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The Publication on AIDS Vaccine Research

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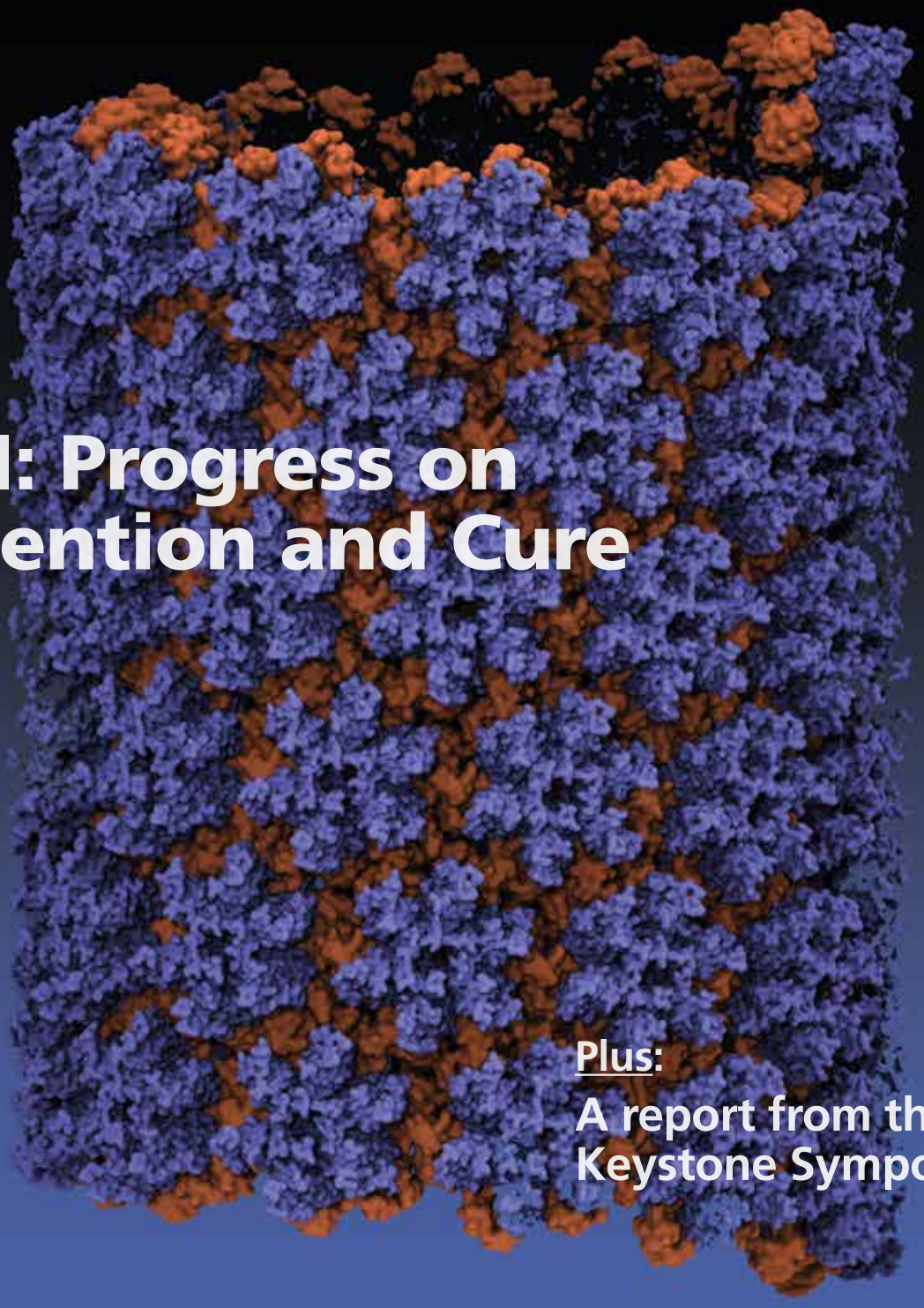

CROI: Progress on Prevention and Cure

Plus:

A report from the Keystone Symposium

EDITOR'S LETTER

It's déjà vu all over again, as American baseball star Yogi Berra once said. In 2012, I left my post as managing editor of this publication to have a baby. And although I won't regale you with stories of my incredibly wonderful daughter, let's just say I'm completely smitten! Now, after a two-year hiatus, I am temporarily back at *IAVI Report* as a contributing editor.

During my time away, the publication was in the very capable hands of Unmesh Kher, who has now taken his considerable writing and editing skills to another organization. Other changes have also occurred. After seven years as a senior science writer, Andreas von Bubnoff also departed to take on other exciting new projects.

While *IAVI Report* undergoes a bit of a changing of the guard, we've put together a great issue with a little, or actually a lot of help from our friends.

The first issue of the year is always dominated by coverage from two of the biggest conferences of the year—the Conference on Retroviruses and Opportunistic Infections (CROI) and the Keystone Symposium on HIV vaccines. Richard Jefferys of the Treatment Action Group, who is not a stranger to anyone who reads about HIV vaccine research, has contributed extensive coverage on the substantial progress on HIV prevention and cure research that was reported at CROI in March (see page 4), and Yegor Voronin of the Global HIV Vaccine Enterprise kindly agreed to write an update from the HIV Vaccines: Adaptive Immunity and Beyond symposium, also held in March (page 12). Together these articles provide an excellent round up of the current state of HIV prevention and cure efforts.

Also in this issue, we pay tribute to Reinhard Kurth, a renowned German retrovirologist and AIDS vaccine researcher, who passed away in February (page 18).

There is also a short article examining the alarming increase in rates of HIV infection occurring in the Middle East and North Africa (page 16), and a news story detailing the passage of new anti-homosexuality legislation in Uganda (page 19), the latest in a string of countries to pass laws criminalizing homosexuality. In a future issue, *IAVI Report* will investigate how these laws are affecting the work of organizations doing or funding research in these countries.

While the contributors may have changed, I trust you will find this issue as informative and wide-ranging as ever, and we can all look forward to the new *IAVI Report* team and their continuing unparalleled coverage of HIV vaccine research.

– KRISTEN JILL KRESGE

The International AIDS Vaccine Initiative (IAVI) is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. Founded in 1996, IAVI works with partners in 25 countries to research, design and develop AIDS vaccine candidates. In addition, IAVI conducts policy analyses and serves as an advocate for the AIDS vaccine field. IAVI supports a comprehensive approach to addressing HIV and AIDS that balances the expansion and strengthening of existing HIV-prevention and treatment programs with targeted investments in the design and development of new tools to prevent HIV. IAVI is dedicated to ensuring that a future AIDS vaccine will be available and accessible to all who need it. IAVI relies on the generous donations from governments, private individuals, corporations and foundations to carry out its mission. For more information, see www.iavi.org.

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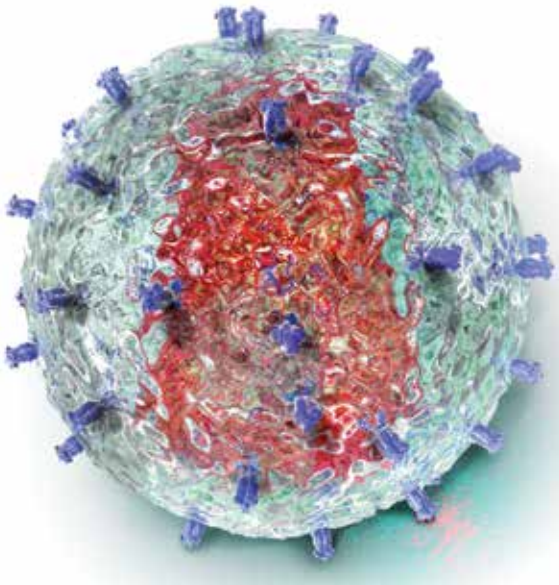

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[ON THE COVER]

The HIV-1 capsid protein associates *in vitro* and *in vivo* into large assemblies of varying shapes. In order to study capsid interactions with drugs or host proteins, atomic-level structures are necessary. In a collaborative effort between experimental researchers at the University of Pittsburgh and Vanderbilt University, as well as computational scientists at the University of Illinois, such detailed structures have been determined. The figure displays a tubular assembly of the HIV-1 capsid protein, comprising 13 million atoms. The N-terminal domains on the outer surface of the tube are depicted in blue and the C-terminal domains on the inside in orange.

Image courtesy of Theoretical and Computational Biophysics Group, Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana Champaign.

Progress on *PREVENTION AND CURE*

The 21st edition of the Conference on Retroviruses and Opportunistic Infections thawed the chilly climes with encouraging news on several fronts

By Richard Jefferys

The headlines from last year's Conference on Retroviruses and Opportunistic Infections (CROI) were dominated by the report of an infant from Mississippi who may have been cured of HIV infection as a result of receiving antiretroviral therapy (ART) soon after birth (see *A Toddler Stole the Show*, *IAVI Report*, Spring 2013). This year, when experts from all avenues of HIV research gathered in Boston March 3-6, there was an echo of this media attention when details on another possibly cured baby were presented, along with an update revealing the child in Mississippi remains off ART with no sign of a return of HIV replication.

