

Immunological Aspects of Ozone

Summary

Ozone is the second strongest natural oxidant and will therefore have an effect on the human immune system. This is based not only on clinical observation and experience, but also on fundamental aspects dealing with the physiology of activated oxygen phases.

Through use of ozone in water hygiene, it is known that this gas has a microbiocidal effect but also of recent it has often been detected in blood. Furthermore there are strong indications that this microbiocidal action of ozone is applicable in living biological systems as witnessed from the results of the chronic aggressive hepatitis study. The dose-dependent immunological effect of ozone as well as the latest theories and clinical results have been elaborated. Elucidation of the correlation between these investigations and based on the already established ozone treatment resulted in the new auto-homologous immune therapy which uses ozonized autogenic blood and cell fractions.

The results from the auto-homologous immune therapy clearly imply that the immune stimulative or suppressive effect of ozone is in no way only based on the dosage, but much more on the selected blood cell fractions used. This is strongly supported by the effects seen of single fractions on the lymphocyte subpopulations in our patients. Additionally, the importance of thrombocytes will definitely come to meaningful light due to the fact that pure platelet concentrations are not ozone resistant which implicates the value of ozonolysis on this blood fraction. A summary concerning the current status of the effects of ozone on the immune system would not be complete without mentioning its secondary product - the oxygen radicals. The present study was concluded with a short review of the latest research concerning oxygen radicals and their effects on the cellular components of the immune system.

Until recently other than the known bacteriocidal, virucidal and fungicidal effects of ozone in water hygiene, there has been no information regarding the killing effects of ozone gas on microorganisms in biological systems. There are certain indications in the literature of the clinical efficacy of the use of ozone with viral diseases (1,2,3) but the direct virucidal effect of ozone in living systems will be discussed especially if one compares the contamination time for the destruction of viruses in water hygiene (see fig. 6). Also of note is the fact that several publications (1) do not offer any special valid confirmation because the described cases (acute viral hepatitis) belong to a group of diseases characterized by extensive self-division. Carpendale, Freeberg, and Wagner have demonstrated (4,5,6) that ozone in general has the ability to destroy viruses in living biological systems in vitro. The extent to which these results can be applied to clinical application remains unclear. Clinical control studies of the treatment of chronic aggressive hepatitis with hyperbaric ozone therapy (2) seems to suggest that ozone functions by means of an activation of the immunological system. In support of this theory is the temporary increase in transaminases observed throughout the series of treatments which are often seen before final clinical cure was established. Of interest is the intracorporal vaccine process which is accomplished with killed viral material applied to the in vitro ozonized autogenic blood. This, though, does not explain the action of ozone on viruses persisting within liver cells. On the other hand, the exclusive ozonization of blood within the I.V. bottle is no counterargument for the extensive action of ozone within the entire organism, especially after Kief proved the catalytic propagation of a chain reaction of events in the peripheral blood of treated patients (7). These results are even more astonishing due to the fact that according to the law of mass effect this should not occur. Furthermore this offers the possibility of a simple cancer screening.

According to Washüttel, Rokitansky and the guidelines of The German Medical Society for Ozone Therapy, low doses of ozone up to 3000 gamma will result in the absence of side effects and have an immune stimulative effect. This seemingly precise amount of ozone, though, is based on a general biochemical consideration, not upon solid clinical data. In contrast to this recommended dosage Kief found a clear immunological stimulative effect of ozone applied in significantly higher doses to cancer patients (9) (see table 01 and graph 01). In these cases vitamin C was also given to the ozone mixture (1g Cebion). These results were again confirmed in a publication with respect to data on the lymphocyte subpopulation of AIDS and ARC patients after hyperbaric ozone therapy in which in general a similar effect was obtained (10). With respect to the discussion of these results, the question arose as to whether the recovery of the lymphocyte subpopulations resulted from a virucidal effect of ozone or due to a general immunological stimulation. With respect to Wagner's results (6), the virucidal effect of ozone in the doses we use is improbable and can be most likely excluded if one also takes into consideration the results of others (2,3). An explanation to this question was produced by means of a comparative study with these types of patients by application of low doses (5000 gamma) and high doses (16,000) of ozone (earlier data published

using 10,000 gamma must be corrected due to the fact that two tubes of the Biozomat 13-T machine were defect. This false ozone concentration was discovered with a control detection device by the Technomed testing instrumentation. The Biozomat only produced 1/2 of the indicated concentration). The patients receiving low dosages showed a clear recovery of the leucocytes whereby the patients receiving high dosages demonstrated a clear tendency of a decrease in these values (see graphs 02 and 03).

