Effective Vaccines against and Immunotherapy of the HIV: A preliminary Report.(1)

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ABSTRACT

The human immune deficiency virus, HIV, causes chronic infections because the immune responses to it fail to kill and eliminate the virus from the body. Our first step was to identify the factor that caused this failure. The HIV has an envelope derived, by budding, from the infected host CD4 cells. For this reason, the virus is perceived by the host immune system as partly self. If the host immune responses were to kill and eliminate the virus with its envelope from the body, they would also kill the host CD4 cells as well, causing a fatal auto immune disease. For its own survival therefore, the immune system is obliged to, or is blackmailed into immune responses that do not kill the virus in order not to kill itself with the virus. The viral envelope of the HIV is therefore the factor that explains the failure of the immune system to eliminate the virus from the body. When the viral envelope is destroyed, the viral antigens left, when introduced into an uninfected person as a vaccine, are perceived by his immune system as non-self and provoke immune responses that effectively kill all the antigens of the HIV except its envelope. When the vaccinated person is then infected with the enveloped HIV, the immune responses kill all the viral antigens of the HIV except its envelope. Empty viral envelopes that remain have no biological function and the person would be immune to the HIV. Therefore, an effective vaccine for the HIV consists of all its antigens with its envelope destroyed before hand. Such a vaccine is safe because, without its envelope, the HIV cannot infect other cells. It is effective because the viral envelope, which blocks an effective immune response, has been destroyed. Autologous vaccines were prepared from the infected bloods of HIV infected persons. These autologous vaccines were then cultured in vitro with their respective peripheral leukocytes in a medium free from host antibodies. Such “vaccinated” cultures were then re-injected into the patients concerned. We had evidence that these autologous vaccines induced some viral destruction in the patients concerned. When confirmed, vaccines prepared on these principles could be used in the way reported, as a simple and cheap form of immunotherapy of HIV. These simple vaccines, if properly organized, could provide cheap and effective protection against the HIV/AIDS through out the world.

Key words: viral envelope, vaccine, HIV, immunotherapy


RESUME

Le VIH est perçu par le système immunitaire humain comme partiellement soit-même (partly-self) parce qu’il porte une enveloppe tirée de la membrane de la cellule lymphocytaire CD4. Si le système immunitaire tue le virus, tel qu’il est, il va tuer aussi tous les CD4. Cela consisterait une maladie auto-immune fatal. Pour éviter cette fatalité, le système immunitaire est obligé de ne pas tuer le virus. Le VIH a donc utilisé son enveloppe et le CD4 comme outil pour faire chantage au système immunitaire de la personne infectée. L'enveloppe du VIH est aussi le facteur qui empêche le système immunitaire d'éliminer le virus. Si l'enveloppe virale du VIH est détruite en vitro, et le reste de ses antigènes restent dans un corps auto-infecté, il sera perçu par le système immunitaire comme non-soit-même (non-self) et provoquera une réponse immunitaire appropriée qui éliminera efficacement tous les antigènes du VIH, sauf l'enveloppe. Ce résultat protégera ce corps efficacement contre le VIH. Donc, un vaccin efficace du VIH consistera en tous les antigènes du VIH après avoir détruit son enveloppe. Ce vaccin est sans risque parce que le VIH, sans son enveloppe, ne peut pas infecter d'autres cellules, et efficace parce que l'enveloppe qui bloque la réponse immunitaire efficace a été détruite. L'efficacité de ce type de vaccin a été testée sur des personnes infectées par la culture in vitro dans un milieu neutre, d'un auto-vaccin préparé à partir du sang du malade lui-même avec ses propres cellules immunitaires. Les cellules ainsi "vaccinées" en vitro, ont été injectées souciées aux malades concernées. Nos observations préliminaires ont montré que ces cellules ont induit la destruction virale chez les malades concernées; la destruction virale est le signe que le vaccin efficace. Après la confirmation de ce travail, il y aura un vaccin simple, moins cher et efficace contre le VIH. En plus, on peut utiliser dans la manière proposée des malades sélectionnés comme une forme efficace d'immunothérapie du VIH. L'exploitation rapide de ce vaccin rendra grand service à la lutte contre le VIH/SIDA dans le monde entier.