While this news stoked enthusiasm over HIV cure research, the field also received sobering news about two individuals in Boston who had experienced a prolonged absence of detectable virus after receiving stem cell transplants for concomitant cancers; HIV remained undetectable for an extended period after ART interruption, but ultimately rebounded to high levels requiring treatment reinitiation.

In the prevention realm, three studies of long-acting antiretrovirals (ARVs) offered hope of more user-friendly pre-exposure prophylaxis (PrEP) options, and additional compelling evidence emerged that for HIV-infected individuals, suppression of viral load by ART massively reduces, or even obliterates, the risk of HIV transmission. Vaccines contributed only quietly to the busy symphony of science this year, primarily through the incremental advances reported in the accelerating quest to induce broadly neutralizing antibodies (bNAbs) against HIV.

Baby makes two?

Following on the apparent success of curing an HIV-infected baby in Mississippi with early initiation of ART, Deborah Persaud, associate professor of pediatrics at Johns Hopkins University School of Medicine, presented the case of a second baby who for now remains on ART, but has no detectable virus.

In this case the baby's mother had relatively advanced HIV infection at the time of delivery, with a viral load of 138,811 copies and a $CD4^+$ T-cell count of 70—she had been prescribed ART but had not adhered to the regimen. Although ART was administered during labor, the baby was ascertained to be HIV infected based on a positive HIV DNA test at four hours of life, with a viral load level of 217 copies/ml of blood at 36 hours, and another viral load measurement of 32 copies in a cerebrospinal fluid sample drawn at day six as part of a work up for sepsis.

Based on knowledge of the Mississippi baby, infectious disease specialist Audra Deveikis at Miller Children's Hospital in Los Angeles County

instituted a treatment regimen including the ARVs AZT, 3TC, and nevirapine at four hours after birth, which was supplemented with lopinavir two weeks later. Nevirapine was discontinued after 3.4 months, and the infant is now nine months of age and remains on AZT/3TC/lopinavir. Persaud, who was at the center of the media maelstrom generated by the first baby reported on last year at CROI, took pains to emphasize that ART has not yet been interrupted in this case.

However, the possibility of a cure is suggested by the results of virological analyses. The baby's viral load became persistently undetectable (<20 copies/ml blood) after 11 days of follow up, and multiple subsequent tests for HIV DNA and replication-competent virus were all negative. Persaud noted that the viral outgrowth assay used to measure replication-competent virus did suggest the presence of low levels of integrated HIV proviruses that did not replicate after stimulation (described as "non-induced proviruses") at one month of age, but not afterward. The technique of digital droplet polymerase chain reaction (PCR) for HIV DNA (see *In Pursuit of a Cure*, *IAVI Report*, Jan.-Feb. 2012) showed levels below 1.6 copies per million peripheral blood mononuclear cells (PBMCs) at the most recent time point. The infant now tests HIV antibody negative. Doctors are considering interrupting treatment if HIV remains undetectable at two years of age.

During the same presentation, Persaud provided an update on the child in Mississippi, who is now 41 months old and has been off ART for close to two years. HIV RNA continues to be undetectable by extremely sensitive viral load tests capable of measuring as little as one to two copies of virus per ml of blood, and no replication-competent virus can be found. However, HIV DNA has been consistently detectable at low levels that border on the detection limits of the assay, averaging around four copies per million PBMCs. To assess whether cells transferred from the mother might be the source of the HIV DNA, samples were evaluated for the presence of maternal alleles. One allele could be detected at an extremely low frequency (0.001%) at 24 months, but not at 40 months of age. Immunological analyses have documented no HIV-specific antibody or $CD8^+$ T-cell responses. Persaud characterized the outcome not as a cure but rather as ongoing "remission" of HIV infection.

Based on the cases of these two babies, the International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT) is planning to launch a study of very early treatment in babies born to mothers who have not received adequate prevention against mother-to-child HIV transmission.

Diminishing reservoir with early treatment

In the vast majority of cases, where HIV is not cleared so dramatically, early treatment may still reduce the long-lived reservoirs of HIV that form quickly after infection, and typically reignite viral replication if treatment is interrupted. In a separate talk in the session on pediatrics, Persaud described results from a cohort study of 144 children with a median age of 14.3 years who were divided into three groups based on when viral load suppression was achieved—14 within one year after birth, 53 within 1-5 years, and 77 after five years. The amount of HIV proviral DNA detected in each group was starkly different: 4.2 copies, 19.4 copies, and 70.7 copies per million PBMCs, respectively. Similarly, earlier suppression of virus was associated with a significantly increased likelihood of indeterminate or negative results on HIV antibody tests—proportions were 86%, 19%, and 3%, respectively. Persaud emphasized that the relatively limited size of the reservoir in early treated children and adolescents makes them good candidates for inclusion in cure research studies because the number of latently HIV-infected cells that need to be cleared is likely very small.

In addition to the excitement generated by the reports of the now possibly two babies cured of HIV infection because of extremely early initiation of ART, a poster presentation about a possible adult equivalent also drew much attention. The case report by Hiroyu Hatano, assistant professor of medicine at the University of California-San Francisco School of Medicine, involved a man who was recruited into a PrEP demonstration project in San Francisco and acquired HIV infection during the 13-day interval between his last screening visit and the initiation of the ARV Truvada for PrEP.

A baseline sample taken on the day he started Truvada showed a viral load of 220 copies. As soon as this result became available seven days later, he was switched to a conventional ART regimen. Viral load at the time of the switch was 120 copies and was followed by a reading of "detected" but below 40 copies after 22 days. A test for cell-

associated HIV RNA measured 4.7 copies per million CD4+ T cells at 22 days. All subsequent viral load tests have been negative, and no HIV DNA or replication-competent virus can be detected now. Hatano described the case as representing treatment of "hyperacute" HIV infection. A treatment interruption is planned after one year to assess if the virus may have been cleared.

ART interruptions followed by long-term clinical monitoring represent the only definitive means for establishing whether a cure of HIV infection may have been obtained.

Does lack of virus equal cure?

While all this news was encouraging, other research suggests the road to an HIV cure will likely still be a long one. Timothy Henrich, associate physician at Brigham and Women's Hospital and assistant professor of medicine at Harvard Medical School, presented data that should give pause to anyone inclined to assume that the absence of detectable HIV equates to a cure.

Henrich previously reported on two HIV-infected individuals from Boston, who after receiving stem cell transplants to treat cancers while on continuous ART showed no measurable levels of virus over a period of several years (*J. Infect. Dis.* **207**, 1694, 2013). Designated patients A and B, one had acquired HIV infection perinatally and required the transplant for recurrent Hodgkin's lymphoma, while the other became HIV infected as a result of sexual transmission and had a diagnosis of myelodysplastic syndrome. Both were originally heterozygous for the CCR5Δ32 mutation, but received stem cells from wild-type individuals, unlike Timothy Brown, the lone adult considered cured of HIV, who received a transplant from a CCR5Δ32 homozygous donor.

Last spring, ART was interrupted in both patients A and B under a careful research protocol involving monitoring for viral load rebound every one to two weeks. Early signs were promising, as multiple tests continued to find no detectable HIV RNA or DNA. But after twelve weeks, patient A had a viral load of 900 copies, which rapidly increased to 127,000 copies just three to four days later. A drop in CD4+ T-cell count was also documented. Although ART was reinstituted, patient A only stayed on therapy for several days due to problems with adherence, resulting in a viral load that peaked at several million copies, along with accompanying symptoms of acute HIV infection including fever, malaise, and aseptic meningitis. ARV resistance was detected and a new treatment regimen was initiated that has since led to HIV suppression and an ongoing increase in CD4+ T-cell counts.

HIV remained undetectable in patient B for a longer period of eight months, but about a week after the last negative virology results, symptoms of acute HIV infection developed and viral load was measured at just under two million copies. ART was immediately restarted and HIV replication was successfully controlled. Sequencing experiments are ongoing, but Henrich noted that there is a clear relationship with the original infecting virus, ruling out any possibility of a new infection or superinfection. Samples taken five days after HIV rebounded in patient A also show an extremely homogenous viral population, suggesting that the source was perhaps just one latently infected cell.

Henrich suggested the rapid development of acute infection symptoms in both of these patients was due to the new, transplanted immune system not having encountered HIV antigens prior to the recrudescence of viral replication. Analyses of HIV-specific cellular immune responses by Marcus Altfeld at the Ragon Institute supported this suspicion—they were undetectable after the stem cell transplants and did not emerge until after viral load rebounded (after 108 days in patient A, and 13 days in patient B).

Henrich highlighted several lessons from these data. The estimated magnitude of the reduction in HIV reservoirs in these two individuals was at least three logs, but this only achieved a delay of viral load rebound, indicating the presence of long-lived sources of replication-competent virus in tissues that were not sampled in the study. And despite the potential risks, analytical ART interruptions followed by long-term clinical monitoring represent the only definitive means for establishing whether a cure of HIV infection may have been obtained.

