 109:33-44

Bocci V. Ozone: A New Medical Drug. Springer, 2005

Bocci V. Oxygen-Ozone Therapy: A Critical Evaluation. Kluwer Academic Publishers, Dordrecht, 2002

Bolton DC, Zee YC, Osebold JW. The biological effects of ozone on representative members of five groups of animal viruses. Environmental Research 1982; 27:476-48

Buckley RD, Hackney JD, Clarck K, Posin C. Ozone and human blood. Archives of Environmental Health 1975; 30:40-43

Cann AJ. Principles of Molecular Virology, Second Edition. Academic Press, New York, 1997

Cardile V, et al. Effects of ozone on some biological activities of cells in vitro. Cell Biology and Toxicology 1995 Feb; 11(1): 11-21

Cech T. RNA as an enzyme. Scientific American 1986 Nov; 255(5): 64-76

Clarke LM, Bromberg K. Human Respiratory Viruses. In Armstrong. Infectious Diseases, First Ed. Mosby, Philadelphia, 2000

Carpendale MT, Freeberg JK. Ozone inactivates HIV at noncytotoxic concentrations. Antiviral Research 1991; 16:281-292

Cross CE, Reznick AZ, Packer L, et al. Oxidative damage to human plasma proteins by ozone. Free Rad Res Commun 1992; 15:347-352

Dailey JF. Blood. Medical Consulting Group, Arlington MA, 1998

Dawson TM, et al. Gases as biological messengers: Nitric oxide and carbon monoxide in the brain. J Neuroscience 1994; 14:5147-5159

Delves PJ, Roitt IM. Encyclopedia of Immunology. Academic Press: San Diego, 1998

Di Paolo, Bocci V, Garosi G, et al. Extracorporeal blood oxygenation and ozonation (EBOO) in man. Preliminary report. Int J Artif Organs 2000; 23:131-141

Dulak J, Jozkowicz A. Carbon monoxide: A "new" gaseous modulator of gene expression. Acta Biochimica Polonica 2003; 50(1): 31-47

Dyas A, Boughton B, Das B. Ozone killing action against bacterial and fungal species: Microbiological testing of a domestic ozone generator. J Clin Pathol (Lond) 1983; 36(10): 1102-1104

Evans AS, Kaslow RA (Eds). Viral Infections in Humans: Epidemiology and Control, Fourth Edition, Plenum, New York, 1997

FukunagaK, Nakazono N, SuzukiT, et al. Mechanism of oxidative damage to fish red blood cells by ozone. IUBMB. Life1999; 48:631-634

Goheen SC, O'Rourke L, Larkin EC. Ozone and the peroxidation of polyunsaturated fatty acids in vivo. Environ Res 1986: 40: 47-57

Gumulka J, Smith L. Ozonation of cholesterol. J Am Chem Soc 1983; 105(7): 1972-1979

Holland J. Replication error, quasispecies populations, and extreme evolution. In: Emerging Viruses. Morse S (Ed). Oxford University Press, New York, 1993

Hurst CJ. Viral Ecology. Academic Press, New York, 2000

Ignarro LJ (Ed). Nitric Oxide: Biology and Pathobiology. Academic Press, 2000

Ishizaki K, Sawadaishi D, Miura K, Shinriki N. Effect of ozone on plasmid DNA of Escherichia coli in situ. Water Res 1987; 21(7): 823-828

Ivanova O, Bogdanov M, Kazantseva V, et al. Ozone inactivation of enteroviruses in sewage. Vopr Virusol 1983; 0(6): 693-698

Kash JC et al. The global host immune response: contribution of HA and NA genes from the 1918 Spanish influenza to viral

pathogenesis. J Virol 2004; 78: 9499-9511

Knipe DM, Howley PM. Fundamental Virology, Fourth Edition. Lippincott Williams & Wilkins, Philadelphia, 2001

Knobler SL, Mack A, Mahmoud A, Lemon SM (Eds). The Threat of Pandemic Influenza. The National Academic Press, Washington, D.C. 2001

Kobasa D et al. Enhanced virulence of influenza A viruses with the haemagglutinin of the 1918 pandemic virus. Nature 2004; 431:703-707

Konrad H. Ozone therapy for viral diseases. In: Proceedings 10th Ozone World Congress 19-21 Mar 1991, Monaco. Zurich: International Ozone Association 1991:75-83

Kourie JI. Interaction of reactive oxygen species with ion transport mechanisms. Am J Physiol 1998; 275:1-24

Langlais B, Rekkow DA, Brink DR. Ozone in Water Treatment: Application and Engineering. CRC Press, 1991

Larini A, Aldinucci C, Bocci V. Ozone as a modulator of the immune system. In: Proceedings of the 15th Ozone World Congress, London, UK, 11th-15th September 2001. Medical Therapy Conference (IOA 2001, Ed.) Speedprint MacMediaLtd. Ealing, London, UK, 1-10

Laskin JD, Laskin DL. Cellular and Molecular Biology of Nitric Oxide. Marcel Dekker, 1999

