REPORT

Report of the Conference Protection from HIV: Targeted Intervention Strategies; March 20-25, 2011, Canada

Enrique Iglesias

Center for Genetic Engineering and Biotechnology, CIGB
Ave. 31 e/ 158 y 190, AP 6072, Cubanacán, Playa, La Habana, Cuba
E-mail: enrique.iglesias@cigb.edu.cu

*I*ntroduction

The Protection from HIV: Targeted Intervention Strategies conference was held at the Whistler Conference Center in Canada, from March 20th to 25th, this year. It was part of the meeting season in celebration of the 40th anniversary of the Keystone Symposia. Two hundred and seven researchers from all continents were present at the meeting. Current topics in viral pathogenesis, therapeutics and vaccine candidates were discussed.

The activities started in the afternoon of March 20th, with a pre-meeting workshop. The opening ceremony was in the evening. The key lecture was delivered by Dr. Haase. It was followed by five days of working sessions for lectures where 56 speakers showed their latest results and also four poster sessions with a total of 1 172 presentations.

*P*re-conference workshop

Four conferences were delivered by prominent investigators. Dr. Weiner from University of Pennsylvania, School of Medicine talked about Vaccines. After discussing the result of the RV144 clinical trial, he stressed the importance of developing vaccine candidates better than recombinant Adenovirus type 5 (Ad5). Dr. Shattock's speech (St. George's University of London) was related to microbicides. The most important results in the field of pre-exposed prophylaxis (PEP) using antiretroviral drugs (ARV) were discussed. Because drugs differ in their ability to access the mucosal tissue, oral prophylaxis has worked only when used immediately before the sexual intercourse or a short time after. If the side effects of the same drugs are considered, it seems impractical to distribute ARV for this application. To solve this issue, new devices for intravaginal use have been developed. A ring that allows the slow release of ARV inside the vagina during several weeks might be the solution. It will be tested in a large phase III clinical trial this year. Dr. Shattock thinks that a successful PEP will be obtained well before a partial protective vaccine and this will impact the design of vaccine clinical trial. In that scenario, a control group treated with PEP will be mandatory and that will increase the cost of trials. Afterwards, Dr. Keele (SAIC-Frederick, Inc. at NCI) talked about virus variation and evolution. Using a very powerful PCR technique, he showed that a lot of mutations accumulate in the founder population time after infection because of the pressure of cytotoxic T lymphocytes (CTL) and neutralizing antibodies (Nab). Dr. Weiner asked him about the influence of the innate immunity. He said that no evidences of the pressure of the innate immunity were detected and two possibilities were considered: First, maybe there is not any pressure of the innate immunity and second there may be some activity that does not allow the development of escape mutants. At the end, Dr. Harris' speech (University of Minnesota) was related to host/pathogen interaction. In his talk he discussed the proteomic results published about the viral cycle of HIV-1. Interestingly, only three proteins were common to all studies. In his opinion, it demonstrates the importance to evaluate different human cell lines and tissues to draw definitive conclusions in proteomic studies.

*T*he conference

In his key lecture "Founder events at the portal of entry in mucosal transmission" Dr. Haase (University of Minnesota, USA) reviewed the mechanisms by which the simian immunodeficiency virus (SIV) recruits a population of target cells after vaginal transmission to fuel its local expansion. Using the microarray technology, he showed that during the first week of infection the activation of the innate immunity promotes local inflammation that stimulates viral replication and the recruitment of $CD123^+$ pDC and other APC which in turn disseminate the virus to distal tissues. Then, he hypothesized that inflammation is the key event to allow viral transmission and dissemination. According to this hypothesis any agent capable of avoiding inflammation will reduce viral transmission and dissemination. To test this idea, glycerol monolaureate, the drug registered by the Food and Drug Administration (FDA) to inhibit the septic shock, was selected. In vitro assays using human vaginal tissue showed the ability of this compound to decrease chemokines secretion and other markers of inflammation. Previous experiments published four years ago in monkeys demonstrated the potential of glycerol monolaureate to protect against intravenous SIV infection. Ending the first part of his speech Dr. Ashley said that this compound should be tested in humans. The second part of the lecture was about the $SIV_{mac239}\Delta Nef$ model of vaccination. He showed that after few weeks of vaccination monkeys were not protected against SIV challenge. However, twenty weeks later sterilizing immunity was developed. Although no one has a positive explanation for this, it seems that some maturation of the immune response is needed to achieve protection. At the end of the talk some prospects of correlates of protection were shown. Correlate 1: no local expansion (small founder population), no influx of target cells and low plasma viral load. Correlate 2: Tissue antibodies (Abs) like IgG and IgA secreted from $CD27^+$ $CD138^+$ plasma cells to abolish inflammation. Also, Dr. Johnson (Harvard Medical School, New England Primate Center, USA) working with the same SIVΔNef model and intravaginal challenge

found something similar. Immunization with SIVΔNef decreased the recruitment of target cells in the vagina. He argued that none neutralizing IgG Abs secreted in vagina might bind to the virion particles interfering with the establishment of a founder population and the innate immunity would be able to control systemic viral dissemination.