Shortcomings of single agents for reversing latency

The central challenge in HIV cure research is eradicating the long-lived pool of cells containing latent, integrated HIV proviruses. A variety of strategies are being explored that might be able

to awaken this slumbering reservoir, thereby facilitating the elimination of the infected cells. One strategy involved the use of so-called latency-reversing agents (LRAs), and among these are histone deacetylase (HDAC) inhibitors, a class of cancer drugs that target the cellular proteins involved in locking down integrated HIV and preventing viral gene expression in latently infected cells. HDAC inhibitors appear able to reverse latency in laboratory models, and human trials have suggested that the US Food and Drug Administration (FDA)-approved HDAC inhibitor, vorinostat, may be able to induce intracellular RNA production by latent HIV in ART-treated individuals (*Nature* 2012, doi: 10.1038/nature11286).

However, at CROI, Gregory Laird, a graduate student in the laboratory of Robert Siliciano at Johns Hopkins University, reported findings indicating single LRAs have essentially no effect on latently infected CD4+ T cells sampled from HIV-infected people on suppressive ART. The rarity of these cells make this type of study challenging—their frequency is around one in a million CD4+ T cells—but sufficiently large volumes for this study were collected from volunteers via apheresis.

Laird tested a panel of candidate LRAs including three HDAC inhibitors (vorinostat, romidepsin, and panobinostat), the acetaldehyde dehydrogenase inhibitor disulfiram, the bromodomain inhibitor JQ1, and a protein kinase C (PKC) agonist, Bryostatin 1.

Results were compared to those achieved by activating CD4+ T cells with phorbol 12-myristate 13-acetate (PMA)/ionomycin, which is known to maximally induce replication of latent HIV. Outgrowth of replication-competent HIV was measurable after PMA/ionomycin stimulation in CD4+ T cells from 11 of 13 donors, whereas, no virus was measurable in any of the samples exposed to LRAs. Similarly, an assay that quantified extracellular HIV messenger RNA (mRNA) in the cultures could detect none after exposure to LRAs, with the sole exception of a CD4+ T-cell sample from one individual in which viral mRNA became detectable in response to Bryostatin 1. The last assay Laird looked at was for intracellular HIV mRNA, a measure that has been used to assess activity in human trials of LRAs. Low levels were apparent at baseline in the majority of cases, and only increased significantly after treatment with Bryostatin 1 and PMA/ionomycin.

Although HIV mRNA increases were documented in the published clinical trial results of vorinostat, Laird pointed out that care needs to be taken with this particular assay because HIV mRNA can be generated as a result of the transcription of the human genes into which HIV has integrated. This phenomenon is distinct from the activation of the virus itself, as it does not lead to HIV replication and the production of virions, the desired goal of LRAs. HIV mRNAs generated by the activity of human genes are referred to as readthrough transcripts. Laird has developed an assay to specifically measure HIV readthrough transcripts, and in an analysis involving five donors, Laird showed that their levels are increased by vorinostat, suggesting that this mechanism likely accounts for the previously reported HIV mRNA increases in recipients of the drug. These analyses were published shortly after CROI ended (*Nat. Med.* 2014, doi: 10.1038/nm.3489).

Because HIV establishes latency in memory CD4+ T cells, their long-lived nature is actually a key obstacle to curing the infection.

Having called into question the value of single LRAs in HIV cure research, Laird concluded his talk on a more upbeat note by offering a glimpse at new data generated with combinations of LRAs. In several cases, significant synergy was observed, particularly when either romidepsin or panobinostat was combined with Bryostatin 1. Laird found levels of latency reversal with these duos were close to that achieved with PMA/ionomycin. While Bryostatin 1 may be too toxic for use in people infected with HIV, several analogs and other compounds from the PKC agonist class are now being studied.

Memory T-cell proliferation expands HIV reservoir

The persistence of memory CD4+ T cells is normally beneficial because these cells are specific for previously encountered pathogens and help prevent disease in the case of second exposure. The induction of CD4+ T-cell memory also makes an important contribution to the lasting effectiveness of many vaccines. But because HIV establishes latency in memory CD4+ T cells, their

long-lived nature is actually a key obstacle to curing the infection. One of the mechanisms by which CD4+ T cells persist is proliferation; the cells divide, making new copies, which can in turn later repeat the process. Several studies at CROI identified proliferation as a mechanism by which latently HIV-infected CD4+ T cells can increase in number and persist.

Mary Kearney from Frank Maldarelli's laboratory at the National Cancer Institute reported in a poster that 7%-55% of HIV DNA sequences identified in 14 study participants were identical, and that the numbers were seen to increase in chronically infected individuals initiating treatment at low CD4 counts (<100 cells). This finding suggests that when CD4+ T-cell numbers increase in response to HIV suppression, some of the proliferating CD4+ T cells are latently infected and so the integrated HIV is copied right along with the genome of the cell. In some cases, Kearney's results indicate, more than half of the detectable HIV DNA reservoir can be derived from the proliferation of a single latently infected CD4+ T cell.

Frank Maldarelli presented a complementary late-breaker poster suggesting that the specific genes into which the virus integrates may also contribute to the persistence of latently infected CD4+ T cells. Thor Wagner, assistant professor of pediatric infectious diseases at the University of Washington, echoed this theme in his presentation. He has also observed preferential integration of HIV into cellular genes associated with proliferation and survival. Wagner also speculated that HIV integration is affecting the function of these genes in ways that promote CD4+ T-cell survival.

Improving PrEP with long-acting ARVs

Last year at CROI, results from an experiment in rhesus macaques offered an encouraging indication that a long-acting analog of the FDA-approved integrase inhibitor dolutegravir, named GSK744, could have a future as intermittently administered PrEP (see *A Toddler Stole the Show*, *IAVI Report*, Spring 2013). This year, three new studies bolstered this possibility, generating considerable excitement and a slew of mainstream media stories highlighting the preventive potential of the approach.

Chasity Andrews, a postdoctoral fellow at the Aaron Diamond AIDS Research Center in New York City, who presented the first macaque study last year, described new work that aimed to establish the minimum protective drug levels of GSK744 using the same model. Twelve macaques received a single 50mg/kg dose of GSK744 followed by weekly intra-rectal challenges with the hybrid simian/human immunodeficiency virus (SHIV)162p3, while four animals served as controls. Protection was sustained for at least five challenges, with between six and 17 challenges required to infect all the treated macaques.

Drug levels of GSK744 were then analyzed based on what is called the protein-adjusted 90% inhibitory concentration or PAIC90 (the concentration required to inhibit HIV replication by 90% *in vitro*, adjusted for the binding of the drug to proteins that occurs *in vivo*). This analysis demonstrated that levels three times the PAIC90 were

Alarming HIV Rates Among Black MSM

Reports have repeatedly shown high rates of HIV infection among black men who have sex with men (MSM) in the US, and at this year's Conference on Retroviruses and Opportunistic Infections, researchers began asking when something will actually be done to address the growing disparity between black and white MSM.

Eli Rosenberg, assistant professor in the department of epidemiology at Emory University, pointed out that the severe impact of HIV infection on black MSM in the US has not been linked to differences in behaviors, suggesting more research is needed to better understand the factors contributing to HIV risk in this population. To this end, Rosenberg and colleagues conducted a prospective study comparing HIV incidence in black and white MSM in Atlanta. Baseline testing documented an HIV prevalence of 44% and 13%, respectively.

An HIV-uninfected cohort of 260 black MSM and 302 white MSM were subsequently followed for an average of about 1.5 years. Rosenberg cited several significant differences between the two groups, including a relative lack of health insurance and higher rates of poverty among the black MSM. The results showed a stark difference in HIV incidence: 6.6 per 100 person-years in black MSM compared to 1.7 per 100 person-years in white MSM.

The differential was even more notable in the 18-24 year old age group: 12.1 per 100 person-years (representing 16 infections) versus one per 100 person-years (one infection). Rosenberg said that this translates to more than 1 in 10 young black MSM in Atlanta becoming HIV infected every year.

In a multivariate analysis, lack of health insurance and having a black partner were factors significantly associated with becoming HIV infected. Rosenberg said these findings add to the evidence that implicating social factors and HIV prevalence in sexual networks, not individual characteristics and behaviors, are the primary drivers of the distressing rates of HIV infection in the black MSM population.

In the question and answer period following Rosenberg's talk, Jeff Klausner from the University of California at Los Angeles called for greater urgency in moving from observational studies to implementing pre-exposure prophylaxis (PrEP) and other interventions that could avert HIV infections in black MSM. Klausner specifically lamented the unwillingness of Gilead—the pharmaceutical company that manufactures the ARVs Truvada and tenofovir, both of which have been shown to be effective PrEP drugs—to market PrEP to vulnerable populations and their providers, and drew applause when he concluded: "We need to think whether we can keep talking about observational studies showing this high incidence and then not doing anything about it." *–RJ*

completely protective, while those above the PAIC90 were 97% protective. Only one animal with drug levels above the PAIC90 became infected. Because human pharmacokinetic studies of injectable GSK744 have shown that levels are maintained at four times the PAIC90 for at least 16 weeks following an 800mg dose, Andrews was able to conclude that protective concentrations can be achieved by quarterly injections that are administered by two shots, one into each gluteal muscle. A Phase II safety and tolerability trial of this drug as PrEP in high-risk men who have sex with men (MSM) is slated to start this spring. Data from 480 volunteers to date suggest the drug is safe, with some volunteers reporting instances of dizziness and grade one rash, but nothing more serious.