The results mentioned thus far raise many questions: "How does one explain the immune stimulative effect on the white blood cell system?"

"What effect does ozone have on the humoral immune system?"

"What type of interrelationship does ozone elicit between these two?"

Parts of these questions can be explained by the auto-homologous immune therapy (AHIT). We know that ozone splits the disulfide bridges of immunoglobulins. This effect is extensively used. A consequence of AHIT was the possibility of the regulation of T helper and suppressor cells (11) which is made possible by the production process of the autovaccine with respect to its various mixtures. Surprising was the stimulation of the T helper cell population which was produced from the products of ozonized washed erythrocytes. These results are attributed exclusively to the application of ozone and not to AHIT. The erythrocyte fraction in AHIT undergoes various stages during its production and in no way represents more ozonized erythrocytes than those above before readministration into the patient. Nevertheless, both original substances are identical.

For the first time autoimmune diseases can be extensively influenced by means of AHIT. Constituents used for the 01 vaccine for autoimmune diseases consist not only of serum from patients, but also dihydroascorbic acid as well as products released from thrombocytes. For ozone experts, it might be surprising to know that thrombocytes are not ozone resistant and can be destroyed by the gas (experiments with pure platelet concentrations)(12). Thrombocytes play an important role in the human immune system which has not as yet been recognized.

The action of AHIT in autoimmune diseases deals with, among other factors, an anti-antibody reaction process which has been extensively discussed (Erfahrungsheilkunde, 3/88). Thrombocyte fragments represent a part of the autovaccine and the question arises as to which physiobiochemical role they play in the treatment. Autoimmune diseases are the main indications for use of thrombocyte fragments.

In this context it would not be proper to elaborate upon the function of blood platelets only with respect to coagulation. Also the role of platelets in defense mechanisms, as well as in allergic

inflammatory reactions, autoimmune diseases and as a growth factor in wound healing and during embryogenesis should not be underestimated.

The intermediate stage of platelets which are derived from bone marrow megakaryocytes are similar in several respects to polymorphonuclear leucocytes; they are capable of phagocytosis and chemotaxis and stimulate an enormous amount of mediators concerned with cell repair during inflammatory processes.

Findings by Clive Page from the Kings College in London have revealed that blood platelets take part in the defense against bacterial infections in which bacteriocidal substances such as Beta-lysin are released which possibly possess the ability of phagocytosis (14). Clinical findings have shown that cancer patients clearly exhibit an activation of platelets. Animal experiments have shown after injection of tumor cell suspensions in rats and mice exhibiting thrombocytopenia that the amount of lung metastasis are significantly smaller which implicates the participation of platelets in the spread of cancer.

Several other pieces of evidence are also found concerning the important role of platelets in the immune system: Platelets possess IgE and IgG receptors. IgE receptors are components in the defence against parasitic infections. For example, if platelets marked with IgE antibodies against certain developmental stages of shistosoma are infused in rats and mice that have not come into contact with this parasite, then these animals will be protected against future infection. IgE-activated blood platelets release cytotoxic free radicals that can destroy parasites.

Antiallergic agents such as disodiumchromoglycate and nedocromile-sodium are able to inhibit the IgE-dependent release of free radicals without disturbing the aggregation function of the platelets. Therefore the functional influences of antiallergic and antiparasitic effects have nothing to do with coagulation processes. Interestingly, it has been shown that aspirin-induced asthma also stimulates the release of free radicals from blood platelets. It has been known since 1955 that asthmatic attacks are accompanied by thrombocytopenia. In the meantime it has been demonstrated that an increased amount of thrombocytic aggregation exists in the blood during these asthmatic conditions. Substantiating this point is also the fact that an activation and release of thrombocytic factor 4 and beta-thromboglobulin occur in connection with bronchoconstriction. It has furthermore been observed that the life span of platelets in atopic asthmatic patients is greatly decreased which, though, can be rectified by means of administration of glucocorticoids and ketotifens. During an acute asthma attack, the consumption as well as the regenerational ability of platelets are accelerated which, in some atopic asthmatic patients, can lead to a delay in hemostasis. Lungs of patients who have died of an acute asthmatic attack have demonstrated a

great amount of megakaryocytes. Latest findings have disclosed that the lungs participate in the production of platelets.

