REPORT

Report of the V Conference of IAS on HIV pathogenesis, treatment and prevention of HIV

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Introduction

The Conference of the International AIDS Society (IAS) [1] was held at Cape Town International Convention Center (CTICC) in South Africa, from July 19th to 22th, 2009. This is a country where the prevalence of the human immunodeficiency virus (HIV) reaches an average of 20% of the adult population. The most current topics of AIDS investigations were discussed, also including debates on social aspects. Topics under analysis comprised up to 162 working sessions and 1491 oral presentations and posters.

The congress was focused on therapeutics, but just a few novel presentations were related to vaccines. This reflected the skepticism about the successful development of a vaccine against HIV and the lack of novel strategies since the failure of the Adenovirus 5-based vaccine candidate of Merck in the STEP phase IIb clinical trial. The greatest pharmaceutical companies were also strongly criticized for the unaffordable prices they gave to their antiretroviral therapies (ART) for most of the people needing it.

Concerning therapeutics, the congress contributed to re-settle the criteria to start ART treatment, being proposed a new threshold at earlier stages of infection when the count of CD4+ T cells is approximately of 350 cells/mL. This recommendation has been already adopted in industrialized countries; however, the World Health Organization (WHO) is still cautious to change its current recommendation (< 200 cells/mL) due to the lack of funding for the increase in the number of patients to be treated.

The conference

Now we are going to summarize the most interesting and relevant speeches, according to our knowledge.

In the opening session there were four speakers, among them Dr. Montaner (Canada), President of the IAS, and the Nobel Price of Medicine, Dr. Barré-Sinoussi (France). Dr. Montaner questioned the eight most industrialized countries (G8) for not complying with their promises for funding research and treatments. On the other hand, Dr. Barré-Sinoussi disserted about the existence of viral reservoirs and the need of finding ways to eliminate them, also encouraging participants to attend a European conference to be held on this issue at the end of the year [2].

Plenary sessions began the following morning. Firstly, Dr. Granich (WHO) disserted on the widely known concept about the probability of decreasing the virus transmission by decreasing the viral load, and the increased mortality following a delayed ART start. He also commented on the recent publication in Lancet of a meta-analysis showing results from 18 cohorts [3] evidencing the best time for starting ART as the moment when CD4+ T cell counts are below or equal to 350 rather than 200 cells/mL (the latest proposed by the WHO therapeutic guide). Besides, he also mentioned the fact that tuberculosis is acquired rather easier at lower CD4 counts. With all these background, he presented the novel idea of ART as the only effective method to eradicate AIDS. This idea comes from the real fact that only ART has become an effective way to fight the HIV-1 although it is not completely eliminated, it surely carries the viral load to undetectable levels in blood. On the other hand, it is well known that sexual transmission, the most common route of transmission in Africa, almost disappears at a very low viral load. In this sense, the proposition was to detect all the seropositive people and treat them with ART as a universal method. According to a mathematical model, based on epidemiological data in South Africa, a 95% reduction in the incidence of HIV in 10 years was predicted, accounting for a prevalence of less than 1% by the year 2050. Evidently, this proposition does not take into account the problems with funds, adherence to treatment, human rights or others that significantly limit this proposition. In spite of this considerable limitation, it could still be plausible for some areas in South Africa with a very high incidence of HIV.

In the following dissertation, Dr. Telenti (Switzerland) showed that there are several genetic factors that help to explain the viral load levels reached after ART in about 20% of patients. Moreover, he showed some examples on how the patients can be categorized in *faster*, *extensive* or *lower metabolizers*, a result obtained after studying the metabolic biochemical pathways for drug turnover. They found it is related, at least in part, to genetic variation in cytochrome genes (CYP2A6 and CYP3A4) and other transporters. Then, they achieved an impact in the reduction of ART toxicity (normally leading to altered lipid metabolism, kidney damage, etc.) by adjusting the dose of administered drugs based on the previous findings, and reach an equivalent effectiveness for viral control among all patients in the study. Significantly, they were also able to retrospectively evidence that those patients who showed the highest genetic risk also had the lowest adherence to treatment, and their adherence improved once the dose was adjusted. In our opinion, this result should have a relevant impact on ART, supporting the recommendation for a personalized treatment.