Following Andrews' talk, J. Gerardo Garcia-Lerma, a research microbiologist at the US Centers for Disease Control and Prevention (CDC), debuted findings from a study exploring the efficacy of GSK744 against vaginal transmission in rhesus macaques. An initial pharmacokinetic assessment of a single 50mg/kg dose in six female pigtailed macaques revealed that drug levels were maintained above the PAIC90 in vaginal tissue for at least a month, although the amount of GSK744 in these tissues was approximately four- to five-fold less than seen in plasma. This led to the selection of a monthly dosing regimen.

Garcia-Lerma explained that female pigtailed macaques were used because they have lunar menstrual cycles, allowing efficacy to be tested throughout all phases of the cycle. Six animals received three monthly GSK744 injections and six were given a placebo at the same time points. After the first dose, biweekly, low-dose, intravaginal challenges with SHIV162p3 were administered for a period of 12 weeks, followed by cessation of drug and continued monitoring for infection. All placebo recipients were infected after a median of two to four challenges, but no macaques in the GSK744-treated group acquired infection. Garcia-Lerma concluded that these results strongly support further efforts to establish the efficacy of GSK744 for PrEP in women.

In a complementary poster presentation by Chasity Andews' group, protection against a single, high-dose intra-vaginal SHIV162p3 challenge was reported in macaques. This study employed a somewhat different model involving female rhesus macaques treated with the injectable hormonal contraceptive Depo-Provera to purposely thin the cervicovaginal epithelium and therefore mimic the most vulnerable phase of the menstrual cycle. Eight macaques were given 50mg/kg of GSK744 at the start of the study and four weeks later, and four animals served as controls. The high dose SHIV162p3 challenge was administered one week after the first dose of GSK744, with additional challenges at weeks five and seven in the GSK744 recipients. All control animals were infected after a single challenge, while six of the eight macaques in the GSK744 group remained uninfected throughout. In the two treated animals that became infected, viral load was first detected at three and seven weeks after the last challenge, respectively.

The development of long-acting ARVs that could represent potentially more user-friendly PrEP options is timely given that several trials have recently found that PrEP recipients appear to struggle with adherence to daily regimens. "Daily dosing is burdensome to the user," said Ariane van der Straten, director of the Women's Global Health Imperative at RTI International, an independent, nonprofit institute.

van der Straten described a new analysis of adherence measures in the four-arm VOICE trial, which compared vaginally administered tenofovir gel, oral tenofovir, and oral Truvada to placebo and was unable to show efficacy with any of the interventions. Multiple behavioral measures of adherence were used in the trial, including self-reports, interviews, and monthly returns of unused product, but none showed any correlation with biological measures of drug levels. Rates of non-adherence, defined as no product taken during the previous week prior to drug sampling, were high (>60%), but behavioral measures failed to reflect this reality.

Zeroing transmission

While it's now well accepted that suppression of HIV by ART reduces the risk of sexual transmission of HIV, it is unclear how protective treatment is if condoms aren't used. The PARTNER study, which has recruited over 1,000 discordant couples at 75 European clinical trial centers, was designed to address this question, and results of an interim analysis were presented at CROI by Alison Rodger, senior lecturer and honorary consultant in Infectious Diseases, Infection & Population Health at University College London.

Rodger described data from 767 couples that met criteria for inclusion in the analysis. The criteria included: reporting condomless sex in the partnership, a viral load below 200 copies in the HIV-infected partner, and reporting no PrEP or PEP (post-exposure prophylaxis) use in the HIV-unin-

[IMMUNE ACTIVATION AND HIV TRANSMISSION]

Erin M. Kahle and colleagues from the Partners in Prevention herpes simplex virus (HSV)/HIV Transmission Study and the Couples Observational Cohort Study presented a poster at the Conference on Retroviruses and Opportunistic Infections describing a novel case-control analysis designed to assess whether levels of a variety of cytokines influenced HIV transmission risk.

Researchers compared 120 couples with genetically linked HIV transmission to 321 couples with no transmission events. Significant associations were reported for two cytokines linked with immune activation—interleukin (IL)-10 and interferon γ presenting (IP)-10—both in the partner acquiring HIV infection and the HIV-infected transmitting partner. The researchers suggest that further studies are needed to better understand the role of immune activation in determining both susceptibility to HIV and infectiousness. *–RJ*

fected partners. Of these, 282 were MSM, 245 were heterosexual couples in which the female partner was HIV infected, and 240 were heterosexual couples in which the male partner was HIV infected. Virus sequencing was used to confirm or rule out an epidemiological link for any infections that occurred in HIV-uninfected partners.

In the group of 767 couples, there were zero documented transmissions from HIV-infected partners on suppressive ART. However, Rodger stressed that the limited duration of follow up, an average of around a year per couple, still leaves some uncertainty. Based on the 95% confidence interval, the data are compatible with up to a 4% risk of transmission over ten years for any sex, or a 10% risk over ten years for anal sex. The PARTNER study is now being extended until 2017 for the MSM couples in order to try and narrow the confidence interval associated with the findings. Rodger noted that, in the absence of ART, around 50 to 100 HIV transmissions would have been expected in this group.

Stable trimer advances vaccine research

HIV vaccine researchers recently made a major advance in the pursuit of antibody-based vaccines—the creation of a stable envelope trimer. This work, done by the laboratory of John Moore, professor of microbiology and immunology at Weill Cornell Medical College (see *Keystone in Rio: Breakthroughs, Predictions, and Surprises*, *IAVI Report*, Winter 2013), is viewed as addressing one of the major roadblocks to developing vaccine candidates capable of inducing antibodies against this unstable protein structure, which is the target of the bNAbs that researchers are trying to induce through vaccination. Accurately recapitulating this critical component of HIV has been enormously challenging, with many attempts resulting in disappointment. Loosely speaking, stabilizing HIV's envelop trimer has been the biochemical equivalent of baking a soufflé—researchers would think they had succeeded only to have it collapse into disarray the moment it was removed from the oven.

At CROI, Moore presented the first immunogenicity data generated with the new and better-behaved gp140 trimer protein, which is named BG505 SOSIP.664 and was derived from a clade A virus isolated from a Kenyan infant. Explaining the rationale for the experiment, Moore noted that previous gp140 immunization protocols have used versions of the molecule that unfold and do not retain a homogenous, consistent trimeric form (see diagram, this page). BG505 SOSIP.664 is also sourced from a founder virus, and these viruses have been shown to typically generate strong autologous neutralizing antibody responses during primary infection—in around 25% of cases, these responses eventually evolve to become broadly neutralizing. The hope is that the new trimer will provide a platform for the design of immunogens capable of inducing bNAbs; however, the early data suggests there is still a long way to go.

In collaboration with Hansi Dean and colleagues at the International AIDS Vaccine Initiative, experiments were carried out in rabbits involving BG505 SOSIP.664 proteins generated in different cell lines, with or without digestion of surface glycans. Immunizations with a 30µg dose of the trimeric proteins were given at 0, 4, and 20 weeks, along with the ISCOMATRIX adjuvant (a particulate adjuvant comprising cholesterol, phospholipid, and saponin), with monomeric BG505 gp120 used as a comparator. Assays run by David Montefiore at Duke University demonstrated high titers of neutralizing antibodies in all immunized rabbits against the autologous BG505 virus, which is classified as tier 2 in the system that grades difficulty of neutralization (with tier 1 being the easiest to neutralize). By contrast, antibodies induced by monomeric BG505 gp120 showed significantly less activity. However, responses to heterologous tier 2 viruses with BG505 SOSIP.664 were far lower. Some neutralization was observed of a subtype C isolate, superior to that achieved with the monomeric gp120, but there was no effect against a

Taming the Tricky Trimer

Unlike previous best attempts which are unstable and display a diversity of shapes (*PNAS* 2014, doi: 10.1073/pnas.1319512111 and *PNAS* 2013, doi:10.1073/pnas.1314351110), BG505 SOSIP.644 (far right) shows a regular, homogenous conformation (*PLoS Pathog.* 2013, doi:10.1371/journal.ppat.1003618). Image courtesy of Andrew B. Ward of The Scripps Research Institute and John P. Moore.

subtype B virus. Cross-neutralization was only seen for subtype B viruses classified as tier 1B. For heterologous viruses, Moore said, "We haven't gotten anywhere close to where we want to get."

But it appears researchers are on the right track. A retrospective analysis by Montefiore suggests that the autologous neutralization titers seen with BG505 SOSIP.664 exceed those achieved by any prior gp140 immunogen, including a prior attempt at developing a stabilized trimer from Moore's laboratory. This offered some encouragement that it represents a platform that can be built upon.

Efforts are now underway to map the epitopes being targeted by the neutralizing antibody response against BG505, and to identify means of improving the immunogenicity of BG505 SOSIP.664. Moore said one possibility for improvement is lessening the induction of antibodies against the V3 loop of the HIV envelope protein. Future plans also include developing multiple trimers based on viral sequences that appear to have invoked bNAb responses in HIV-infected individuals, and testing trimer cocktails derived from different HIV isolates from the same or different clades. Moore also cited the use of BG505 SOSIP.664 in structural studies as critical to better defining bNAb targets.

