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Commentary Article

Taming the AIDS Epidemic: a Commentary on Vaccine and Non-Vaccine Approaches to Combatting HIV-1

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Early in the 1980's the AIDS era began, with multiple cases of a peculiar and deadly disease marked by immune deficiency appearing simultaneously around the world. Epidemiologists soon traced the disease to Africa, from where it was spread by a particularly active and well-travelled air steward. The causative agent having been identified as a retrovirus, researchers throughout the world, and especially members of the gay community, who were particularly affected due to certain characteristics of the virus as well as their lifestyle, called out for the production of a vaccine to protect against transmission of this virus [1]. Definition of the requirements for such a vaccine has proven to be an elusive target, however.

The cover of *Science* 10 July 2015 was graced by an artist's rendering of B cells producing antibodies; a change in their colors indicates evolution of broadly neutralizing antibodies with a high affinity for the HIV-1 envelope protein. Three articles in the Journal [2,3,4] describe how scientists have recently engineered immunogens that prime a first step of the antibody maturation pathway and have successfully immunized animals against HIV-1. These scientists are to be commended for their efforts, but it is important also to note that the caption on the cover reads "Toward an HIV Vaccine; Eliciting antibodies by rational design". There have been at least two previous covers of *Science* over the last twenty years that have also touted advances in HIV-1 vaccine research, but always the bottom line is "Toward an HIV vaccine". Always toward, but never yet arriving at the goal.

Development of a protective HIV-1 vaccine for use in humans has proved to be a formidable task. The elements of the rational design in the present studies hinge on the slowly growing appreciation of the fact that the HIV-1 envelope protein presents as a tree trunk-like trimer on the surface of infected cells; that the presentation of the protein depends on a cleavage of the gp160 chains that causes both extensive structural changes and leave the gp120 chain attached noncovalently to the gp41 membrane-inserted portion; that the most antigenic region, the V3 region which projects cleanly and far from the cell surface and is easily accessible to both antibodies and to immune cell surface sensors, is also hypervariable and thus constitutes a decoy target that changes faster than the human immune system can successfully keep up with; that the more stable antigenic regions are nearer the cell surface and are thus accessible with more difficulty and only by antibodies; and that there are several variants of HIV-1 which are differentially sensitive to individuals of different immunotypes.

Thirty years ago, this author was in the forefront of some of these emerging insights. Working first with negative-strand RNA viruses, I was the first to determine the gene sequence of the paramyxovirus (Sendai virus) fusion and attachment proteins [5,6] which along with the previously determined influenza virus surface glycoproteins were good models for the structural determination of the HIV-1 envelope glycoprotein that combines in a single polypeptide chain both the attachment and the fusion elements of the Sendai and influenza virus surface glycoproteins. Then, switching my orientation to HIV-1 in brains and other tissues of young children as well as adults in a Department of Pediatric Neurology at the outset of the AIDS epidemic in the United States, my lab sequenced the immunodominant V3 and the pathogenesis-associated NEF region, both by cloning and by direct sequencing of RNA and DNA extracted from brains and other tissues of these children, which enabled us to derive an appreciation for the extreme degree of variablity of HIV-1 in tissues [7].

Unsurprisingly, we found our brain sequences to be mainly the same as sequences from peripheral compartments of children and adults with HIV-1 infection. However, our unique focus on HIV-1 infected brains also provided us with a unique insight: in the brain, HIV-1 infects astrocytes as well as microglia and transiting lymphocytes and macrophages. And in astrocytes, HIV-1 undergoes a unique sort of reproductive cycle: the NEF region of the viral genome is over-transcribed and over-translated, while transcription and translation of later gene regions such as GAG and ENV are delayed (we called this "restricted infection" [8]). Thus slowly-maturing HIV-1 lurks inside astrocytes, waiting to seed renewed infection of passing immune cells. This also led us to realize why antibodies against the HIV-1 ENV protein were so useless in brain tissue; there was little ENV expressed in brain, and because of the noncovalent attachment of gp120 to gp41, the antigenically dominant epitopes such as V3 tended to be washed away during immunocytochemistry. In vivo, engineered antibodies

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against ENV would have no better success, and worse, if effective antibodies against HIV-1 in astrocytes could be developed and could destroy the infected astrocytes, the result would be disruption of the structure of the brain and its functions - not an appealing prospect.

