Deadly SYNERGY

A Keystone symposium on TB and HIV emphasized the need to tackle the diseases in tandem

By Andreas von Bubnoff

Although tuberculosis (TB) is thought to be the leading cause of death among people with HIV/AIDS, scientific conferences often focus on these two deadly diseases separately. But this changed recently, when over 300 scientists and clinicians from all over the world gathered in Arusha, Tanzania, from October 20-25 at a Keystone Symposium on "Overcoming the Crisis of TB and AIDS."

TB is responsible for between one-third to a half of AIDS deaths, and at least a quarter of the approximately two million people who died of TB last year were coinfected with HIV, conference co-organizer Anne Goldfeld of Harvard Medical School said in the opening session. "Each infection and its solution cannot be separated from each other," she added. "By bringing together scientists and clinicians who work at the cutting edge of each disease, it's the aim of this conference to serve as a catalyst to generate new ideas and to identify new ways of solving our global humanitarian disaster."

The presentations at the Keystone symposium spanned many topics including the natural history of the two diseases, the mechanism of their synergy, and their treatment and prevention. The meeting's location in Africa was also important to help build partnerships, said co-organizer of the conference Stefan Kaufmann of the Max Planck Institute for infection biology.

A lethal dance

Given that the meeting convened both TB and HIV experts, one central topic was how the two infections synergize in individuals that are coinfected. In such patients, each infection enhances the other infection's pathogenicity: HIV by compromising the immune system, and TB by driving HIV replication.

Goldfeld showed the results of *in vitro* studies that address how TB infection increases viral load of HIV by inducing virus replication. Her data suggest that TB infection in monocyte-derived macrophages directly induces a novel transcription factor that binds to the long terminal repeat enhancer element of HIV, thereby activating HIV replication. This is just one of several transcription factors induced by TB infection that activates HIV replication.

Goldfeld is also involved in the CAMELIA clinical trial in Cambodia that is studying the timing of HIV therapy in coinfected individuals who are already on TB therapy. Starting coinfected people on highly active antiretroviral therapy (HAART) too late results in high mortality because they become too immunosuppressed, but there have also been concerns that starting HAART early might result in adverse drug reactions and interactions. "[There] was a tremendous bias that people couldn't take seven different types of medicines at once," Goldfeld said. "So the recommendation was to wait until after the intensive

phase of TB therapy was finished to initiate HAART."

Another concern is the worsening of TB symptoms in people who start HAART during TB treatment as a result of immune reconstitution inflammatory syndrome (IRIS), an inflammatory disease thought to be associated with the increase in the number of $CD4^+$ T cells that occurs once people start HAART. It typically occurs in 7-34% of coinfected people according to the published literature, six to eight weeks after initiation of HAART, Goldfeld said.

The aim of the CAMELIA trial, which just finished enrolling 661 coinfected volunteers, is to see if early HAART initiation will increase survival in coinfected, immunosuppressed individuals despite the perhaps more complex initial clinical management that might involve dealing with IRIS, Goldfeld said. In the trial, some coinfected volunteers are started on HAART two weeks after starting TB therapy, while others delay initiation of HAART until two months after starting TB therapy. Results of the trial, which evaluates survival one year after initiation of TB therapy, are expected in mid-2010.

Some volunteers enrolled in CAMELIA are also enrolled in another trial called CAPRI-T, which is designed to evaluate whether distinct characteristics of $CD4^+$ T cells are involved in causing IRIS. Investigators are prospectively analyzing blood samples from participants at several time points after they start TB therapy and at the time IRIS occurs, if this condition develops. Results of this trial are also expected in 2010.

Alan Sher, chief of the laboratory of parasitic diseases at the National Institute of Allergy and Infectious Diseases (NIAID), presented data from a study of IRIS in mice. He said that recent studies suggest that IRIS might be caused by an enhanced expansion of TB-specific effector memory $CD4^+$ T cells, although longitudinal studies have called this association into question.

But to his surprise, Sher did not observe expansion of T cells in a mouse model for IRIS. He infected T-cell deficient mice with *Mycobacterium avium*, which causes a TB-like infection in these mice. When given $CD4^+$ T cells from a normal mouse, the mice indeed got a rapid IRIS-like wasting disease and died. But the injected T cells did not expand more in infected mice compared to uninfected mice. Instead, they became more activated. This may induce chemokine production that leads to recruitment of myeloid cells, such as macrophages, from blood into tissues including the lung, where they produce the inflammatory cytokine TNF-α and cause damage.

"[The myeloid] cells are what causes disease," Sher concluded. "T cells themselves don't cause disease." This suggests that the expansion and tissue recruitment of myeloid cells by these activated T cells might also be a potential target for the prevention of IRIS, Sher said, adding that the current treatment with steroids is not ideal.