Ian Wilson, Hansen Professor of structural biology at The Scripps Research Institute, sounded the latter theme in a symposium session on visualizing HIV. Wilson covered the X-ray crystallography and cryo-electron microscopy (EM) work described in the last issue of *IAVI Report*, and published recently (*Science* 2013, doi: 10.1126/science.1245625, *Science* 2013, doi: 10.1126/science.1245627). Wilson stressed that SOSIP trimers are providing a new framework for studying bNAb interactions with the HIV envelope, for example, revealing how various glycan-dependent bNAbs can target the same site in different ways. These observations are cause for optimism because they suggest there are multiple opportunities for the immune system to successfully attack vulnerabilities in HIV's armor.

Following Wilson, Walter Mothes, associate professor of microbial pathogenesis at Yale University School of Medicine, outlined his laboratory's efforts to elucidate the dynamic behavior of the HIV envelope trimer, with a view to better understanding how bNAbs exert their inhibitory activity. The work is being conducted in collaboration with Scott Blanchard at Weill Cornell Medical College and Jason Gorman and Peter Kwong at the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases. Mothes has employed a method called single molecule fluorescence resonance energy transfer (smFRET), which is capable of measuring microscopic movements over millisecond timescales. Molecules of interest are tagged with dyes and the distance between the tagged molecules can be measured based on computerized assessments of the transfer of energy between the dyes. For these studies, three HIV viruses were generated with one dye in the V1/V2 region and a second in the V4 and V5 loops of gp120. Two of the viruses were derived from neutralization-sensitive NL4-3 laboratory-adapted isolates and one represented a neutralization-resistant clinical isolate, JR-FL.

Analysis of the movement of the tags by smFRET showed that HIV envelope, unbound to any ligands, is dynamic and transitions between three distinct states based on the distance between the tagged variable loops. Mothes described these states as low, intermediate, and high, with low representing a "ground state." Comparing the neutralization-sensitive and neutralization-resistant viruses revealed interesting differences: NL4-3 transitioned between states more frequently and spent more time in an intermediate form in which the envelope is more open, whereas JR-FL mostly remained in a ground state. "It opens up much less frequently," Mothes explained. A similar reticence to remain in a potentially vulnerable conformational state was seen when JR-FL was studied interacting with ligands on target cells (CD4 and co-receptors).

Mothes next looked at how a panel of different bNAbs (including VRC01, PG16, and 2G12) affected the dynamics of the HIV envelope, finding that they all stabilized the ground state, and thus inhibited the transition to other conformational states required for the virus to successfully infect cells. A potent entry inhibitor drug in development by Bristol-Myers Squibb achieved the same effect. Mothes' work offers a novel complement to techniques such as X-ray crystallography and electron cryomicroscopy (cryo-EM), which capture immobilized forms of the HIV envelope, by tracking the same molecules in flux. ■

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Building an IMMUNE TOOLBOX

Researchers showcase efforts to apply basic immunological principles to the design of antibody-based vaccine candidates

By Yegor Voronin

For many years when leading HIV vaccine researchers gathered for the annual Keystone Symposium, the most befitting analogy to the state of the field was attempting to scale the snow-covered mountain peaks that serve as the meeting's backdrop.

At this year's symposium, HIV Vaccines: Adaptive Immunity and Beyond, which was held in the Canadian Rockies in the picturesque town of Banff Mar. 9-14, researchers acknowledged that although there is still a steep climb ahead, employing a better understanding of basic immunology might help ease the climb.

The focus on basic immunology was the choice of the conference co-organizers—Galit Alter, associate professor of medicine at Harvard Medical School; Susan Barnett, director of vaccines research at Novartis; and Nicole Frahm, associate director for laboratory science at the HIV Vaccine Trials Network—and it resulted in a different type of symposium. New faces were seen at the podium, discussions circled around understanding the immediate events after vaccination, and new collaborations were formed as researchers realized they are working on the same problem from very different angles.

Antibodies were the center of attention once again, but neutralizing antibodies shared the stage with molecular signaling, germinal centers, T follicular helper (Tfh) cells, and so-called functional antibodies (non-neutralizing). Researchers still have much to learn about the intricate process of guiding antibody responses at molecular and cellular levels. Still, recent progress holds promise for development of a new generation of vaccine candidates that are more grounded in basic immunological principles than their predecessors.

Starting with neutralization

In 2009, a collaboration led by Dennis Burton, professor at The Scripps Research Institute in La Jolla, published a paper describing isolation of two highly potent antibodies, from an HIV-infected donor, which could neutralize a broad swath of HIV isolates (*Science* **326**, 285, 2009). Compared to previously isolated broadly neutralizing antibodies (bNAbs), the newcomers were active at 10- to 100-fold lower concentrations and were capable of neutralizing a wider spectrum of viruses. Over the past five years, dozens of bNAbs have been isolated from HIV-infected individuals, enabling a whole new wave of antibody research that has led to many discoveries about how HIV-specific antibodies develop in infected individuals.

Researchers have found that these bNAbs occur more frequently in HIV-infected individuals than was previously believed and that there is a smooth continuum between HIV-infected people that develop only weak neutralizing antibody responses and people that develop bNAbs, with a large majority having responses of intermediate potency and breadth (*AIDS* **28**, 163, 2014). Studies have also shown that antibody gene variants conducive to generation of certain kinds of bNAbs are quite common among humans (*PNAS* **109**, E2083, 2012). And recently, several groups have started tracking the arms race between the virus and the immune response in real time, trying to understand the details of the processes that lead to bNAbs appearance in some people (*Nature* **496**, 469, 2013).

Delving into the germinal center

Following the discovery of the improved crop of bNAbs, researchers began studying the unique sequences and structures of these antibodies to identify what makes them so potent and broadly neutralizing. They soon realized that these bNAbs often have variable regions with unusually high levels of somatic hypermutation, genetic changes that allow them to bind more strongly to the specific pathogen.

It happens like this: Naive B cells activated by an antigen travel to lymph nodes and the spleen where, together with helper T cells, they establish special structures called germinal centers. Within germinal centers, B cells multiply and undergo the process of affinity maturation that results in higher affinity antibodies. Affinity maturation is an evolution-like process that is divided into two alternating stages. During the somatic hypermutation stage, a special enzyme mutates the antibody gene in each cell. Then, during affinity selection, the cells with mutated antibodies compete with each other for binding to the pathogen that is presented on the follicular dendritic cells (FDCs), and also for activating signals from Tfh cells. The B cells that win the competition may undergo additional rounds of mutation and selection, further improving their affinity to the pathogen.

The fact that genes coding for bNAbs against HIV have extensive somatic hypermutation suggests that B cells in germinal centers went through an unusually high number of these rounds of mutation and selection, likely reflecting a long period of exposure to HIV proteins in chronically infected people. This raised concerns that such bNAbs can only develop over prolonged periods of time in HIV-infected people and that it would be difficult for a vaccine to recapitulate this process.

However, researchers are now studying ways to either accelerate or guide the process of affinity maturation as part of a vaccination strategy. To do that, they need to first understand the processes that take place inside germinal centers.

Germinal centers and their functions have been extensively studied in mice, where it has been shown, among other things, that each center is founded by three to four B cells, which remain in that particular center and don't migrate to neighboring germinal centers.

The laboratory of Gabriel Victora, a fellow at the Whitehead Institute for Biomedical Research, has been studying, in particular, how helper T cells behave in these structures (*Science* **341**, 673, 2013), work they presented at Keystone. After transgenic mice, deficient of their own T cells, were transplanted with T cells randomly labeled with three different fluorescent markers, Victora and colleagues observed that cells labeled with different colors are always evenly distributed among lymph nodes and among germinal centers within a lymph node. These results suggest that T cells behave differently from B cells, in that multiple T-cell clones are engaged during formation of a germinal center or that they can freely migrate between centers.

This hypothesis was confirmed in another experiment in which T cells within a single germinal center were labeled by photoactivation and then traced to other germinal centers within the same lymph node. Moreover, Victora was able to directly show that new T-cell clones may invade existing germinal centers. T-cell-deficient mice were populated with a mixture of T cells recognizing two different antigens. Injection of one of these antigens led to establishment of germinal centers with T cells specific for that antigen. After injection of the second antigen, T cells with the new specificity were observed entering these germinal centers and providing help to B cells.

This difference in behavior between B and T cells makes sense during an immune response to a chronic mutating pathogen such as HIV. Spatial isolation of B-cell clones during the affinity maturation process prevents competition for antigen between B cells producing antibodies that target overlapping epitopes and a "winner takes all" scenario, which would limit the diversity of the antibody response. Whereas T cells are not competing with each other, and therefore the T-cell immune response benefits from wide dissemination of a new clone that is able to recognize escaping viral variants and continue to assist B cells.

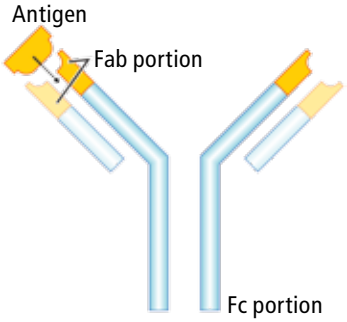
Other research on germinal centers presented at Keystone suggests the events that occur at these sites may have an extremely long-lasting effect on humoral immunity. Mark Slifka, a senior scientist at the Oregon Health & Science University, and colleagues are focusing on understanding the mechanisms behind decades-long production of antibodies by plasma cells observed in response to some natural infections and live-attenuated vaccines, including the yellow fever 17D vaccine.