Dr. Anton (University of California) spoke about the anatomy of the rectum versus the vagina. The rectum has higher epithelial fragility and high concentration of immunocytes which made the tissue highly susceptible to HIV infection. Nevertheless, it also has a high rate of regeneration. Then, he talked about UC-781 microbicide which is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that was already tested for safety in a phase I clinical trial with promising results. In his group they developed an ex vivo model for HIV-1 infection using rectal biopsies. In this model, they obtained that UC-781 is able to avoid infection up to 30 min after HIV exposure. Because of that, it should be use repetitively to achieve protection in the real situation. Fortunately, it was also shown that in vivo the drug does not increase its concentration in plasma after several rectal applications. Compared with the use of oral prevention approach using ARV, the use of rectal microbicides seems to be advantageous because drugs taken orally are detected in rectal mucosa only during 30 min. Many questions are still open to discussion like the influence of trauma, age, etc. Dr. Heneine (CDC) talk was in line with Dr. Anton's opinion regarding the limitation of oral PEP to prevent vaginal or rectal acquisition. Using the Rhesus macaques model with application of a gel with Truvada in vagina, it was shown a decay of the efficacy down to 63% to rectal challenge in three days. This is far better than oral Truvada. At the end of his speech Dr. Heneine said that it would probably be a wise idea to combine RT inhibitors with IN inhibitors because considering the viral cycle, the first one will act during the first two hours of infection meanwhile the second one will still inhibit the virus 10 hours later and this approach might be more effective.

Dr. Lagenaur (Osel, Inc., USA) showed results of the first proof of concept that live microbial prevention approach could be use to reduce heterosexual HIV acquisition. Its group engineered the *Lactobacillus jensenii* to produce cyanovitin-N (LB-CVN) which is a protein from a cyanobacterium that inhibits viral entry. After vaginal colonization with the LB-CVN, they showed 62% reduction in simian-human immunodeficiency virus transmission using the Chinese rhesus macaque model. Additionally, for macaques with breakthrough viral infection a 10-fold reduction in peak viral load was observed. However, the study lacked a control group infected with a non-recombinant Lactobacillus to discard any influence of the bacteria itself.

Dr. Lu (university of Massachusetts) showed that only few sequences of Env are good enough to elicit neutralizing antibodies (NAb). Working with a prime-booster approach using DNA and soluble recombinant proteins, she tested a high number of sequences extracted from a database of primary isolates. She observed an increase in the Nab titers with the doses of naked DNA before the boosting with soluble gp120. All sequences were selected because of their functionality. Dr. Weiner (university of Pennsylvania) talked about a new platform base for electroporation to increase the delivery of naked DNA. He compared this delivery system with rAd5 obtaining better immune responses. He suggested it as the best approach to combine priming with DNA and boosting with Ad5. Up to now this new platform has not been tested in humans. On the other side, Dr. Johnson (NIAID, NIH) studied the causes of the low immunogenicity of Ad28 and Ad35 compared with Ad5. He showed that Ad28 and Ad35 (not Ad5) induce maturation of mDC and pDC and also secretion of IFN-α with other pro-inflammatory cytokines. In mouse IFN-$\alpha^{-/-}$ knock-out model they obtained similar immune response for all Ad. It suggested that IFN-α control on the Ad28 and Ad35 infections is so effective that expression of recombinant genes does not attain the levels required to elicit a good immune response.