Four works were discussed in the session dedicated to Acute Infection and Correlates of Immune Control. Dr. Keele (USA) in his lecture entitled "Understanding acute infection" studied the viral transmission and characterized the intra-patient viral evolution, evidencing that 75-80% of the mucosal infections (vagina and rec-

1. IAS conference website. 2009 Available from: http://www.ias2009.org/start.aspx

2. Fourth International Workshop on HIV Persistence during Therapy, 2009. Available from: http://www.informedhorizons.com/persistence2009/

3. When to Start Consortium. Timing of initiation of antiretroviral therapy in AIDS-free HIV-1-infected patients: a collaborative analysis of 18 HIV cohort studies. The Lancet 2009;373:1352-63.

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tum) are produced by a single viral isolation. This was evidenced by sequencing more tan 30 viral genomes per patient, in patients infected with different subtypes. They further observed that mutations were accumulating due to selective pressure of the immune response and progressively increasing the viral diversity. He corroborated these results by studying viral transmission and viral evolution in macaques artificially infected with the simian immunodeficiency virus (SIV), with similar findings. This work demonstrated that HIV-1 has to pass through a "bottleneck" that efficiently selects certain viral variants for mucosal infection. Evidently, this does not happen in intravenous drug users who become infected directly through bloodstream with a higher diversity of isolates that do not find physical or chemical barriers of any type. Answering a question, Dr Keele explained that in all the patients, except one, the transmitted isolations were of R5 type.

In the same session, Dr. Haddad (Canada) spoke about "Profiling used the microarray technology acute and chronic HIV-1 infection". His group, using the microarray technology, studied chronic patients in acute phase, and compared them to control patients infected with human cytomegalovirus (hCMV). They found a differential profile of activated or deactivated genes between acute and chronic phases of infection. For example, they found over-expressed genes in the acute phase related to cell proliferation, pro-apoptotic genes and also found a higher activation of effector memory T cells with low expression of PD1. This contrasted to what happened in the chronic phase.

In the Control of HIV-1 by Cellular Immunity session, Dr. Miura (Japan) presented the work entitled "Characterization of HIV-1 in the acute and chronic phases in individuals who subsequently become viremia controllers". In 18 patients he did not find any association between the HLA haplotype and blood viral load levels. However, he found a significant association between the presence of mutant isolates for ART with a decreased replication capacity and the evolution to viremia controller (< 2000 copies viral RNA/mL). They also cloned the *gag-pol* sequence of the isolation to obtain molecular clones, evidencing its lower fitness.

In the section "Future directions in Biomedical Prevention Research", Dr. Haynes, from the USA with the lecture entitled "The B cell response to HIV: what does a successful vaccine need to do", showed evidences on the difficulties to achieve protection only based on neutralizing antibodies. By using the SIV infection macaque model, it was shown that a vaccine immunogen is required to generate an antibody response in a time as short as 1-2 weeks after mucosal infection, to contain virus spread and to avoid the establishment of viral reservoirs. Searching for that immunogen among currently tested candidates and even evaluating prime-boosting strategies, he found that 2 weeks was the shorter time for generating a functional antibody response. He also showed that the SIV could be completely erased within the first week of infection by generating simultaneous humoral and cellular responses, but this is almost impossible to achieve at present.

During the plenary lecture on Tuesday 21st, Dr. Gray (UK) disserted about "Biomedical Prevention Including Microbicides, Vaccines, Circumcision and PrEP". He explained that twenty nine clinical trials had been counted up for the prevention, but only four were positive (three of them based on circumcision) and nine showing clearly negative results. Seven out of eight trials intended to decrease the incidence of sexually transmitted diseases as a measure to reduce HIV-1 transmission, seven were negative, and one led to a doubtful positive result due to a lower incidence in the studied population. In general, it is considered almost null the probability to decrease HIV-1 transmission by decreasing the incidence of sexually transmitted diseases. Concerning microbicides, he commented that four studies were conducted with negative results and another one gave a doubtful positive result. He also explained that microbicides are tested *in vitro*, showing encouraging results, but fail in the field because the seminal plasma and vaginal secretions decrease their efficacy. Therefore, he recommended to develop in *vitro* tests that also consider both factors. On the pre-exposure prophylaxis (PrEP) with ART, he said that the results in animals were really encouraging and that human studies are being carried out at this moment. In the case of vaccine clinical trials he explained that there is a huge deception since two trials testing subunit vaccines based on viral envelope proteins (developed by Vaxgen) and the STEP trial with the Adenovirus 5 vector (Merck) failed, recommending to move toward vaccine candidates aimed at developing mucosal immunity. About circumcision as a preventive method, he explained that it only provides about 57% of protection against HIV-1 infection, but this strategy does not work in the case of *Neisseria gonorrhoeae*, *Trichomonas* sp. and *Chlamydia* sp. infections. Besides, if the viral load is higher than 50 000 copies in man, circumcision will not prevent transmission to women. On the other hand, it was notorious a study in Uganda, where circumcision was ineffective for protecting man from HIV-1 transmission.