Some researchers believe that plasma cells are short lived and may be repopulated by memory B cells either via homeostatic mechanisms or in response to reinfection, but experiments by Slifka's group strongly suggest that some plasma cells have a very long lifespan.

In one study, researchers prevented the repopulation of plasma cells by B cells by treating rhesus macaques with an anti-CD20 antibody, which deletes more than 99% of peripheral B

cells, and by removing the spleens of these animals. Despite the lack of memory B cells, the animals maintained antibody titers against tetanus for more than 10 years, indicating continuous production of these antibodies by plasma cells that were formed before B-cell depletion occurred.

But just what makes these plasma cells so long lived is unknown. Slifka proposes that specific signals received by B cells in germinal centers, such as activation by multi-meric antigens and strong Tfh help may result in imprinting of the "long lifespan program" on the resulting plasma cells. The ability of HIV vaccine candidates to employ such signals, according to Slifka, and to trigger formation of plasma cells continuously producing neutralizing antibodies against the virus will be essential for long-lasting, sterilizing immunity.



Schematic diagram of an antibody

Even in the absence of high neutralizing antibody titers, the ability to elicit long-lived plasma cells could be extremely beneficial. In the RV144 trial in Thailand, the first to show any protection against infection, the level of protection achieved by the prime-boost regimen (a canarypox vector expressing HIV *env*, *gag*, and *pro*, followed by a boost with B/E gp120 recombinant protein) was 60% in the first six months, but then rapidly declined (*N. Engl. J. Med.* **361**, 2209, 2009). Some policymakers have suggested that if the 60% level of protection could be extended to last a decade or more, such a vaccine could be licensable in many countries.

Not just neutralizing

The idea that signaling during B-cell activation may have a long-lasting impact on antibody responses also intrigues Alter. Her group studies what other antibody functions, besides neutralization, may play a role in protection against HIV, especially those that engage the innate immune responses. The potential importance of these so-called functional antibodies was highlighted by the results of the RV144 vaccine trial that showed protection against HIV in the absence of neutralizing antibodies (see *Antibodies: Beyond Neutralization*, *IAVI Report*, Jan.-Feb. 2010; *Sci. Transl. Med.* **6**, 228ra39, 2014). New and better assays for other antibody functions, such as antibody-dependent cellular cytotoxicity (ADCC), complement activation, and phagocytosis are being developed and applied to look for correlates of protection or of viral control in animal studies and clinical trials.

Antibody binding may activate the complement cascade, a complex system composed of over 30 interacting proteins in the blood, which immobilizes the pathogen and may lead to its lysis or capture by macrophages. In addition, binding of antibodies to the surface of an HIV-infected cell attracts natural killer (NK) cells (see *Rethinking the Natural Killer*, *IAVI Report*, Winter 2013), which may kill the cell via ADCC. The so-called Fab portion of an antibody is where antigen recognition occurs, while the Fc portion triggers other functions such as ADCC (see antibody image, at left).

The Fc portion of an antibody does not undergo recombination and hypermutation and, therefore, is usually viewed as being constant, but there are distinct variants of it, which are referred to as subclasses. Alter and others have shown that antibody subclass may have a major impact on an antibody's ability to initiate various innate immune responses.

For example, within the immunoglobulin (Ig)G isotype antibodies, the IgG3 subclass is much better at triggering ADCC than the IgG4 subclass. Alter and colleagues reported recently that the vaccine candidates in the RV144 trial elicited primarily IgG1 and IgG3 antibodies, while the vaccine evaluated in the VAX003 trial (a B/E gp120 recombinant protein) elicited primarily the IgG4 subclass of antibodies, and these responses correlated with protection in RV144 or the lack thereof in VAX003 (*Sci. Transl. Med.* **19**, 228ra38, 2014). This evidence suggests that vaccination regimens may impact the subclass of antibodies induced, and that the subclass of antibodies could possibly account for the difference in efficacy observed in the two trials.

Of course it's not as simple as just a difference in subclass. Even within a specific subclass of antibodies there are differences in the functionality of the antibodies, which are determined by the composition and structure of glycans attached to the Fc portion of the antibody, as Alter discussed at Keystone. These modifications can make the antibody better, or worse, at a particular type of activity.

The exact mechanisms behind selection of a particular type of Fc glycosylation pattern are not well understood, but Alter believes that early signals during B-cell activation play a major role. And once the plasma cell adopts a particular glycosylation pattern for the produced antibody, it retains it for the rest of its lifespan. Therefore, just like with imprinting long lifespan on plasma cells, appropriate signaling during vaccination may be key to the development of antibodies with the desired functions.

Vaccines and germinal centers

While the knowledge of what signals are needed to drive the immune response in a particular direction is important, researchers are still a

long way from being able to provide these signals in the right place at the right time during vaccination. Diego Farfan-Arribas, a postdoc from Shan Lu's laboratory at University of Massachusetts Medical School, presented their efforts in collaboration with Alex Dent from Indiana University to track and tweak the events occurring in germinal centers in response to vaccination (*Hum. Vaccin. Immunother.*, in press). In a study comparing two different types of vaccines in mice, Farfan-Arribas and colleagues found that the vaccine administered as a prime had significant effects on cellular composition of germinal centers, which correlated with elicitation of different immune responses and modified the potency of the booster vaccination.

In germinal centers of mice primed with either HIV gp120-expressing DNA or a gp120 protein subunit vaccine, researchers observed approximately three-fold more B cells after vaccination with DNA. The effect was transient but clearly indicate these two vaccines resulted in different immune signals.

Also, priming with DNA had a delayed effect on Tfh cells. The number of observed Tfh cells was similar between the two vaccines after the prime, but boosting the DNA-primed responses resulted in a higher number of effector memory Tfh cells, an important subset of $CD4^+$ T cells indicative of established immunity and recall response. Finally, priming with DNA and boosting with protein resulted in higher antibody titers, suggesting that the differences in the numbers of cells in germinal centers have downstream consequences for the overall immune response.

This effect was further explored by supplementing the DNA gp120 vaccine with plasmids coding either for ICOS-L or BLyS, two proteins involved in B-cell stimulation signaling. Expression of these signaling proteins in the vicinity of the gp120 expression was expected to have an adjuvant-like effect and boost immune responses to the viral protein. Also, local expression at the site of injection is preferable to systemic administration, which may lead to side effects and safety concerns.

Researchers reported that co-expressing ICOS-L resulted in slightly increased numbers of Tfh cells in germinal centers, while BLyS expression increased the number of Tfh cells by approximately 50%, while reducing the number of B cells. At the moment, the ability to rationally modulate B-cell responses is very limited, so researchers were surprised by the effect supplementing the vaccine with BLyS had on B cells, but such experiments are critical to inform future attempts to tweak the immune system via vaccination.

From mice to men

An obvious limitation of many of the basic immunology studies described above is that they were done in animal models. While mouse models allow genetic manipulation as well as the invasive sampling necessary to dissect the processes occurring in germinal centers, the mouse immune system is obviously very different from the human immune system, especially with regard to innate immune responses. Therefore, studies in humans are necessary to confirm these findings. However, to do such studies in humans, researchers will likely have to rely on samples of peripheral blood.

For this reason, some researchers are developing assays that can use blood samples to help deduce what is happening in lymphoid organs. Shane Crotty, a professor at the La Jolla Institute for Allergy & Immunology, and colleagues were studying $CD4^+$ Tfh-like cells (generally defined by the presence of CXCR5 on the cell surface) in blood when they noticed that a subset of these cells was highly reminiscent of resting memory Tfh cells obtained from germinal centers (*Immunity* **39**, 758, 2013). Compared to other CXCR5-positive cells, gene expression profiles of cells of this subtype (defined by expression of low levels of PD-1 and the lack of CXCR3 surface marker) was much more similar to the gene expression profile of Tfh cells. And *in vitro*, these cells behaved just like Tfh cells do *in vivo*—they activated memory B cells, stimulated expression of IgG, and led to conversion of B cells into plasma cells.

Moreover, the frequency of these $CD4^+$ Tfh-like cells in blood samples from HIV-infected volunteers was associated with the development of bNAb responses. This indicates that it may be possible in the future to track immune responses in blood samples soon after vaccination to ensure they are developing in the desired direction.

Although there is still a long way to go in the design and development of HIV vaccine immunogens, this Keystone meeting highlighted the increasing importance of the convergence of developing a basic understanding of the immune signaling pathways with the empirical testing of various vaccine candidates. Working from opposite directions, these efforts complement each other more than ever before, with vaccine candidates getting more sophisticated via employment of the latest discoveries in immunology, and clinical trials serving as hypothesis-generating factories for basic research. ■

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[BEING FRUGAL WITH INFLAMMATION]

The search for vaccines that are safer and have fewer side effects has led to the development of subunit vaccines composed of a single protein. These vaccines, however, are not very immunogenic on their own and usually have to be supplemented by adjuvants—compounds that boost the adaptive immune responses by activating the innate inflammatory responses.