Insights on HIV transmission

The meeting also featured many presentations that dealt with HIV and TB separately. George Shaw, a professor of medicine and microbiology at the University of Alabama, presented an update on his analyses of tracing clinically productive HIV infections to the transmitted founder viruses that caused them (see *HIV Transmission: The Genetic Bottleneck*, *IAVI Report*, Nov.-Dec. 2008). The additional data confirmed previous results that showed that the majority of heterosexual infections can be traced back to a single transmitted founder virus, while about 20% involve more than one transmitted founder virus. By contrast, more than one transmitted founder virus is evident in about 40% of men who have sex with men (MSM) and in about 60% of injection drug users (IDUs; see *Capsules from Keystone*, *IAVI Report,* March-April 2009).

Shaw reported that the highest number of transmitted founder viruses observed in IDUs is now at least 17. "There [are] so many we can't count [precisely]," he said. He pointed out that in light of the observation that heterosexual infections result in a lower number of transmitted founder viruses than MSM or IDU infections, it was interesting that in the recently published results of the RV144 HIV vaccine trial, the vaccine may have been more effective in subjects at lower risk of HIV infection (*N. Engl. J. Med.* 2009; doi:10.1056/NEJMoa0908492). "It will probably be interesting to [quantify] the numbers of transmitted viruses [in the vaccinees] and their neutralization susceptibility [and] correlate that with the [risk behavior]," Shaw said. "Something might come out."

Since the majority of HIV/AIDS infections are the result of sexual transmission, researchers are also focusing on the role of semen in HIV transmission. Frank Kirchhoff, a professor of virology at the University of Ulm, gave an update on his studies of the enhancing effect of semen on HIV transmission. Previously, he had found that the semen peptide prostatic acidic phosphatase forms amyloid fibrils called semen-derived enhancer of virus infection (SEVI) that can capture HIV virions and promote

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– Anne Goldfeld

[SICK CHIMPS]

Tanzania's national parks were the safari destination for many attendees of the Keystone symposium on "Overcoming the crisis of TB and AIDS" in Arusha. One, Gombe National Park, is also home to the wild-living chimpanzees that Beatrice Hahn, a professor of medicine and microbiology at the University of Alabama, and her colleagues have been studying for more than nine years. At the conference, Hahn presented the results of her studies with chimpanzees infected with simian immunodeficiency virus (SIV)cpz, which is thought to have given rise to HIV-1 group M in humans, which accounts for the majority of HIV-1 infections. She found that wild-living SIVcpz-infected chimpanzees are 10-16 times more likely to die than uninfected chimpanzees (*Nature* 460, 515, 2009). "[There] is no doubt that the infected group died faster," Hahn said. This challenges the prevailing view that all natural SIV infections are non pathogenic (see *SIV May Be Much Younger Than Previously Thought, Research Briefs, IAVI Report*, May-June 2009).

The study also found that SIVcpz-infected females are less likely to give birth and had a higher infant mortality rate than uninfected females. In addition, the spleens and lymph nodes of three infected animals showed CD4+ T-cell depletion, and one female had histopathological signs of end-stage AIDS. The same analysis in sooty mangabeys, which are thought to not get sick from SIVsmm infection, confirmed that indeed, being infected does not affect the lifespan of sooty mangabeys. However, Hahn said there may be other nonhuman primate species that get sick from SIV infection. "I think that gorillas are probably also negatively impacted," she said. "I have no proof [but] if I had to bet I would say yes." *—AvB*

their attachment to target cells. This can enhance the infection rate of HIV in limiting dilution assays by up to 100,000-fold (see *Clues from CROI, IAVI Report,* Jan.-Feb. 2008).

Kirchhoff found that semen itself also increases the infectiousness of HIV by about two- to 50-fold, a variability that was first observed when Kirchhoff compared semen samples from different members of a local soccer team. This variable enhancement correlated with the amount of SEVI. But in contrast to SEVI fibrils, the effect of semen cannot be tested under ideal conditions because it contains cytotoxic compounds, Kirchhoff said. To protect the target cells from the cytotoxic effect, he removes the virus and semen from the target cells after two hours. As a result, however, much of the virus is also removed, which is part of the reason why the enhancing effect of semen is smaller than the effect of isolated SEVI fibrils.

The cytotoxic effect of semen could also explain why a recent study found that sperm appears to inhibit, and not enhance, HIV infection (*FASEB J*. **23**, 3609, 2009). Kirchhoff said that the researchers involved in that study incubated target cells with seminal plasma for one day, long enough for the cells to be harmed. "Some people push the system so that they work at the threshold of cytotoxicity," he said, adding that many common semen treatments such as heating or preincubation of semen with target cells also reduce the ability of semen to enhance HIV infection and do not reflect the *in vivo* situation.

Kirchhoff also presented data suggesting that the presence of semen counteracts the inhibition of HIV transmission by microbicides, potentially eliminating their protective effect. He found that semen generally reduces the efficiency of microbicides and antiretroviral agents. In some cases this means that they are inactive at concentrations applied *in vivo*. But in the case of microbicides containing the CCR5 inhibitor Maraviroc, Kirchhoff said it should still be possible to achieve the effective dose that inhibits HIV even in the presence of semen. "I think Maraviroc is still very promising because it's relatively efficient even in the presence of sperm," he said.