We (my boss, Leon Epstein MD, now Chief of Neurology at Children's Hospital in Chicago, and I) therefore sent a Letter to the Editor of Science pointing out this distressing situation, and warning that we thought it would be unhelpful, if not downright dangerous, to concentrate overmuch on developing a vaccine against HIV-1. We also pointed out that no effective vaccine had ever been developed against any retrovirus. The Editor of *Science* passed over our Letter in silence. I call this the Blumberg Who-He? Factor. Two years later David Baltimore submitted a very similar Letter to the Editor of Science; his letter was published. Did David Baltimore plagiarize our work? – of course not, since it was never published. He clearly knew of and understood the significance of our work, but even with His name on his Letter, the concept that an AIDS vaccine might not work out as planned disappeared from view.

I must point out here, that this was a period of intense ferment on many fronts in Science in general and on HIV-1 research in particular. Craig Venter was becoming practically the owner of the pages of *Science* with his dramatic efforts at sequencing the human genome, while the HIV-1 NEF gene gained a large measure of publicity when certain HIV-1 infected individuals were found to have resisted the onset of AIDS because of a defect in their NEF genes [9]. This story gained additional weight when studies on SIV-infected monkeys also showed that disruption of the SIV NEF gene destroyed the ability of this virus to cause sickness and death in the monkeys [10]. Meanwhile, the gay lobby had seized at least partial control of NIH funding of research on HIV-1, because AIDS was an acutely painful subject to them. They did many helpful things in the field of AIDS research, among them a revision of the way controls were implemented so as to move the course of research along as rapidly as possible. But they insisted that development of an AIDS vaccine be a priority (a priority which persists even today).

Fortunately for those infected with HIV-1, a number of non-vaccine approaches to a cure for AIDS were successfully worked out. First were the nucleotide reverse transcriptase inhibitors such as AZT and ddI, then the non-nucleotide reverse transcriptase inhibitors, then the GAG-protease inhibitors, then inhibitors of other regulatory proteins such as the integrase, and finally came inhibitors of HIV-1 entry or fusion with target cells. When three (e.g. Atripla® = tenofovir+emtricitabine+efavirenz) or four (e.g. Stribild® = tenofovir+ emtricitabine+ elvitagravir+ cobicistat) of these 5 classes of inhibitors are combined in various ways, they make effective anti-HIV-1 cocktails, now acronymmed as cART (combined anti-retroviral therapy). These cocktails are now able to provide individuals in the developed world a lifespan similar to that of HIV-1-uninfected individuals, but are mostly unavailable and carry prices unaffordable in the third world. So the pressure to produce a vaccine quickly was somewhat relieved, but research on a HIV-1 ENV vaccine still consumes a major slice of AIDS funding.

As always, I chose the contrarian way, and continued to work on the NEF story. It took a long time to work out the functions of NEF in the HIV-1 life cycle, and I am not certain that even now we know the full story [11,12,13,14,15,16]. I had noted that the *nef* gene ends in a UGA termination codon, which allows readthrough of the terminator and insertion of a selenium-containing tRNA (SeCys-tRNA) into an extension protein. I hypothesized that this extended protein would be misfolded and therefore inactive, and that the ability to produce an inactive NEF protein in an HIV-1 infected person might therefore have the same effect as the mutated NEF in the individuals and monkeys who resisted development of AIDS and its simian equivalent.

Two individuals were extremely important and helpful in this line of research. First came papers from Ethan Will Taylor PhD, at the time Professor of Nutrition at the University of Georgia, showing that selenium, a trace metal, was important in the regulation of oxidation-reduction processes and that HIV-1 encoded genes with similar structure and activity to cellular glutathione peroxidase and thioredoxin reductase [17]. Taylor had previously published a marvelous paper showing that novel genes in HIV-1 were expressed by ribosomal frameshifting and termination suppression, and suggested how dietary selenium supplementation could provide a new approach to AIDS chemotherapy [18].

Second was Marianna Baum PhD, at that time Professor of Nutrition at the University of Florida in Miami, who published the important finding that a low level of selenium found in the blood of HIV-1 infected individuals was THE single strongest correlate with progression to AIDS [19]. I submitted NIH RO1 grant applications by myself and with Prof. Taylor, and tried to visit Prof. Baum to discuss her research, since the amount of bio-available

Selenium in the body is strictly controlled by the limited availability of selenium carrier proteins which function like the more familiar iodine carrier proteins, the thyroxins, thus making it difficult to attain high levels of blood selenium. But first the NIH and then the World fought back.

