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Do Clades Matter for HIV Vaccines?

As thousands of people prepare to gather in Nairobi and New York for September's AIDS meetings, a key question for vaccine developers is how to contend with the huge diversity of HIV strains circulating worldwide

BY PATRICIA KAHN

HIV is famously the most genetically diverse viral pathogen known—nowhere more so than in Africa—as well as one of the most rapidly mutating. That, plus the uneven global distribution of its nine genetic subtypes, or clades, poses one of the biggest scientific unknowns facing AIDS vaccine developers: is a single, “universal” vaccine against all strains possible? Or will it be necessary to make a slew of different vaccine formulations, each tailored to the most common strains in a given region? Even worse, could it mean that new formulations might be needed regularly, as with flu vaccines?

The answer will be key to how quickly, and at what cost, an AIDS vaccine can be widely distributed around the globe once a successful candidate is identified. Manufacturing even a single formulation and getting it out quickly to adults and adolescents soon after it is licensed will be far more complicated and costly

than anything the public health field has ever attempted. Doing it with many different formulations, or with repeated updates, would be even more challenging.

Nailing down the impact of HIV diversity on vaccine responses is difficult, for several reasons. One is that the current system for classifying HIV diversity is based on genetic sequence, not immune properties, and hasn't been translated into distinct “immunotypes”—which is what really matters for vaccines. While that task is slowly being tackled for epitopes targeting cellular immunity, it may be impossible for neutralizing antibodies (NAbs), where clades don't seem to correlate with immune recognition.

Another complication is that the clade issue has become highly politicized. Until recently, vaccine development has had a lopsided focus on clade B strains, which dominate the epidemic in industrialized countries but cause only about 12% of infections glob-

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NIH DETAILS PLANS FOR ANALYZING VAXGEN PHASE III DATA

BY EMILY BASS

At the 24 June meeting of the National Institutes of Health (NIH) AIDS Vaccine Research Working Group, Peggy Johnston, head of the NIH AIDS vaccine research effort, outlined a three-pronged plan for involvement of NIH and the Centers for Disease Control (CDC) in VaxGen's completed and ongoing Phase III trials. The plan emerged after VaxGen released data from its US-European Phase III study and made the controversial claim that the vaccine showed efficacy in non-white minority groups.

In the plan's ongoing first phase, an independent team will re-analyze existing data, looking at factors such as race, risk category and the viral strains that infected volunteers. A second team is discussing potential additional analyses of blood samples, such as broader characterization of antibody responses, and HLA typing. The goal of the third component is “to ensure completion” of the ongoing Phase III trial in Thailand, Johnston said. To achieve this, NIH, CDC and an as-yet unnamed donor will work on statistical

analysis of data with Thai investigators and VaxGen.

Johnston said that NIH developed this strategy after VaxGen expressed reluctance to invest additional funds in the Thai trial. At the same meeting, VaxGen representative Marc Gurwith said that the company—which already has many samples from the nearly-completed Thai study—was collaborating fully with the independent data analysis. At press time, precise details of the NIH-initiated consortia, and of VaxGen's role, had not been specified. ♦

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In Memoriam: Balla Musa Silla (1955-2003)

Balla Musa Silla, founding vice-President for Vaccine Preparedness at the International AIDS Vaccine Initiative (IAVI), and a visionary leader in the fields of population and international development, died on 27 June, 2003 at his home in Ossining, New York. He was 48. The cause of death was T-cell lymphoma.

Mr. Silla was born in The Gambia but was truly a citizen of

the world, having lived and worked in over 27 countries. He was a tireless advocate for the needs and potential of the developing world, a theme that runs through his professional and personal life. His career began in The Gambia, first as a volunteer working alongside the international community, and eventually as the government's Director for Population Affairs, where he was a direct advisor to the President.

In 1996, he was approached to lead an ambitious and untested venture, Partners in Population and Development, an intergovernmental alliance of Southern countries addressing issues of family planning and reproductive health through South-to-South collaboration. Partners was a unique organization of Southern governments working closely with each other and with a variety of NGOs and community-based groups toward a shared goal. The pace at which the program grew bore witness to Mr. Silla's deft diplomacy and his unstinting commitment to South-South cooperation. His vision was grounded in the belief that the solutions to development lay in developing countries themselves.

Tall, elegant and well-spoken, Mr. Silla had a stately, captivating presence. A colleague at Partners recalls a plenary speech he once gave. "Unlike the others on the podium, he had no slides; he simply spoke and told the life story of a girl growing up in poverty. The tale flowed naturally and illustrated the realities of injustice and the challenges to development, but on a deeply personal level. He put the sentiment back into that auditorium in Geneva—he brought poverty into the room. Colleagues from different parts of the world thought of their own people. This was his gift."

Mr. Silla joined IAVI in September 2001, where he founded a department tasked with building international support and awareness for AIDS vaccine development and helping to build developing country capacity for clinical testing. Once again, he proved to be equally at home power-brokering with world leaders and listening to the needs and concerns of underserved communities desperately in need of an AIDS vaccine. Most of all, he understood the centrality of AIDS in undermining development, as well as the potential of vaccination to reverse the epidemic's devastation in the world's poor countries.