In the following speech, Dr. Walker (USA) delivered his lecture entitled "Immune control of HIV-1 replication", demonstrating that the anti-Gag (p24) cellular response correlates to a decreased viral load when involving more than one epitope, what is also related to the presence of the HLA-B*5801 haplotype. This has been observed even by comparing patients with similar CD4 counts. In children, a strong immune response against Gag is not common, and that could help to explain why they evolve so fast, said Dr. Walker. This anti-gag "protective" response is not only limited to the activity of cytotoxic T lymphocytes, but it is also mediated by soluble factors.

At the Viral Fitness symposium, Dr. Hunter (USA) revisited the topic of "Tropism and fitness of newly transmitted strains". He explained that in Gambia, about 20% of couples attending consultation are discordant. He found by studying recent infections that 80 to 90% of the viral diversity observed during the chronic phase comes from a single viral isolate that is generally an R5 isolate (as demonstrated *in vitro*). Besides, he pointed out that genetic polymorphisms on the donor viral isolate are transferred to the receptor and there are also mutations conserved along a chain of transmission. This work is in agreement with the abovementioned idea of Dr. Keel on a mucosal "bottle-

neck" for transmission. Answering questions, Dr. Hunter quoted that such a «bottleneck" disappears when inflammation of the vaginal mucosa is present, and a variety of isolates can be transmitted in those conditions. To demonstrate this he conducted experiments in monkeys. He also said that the strain that is transmitted is commonly under-represented in plasma and it may be adapted to mucosal replication.

The topic of "Determinants of mucosal transmission" was presented by Dr. Dinh (USA). Her group developed an *in vitro* model with human vaginal mucosa explants, demonstrating that HIV-1 penetrates by passive diffusion through intercellular spaces. This observation was also corroborated in *in vivo* macaques. The R5 and X4 tropic isolates penetrate similarly by diffusion, but R5 isolates showed higher penetrability through the endocervix where the tissular layer is coated with carbohydrates. This could explain the very low transmission of X4 isolates through mucosa. Concerning circumcision, she referred that it provoke anatomical changes in the skin recovering the penis, but those changes were not proven to generate differences in viral penetrability compared to the uncircumcised tissue.

Dr. Martin (UK) frankly disagreed with the previous point of view of diffusion as the main route for transmission (infection) of the virus, favoring the relevance of infected-to-receptor cell transmission by a virological synapse. She showed images of nanotubes projected by an infected cell toward another uninfected cell (so-called infectious synapse) for virus transfer. According to that evidence, neutralizing antibodies like 2F5 and B12 are capable of penetrating the nanotube to inhibit the fusion of membranes.

In the session of "Prospects for Eradication: determinants of viral reservoirs", Dr. Peterlin (USA) talked about two new developed drugs that induce the activation of latent proviruses, the Hexamethylene bisacetamide (HMBA) and Suberoylanilide hydroxamic acid (SAHA, recently approved to treat leukemia). Studies with Rhesus macaques have not been conducted yet to initiate trials in humans.

In the session "Hyperimmune activation and HIV", Dr. Dandekar (USA) confirmed that long term ART treatment restore mucosal populations of Th17 cells and decrease blood lipopolysaccharide levels by reestablishing immune control over microbial intestinal flora. Moreover, Dr. Brenchley (USA) presented a series of immunohistochemical evidences obtained in the SIV model in macaques that immune hyperactivation takes place by the passage of intestinal microbes to blood after loosing the Th17 cell population.