Lincoln J, Hoyle CH, Burnstock G. Nitric Oxide in Heath and Disease. Cambridge University Press, 1997

Matus V, Nikava A, Prakopava Z, Konyew S. Effect of ozone on survivability of Candida utilis cells. Vyestsi AkadNavuk Bssr Syer Biyal Navuk 1981; 0(3): 49-52

Matus V, Lyskova T, Sergienko I, Kustova A, Grigortsevich T, Konev V. Fungi; growth and sporulation after a single treatment of spores with ozone

Max J. Antibodies kill by producing ozone. Science 15 Nov 2002; 298: 1319

Menzel D. Ozone: An overview of its toxicity in man and animals. Toxicol and Environ Health 1984; 13:183-204

Morse SS (Ed). Emerging Viruses. Oxford University Press, Oxford, 1993

Mudd JB, Leavitt R, Ongun A, McManus T. Reaction of ozone with amino acids and proteins. Atmos Environ 1969; 3:669-682

Olinescu R, Smith TL. Free Radicals in Medicine. Nova Science Publishers, Inc. Huntington, New York, 2002

Paulesu L, Luzzi L, Bocci V. Studies on the biological effects of ozone: Induction of tumor necrosis factor (TNF-alpha) on human leucocytes. Lymphokine Cytokine Research 1991; 5:409-412

Potter C (Ed). Influenza. Amsterdam, Elsevier, 2002

Razumovskii SD, Zaikov GE. Ozone and its reaction with organic compounds. Elsevier, New York, 1984

Rice RG. Century 21 – Pregnant with ozone. Ozone Science and Engineering 2002; 24: 1-15

Riesser V, Perrich J, Silver B, McCammon J. Possible mechanisms of poliovirus inactivation by ozone. In: Forum on Ozone Disinfection. Proceedings of the International Ozone Institute. Syracuse, NY, 1977:186-192

Rilling S. The basic clinical applications of ozone therapy. Ozonachrichten 1985; 4:7-17

Rilling S, Viebahn R. The Use of Ozone in Medicine. Haug, New York, 1987

Roy D, Wong PK, Engelbrecht RS, Chian ES. Mechanism of enteroviral inactivation by ozone. Applied Environmental Microbiology 1981; 41: 728-733

Roy D, Engelbrecht RS, Chian ES: Comparative inactivation of six enteroviruses by ozone. Am Water Works Assoc J 1982; 74(12): 660-664

Shinriki N, Suzuki T, Takama K, et al. Susceptibilities of plasma antioxidants and erythrocyte constituents to low levels of ozone. Haematologia 1998; 29; 229-239

Snyder S. Drugs and the Brain. Scientific American Library Series, 1996

Sunnen GV. Ozone in Medicine. Journal of Advancement in Medicine. 1988 Fall; 1(3): 159-174

Sunnen G. Possible mechanisms of viral inactivation by ozone. Townsend Letter for Doctors. Ap 1994: 336

Thanomsub B. Effects of ozone treatment on cell growth and ultrastructural changes in bacteria. J Gen Appl Microbiol 01 Aug 2002; 48(4): 193-199

Taubenberger JK, Reid AH, Lourens RM, et al. Characterization of the 1918 influenza virus polymerase genes. Nature 2005 Oct; 437(6): 889-893

Valentine GS, Foote CS, Greenberg A, Liebman JF (Eds). Active Oxygen in Biochemistry. Blackie Academic and Professional, London, 1995

Vaughn JM, Chen Y, Linburg K, Morales D. Inactivation of human and simian rotaviruses by ozone. Applied Environmental Microbiology 1987; 48: 2218-2221

Vaughn JM, Chen YS, Novotny JF. Effects of ozone treatment on the infectivity of hepatitis A virus. Can J Microbiol 1990; 36: 557-560

Viebahn R. The Use of Ozone in Medicine. Odrei Publishers, Iffezheim, 1999

Wells KH, Latino J, Gavalchin J, Poiesz BJ. Inactivation of human immunodeficiency virus Type 1 by ozone in vitro. Blood 1991 Oct; 78(7): 1882-1890

Wentworth P, McDunn JE, Wentworth AD, et al., Evidence for antibody-catalysed ozone formation in bacterial killing and inflammation. Science 13 Dec 2002; 298: 2195-2199

Werkmeister H. Subatmospheric 02/03 treatment of therapy-resistant wounds and ulcerations. OzoNachrichten 1985; 4:53-59

White DO, Fenner FJ. Medical Virology, Fourth edition. Academic Press, New York, 1994

Wink DA, Grisham MB, Mitchell JB, Ford PC. Direct and indirect effects of nitric oxide in chemical reactions relevant to biology. Methods Enzymol 1996; 268:12-31

Wolcott J, Zee YC, Osebold J. Exposure to ozone reduces influenza disease severity and alters distribution of influenza viral antigens in murine lungs. Appl Environ Microbiol 1982; 443:723-731

Yu BP. Cellular defenses against damage from reactive oxygen species. Physiological Reviews 1994 Jan; 74(1): 139-162