It seems that selenium is a very touchy subject at the NIH, ever since a senior Federal bureaucrat actually lost his job over a misunderstanding of the effects of selenium supplements in agriculture that almost destroyed the wildlife habitat of the San Francisco Bay wetlands area (read Tom Harris' book, Death in the Marsh, Island Press, 1991). Selenium can be toxic when ingested, and its radioactive isotope is extraordinarily difficult to work with in the lab, as it emits both strong beta and gamma radiation. I was able to show that ^{75}Se was indeed incorporated *in vitro* into an extended NEF protein, using cloned *nef* genes from our pediatric HIV-1 isolates (unpublished data), while Dr. Taylor showed frameshift errors in *env* expression due to selenium. Nonetheless, the NIH turned down these RO1 grant applications from myself and Dr. Taylor, then at the University of North Carolina, to further

study the effects of selenium on the *nef* and *env* genes in HIV-1 replication.

Dr. Baum more successfully hypothesized that simply supplying extra selenium as selenium yeast in the diet of individuals with HIV-1 infection would be helpful in limiting the onset of AIDS, and received grants to test this hypothesis in Botswana, Africa. In trying to visit her, I made the mistake of first seeking to visit a publication colleague of hers, Dr. Barry Hurwitz, at the University of Florida in Miami, who apparently doubted the efficacy of selenium supplementation in early trials [20]. A weeklong illness that prevented him from meeting with me plus the smirk on the face of his laboratory manager led me to surmise that Dr. Hurwitz had something to do with a long delay of her plans and the move of Dr. Baum's Professorial appointment and laboratory to Florida International University. Ultimately, Dr. Baum was able to complete her studies in Botswana, which yielded the interesting and important result that dietary selenium supplementation was statistically effective against progression to AIDS in HIV-1 infected adults, but only when given in conjunction with multivitamins [21].

Recently, the development of CRISPR-Cas9 systems [22,23] and "toehold gene switches" [24,25] have offered a real means of manipulating both the selenium carrier protein genes and their promoter and enhancer elements, in both animals and in humans, thus potentially significantly increasing the bio-availability of selenium in the blood and body. Despite its toxicity, if selenium levels can be rapidly and greatly increased as a bolus, and then be allowed to fall to normal levels (like classical cancer chemotherapy, where a toxic dose of methotrexate is given followed by the antidote calcium leucovorin just before it kills the patient), there is a chance that a curative dose can be achieved, in HIV-1 infected peripheral tissues and cells such as lymphocytes and macrophages, and even in microglia and astrocytes within the central nervous system.

One recent non-vaccine approach that offers considerable promise is the targetting of the CCR5 and CXCR4 co-receptor molecues [26,27]; by shielding them with vaccine antibodies or removal of CCR5 from the cell surface; this seems not to hurt the metabolism of the cell. This approach is also being tested in a monkey model [28]. Other, even newer approaches that may have even more promise is the use of stem cells, either from the lucky "elite controllers" who have natural resistance to HIV-1 infection, or engineered to lack CCR5 or other molecues at their surface [29,30,31,32]. This technique is dangerous, because it requires replacement of the patient's immune cells, but is by now a well-worked-out therapy for certain types of cancer such as multiple myeloma. Finally, one group, which includes David Baltimore among its researchers, is using both stem cells and the Cal-1 molecule to block CCR5 at the cell surface [32].

In conclusion, imagine, if you will, a group of shipwrecked sailors from the stricken vessel *Nutrition*, bobbing in the stormy seas of AIDS research. This group includes Leon Epstein, Blumberg Who-He?, Will Taylor and Marianna Baum. Every so often a head appears at the surface, most importantly Dr. Baum's, but all are in danger of permanently sinking from sight in the wake of the unheeding passing liner AIDS vaccine, speeding on its way to port with the cover of Science flying proudly from its masthead. I assert that this liner will never reach the port of a successful HIV-1 vaccine, yet it is an awfully long swim for us shipwrecked Nutritioners. It is my hope that this commentary, for the inaugural issue of BioAIDS Journal, may help throw a life jacket to those who favor a nutritional approach to AIDS therapy, and possibly even help redirect the course of the great liner AIDS vaccine.

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