Mr. Silla left an indelible impression on those who worked with him, and will be remembered for his kindness, generosity of spirit and dynamic personality. "My memories are of a big, strong, proud man, with a wide, mischievous grin, and a gracious manner beyond any I have encountered," says Craig McClure, who worked with him at IAVI on community mobilization. To his former colleagues, he leaves a legacy of commitment to a vision of a better future.

Mr. Silla is survived by his wife, Joan Millsap, and their two sons, Christopher and Andrew.

BONNIE BENDER AND FAWZIA RASHEED

Bonnie Bender, IAVI's Program Manager for Vaccine Preparedness, worked with Mr. Silla during his tenure with the organization. Dr. Fawzia Rasheed was his colleague at Partners, where she served as Senior Advisor on HIV/AIDS & STD and Policy Advisor.

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ally. That disconnect helped mobilize developing countries to get involved with HIV vaccine testing, and spurred development of non-clade B candidates—which now greatly outnumber new clade B-based ones. But it also helped create a political logjam: Fears that testing vaccines based on "unmatched" strains exploits trial volunteers in developing countries sometimes engendered resistance even to early-stage trials of non-local clades, and raised pressures to tailor vaccine candidates to ever-finer, single-country levels.

Yet only by comparing vaccine efficacy in matched settings to partially or completely unmatched ones can the impact of HIV diversity ultimately be resolved. Moreover, "a country-by-country approach to vaccine development would be crippling," says Francine McCutchan (Henry M. Jackson Foundation, Rockville), who leads the US military's HIV global surveillance program. "It would make it very, very slow to get vaccines suitable for some hard-hit regions, espe-

cially in Africa."

But despite these challenges, there are promising developments, along with some sobering ones. A growing body of data shows that immune responses to T-cell-based HIV vaccines, and to natural infection, often recognize HIV proteins from different clades—fueling optimism that, at least for vaccines targeting cellular immunity, some cross-clade protection can be achieved. And studies in infected people are revealing that a few antibodies which neutralize primary HIV strains also work against other clades, findings that are renewing hopes of designing immunogens able to elicit NAbs, a task that has long seemed intractable.

Things are also moving on the political front. "I see a major shift," says Jose Esparza, coordinator of the WHO-UNAIDS HIV Vaccine Initiative, pointing as an example to a consensus document on clades released by the African AIDS Vaccine Programme at its June meeting (see p. 20). "There's now widespread recogni-

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Setting a Scientific Agenda for a House on Fire

AIDS PREVENTION RESEARCH MOVES AHEAD IN RUSSIA

BY EMILY BASS

There's nothing eye-catching about the 8 Plus Clinic. No shingle outside gives away the building's identity; the sole identifier is a small, colorful sign tucked behind the bars that cover all the windows. A muddy yard separates the one-story building from Gazovaya street in St Petersburg, and an improvised stepping stone pathway offers minimal protection from the slippery surface, which is particularly treacherous in late April when the long winter begins to thaw.

But as modest as it looks on the outside, the clinic is a landmark in the Russian AIDS landscape. The clinic—a project of the Biomedical Center, a non-profit research institute led by molecular biologist Andrei Kozlov—is conducting Russia's first prospective incidence study of HIV in intravenous drug users (IDUs). For over a year, the clinic, which is named for the number of required study visits, has tracked the spread of the virus which infects more than 30% of St Petersburg's IDUs. It has also established itself as a trusted source of voluntary counseling and testing, harm reduction and basic medical care to IDUs, who generally risk being reported to the authorities, or arrested, each time they seek treatment. They also face stigma in the general population: St Petersburg residents were so unwilling to have a clinic serving IDUs in their backyards that “it took us a year just to find a building,” says project coordinator Alla Shabolta.

Inside the small building, Kozlov, Shabolta and the clinic team are on the frontlines of an exploding epidemic. Since 1998, the number of reported HIV infections has doubled each year—a frightening, exponential growth curve. Similar crises are building in neighboring countries, including Ukraine (which has the

highest prevalence of any European country), Belarus and, more recently, Kazakhstan.

Today, there are 230,000 documented cases of HIV in Russia—and the actual number is estimated at 800,000-1.5 million. The face of the epidemic is young: 60% of all infections are in 21-40 year olds. Recent data show that increasing numbers of women are being infected, and that rates of heterosexual transmission are on the rise (see box, page 6). These trends are indices of an ominous trajectory: an epidemic spreading from high-risk groups to the general population.

“Recently we attended parliamentary hearings where the Russian authorities all started their speeches by saying, ‘Attention, attention, we are on the edge of a terrible disaster. Our house is on fire,’” says Eduard Karamov, an outspoken virologist at Moscow's Ivanovski Institute for Virology. “I said, ‘Calm down, sit back, the fire started many years ago.’”