Mots clés: L'enveloppe virale, vaccin efficace de VIH, immunothérapie

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1. Introduction
An effective vaccine for the HIV has long been awaited by the whole world but especially by Sub-Saharan Africa which is being ravaged by AIDS with over 2/3 of all world cases of HIV or nearly 23 million persons in December 1999 (UNAIDS Report, Dec. 1999). Therefore, any proposals that can contribute to finding an effective vaccine against this plague deserve careful study.

2. An ideal vaccine for the HIV should:
- Provoke in vaccinated persons, immune responses that actually kill the virus in a natural infection following the vaccination.
- In the absence of an established hierarchy for viral antigens, contain all the antigens of the HIV so that the immune responses provoked should act on the whole virus.
- Be simple, cheap to produce and easy to distribute and accessible to Third World countries with the greatest needs but also with the weakest economies.

3. Our search for an effective vaccine for the HIV started by trying to understand why the natural infection with the virus provoked immune responses that fail, in practice, to kill and eliminate the virus from the body. Understanding the structure of the virus and its relationship to the host immune system should provide insights into the factor that caused this failure. Removing the inhibiting factor should provide a basis for provoking effective immune responses that killed the virus, the sine qua non of an effective vaccine for the HIV.

4. The structure of the HIV.
It had been known, since the discovery of the virus (Sinooussi et al 1983) and a study of its fine structure (McKeating and Willey 1989), that the HIV is an enveloped virus and that its envelope is derived, by budding, from the cell wall of an infected CD4 cell (see fig 1) as the virus emerges from the cell. This makes the HIV a 'hybrid' virus with elements that are truly viral and specific to it and therefore foreign to the body, but with an envelope that is of host or human origin and therefore not foreign to the body. The presence of host cell wall as the viral envelope has obvious and important immunological implications for the virus and for the body, but the scientific community has, apparently, overlooked these implications or has not taken them into full account.

5. The immune system and the structure of the HIV.
The ability of the immune system of the body to destroy any given virus will depend on how the immune system 'perceives' the virus in question: whether as completely foreign to that body i.e. non-self or whether as only partly foreign i.e. partly-self because the virus carries on itself some of the body's structure. The immune response to a virus that is perceived as non-self, all things being equal, will be considerably stronger and more effective in killing such a virus than a response to a virus that is perceived as partly-self. The HIV has an envelope of host origin; it is therefore perceived by the host immune system as partly-self and therefore provokes immune responses that are, so to speak, partial and are considerably less effective in killing the virus than would have been the case if the HIV been without an envelope of host origin i.e. had the HIV been completely non-self.

6. The HIV ‘blackmails’ the host immune system.
If the host immune system were somehow to ignore the presence of its own cellular membrane on the viral envelope and were to perceive the HIV as non-self (instead of partly-self), the corresponding immune responses provoked would destroy not only the virus with its envelope but also all the CD4 cells from which the viral envelope was derived. This would constitute a serious, and a rapidly fatal autoimmune disease. If the immune system is to avoid such a fatal outcome and preserve itself, it would “be obliged to” that is, "blackmailed into" producing immune responses that do not destroy the virus in order not to destroy itself with the virus. If, in fact, the immune system has not killed the patient with the virus, it is because of this 'blackmail' exercised by the HIV on the host immune system using the viral envelope and the CD4 cell wall membrane as 'hostage'. The viral envelope is unquestionably the most powerful secret weapon of the HIV with which it frustrates and blocks those immune responses that could effectively kill the virus in the body. It is the viral envelope, more than any other factor, that ensures the survival of the HIV in the body and blackmail is its modus operandi.