It is apparent that an interaction exists between blood platelets and eosinophils in irritated asthmatic bronchial tissue. This is supported by the fact that the concentration of platelet-activating factors PAF as well as eosinophilic infiltration of lung tissue can be decreased if the number of platelets is lowered by means of specific antibodies. It is obvious that platelets attract eosinophils through chemotaxis. Furthermore, eosinophilic infiltration can be inhibited by PAF antagonists. One of the first steps in the reaction chain of the thrombocyte-eosinophil interaction is the antigen-induced release of PAF.

Very little is known to date concerning the growth factors produced by platelets. The thrombocyte growth factor PDGF apparently plays a role during wound healing. For example, in vascular walls that are constantly damaged, PDGF probably contributes to the vascular muscular hypertrophy which is a typical characteristic in cardiovascular disease. This phenomenon of smooth muscle hypertrophy is also known to occur in the bronchioles of asthmatic lungs. A consequence of these findings is the newest discovery that blood platelets release growth factors during very early phases of embryogenesis which stimulate the reproductive potential of cells. This function is correlated to that described above concerning the effect on the smooth musculature.

These functional interrelationships explain the therapeutic efficacy of the ozonized autovaccine of AHIT. Ozone experts as well as users of AHIT might be interested in knowing that by means of a special purified form of leucocytes within the vaccine, that the therapeutic reaction of AHIT can be greatly accelerated. Healing of dermatological pathology in neurodermititis patients as well as improvement of ventilation in obstructive lung disease occur within days and in several patients in 24-48 hours after administration of this new vaccine. It is feasible to assume that this new vaccine can be even more perfected if one considers the fact that quick reacting mediators can elicit pathological conditions in seconds or minutes in type 1 immune diseases (i.e. atopic dermititis or asthma).

The autovaccine for AHIT undergoes enzymatic steps incubating for long periods at 37 C and lead to an observation which is most relevant to ozone therapy as well. That is, bacteria are often cultured from the fractions derived from the autogenic blood for the AHIT method in which the genetic potential becomes obvious only after the division into the subcellular fragments. Based on our experience to date, it is reasonable to assume that these bacteria survive intracellularly. The bacteria are not cultured from full blood and almost never from serum.

These bacteria have a disease-specific correlation. For instance the most common bacteria is staphylococcus epidermitis in which according to the latest investigations, occurs in the skin of neurodermitis patients up to $10^7 / \text{cm}^2$ (15).

Streptococcus viridans is often encountered in chronic recessive coronary occlusion and streptococci populations and klebsiella in polyarthritis patients. In cases of atopic dermatitis, candida albicans and klebsiella are known to be etiological factor as difficult-to-define staphyloid bacteria for malignant diseases. The bacteria are automatically processed for the vaccine during the laboratory procedures for AHIT and reintroduction into the patient in dilutions of 1 : 102 is well tolerated. In concentrations of 1 : 102 lightly increased temperatures occasionally occur. These surprising mild reactions are most probably based on the adaptation of the host organism. The intracellular survival of spirochetes and diplococci in the intestinal mucosa of HIV + homosexuals is known (16) and camphylobacter as the pathogen in gastritis is hailed in classical medicine as the discovery of the century (17).

The knowledge concerning the generation of activated oxygen phases was the basis for our fundamental comprehension of the immunological effects of ozone. The significance of activated oxygen phases is especially evident in chronic granulomatosis which is characterized by bacteria (mostly staphylococcus aureus) and fungal infection. The leucocytes of these patients are able to phagocytize normally, but are not capable of destroying the pathogen because they are not able to activate the respiratory burst (Klebanoff,18). Similarly, the incapability to activate the respiratory burst is responsible for the intracellular survival of organisms, for example toxoplasmosis gondii within phagocytes (19). Hydrogen peroxide represents a reaction product of the respiratory burst and is capable of disrupting the function of lymphocytes and to influence the cell membrane of these cells (20). H_2O_2 though, can stimulate the in vitro lymphocyte mitogenesis in which an inhibitory effect is seen in higher concentrations. This immune modulation of lymphocytes is typical for hydrogen peroxide (21). The dose-dependent effect of ozone on the immune system partially stems from the influence of H_2O_2 on the lymphocytes.