In his speech Dr. Michael (Walter Reed Institute) talked about the RV144 clinical trial already finished in Thailand. The immunogenicity studies showed no induction of CD8+ cells but CD4+ cells against the V2 loop of gp120 in 25% of volunteers (it is very rare in natural infection). He explained the unique characteristics of the gp120 (isolate A244) used in the trial. Because this recombinant protein had the gD peptide of HSV as a Tag in the Ct for the purification process, its 3D shape was unique since the V2 loop was better expose than in the natural gp120. Both CD4 T-cell and antibody responses to the V2 loop of HIV-1 envelope are prevalent in vaccinees. Neutralizing antibody responses in vaccinees were low. He also explained that the gp120 sequence inserted in the recombinant ALVAC use in the priming was slightly different to the gp120 used at the booster doses because it exposed an antibody-dependent cell-mediated cytotoxicity (ADCC) site. In comparison, schedules using naked DNA plus Ad5 do not induce any response to the V2 loop. There are new clinical trials underway in Thailand codified as RV305 and RV306 where some participants in RV144 will be re-inoculated to boost the immune response. Another speech delivered by Dr. Tomaras (Duke University) about the trial RV144 reaffirmed that approximately 88% of vaccinees developed ADCC and a high percent of them also had binding Ab (at a level similar to acute infected people) without neutralizing activity. All vaccinees developed IgG against the gD peptide.

Dr. Barnett (Novartis Vaccines & Diagnostic Inc.) started her conference with an overview of the results of all clinical trials using Env immunogens. She concluded that priming with a viral vector and boosting with soluble gp120 is the best approach to induce a protective immunity. A conclusion clearly influenced by the results of RV144. Then, she showed a new approach using alphavirus replicon particles for priming plus gp120 adjuvated in MF-59 in the boosting. To evaluate the approach in monkeys the gp120 of SIV_{mac239} was selected and 100% protection against low dose intrarectal challenge was obtained using mucosal-parenteral coadministration. There are studies underway to select the best Env sequences from R5 isolates to induce Nab based on their binding to broadly neutralizing Ab (bNAb). It is a previous step

before testing the approach in humans. Dr. Barnett also talked about the development of a new immunogen to simulate the virion binding to the CD4 molecule. Her group has produced a mini-CD4 molecule fused to gp120 (gp120-CD4) but results obtained in rabbits and monkeys are disappointed considering the induction of NAbs.

Dr. Mascola (NIAID, NIH) presentation was about the ontogenesis of bNAb VRC01, VRC03 and VRC-PG04. The 3D structure of these Abs fused to gp120 was obtained after crystallization. It is noteworthy that all of them bind to gp120 in a very similar way through the heavy chain; although their primary sequences are quite divergent (up to about 50%). To describe the process of maturation from germinal lines to produce these Abs, they used 454 pyrosequencing technology. Many mutations are required to produce these bNAbs over a long period of time. In the case of VRC01, 93 mutations were described. It suggests that long schedules of vaccination might be needed to develop similar NAbs.

Regarding the pathogenesis of HIV infection there were several interesting aspects in discussion. Dr. Galit (Massachusetts General Hospital) talked about the natural killer (NK) cells activity and showed evidences supporting a differential state of glycosylation of Abs in progressors, long-term non-progressors (LTNP) and elite controllers. He argued that it is the permanent state of inflammation in progressors the cause of such a differential glycosylation. In long-term non-progressors, glycosylation patterns have less fucose and sialic acid which make the Abs more prone to ADCC activity. Consequently, NK cells are more efficient to control replication.

Perhaps Dr. Foley (Massachusetts Institute of Technology) delivered the most spectacular presentation. She showed videos where CTLs from elite controllers were followed for hours destroying HIV-infected CD4+ T lymphocytes. They expend approximately 3 h to completely disengage from the target cells. Although in 2 h the target is already destroyed the CTL still secretes chemokines and cytokines during the next hour. In contrast, CTLs from progressors only expend 10-20 min with their targets. In the videos, CTLs from long-term non-progressors were persistently chasing the targets until their total destruction. In contrast, CTLs from progressors were unable to restrain the target cells from dissociation and to kill them. Avidity seems to be a crucial factor for the effectiveness of CTLs.

Dr. Keele (SAIC-Frederick, Inc., National Cancer Institute) discussed HIV Transmission and Early Evolution in Nonhuman Primates. He explained that transmitted isolates tends to recapitulate the pathogenic events of their parenteral isolates. In this work, they used rhesus macaques monkeys infected with SIVmac251 and SIVsmE660 and they tracked the viral variants in every region of the female reproductive apparatus. They found a limited number of variants with a diverse distribution along the tissues but a single isolate was always the predominant. At the end of the speech he commented that viral isolates are more diverse when transmission is associated with sexually-transmitted diseases. Probably, the infectious dose is also playing a role, because at higher doses diversification increases, but he thinks that to simulate the human situation a low dose model would be more accurate, and in this setting the transmitted isolates will suffer a bottleneck stage where inoculums variation will decrease dramatically.