Poster session

Such amount of posters presented in this Conference did not have enough room, so some were submitted by electronic billboards that could be viewed through computers installed in the exhibition area. Now we are going to comment on those that we consider the most interesting.

The work entitled "Development of intra- and intersubtype cross-neutralizing antibodies in HIV-1 subtype C infection" by Madiga and co-workers (South Africa) was the most relevant of the two presented at the "Virus-specific humoral immunity" session. They studied the presence of wide spectrum neutralizing antibodies against a panel of isolates from several subtypes (A, B and C) in 18 women after three years seroconversion. They found that about 20% of sera was moderately or widely cross-neutralizing, concluding that this is a rare event. However, they also showed that all these individuals are useful to obtain monoclonal antibodies directed against wide spectrum epitopes. This could lead to more effective immunotherapies.

In the "Virus-specific cellular immunity" session, Mussini and co-workers (Italy) presented the work entitled "Specific anti-Gag response during and after structured treatment interruption". Here they proved that neither the cellular anti-Gag response is affected nor irreversible damage of the immune system is reported in patients subjected to treatment interruptions based on CD4+ T cell counts.

In another poster, results presented by Dr. Eyzaguirre and co-workers (USA) in the section "Immune responses in resistant cohorts: elite controllers and exposed uninfected" were in accordance to what Dr. Miura said in his lecture (see above). They showed a correlation between the levels of defective mutant proviruses and low viral loads in individuals classified as elite controllers; although they could not conclude this as a cause or consequence of their status of infection.

Results shown in chronic patients by Zalar and coworkers (Argentina) in the work entitled "Perforin, granzyme-A and IFN-γ expression in duodenal CD8+ T cells of HIV-1 chronically infected patients", at the "Mucosal immunity/defenses: responses and dysfunctions" session, were very interesting. They observed that CD8+ T cells at the duodenal region are dysfunctional (low perforin, granzyme A and IFN-γ expression), probably explaining its inability to contain the virus and to eliminate viral GALT reservoirs.

Another study confirmed the idea of HIV-1 subtypes with differential pathogenic behavior, as presented by Dr. Kiwanuka and co-workers (Uganda-USA) on their work entitled "HIV-1 viral subtype differences in the rate of CD4+ T cell decline among HIV seroincident antiretroviral naïve persons in Rakai district, Uganda", at the "Viral determinants of HIV pathogenesis" session. They showed that patients infected by subtype D isolates had low CD4+ T lymphocyte counts, and consequently, they progress to AIDS faster than in patients infected by subtype A isolates.

Another research group from Brazil studied polymorphisms of the α4 chain of α4β7 integrin in New World primates. It is known that α4β7 integrin acts as homing receptor for lymphocytes in the gastrointestinal mucosa and this molecule has been also described as a receptor for HIV-1 in GALT. This study was presented at the "HIV-1: Attachment, receptors and coreceptors, penetration and tropism" session, showing that there is a wide polymorphism that changes the surface charge of this molecule, an effect presumed as significantly affecting its interaction with the gp120 protein and what may account for the inability of HIV-1 to infect these animals.The study of Dr. Waters and co-workers (UK), entitled "The evolution of co-receptor tropism in patients interrupting suppressive HAART" was also presented in this session. They

observed that those patients under ART that suppress viral load until undetectable levels retain the same tropism of the pre-existing virus once after interrupting ART (due to adverse events) and switch to new drugs.

Co-infection of HIV-1 together with other viruses that share the same transmission route (like HBV) was studied in the work entitled "Prevalence of hepatotropic viruses HBV + HCV + HDV in HIV infected clients from Northern Nigeria" by Dr. James and co-workers (Nigeria). They carried out a retrospective study in 200 serum samples and found infection markers of HBV in 33% and 66% of samples corresponding to HIV positive man and women, respectively. This was representative of complexities faced in the African pandemic. As previously known, HBV co-infection affects adherence to ART.