At this year's Keystone Symposium, Nick Valiante, site head of immunology/global head of immuno-therapy at Novartis Vaccines, presented the ongoing work of the Swiss pharmaceutical company to develop new small molecule adjuvants that are safer and more potent.

Their hypothesis was that most of the inflammation that occurs in response to a pathogen is to non-specifically block replication of the pathogen and limit its spread in the body, while only a small portion of these inflammatory responses are needed to activate the adaptive immune system. Because subunit vaccines do not contain replicating pathogens, a large portion of the inflammation caused by adjuvants is therefore "wasted," according to Valiante.

Starting with early prototypes, Valiante and colleagues iteratively tested several variations of adjuvants that had different physicochemical properties, comparing the amount of inflammation with the resulting immune responses. The lead candidate resulted in little to no systemic inflammation, while eliciting the highest level of antibodies. This work shows that adjuvant efficacy can be uncoupled from its toxicity and may lead to adjuvants that trigger just the minimal essential inflammation required to stimulate adaptive immune responses. *–YV*

Better Data Highlight *Growing Problem*

In politically unstable regions of the Middle East and North Africa, a weak AIDS response is stoking an alarming rise in HIV incidence and AIDS-related deaths

By Regina McEnery

In last year's annual report on the status of the pandemic, the Joint United Nations Programme on HIV/AIDS (UNAIDS) reported a 33% decline in new HIV infections since 2001, with the most dramatic declines occurring in sub-Saharan Africa and the Caribbean.

Unfortunately, the epidemic is on the upswing in another region: the Middle East and North Africa (referred to as MENA). In 2012, an estimated 32,000 individuals from this region acquired HIV—a more than 50% higher rate of new infections than reported in this region in 2001, according to UNAIDS. Moreover, the number of people dying from AIDS-related illnesses in this region more than doubled between 2001 and 2012, again an opposite trend compared to what has been occurring in sub-Saharan Africa and most other regions thanks to the success of increased accessibility to antiretroviral drugs (ARVs).

MENA, which encompasses a swath of 23 countries stretching from Morocco to the west, Pakistan to the east, and Somalia to the south, is not the only region experiencing a surge in new infections. UNAIDS has also seen a 13% increase in new HIV cases in Central Asia and Eastern Europe between 2001 to 2012, which the agency has attributed to both a lack of access to ARVs and the criminalization of injection drug users (IDUs).

Compared to sub-Saharan Africa, the HIV burden in MENA is still quite small—the HIV/AIDS prevalence is thought to be less than 0.2% in most countries. Meanwhile, despite the dramatic decline in HIV incidence in sub-Saharan Africa, there were still an estimated 1.5 million new HIV infections and 1.2 million AIDS-related deaths in this region in 2012. But the emerging epidemic in MENA over the past decade, which is just coming into better view because of improved surveillance, is concerning none the less because it runs counter to a global effort to end the AIDS epidemic by stopping HIV transmission and halting AIDS-related deaths.

The data and the response

The paucity of quality HIV/AIDS data to emerge from the MENA region makes it difficult to know for sure whether the spike in HIV incidence over the past decade is a recent phenomenon or evidence of a longer-term trend that countries either ignored, or lacked the resources to detect. One recent study conducted by the World Health Organization (WHO) suggests only three of the 23 countries that comprise MENA have HIV surveillance programs capable of tracking their epidemics.

In the past, data being collected in many countries in MENA often resulted in questionable estimates of HIV incidence, and even when the estimates seemed plausible, they often came with wide confidence intervals, noted Laith Abu-Raddad and two colleagues from the WHO and Weill Cornell Medical College (*Curr. Opin. HIVAIDS* **9** 183, 2014). It's only been in the last four to five years that that various global health organizations, academic scientists, and non-governmental organizations (NGOs) in these countries have begun collecting data that more reliably illustrates the status of the epidemic in this region, particularly within specific high-risk populations, which vary by country. The 2010 data for Libya showed that this country had the highest reported HIV prevalence among IDUs in the MENA region at 87%. Data also indicate emerging HIV epidemics among IDUs in Iran, Afghanistan, Egypt, Morocco, and Pakistan. In Lebanon and Tunisia, the highest risk group is men who have sex with men (MSM), and there is also evidence of concentrated

epidemics among MSM in Egypt, Morocco, Pakistan, Sudan, and Yemen. In Somalia and Djibouti, there is evidence of a concentrated epidemic among female commercial sex workers (CSWs).

In some respects, MENA is not all that different from the AIDS-battered region of sub-Saharan Africa, where HIV stigma and discrimination are high and the behaviors associated with HIV transmission are either culturally prohibited or illegal, such as drug use, commercial sex work, or homosexual sex. But unlike sub-Saharan Africa, MENA stands out as the "only region where knowledge of the epidemic continues to be very limited and subject to much controversy," according to a report released in 2010 by the World Bank.

This could partly be due to the widespread political instability and extensive poverty in the region, which is home to the highest number of refugees and internally displayed persons in the world, according to the World Bank report. Vulnerability of women and girls could also be a factor. Some of the highest rates of HIV in MENA have been reported among females, and in three countries where HIV incidence among females is dramatically rising—Yemen, Sudan, and Somalia—child marriage is still common, wrote Navid Madani, an Iranian biochemist and AIDS researcher at the Dana-Farber Cancer Institute in Boston and Harvard Medical School who travels frequently to the MENA region, in a recent commentary (*JAIDS* dx.doi.org/10.7448/IAS.17.1.19074).

Lack of awareness of one's HIV infection status is also high. A report released last year by the WHO and UNAIDS estimates that about 80% of people living with HIV in the MENA region are not aware they are infected with the virus.

An overall weak response to the epidemic in this region may also be to blame. Various groups, including UNAIDS, the World Bank, the WHO, and The Global Fund to Fight AIDS, Tuberculosis and Malaria are working to address the epidemic in MENA, but with a few exceptions—such as Morocco and Iran—the responses have been pretty weak, some researchers contend.

Abu-Raddad, an associate professor of public health at Weill Cornell Medical College in Qatar, who has studied the HIV/AIDS epidemic in MENA extensively, says in countries where NGOs are strong, the HIV response has also been strong. "In a few countries though, and this is worrying, civil society organizations are very weak and barely exist in relation to HIV," he says.

Some of the countries with the fastest growing rates of HIV/AIDS are Afghanistan, Egypt, and Pakistan, where war and civil unrest reign. But Abu-Raddad doesn't think that political instability in those countries is the main cause of the rising HIV/AIDS burden. "It is too early to link the political instability to the epidemic, and the link may not be a one-directional one," said Abu-Raddad. "Some of the countries that are witnessing large epidemics, such as Iran, are largely stable politically."

Nor is Abu-Raddad convinced that sociocultural factors, including religious beliefs, are the cause. "One can see examples where sociocultural factors, well beyond religion, have in fact benefited the HIV response," he said.

How effectively countries have responded to the HIV/AIDS crisis varies as well. Madani notes in her recent JAIDS commentary that the NGO El Hayet in Algeria is coordinating projects that are designed to ensure the "socio-economic reentry of women affected by HIV into the workforce," while across the region female religious leaders and imams have also been trained to reach out to women in religious institutions about HIV prevention and awareness. Morocco, meanwhile, has expanded voluntary counseling and testing services across the country, provided a range of HIV services aimed at high-risk groups (female CSWs, MSM, and IDUs), offered harm reduction programs, and expanded access to ARVs.

"The extent of the HIV epidemic in the region is now undeniable, and governments, some reluctantly and others readily, are now beginning to address the problem," wrote Egyptian journalist Pakinam Amer in a recent commentary (*Nature Middle East* doi: 10.1038/nmiddleeast.2013.228). ■

Obituary: Reinhard Kurth

Distinguished retrovirologist and calming German voice during AIDS crisis years

By Michael Dumiak

Ten years after an experiment by Reinhard Kurth and virologist Steve Norley began to probe the protective mechanism of live attenuated viruses, particularly an attenuated hybrid simian/human immunodeficiency virus, data are still being pored over at the Robert Koch Institute in Berlin.

The analysis continues but Kurth will not be there to finish it. Perhaps Germany's pre-eminent retrovirologist, a longtime AIDS vaccine researcher, effective science communicator, and former scientific advisory committee chair and board member of the International AIDS Vaccine Initiative (IAVI), Reinhard Kurth died in February following a long struggle with cancer. He was 71.

As acting director from 1996 and then president from 2001 to 2008, Kurth headed the German Ministry of Health's Robert Koch Institute, a leading epidemiological and research center headquartered in Berlin that is responsible for disease control and prevention in the country. Previously Kurth was director of the Paul Ehrlich Institute, a government institution somewhat analogous to the US Food and Drug Administration that is responsible for the safety of medicinal products, blood safety, clinical trial approval, vaccines, and other public health issues.

Kurth was born in Dresden in 1942 and as the Berlin daily newspaper *Tagesspiegel* reports, he grew up in the firebombed city ruins. He later overcame tuberculosis, studied medicine and philosophy in Germany and the US, and became a clinician in 1969. He soon focused on research into the pathogenesis and immunobiology of retroviruses.

