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Medical Hypotheses xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

The failure of HIV vaccines: A new autovaccine may overcome some problems

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ARTICLE INFO

Article history:
Received 19 November 2008
Accepted 22 December 2008
Available online xxxx

SUMMARY

The hypothesis of an autovaccine for HIV is borne out by: (1) the present lack of a valid vaccine; (2) by a remarkable improvement of the HAART, which however does not prevent HIV mutagenicity and a consequent valid immunological response and (3) the persistence of a hidden infection ready to thrive again. The preparation of the autovaccine is described as well as the administration schedule but only a clinical study will define its validity.

Introduction

In 1983, after the discovery of the human immunodeficiency virus (HIV), it was hoped to have a vaccine within two years. However, after 25 years of intensive and costly efforts by Health Authorities and Pharmaceuticals, there is no vaccine because this unique virus has an incredible genomic heterogeneity, undergoes frequent antigenic variations and is specialized in impairing the immune system leading to the acquired immune deficiency (AIDS). Baltimore [1] has recently voiced his pessimistic view for producing an effective vaccine to be proficiently used in different countries because there are conspicuous HIV variants in Asia and South-America. Fauci [2] has recently cancelled a new trial of a DNA plasmid vaccine and recommended the evaluation of new approaches. This situation is strikingly different from the successful *polio* and *flu* vaccines to name a few serious diseases, which are now under control.

Besides the actual availability of a dozen drugs, there is a constant search for novel therapeutic compounds able to hit HIV at multiple targets such as either blocking the CCR5 molecular receptor or preventing the release of the virus from infected cells. Unfortunately, all the time the virus, under the drug pressure, invents a new protein such as the virion infectivity factor [3] or HIV-1 accessory protein U [4] able to maintain HIV spreading. Although the constantly renewing highly active antiretroviral therapy (HAART) has remarkably improved the prognosis and prolonged survival, the infection remains with the possibility of a relapse owing to the development of a drug resistance and the risk of transmitting the disease to other subjects. Besides the millions of people already dead, one must consider the stressful and dispirited life of some 33 million infected people worldwide. Moreover, the socio-economical burden is a matter of grave concern and even more the fact that only less than one-third of the patients can undergo some form of therapy. The various ill-effects of intensive treatment that often reduce the compliance and the imperfect therapeutic results have also to be kept in mind.

The HIV disease is especially harmful because the progressive destruction of the immune system prevents both the ability of forming specific antibodies and maintain an efficacious killer T cell activity [5,6]. This situation is conceptually dooming the vaccine proposal because, even assuming to produce an effective vaccine, unless we act at a very early stage or we are able to restore the efficiency of the immune system, the vaccine cannot succeed in clearing the virus. Moreover the HIV, by quickly modifying its antigens, is always ready to evade any immunologic response and possibly accelerate the progress of the disease. Having now realized the presence of a high variability of HIV strains in different countries, the possibility of preparing a universal vaccine becomes a nightmare.

May an autovaccine alleviate the problem?

In spite of great therapeutic improvement, most of the 2.2 million people dying each year are untreated patients. Consequently, the possibility of testing a newly conceived, inexpensive autovaccine ought to be evaluated. The idea of an autovaccine is not novel: Bruster et al. [7] reported their results after five years experience in pre- and terminal patients The procedure was well described but was complex and the two vaccine components had to be reinfused into patients. Only seven patients out of 220 reached a survival rate of more than 60 months. No adverse effects were observed. Ngu [8] proposed an autovaccine composed of only viral core antigens and claimed to have determined a seroconversion in a dozen patients. These results have not been confirmed.