It should be pointed out that this blackmail is not total. If it had been total, there would be no immune responses present in infected persons and the virus would then have a free hand and its rapid and undisturbed action in the body would kill the patient in a relatively short time. The relatively long natural history of this infection and the presence of immune responses in patients is evidence that there is partial immune control of the virus but this is greatly hampered by the presence of the envelope.

7. The ideal solution for the body.
If the immune system had a choice in the matter, for its own survival, it would prefer the situation whereby the responses kill only the virus without any damage to the viral envelope or to the CD4 cells. To provoke, in an uninfected person, such ‘ideal’ immune responses that kill only the virus sparing its envelope, one must start with HIV antigens from which the viral envelope has been deliberately destroyed before hand, transforming it into non-self antigens. Non-self antigens, perceived as completely foreign to the body, will provoke immune responses that effectively kill the virus concerned and in an uninfected person, will therefore kill an invading HIV, sparing its envelope and CD4. These will be spared because the envelope was absent, from the start, from the viral antigens used to inoculate or vaccinate the uninfected person. Such antigens should be effective as a vaccine because they provoke responses directed exclusively against the non-self part of
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N. Chuckman and R. Mitsuyasu, 1998
the virus only, with no risk to its envelope. The empty envelope that remains after this has no biological significance.

Therefore all HIV antigens, minus its envelope, constitute an effective vaccine for the HIV! As there is, as yet, no established hierarchy for HIV antigens, it is prudent to have all its true antigens present in the vaccine. (It is significant to note that in HIV infected persons, there are no immune responses to the viral envelope itself because of its host origin. Its presence on the virus is simply to give the virus the status of partly self in relation to the host immune system and so ensure the survival of the virus).

8. Preparing an effective vaccine for the HIV.
To prepare an effective vaccine for the HIV, therefore, one destroys the viral envelope with lipid solvents acting on the lipid part of the phospho-lipo-proteins of the envelope. The solvent is removed and the residue constitutes the vaccine. It is a safe vaccine because without its envelope, the HIV cannot infect other cells. Such a vaccine would thus consist of non-self, non-life and all the non-enveloped HIV antigens only.

A vaccine produced on the above principles, including any further refinements and purification that may be later carried out, will be relatively easy and cheap to produce and, being a non-life vaccine, should not be very demanding on the cold chain for its distribution. Above all, it should be effective because it is based on the correct understanding of the role of the viral envelope in the life and structure of the virus.

9. Pre-testing the vaccine as an autologous vaccine.
It had been proposed that this simple vaccine be considered for the 3-phase trial to which other HIV vaccines are being subjected. Its production in sufficient quantities for such a trial would pose minimal logistic problems. Lacking the scientific 'credibility' to persuade competent organisations, funding bodies, the media and the public to accept such a trial, we were obliged to take the unprecedented step of pre-testing autologous vaccines on the infected persons concerned. The purpose was to show that the vaccine did indeed provoke immune responses that actually killed the HIV. The immune killing of the HIV, whether occurred in normal or in infected persons, is the ultimate sine qua non of an effective vaccine against the virus. Convinced that the scientific basis of this approach was sound, we were quite prepared to accept any criticism that this new and unusual approach might provoke among traditional immunologists!

i). The scientific basis.
The scientific and immunological basis for pre-testing this vaccine on infected persons was given in a previous paper (Nga 1997). It is summarised as follows: circulating in the peripheral blood of all persons, are various groups of immune cells or immunocytes — macrophages, monocytes, lymphocytes including CD4 and CD8 — assigned to deal with the various pathogens - viruses, bacteria, parasites, fungi etc. that invade the body from time to time. In an immune competent person, there will be other groups of immunocytes, fresh from the bone marrow, on 'stand by' as it were, as yet uncommitted to any specific pathogen, but ready to deal with any new pathogen that may invade the body at that moment. That such uncommitted immunocytes exist in HIV infected persons can be deduced from the fact that, at the start of the HIV infection, most other pathogens are held in check. The progressive destruction of the immune system by the HIV reduces, in time, the pool of uncommitted immunocytes available to deal with new pathogens, which, as opportunistic infections, eventually overwhelm the patient. The available uncommitted immunocytes in an immune competent HIV patient would perceive the vaccine as a new pathogen (antigen) as they would any other new pathogen (antigen) and should deal with it as such. The vaccine is new because it is completely non-self whereas the HIV is partly self.