Longtime colleagues, Reinhard recruited Norley in 1987 as a postdoc. "If something sounded off the wall, he wouldn't reject it out of hand," Norley says. "There was a good chance he would say try it. Not just that—he would invest time and money. He was very enthusiastic about attacking the AIDS problem, and very keen on putting a large group together." One of this group's main focuses was the protection afforded by replicating viral vectors, work that continues today at the Koch Institute. "Sometimes he'd grin and say you expect it to start off complex and get easier. In AIDS vaccines, it started simple and got harder," Norley recalls.

Today, there is a robust pipeline of replication-competent viral vectors being explored in HIV vaccine candidates, both pre-clinically and in clinical trials, according to Wayne Koff, IAVI's chief scientific officer. "Reinhard was right in the center of that," Koff says. "It's a real loss."

By all accounts Kurth was a rigorous scientist. He also had a calm air of rationalism, which he used in communicating complex and vital issues of science both to the general public and in the halls of government. "A lot of scientists would shy away from this or be ineffective," says Robert Gallo, the co-discoverer of HIV and co-founder and director of the Institute of Human Virology in Maryland, who became close friends with Kurth over 40 years. The disconnect between the research community and public policymakers is not only because of a lack of communication skills on the part of lab scientists, Gallo says, but because it is potentially risky to dive into waters made murky by politics. "Reinhard knew that someone had to, and he did it. And did it well."

Kurth took action during the early years when HIV infection was a death sentence and fear, anger, and hysteria ran high. He was already one of the top retrovirologists in Germany when the pandemic began. This "Champion of Reason," as *Tagesspiegel* called Kurth, advised the German health ministry on how to respond to HIV/AIDS, worked with patient and support groups, and pressed for a rational approach as head of the agency responsible for the country's blood supply.

Koff recalls Kurth as a warm person and a disciplined but open scientist, not one to be satisfied with received wisdom. "Bring me data, not dogma," Koff recalls Kurth saying. The German retrovirologist was unassuming but moved in the inner circle of AIDS vaccine research, a stalwart at Gallo's annual research meetings. Gallo, Kurth, and Billy Hall, professor of microbiology at the University College of Dublin, co-founded the Global Virus Network to coordinate medical research and response to viral pandemic threats and viral causes of human disease.

As early as the mid-70s, Gallo says, Kurth was making his name doing immunochemistry, first under Werner Schäfer in Tübingen and then in his own lab. That's when Gallo first got to know him. "We went to a disco, ate and drank together, and talked about retrovirology," he says. Kurth introduced him to immunochemistry, a then-emerging field. "It was an area I barely knew existed," Gallo says.

Gallo would eventually bring immunochemistry to his studies with animal retroviruses and, eventually, to human retrovirology. Six weeks before he died, Kurth found the strength to fly to the US and speak at Gallo's investiture—even getting in a couple zingers. "He was the only one to roast me!"

In honor of Kurth, the Global Virus Network has launched a fund to endow a scholarship in Kurth's name (http://gvn.org/gvn_remembers_reinhard_kurth/).

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In BRIEF

New anti-homosexuality laws raise international concerns

The East African country of Uganda has become the latest to increase the penalties for homosexual behaviors, raising strong concerns among international aid organizations, and HIV/AIDS groups in particular, that the new legal framework in the country could have devastating effects on public health efforts, in addition to violating human rights.

The Anti-Homosexuality Act, as the bill in Uganda is called, allows for life sentences for HIV-infected men who have sex with other men and criminalizes the "promotion" and "recognition" of homosexual relations by individuals and groups. This policy, signed into law by Ugandan President Yoweri Museveni on Feb. 24, passed the Uganda Legislature with broad support from political leaders and the public.

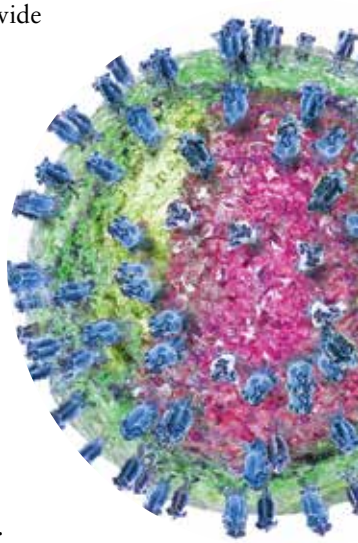
Uganda's Parliament first introduced the amended bill in 2009, but withdrew it over objections that the penalties, which then included the death penalty, were too harsh. A revised version was finally passed by the Parliament in December 2013. Initially, President Museveni declined to sign it, but changed his mind after reviewing the findings of a committee appointed by the Ugandan Health Ministry to review the scientific evidence about the causes of homosexuality. "Their unanimous conclusion was that homosexuality, contrary to my earlier thinking, was behavioural and not genetic. It was learnt and could be unlearnt," Museveni wrote to US President Barack Obama on Feb. 18, according to a recent article in *Science* magazine (see *Science Misused to Justify Ugandan Antigay Law*, *Science* **343**, 956, 2014). However, some scientists on the 11-member committee said their findings were misrepresented, according to the *Science* article.

Laws prohibiting homosexual behaviors are hardly unique—homosexual activity is prohibited in 38 of 54 countries in Africa, according to the International Lesbian and Gay Association, and according to estimates by the United Nations, in 78 countries worldwide. Both Russia and the West African country of Nigeria have recently passed new anti-homosexuality legislation. Nigeria's law, passed in January, carries up to 14-year prison sentences for anyone entering a same-sex union, and 10-year sentences for persons or groups that support gay-related activities or organizations. Last December, India's Supreme Court reinstated a colonial-era law making homosexuality a crime, while Australia's high court recently overturned laws allowing same-sex marriages.

However, the Ugandan law is particularly disconcerting to HIV/AIDS researchers as the country is a major recipient of both international research and development money. Uganda's response to the AIDS pandemic was highly lauded internationally and was often cited as an example for other African countries. The country was one of the original recipients of the President's Emergency Plan for AIDS Relief (PEPFAR), and is slated to receive around US$324 million in PEPFAR money this year.

The US, Norway, Denmark, Sweden, and the World Bank have already announced they are withholding or diverting some foreign aid from Uganda, and the US National Institutes of Health—the largest public provider of research funds in the world—is reportedly contemplating whether it is too risky to fund research in these countries that involves people engaging in "criminalized" behaviors, said Chris Beyrer, director of the Johns Hopkins Center for Public Health and Human Rights.

A letter signed by nearly 1,000 HIV physicians, researchers, nurses, and health care workers, many of whom work in Uganda and Nigeria, urges the US to provide legal aid and other services to protect patients, providers, and organizations serving gay people and to facilitate asylum for individuals affected by these laws.



"Let me just say that the Ugandan LGBT (lesbian, gay, bisexual, transgender) community does not want to see restrictions in humanitarian assistance such as PEPFAR," added Beyrer.

But it is too soon to discern just how the Anti-Homosexuality Act will impact HIV/AIDS funding in Uganda, or the long-standing research projects that have been established in the country. Many organizations that do HIV/AIDS work in Uganda are now sorting out the legal complexities, and their responses will become clearer in coming months.

Ron Gray, a professor of epidemiology at the Johns Hopkins Bloomberg School of Public Health, recently returned from his latest trip to Uganda, where he has been conducting research for over 27 years. "Personally, I feel the [anti-homosexuality] legislation is inappropriate and discriminatory," said Gray, whose primary focus has been HIV prevention, including the study of adult male circumcision in heterosexual men, with much of the work carried out in the Rakai district of Uganda. As for the future, "everything is up in the air," he said. ■

Upcoming HIV-Related Meetings

APRIL 2014

17th Annual Conference on Vaccine Research
April 28 - 30, 2014; Bethesda, Maryland
More information: www.cvent.com/events/17th-annual-conference-on-vaccine-research/event-summary-742976fb42dc43849867074b2754bed7.aspx

MAY 2014

IMV 2014 – Immunopotentiators in Modern Vaccines
May 7-9, 2014; Sao Rafael Atlantic Hotel, Albufeira, Algarve, Portugal
More information: meetings.cshl.edu/meetings/2014/retro14.shtml

Cold Spring Harbor Laboratory - Retroviruses
May 19 - 24, 2014; Cold Spring Harbor, Long Island, NY
More information: www.meetingsmanagement.co.uk/index.php?option=com_content&view=article&id=147&Itemid=306

JUNE 2014

VLPNPV 2014 – Virus-Like Particle & Nano-Particle Vaccines
June 4-6, 2014; Salk Institute, La Jolla, California
More information: www.meetingsmanagement.co.uk/index.php?option=com_content&view=article&id=142&Itemid=275

World Vaccine Congress Asia 2014
June 9-12, 2014; Singapore
More information: www.terrapinn.com/2014/world-vaccine-congress-asia/index.stm

JULY 2014

AIDS 2014
July 20 - 25, 2014; Melbourne, Australia
More information: www.aids2014.org

SEPTEMBER 2014

Cold Spring Harbor Asia Conference on Frontiers of Immunology in Health and Diseases
September 2-6, 2014; Suzhou, China
More information: www.csh-asia.org/2014meetings/immune.html

OCTOBER 2014

HIV Research for Prevention 2014 (HIV R4P)
October 28 - 31, 2014; Cape Town, South Africa
More information: hivr4p.org

For a full list of meetings and their descriptions, go to www.iavireport.org/meetings.