The respiratory chain can be activated by means of various immune-reactive substances (immunoglobulins, immune complexes, complement factors and lymphokines). This does not necessarily lead to degranulation, cell migration and an activation of phagocytosis (22,23,24,25). Oxygen radicals (hydrogen peroxide and others) are, as mentioned above, capable of modulating lymphatic activity. Superoxide anions inhibit the activity of T lymphocytes. This explains why superoxide dismutase activation is necessary to maintain normal T lymphocytic function (26, 27). Oxygen radicals inhibit, through as yet unknown factors, the migration of macrophages (MIF). MIF apparently activates the respiratory chain of macrophages through a reversible autotoxic O_2 radical which is produced from the hydrogen peroxid (MPO) halogen system (HOCC). These latter

are most likely responsible for the self-inhibitory effects. It is known that the oxygen radicals which are activated by the respiratory chain of neutrophilic leucocytes inhibit the spontaneous and chemotactic migration of cells (28). Furthermore, there is a connection between arachidonic acid metabolism and the reaction free radical of the immune system whereby the end product of the former and the products of lipogenesis are potent immune modulators. The transient leucopenia (granulocytopenia and monocytopenia) and the respiratory functional disorder in dialysis patients are, according to Craddock and others, based on a mechanism of a connection between these two systems. The plastic membrane that is used in hemodialysis causes a complement activation through an alternative pathway in which C5a activates the respiratory burst and thereby intermediate products are liberated which initiate the generation of superoxide-dependent chemotactic factors (LTB₄, SRSA and others). This subsequently creates the connection to arachidonic acid metabolism. These chemotactic factors play a role in the increased sequester production through granulocytes as well as the adherence of granulocytes on endothelium after injury. Similar mechanisms probably exist in other disease whereby, though, further factors most likely play a role (immune complexes, endotoxins, etc.).

These factors are apparently responsible for the leucopenia in autoimmune diseases (systemic lupus erythematoses, Felty's syndrome, rheumatic arthritis) and for the transient leucopenia of donors with respect to leucaphoresis. Also the symptoms of acute respiratory distress syndrome after trauma, during gram negative sepsis, as well as throughout pancreatitis are based on these mechanisms (29, 30, 31, 33). Circulating immune complexes activate the respiratory burst of granulocytes in autoimmune diseases in which the concentration of oxygen radicals that are continuously being liberated into the extracellular space increases. The arterial activation, based on Craddock's mechanism can take place in vivo and is most likely responsible for the pathological mechanism in autoimmune vasculitis. These theories have, in the meantime, been experimentally confirmed (31,34,35,36).

It is known that in several autoimmune diseases (SLE, rheumatic arthritis, sclerodermia, dermatomyositis, periartthritis nodosa, ulcerative colitis, Crohn's disease) that a chromosomal instability occurs. This process can be seen in only 1/3 of all cases of rheumatic arthritis. A "clastogenic" factor has been found in sclerodermia, SLE and other rheumatic arthritis patients which is the cause of chromosomal fragility in these diseases. The exact composition of this "clastogenic" factor has not as yet been determined but probably is secreted from monocytes. An increased frequency of tumors with a common occurrence of chromosomal fragility and an abnormal immune response is well known. This can be collectively seen in Bloom's and Werner's syndrome, Fanconi's anemia, ataxia telangiectasia, and Down's syndrome (37,38,39). A decreased concentration of superoxide dismutase also occurs in the erythrocytes in Fanconi's

anemia(40). In Down's syndrome the amount of manganese superoxide dismutase in mitochondria is smaller while the amount of copper and zinc superoxide dismutase is higher in the cytoplasm. These differences are due to a genetic defect (CuZnSOD is coded from chromosome 21) (39,41,42,43). A decreased amount of superoxide dismutase in the granulocytes of juvenile rheumatic patients has also been found (44,45) while no significant discrepancy could be ascertained in adults. In contrast, the amount of manganese superoxide in the cytoplasm of granulocytes is lower (46).

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