The following is a preliminary report on the outcome of testing autologous vaccines in HIV infected persons most of whom had clinical AIDS.

i) The patients
Table 1 shows a sample of 20 patients seen between January 1996 and June 2000 because they had had CD4 cell counts done (CD4 were first done in Yaoundé in 1996 and few patients could afford this test). Based on the counts, patients' immune status was graded as shown in the table. Clinically, all persons except the first two had symptoms of AIDS of varying degree of severity. There were 10 males and 10 females and their ages varied from 23 to 55 years. We had received ethical clearance from the Ministry of Health and patients were informed of what was being done. There was no formal control group of patients for the study who received placebos instead.

ii) We prepared the autologous vaccine as described in 8 above from the blood of patients concerned. Some vaccines were prepared using either as the solvent and others used chloroform. In 2 patients vaccines prepared with ether and chloroform were used in the same patient on different occasions. (See table I last column for more information). The vaccines were to be tested as autologous vaccines in the very persons providing the viruses for the vaccines. They were used to inoculate or 'vaccinate', in vitro, cultures of peripheral leucocytes of the patients concerned and these cultures were then re-injected into them.

iii) The culture of peripheral leucocytes with autologous vaccine:
20 ml of venous blood was withdrawn from the patient into a syringe with heparin and allowed to stand in an incubator at 37 C for 30-45 minutes in a long sterile glass tubes. The leucocytes rich plasma above the red blood cells sediment was transferred into sterile centrifuge tubes and centrifuged at 2000 rpm for 5 minutes. The supernatant plasma was discarded and the cell deposit resuspended in 5 ml of normal saline. After thorough mixing, the cell mixture was again centrifuged and the supernatant discarded. The procedure was repeated 3 times in all to remove all traces of the pa-
Table 1
PATIENTS RECEIVING AUTO VACCINES CULTURED WITH THEIR LEUCOCYTES