Dr. Roederer in his talk showed data about a new subpopulation of CD8+ memory cells identified in mice, non-human primates and humans with the capacity to differentiate in T cell 'central memory' (T_{CM}), 'effector memory' (T_{EM}), and 'terminal effector' (T_{TE}) subsets, etc. This subpopulation represents around 2% of CD4+ and CD8+ T cells. They have a naïve phenotype and have been termed 'stem cell memory' (T_{SCM}). Although they are few in numbers, the stem cell memory phenotype has a huge expansion capacity and they can reconstitute immunodeficient hosts. These cells have been localized in lymph nodes (including mesenteric LN) and blood; they are absent in spleen, bone marrow and other lymphoid tissues. This finding brings hope and open new possibilities in the development of therapeutic immunization strategies for the treatment of HIV disease, and also in other chronic viral diseases.

Poster session

The graduate student Shannon Allen (Northwestern University) showed a poster (selected for short oral presentation) with preliminary experiments using cervical mucus to model HIV mobility as a correlate of infection. She found a slower diffusion rate of HIV particles in the mucus when Abs against gp120 or gp41 was exogenously added to the fluids compared with no Ab treatment. A control cervical mucus from an HIV-infected woman totally blocked HIV movement. This study suggests the importance of biding Abs within the female genital tract to prevent viral interaction with target cells thus avoiding HIV infection. It also suggests that induction of biding Abs and not only neutralizing Abs might be a good approach in vaccination to prevent HIV infection.

In her poster, Dr. Aldovini (Harvard University) showed results of efficacy in female Rhesus macaques immunized intramuscularly with two SIV DNA + rMVA nasal vaccine regimens, one combined with plasmids expressing IL-2 and IL-15, the other with GM-CSF, IL-12 and TNF-α plasmids. Cell mediated responses were efficiently elicited without antibody response. Although almost all animals became infected after intravaginal challenge three log reduction in viral load was observed in vaccinated animals. The median survival was 72 weeks for the control group while more than 50% of the vaccinated animals were still disease-free 130 weeks post-challenge, when the trial closed.

Dr. Sung An (University of California) showed promising results with a model of knock out mice for CCR5 using interference RNA. Transduced human stem cells were implanted in mice and they showed efficient down-regulation of CCR5 without any adverse effect observed. When mice were challenged with CCR5 HIV isolates the CD4+ T cells were protected from infection. This result emphasizes the possible application of gene therapy to achieve viral clearance considering the results obtained by German physicians after transplantation of bone marrow cells

from a HLA compatible CCR5$^{-/-}$ donor to a seropositive patient who clear HIV infection later.

Poster presentation by Dr. Dugast (Ragon Institute) demonstrated that Abs from elite controllers have a robust antiviral control in vitro compared to Abs from progressors. These Abs have higher gp120-binding titers and the titers correlate with the ADCC activity but no neutralization. Also, these Abs promote a potent recruitment of NK cells and monocytes. This result suggests that activation of the innate immunity through the induction of Abs with ADCC activity should be reconsidered in vaccination against HIV-1.

Dr. Hanke (University of Oxford) demonstrated several ways to redirect the CD8+ T cell response to subdominant epitopes. His results evidenced that inactivation of dominant epitopes up-ranks the remaining epitopes. Also, the hierarchy can be modulated depending on the viral vaccine vector to express recombinant protein. This work suggests ways to modulate the immune response in vaccination to elicit responses against more conserved subdominant epitopes.

Conclusions

A lot of work related to the virological and immunological events following HIV-1 acute infection after mucosal transmission is ongoing to understand the viral pathogenesis. Scientists are confident that positive results in these areas will drive the research to the development of an effective prophylactic vaccine. It seems that interest in the therapeutic scenario has waned, at least for the moment. There are also intense studies to have an array of microbial vectors ready for the delivery of potential vaccine antigens. These investigations are mainly directed to identify the best routes, schedules of immunization and the immune mechanisms of innate immunity stimulated after vector inoculation. Heterologous prime-boosting approach is also an intense area of investigation in strategies directed to stimulate humoral (neutralizing antibodies, ADCC, blocking antibodies) as well as cellular HIV-specific immune responses (CTL response and CD4+ T cells).

Microbicides are considered the best approach to deal with prevention of HIV infection in the near future. It is possible that new devices for long-term controlled release of antiretroviral drugs in vagina be tested in a large scale trial in the near future. It is expected that success in this field will impact the vaccine research. If spectacular results are obtained in the prevention of transmission, we expect that financial support for prophylactic vaccination will decline at very low levels.

Acknowledgments

The author thanks Mr. Jeff Lehman, Scholarships Coordinator, and The Bill and Melinda Gates foundation for their financial support.