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0306-9877/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.mehy.2008.12.034

The preparation of the autovaccine needs to be well standardized, reproducible and without side-effects. The autovaccine needs to be prepared and administered to the donor patient at least every month for at least a year before evaluating its effectiveness. This is a limitation but, the need to keep repeating the autovaccination is imposed by the frequent variations of HIV antigenic constituents, Thus, in principle, each patient, after signing an informed consent describing the methodology and the aim, should be ready to accept the monthly treatment. Another caveat is the great patient's variability because the frequency and intensity of HIV mutagenicity, depends either upon the lack of treatment or the type of HAART combination and compliance. This means that each patient, after the initial surge of viral replication [5], has an extremely variable level ranging between 2000 and several hundred thousands virus copies/ml of plasma with hardly a predictable of HIV diversity in terms of virus mutation and recombination. The suggestion of using an autovaccine is reasonable because it implies a personalization of the treatment and the monthly presentation of newly expressed antigens to the immune system. Only a prolonged and accurate clinical experimentation will be able to define its value.

The methodology with potential advantages and disadvantages

(1) By using a Vacutainer system (Becton Dickinson, CE 0050 Plymouth, UK) only about 2.5 ml blood are collected in a 2.7 ml sterile BD Vacutainer (9NC 0.129 M – Ref: 363079) containing the necessary Na citrate.
(2) After mixing, blood is centrifuged at +4 °C at 1500 g for 15 min to sediment all blood cells.
(3) Under a laminar flow cabinet, 1 ml of anticoagulated plasma containing variable HIV–RNA loads, is collected in a sterile silicon-coated polypropylene disposable 5 ml syringe (A).
(4) After connecting the syringe (A) containing plasma to a sterile, disposable multidirectional stopcock for infusion (Discofix-3 Ref: 4095111, Braun, Melsungen, Germany), by means of a second 5 ml sterile silicon-coated polypropylene syringe (B), a mixture of 4 ml gas composed of oxygen–ozone (95% and 5%, respectively) with an ozone concentration of 80 ± 5 μg/ml is insufflated into the syringe (A). The stopcock is immediately closed and 1 ml of plasma containing an ozone dose of 320 μg is then mixed in a monodirectional oscillator (60 cycles/min) for 15 min. In order to obtain reproducible results, the use of a medical ozone generator with the photometric determination of the ozone concentration, periodically checked against the iodometric titration of ozone [9], is recommended. The 15 min mixing period is sufficient to allow the ozone dose to completely react with the plasma with minimal foaming.
(5) Under sterile conditions, the standard quantity of the adjuvant MF59™ is aspirated into the syringe containing the ozonated plasma. The adjuvant is constituted by squalene and two emulsifiers such as sorbitan monooleate and sorbitan trioleate [10]. The formula is based on a Novartis patent and this adjuvant can be used only under Novartis permission.
(6) About 2 ml of sterile human albumin (for medical use) at 25% concentration (dose: 500 mg) are aspirated into the syringe.
(7) Finally, by quick hand mixing, all the components of the autovaccine are emulsified with the adjuvant and the whole content of the syringe is administered into the donor patient at a subcutaneous site including the lower limbs, every month, for at least one year.

What types of reactions happen between plasma and ozone?

Human plasma contains 60–70 mg/ml proteins, of which 40–45 mg are constituted by albumin, and 3–6 mg/ml lipids. Mixing the plasma with the gas phase allows ozone to dissolve in the water and to react immediately with both the natural hydrophilic antioxidants and with omega-6 acyl groups of polyunsaturated fatty acids (PUFAs) either albumin-bound or present in phospholipids and cholesterol exposed in the viral membrane. In previous studies [11–13] of plasma ozonation performed with a variety of ozone doses, it has been determined that the peroxidation reaction with PUFAs generated hydrogen peroxide and a number of aldehydes among which malondialdehyde and 4-hydroxynonenal. (4-HNE). The selected dose of 320 μg of ozone per ml of plasma is 4-fold higher than the highest therapeutic dose used during ozonetherapy [9]. It is totally exhausted within 15 min mixing and leads to total oxidation of the plasma antioxidants, of plasma proteins as well as viral inactivation mostly due to the peroxidation and breakdown of the lipid envelope. 4-HNE, which represents the bulk of alkenals forms adducts mostly with albumin, which has 11 nucleophilic groups and acts as a detoxifying molecule [14]. Hydrogen peroxide has a half life of about 2 min and is reduced to water by antioxidants and traces of catalase [9]. At the end of the reaction the autovaccine is composed of:

(a) Inactivated plasma proteins, which, after administration will be quickly taken up and catabolized by macrophages [15];
(b) Albumin-4-HNE adducts which can stimulate the synthesis of heme-oxygenase-1 [16], an exceptionally protective enzyme;
(c) The final addition of 500 mg albumin serves to increase the oncotic pressure of the autovaccine. After subcutaneous administration, the increase of the interstitial fluid pressure will enhance the absorption of the immunogens via the lymphatics and this strategy may improve the immune response [17];
(d) Partly broken down and possibly oxidised viral components that, with the help of the adjuvant, may act as immunogens. The actual ozone dose has been selected because while it inactivates the virus, it hardly should oxidize the viral antigens. It would be interesting to evaluate their proteomic profile after ozonation, but this planned research has not yet been funded.

Discussion

Several types of prophylactic as well as therapeutic vaccines have been tested: side effects have been minimal but virological and immunological results have been unsatisfactory. In contrast, after the advent of the HAART and recently by using an even more complex combination of drugs, after 6 months treatment, some 60% of patients have shown an increase of CD4 count and a minimal viral load in the plasma [18–20] although the results show the efficacy of the antiretroviral therapy, the infection persists in anatomical sanctuaries [21,22] and, if the medication is stopped, the viral load tends to increase again within weeks or a few months. Moreover, even during successful treatments, under drug pressure, HIV mutates fairly frequently and new variants may become drug-resistant. This vicious circle could be interrupted if the immune system was able to respond to new antigens and therefore, a personalized autovaccine may be useful. Almost needless to say that the personalized autovaccine is limited to the donor patient, it is somewhat cumbersome to prepare, and it cannot be used in other HIV patients owing to the risky presence of other viruses.

Please cite this article in press as: Bocci V et al. The failure of HIV vaccines: A new autovaccine may overcome some problems. Med Hypotheses (2009), doi:10.1016/j.mehy.2008.12.034

In the hope to stimulate the immune system after an extensive search, only the MF59™ appears viable and acceptable today [23,24]. It remains to be seen if, even in the presence of the adjuvant, the patient's immune system is able to adequately respond to the variable antigenic stimulation. The strategy of increasing the absorption of immunogens after SC administration via lymphatics may be helpful.

HIV is known to be easily inactivated (56 °C for 30 min) but another unanswered question is whether the exposure to ozone is better than heat, or chlorine, or radiation. Far lower ozone doses than the one suggested here have proved to inactivate HIV particularly when the virus is suspended in protein–free physiological solution, while the potent antioxidant capacity of plasma can partly protect it [25,26]. However, it remains unknown whether any of the inactivating agents are able to increase the antigenicity of HIV possibly exposing hidden epitopes. At this stage ozone has been preferred because it is an excellent antiviral agent and because the production of albumin adducts induces heme-oxygenase-1, which may enhance the immune response. On the other hand, although the therapeutic use of ozonated autohemotherapy has appeared to stimulate type-1 cytokines [27] which in comparison to type II, may enhance the activity of the immune system, it has not procured a therapeutic advantage in preterminal HIV patients [28] mostly because *in vivo* the necessarily mild ozone treatment cannot inactivate HIV.

The validity of the autovaccine remains uncertain because if the patient undergoes HAART therapy a minimal viral load may yield a too low level of immunogens. Conversely, an untreated or a drug resistant patient may have a high viral load in conjunction with an unresponsive immune system. *In vivo veritas,* is to say that only a clinical trial can give the final answer. We would be glad to assist anyone interested in performing a trial.

Acknowledgment

The University of Siena PAR Progetti 2006 is acknowledged.

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