<table>
<thead>
<tr>
<th>NO</th>
<th>NAME</th>
<th>AGE</th>
<th>SEX</th>
<th>DEGREE OF IMMUNE DEPRESSION</th>
<th>PRE-RX DATE</th>
<th>PRE-RX CD4</th>
<th>POST-RX CD4</th>
<th>PRE-RX WT/KG</th>
<th>POST-RX WT/KG</th>
<th>POST-RX DATE</th>
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<td>F</td>
<td>Normal</td>
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<td>760</td>
<td>787</td>
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<td>70</td>
<td>13/11/97</td>
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</tr>
<tr>
<td>2</td>
<td>OE</td>
<td>37</td>
<td>F</td>
<td>Normal</td>
<td>17/11/96</td>
<td>580</td>
<td>691</td>
<td>74</td>
<td>76</td>
<td>8/1/98</td>
<td>30mn Chloroform - centrifuged</td>
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<tr>
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<td>34</td>
<td>M</td>
<td>Severe</td>
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<td>243</td>
<td>74</td>
<td>74</td>
<td>14/9/99</td>
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</tr>
<tr>
<td>4</td>
<td>AJ</td>
<td>32</td>
<td>F</td>
<td>Moderate</td>
<td>23/6/97</td>
<td>338</td>
<td>416</td>
<td>70</td>
<td>77</td>
<td>29/9/97</td>
<td>2mn Ether - evaporated</td>
</tr>
<tr>
<td>5</td>
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<td>42</td>
<td>M</td>
<td>Moderate</td>
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<td>465</td>
<td>66</td>
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<tr>
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<td>37</td>
<td>F</td>
<td>V. Severe</td>
<td>15/4/98</td>
<td>37</td>
<td>865</td>
<td>53</td>
<td>57</td>
<td>5/6/98</td>
<td>2mn Ether + 1.5hr Chloro- evap.</td>
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<tr>
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<td>55</td>
<td>F</td>
<td>Moderate</td>
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<td>324</td>
<td>54</td>
<td>61</td>
<td>10/1/00</td>
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</tr>
<tr>
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<td>Moderate</td>
<td>5/8/98</td>
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<td>488</td>
<td>49</td>
<td>60</td>
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<tr>
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<td>36</td>
<td>F</td>
<td>Severe</td>
<td>11/8/98</td>
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<td>106</td>
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<td>F</td>
<td>V. severe</td>
<td>9/12/98</td>
<td>10</td>
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<td>76</td>
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<td>26/1/00</td>
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<tr>
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<td>29</td>
<td>M</td>
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<td>16/1/99</td>
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<td>29</td>
<td>F</td>
<td>Moderate</td>
<td>17/6/99</td>
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<td>313</td>
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<td>74</td>
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<td>NSN</td>
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<td>Severe</td>
<td>8/10/99</td>
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<td>77</td>
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<td>11/4/00</td>
<td>1hr Chloroform - centrifuged</td>
</tr>
<tr>
<td>16</td>
<td>AM</td>
<td>47</td>
<td>M</td>
<td>Moderate</td>
<td>9/10/99</td>
<td>480</td>
<td>528</td>
<td>_</td>
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<td>29/12/99</td>
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<tr>
<td>17</td>
<td>AN</td>
<td>30</td>
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<td>Moderate</td>
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<td>339</td>
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<td>18</td>
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<tr>
<td>19</td>
<td>AT</td>
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<td>Moderate</td>
<td>24/2/00</td>
<td>276</td>
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<td>87</td>
<td>7/6/00</td>
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<tr>
<td>20</td>
<td>JA</td>
<td>32</td>
<td>M</td>
<td>Severe</td>
<td>7/3/00</td>
<td>88</td>
<td>152</td>
<td>75</td>
<td>78</td>
<td>13/6/00</td>
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GRADING THE IMMUNE DEFICIENCY

Normal - CD4 >= 500 Nb C/ μl  
Moderate - CD4 200 - 499 Nb C/ μl  
Severe - CD4< 200 Nb C/ μl  
Very Severe - CD4 < 100 Nb C/ m l
tient's serum from the cell deposit. (No attempt was made to separate the lymphocytes from the other leukocytes using ficoll) The final cell deposit was re-suspended in 5 ml of a tissue culture medium (RPMI 1640 Gibco Scotland) to which 1% antibiotics (Penicillin, Streptomycin, Neomycin) and 1% L-Glutamine had been added. Human cord serum screened for HIV, hepatitis and syphilis was added to enrich the medium. To this cell mixture, 1ml of 1 in 1000 dilution of the autologous vaccine was added. The leukocytes vaccine culture, LVC, was incubated at 37 °C for 3 to 4 hours. The mixture was then re-injected subcutaneously into the patient concerned. The rest of the autologous vaccine was kept frozen until required 3-4 weeks later when the procedure was repeated 2-3 times in all. This was to reinforce the 'immunisation or vaccination of the patient by exposing his/her peripheral leukocytes in vitro to their autologous vaccines in a medium free from the patient serum. 

iv) Preliminary result.