In the work entitled "The prevalence of drug-resistant HIV-1 in antiretroviral-naive adults at a treatment center in Uganda" by Kityo and co-workers (Uganda-The Netherlands), the authors showed there were isolates resistant to at least one first line ART drug in 8.1% of individuals in a cohort of 187 patients in phase III/IV of infection according to WHO classifications (mean CD4 counts of 131 cells/mL). This result is overwhelming, because it means that extensive treatments using generic drugs in populations affected would be ineffective in the future. In this sense, the work entitled "Factors associated with increasing HIV-1 resistance to antiretroviral therapy in an urban cohort in Kampala, Uganda" by Sendagire and co-workers (Uganda-United Kingdom-USA) complemented this information. As the authors of the previous study, they showed the presence of mutants resistant to ART is associated to low CD4+ T lymphocyte counts and high viral loads. This means that recommendations of treating at CD4 counts below or equal to 350 cells/mL are the most adequate, as previously considered in this report. Another side on this topic was presented by Lurie and co-workers (USA-Switzerland) in their work "The impact of antiretroviral therapy on the basic reproductive number, R0" at the "When to start therapy" session. Using a mathematical model they evidenced that the number of new infections produce by a seropositive (R0) is 6.9 in an untreated population. This number decreases to 3.8 when the seropositive is treated when CD4 counts reach or are below 200 cells/mL (AIDS), as recommended by WHO, but farther decreasing down to 2.8 when treated at CD4+ counts equal or below 350 cells/mL (CDC criteria), this means a reduction in 74%.

The session "Therapeutic vaccine and immune based therapy trials" was underrepresented by only two works. One of them, by Routy and co-workers (Canada-USA) and entitled "Assessing risk of a 12-week antiretroviral therapy discontinuation as a read out of viral control in immune-based therapy (AGS-004-001 study)", showed that ART interruption during studies intended to evaluate the antiviral effects of immunotherapies (*e.g.*, vaccines) is a pretty safe procedure for patients for at least 16 weeks when CD4 counts are above 350 cells/mL, and even when such an immunotherapy has no effect on viral load. Undoubtedly, this type of procedure has to be carried out under strict monitoring viral loads and CD4 counts in patients, to reintroduce ART if necessary.

Finally, in the category of "Novel vaccine design" only six works were presented, two of them from the CIGB (Cuba). Briedge and co-workers (UK) presented the work entitled "Complex poxvirus recombinants as HIV vaccine candidates", concluding that Poxviruses does not seem to be effective on generating immunity against HIV-1. Dr. Uzhachenko (Russia) presented a comparative study of immunization routes by using a vaccine candidate composed of a naked DNA and a recombinant protein (with gp120 and Gag fragments). Their work entitled "Immune response induced by combined HIV-1 vaccine administered by different routes" concluded that the intradermal route was the most effective for inducing cellular and antibody responses simultaneously. Iglesias and co-workers (CIGB, Cuba) presented two posters "No significant differences between two mucosal-parenteral co-administration schedules of a multiantigenic formulation against HIV-1" and "Increased immunogenicity and cross-reactivity induced by conjugation of V3 based multiple antigen peptides to carrier proteins". In the first poster the authors concluded that the schedule of nasal (IN)-subcutaneous (SC) co-administration, SC + IN or 2IN & 2SC, of an HIV multiantigenic formulation has no impact on the immune response. The multiantigenic formulation was based on a recombinant multiantigenic protein for HIV-1 (CR3), the surface (HBsAg) and the nucleocapsid (HBcAg) antigens of HBV. Consequently, any of these schedules might be considered to be tested in humans in the future. In the other work, they showed that it is possible to induce a highly cross-reactive humoral immune response against V3 peptides from several HIV-1 strains after parenteral administration of formulations containing a sequence of the V3 loop synthesized as multiantigenic peptides (MAPs) and conjugated to HBsAg.

Conclusions

This Conference was attended by many researchers from poor countries affected by the pandemic. The level of discussions on antiviral therapies was the highest among the topics presented, and important consensus were achieved in therapeutic guidelines.

Unfortunately, results from vaccine studies of high impact were not presented at this Conference. Most of the leading researchers in this field are currently focused on basic research related to mechanisms correlating with protection in individuals who progress slowly or naturally viremia controllers. We expect that these studies will effectively bring relevant results for the development of effective immunotherapies and vaccines.

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