That's good news for Ronald Veazey of Tulane University, who provided an overview of microbicide research. He and his collaborators are currently evaluating a microbicide containing Maraviroc in rhesus macaque experiments. Veazey said that unpublished data suggest that the microbicide shows "remarkable efficacy" in preventing transmission from vaginal challenge with an R5-tropic SIV/HIV hybrid (SHIV) challenge virus in rhesus macaques.

When asked if the effects of sperm should be measured in nonhuman primate experiments testing microbicides, Veazey said it would be difficult to get semen from macaques, and using human semen in macaque experiments would be like "dealing with apples and oranges." Kirchhoff disagreed. "The studies in the monkey models are a model for HIV transmission in humans," he said, "so I think it would actually be good to use human semen because we want to know how human semen affects virus transmission."

Targeting with aptamer-siRNAs

One potential strategy to prevent or treat HIV infection in the future is the use of siRNAs, small RNA molecules that can be designed to inhibit HIV replication by silencing certain genes in HIV or in host cells infected with HIV (see *Interfering with HIV, IAVI Report*, Sept.-Oct. 2006). But introducing siRNAs into HIV target cells is difficult, said Judy Lieberman, a professor of pediatrics at Harvard Medical School, who is developing vaginal microbicides that contain siRNAs to prevent HIV infection. However, she reported that using aptamers could overcome that obstacle. Aptamers are RNA molecules that fold in such a way that they bind to specific target proteins. Lieberman uses aptamers that bind to the CD4 receptor and has fused siRNAs that silence HIV or host cell genes to these aptamers. She said that the resulting aptamer-siRNAs enter HIV target cells such as macrophages and CD4+ T cells, and can inhibit HIV replication in human cervicovaginal tissue isolated from hysterectomy patients. Initial experiments in humanized mice look promising, she added. "At least in a few mice it looks like we are getting gene silencing *in vivo* in the genital tract," she said. "We are very excited about this new approach to microbicides."

Ramesh Akkina, a professor of microbiology, immunology, and pathology at Colorado State University, is also using aptamer-siRNA molecules, but in this case to treat HIV infection. Akkina, along with John Rossi of the Beckman Research Institute of City of Hope, and colleagues are using a gp120 binding aptamer that allows entry into HIV-infected cells. They fused the aptamers to siRNA, which represses HIV replication by silencing the HIV *tat* and *rev* genes (*Nucleic Acids Res.* **37**, 3094, 2009). Akkina said that intravenous injection of these aptamer-siRNAs lowers viral load in HIV-infected humanized Rag-hu mice from 100,000 viral parti-

cles/ml to less than 50 within one week. It also reversed CD4+ T-cell loss. Akkina compared the approach to a guided missile. "The missile head is the aptamer, the real bomb is the [siRNA tail]."

According to Akkina, such targeting of specific cells makes it possible to use 100 times lower siRNA doses than previous approaches using untargeted siRNAs. Akkina said there are plans to do Phase I clinical trials with aptamer-siRNAs as a potential treatment.

Antibody PrEP

A much discussed strategy to prevent HIV transmission is the administration of antiretrovirals to uninfected individuals, an approach known as pre-exposure prophylaxis (PrEP). David Ho, a professor at Rockefeller University and the director of the Aaron Diamond AIDS Research Center in New York City, is developing a novel PrEP strategy which involves infusing people with a monoclonal antibody called Ibalizumab. The antibody binds the CD4 receptor—the primary receptor used by HIV—thereby preventing HIV from infecting CD4+ T cells. This strategy, he said, requires much less frequent dosing than with ARVs and is also typically associated with fewer adverse events. So far, Ibalizumab has been extensively tested in HIV- infected people as a therapeutic agent, Ho said. Trials involving more than 200 HIV-infected people who were largely on salvage therapy regimens have shown that Ibalizumab given intravenously is safe, can decrease viral load by about 1 log, and can increase CD4+ T-cell counts by about 50 cells/ml. "The effect is quite reasonable since it is virtually monotherapy in advanced-stage patients," Ho said. Currently, Ibalizumab treatment is being tested in Phase IIb clinical trials.

But Ho said the antibody should also be tested in healthy, uninfected people to see if it has any utility in HIV prevention. One concern with antibodies that bind to the CD4 receptor is that they could inhibit normal immune function, for which the CD4 receptor is important. But Ibalizumab doesn't seem to interfere with normal immune function, Ho said, probably because it binds to a face of domain 2 of CD4, which is opposite the side of domain 1 where the immune function is carried out. In addition, it is an immunoglobulin (Ig) G4 antibody, which means that it almost has no Fc receptor binding ability and can therefore not recruit immune cells that are active in antibody-dependent cellular cytotoxicity. Because gp120 binds to domain 1, Ibalizumab doesn't directly interfere with gp120 binding, so just how it blocks HIV infection is still unknown.

Ho is now testing the ability of Ibalizumab to prevent infection in monkey experiments and is planning to launch a Phase I study in healthy volunteers. He also plans to further improve the potency and the pharmacokinetic profile of the antibody so that it would only need to be administered every few months, and wants to deliver the DNA encoding the Ibalizumab antibody by using adeno-associated virus as a vector. ■