The leukocytes vaccine culture, LVC, when injected under the skin of the forearm of the person concerned caused slight local pain at the site of injection lasting 2 to 24 hours with some induration in some patients. Seven to 10 days later, most patients felt some slight fever of about 37.5 degrees Cent. Those with low CD4 had fevers that were slightly higher, - about 38 to 39 degrees Cent. The fevers were accompanied by body aches and loss of appetite. Such reactions to the vaccines passed by the third or fourth week.

Table 1 lists the 20 patients included in this study. Attention was focused mainly on the degree of immune depression graded as proposed by Centre Pasteur Cameroon, on the pre-treatment CD4, the post-treatment CD4, pre-treatment weights and post-treatment weights. The parameters of CD4 and weights were singled out because at the time, apart from clinical and haematological parameters which all showed improvements but could not be included in this table, they were the best objective indicators of the response of the patients to their vaccines. There were then no facilities for viral load estimates.

All patients showed clinical improvements - diarrhoea stopped, haematological parameters improved and patients gained weight. All patients showed a rise of the CD4 (compare columns marked PRE-RX CD4 and POST-RX CD4). This rise was slight in the first 2 patients with no objective immune depression, while in most other patients and was quite high in case No. 6 for reasons that we were unable to explain.

10. Discussion.

The scientific community has known for some time that the HIV has an envelope taken from the wall of the infected CD4 as it emerges from the infected cell and that the virus is therefore a kind of 'hybrid' being partly foreign to the body and partly self. It is remarkable that the evident and important immunological implications of this fact have not been taken into account in the various attempts to produce a vaccine against it.

Our explanations above clearly show the central role that the viral envelope plays in the life and survival of the virus in the body by presenting the virus to the host immune system as partly self. The immune responses provoked, although quantitatively abundant, are qualitatively partial and ineffective in completely eliminating the virus. They are partial in the sense that they are directed against both the non-self and the self parts of the virus at the same time, (this is how the virus is perceived) the self part or the viral envelope thwarting, so to speak, the responses which should have been effective against the non-self parts of the HIV. It is evident from the foregoing that the only immune responses that can effectively kill the HIV are those provoked against the non-self part of the virus alone (if the absence of the viral envelope). This is the basis for the proposal to produce an effective vaccine for the HIV simply by destroying the viral envelope of the virus. The viral antigens left after this constitute a safe vaccine because without its envelope the HIV antigens cannot infect other cells and it is effective because the envelope has been removed from the antigens.

As indicated above, we were obliged to show that the vaccine was effective by testing it as auto-vaccines in the HIV infected persons concerned. The preliminary study reported above had several defects of design and reporting. Patients numbers were small, selection was poor (sero-positives only would have been preferred to AIDS patients). The intervals for reporting the CD4 were irregular. Viral load estimates would have been very useful but were not available at the time.

These shortcomings, caused in part, by logistic problems that will be addressed in a follow-up study do not invalidate the results. These show in all cases, improvements in the clinical status of patients as judged by physical and haematological indicator (not recorded) and were accompanied by a rise of CD4 in all cases. In the absence of viral load measurements, these changes strongly suggest that the virus was, indeed, being killed by the immune response to the auto-vaccines in the presence of some immune deficiency.

When confirmed, vaccines prepared on the above basis should have 2 very important applications. First, they can be used in the manner indicated above as a form of immunotherapy for early HIV sero-positives and for patients with functional immune systems. (One of our AIDS patients received immunotherapy in 1994, not included in this study, has remained in good health and viral particles were not detected in his blood in a New York Laboratory in May 2000).

The second and most important application for this vaccine is obviously to provide complete immune protection for non-infected persons. As they are easy and cheap to produce, the vaccines could be produced on a regular or sub-regional basis using the viral types or sub-types prevalent in the region concerned. There should be no need to await the discovery of a single vaccine that covers the whole world. The simultaneous production and use of this simple
vaccine in the various regions could protect uninfected persons through out the world in a relatively short time and so break the further spread of this virus! These are proposals worthy of serious consideration by those who are engaged in the fight against the HIV/AIDS.

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