Russia is not without resources to combat this new plague. Despite the Perestroika-era brain-drain which emptied entire labs, the country has retained a cadre of highly-trained research and medical professionals. It also possesses a well-established regulatory system for approving trials and products, and internal capacity to manufacture pharmaceuticals and vaccines (see sidebar, page 5). Marshalling these resources into projects like the 8 Plus clinic could benefit Russia—and the world. IDU cohorts are a rarity in most countries with injection-driven epidemics, as are models for IDU-friendly services.

Unfortunately, the clinic is the exception rather than the rule. By all accounts, Russia's steps to combat the epidemic have been ineffective, hampered by underfunding and lack of

political support for rapid, innovative interventions in high risk groups like IDUs and sex workers. President Vladimir Putin has yet to make a public statement calling for a rapid response to the epidemic. Complicating matters, AIDS must compete for attention with other diseases, including hepatitis, multi-drug resistant tuberculosis, alcoholism and heart disease, which have run rampant since the disintegration of Soviet-era medical services.

The lack of political will has financial and policy implications: the country's annual AIDS budget of US\$5.5 million is primarily used for mandatory HIV testing. Active IDUs are ineligible for treatment for HIV or common co-infections such as hepatitis or TB, and drug-replacement programs which swap methadone or buprinorphen for injected drugs are illegal. The HIV care that does exist is handled by centralized Federal AIDS Centers. In the major urban centers of Moscow and St Petersburg, these centers each provide antiretroviral therapy (ART) to fewer than 2,000 people; in the more remote areas, state-of-the-art therapy is simply unheard of.

Against this bleak backdrop there are scattered points of light, mostly in the form of research sites and NGO-initiated projects. International funding supports most, if not all, of the targeted efforts to respond to the Russian epidemic—from needle exchange



The entryway to 8 Plus Clinic.

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clinics to prison-based prevention programs. The 8 Plus Clinic is no exception. The project is supported by the the Biomedical Center (which receives both international and domestic funding) and the HIV Prevention Trials Network (HPTN), partnering with the University of North Carolina. Too small to make a dent, too necessary to be abandoned, these projects beg the question of what internationally-funded AIDS research can accomplish in the absence of political will to create programs for the most affected groups—and what the cost will be if the world fails to find out.

Growing research activity

The question of whether or not research can play a catalytic role in an AIDS response is not unique to Russia. But unlike many other regions of the world where AIDS is spreading unchecked, Russia's pre-existing infrastructure provides a solid foundation for initiating interventions. Several groups are already building on this foundation, including London's Imperial College, Johns Hopkins University, Yale University and the University of North Carolina. Russia has also received roughly \$12 million from the US Biotechnology Engagement Program (BTEP), which aims to convert bioweapons capacity into public health-related research. A small portion of this—less than \$1 million—is earmarked for AIDS vaccine research and development, which is underway at a handful of sites, including the Biomedical Center.

Jonathan Weber (Imperial College, London) is a co-investigator on a Moscow-based study of the long-term impact of short-course ART treatment during acute infection; he hopes to launch a microbicide trial in the Urals. Weber says that his experience with protocol approval, site development and staffing have been uniformly positive. Based on this infrastructure, "Russia is an ideal place to do clinical tri-

als," he says.

Russia has also landed on the international radar screen as a potential site for efficacy trials of AIDS vaccines. One compelling reason: with the exception of Thailand, almost no countries with IDU epidemics have taken steps to develop cohorts or conduct research with these groups. With its mix of sexual and IDU transmission, Russia's epidemic would also make it possible to evaluate whether and how candidate vaccines protect against intravenous compared to sexual exposure (see *IAVI Report*, May-June 2002, p.6). Another key point: for reasons that are still poorly understood, the clade A strain that has spread like wildfire in Russia shows astonishingly little genetic diversity, with an average of just 3% difference among viral isolates from far-flung regions of the country. It's a situation that "eliminates one of the variables [viral diversity] from a vaccine trial," says molecular epidemiologist Francine McCutchan (Henry M. Jackson Foundation, Rockville, Maryland).

In fact, Russian scientists were advocating for AIDS vaccine research before the country's own epidemic exploded. Biomedical Center head Andrei Kozlov, virologist Eduard Karamov, and Igor Sidorovich from Moscow's Institute of Immunology were among those scientists who began lobbying for state-funded AIDS vaccine research in the late 1980s, when the first handful of AIDS cases appeared in Russia. Their call for action drew on the country's deep roots in vaccine production: in its heyday the Soviet Union was a colossus in the vaccine field, manufacturing 1/3 of the global supply of smallpox vaccine used in the eradication campaign, and producing a wide array of human and veterinary vaccines for its sprawling empire.

In spite of this history, Russian officials did not warm to the call. "The logic was like this. First: An AIDS vaccine is impossi-

ble. Second: It's unnecessary, because there is no AIDS problem in Russia. Third: Who are you going to give it to? Do you want to defend the gays and prostitutes?" recalls Sidorovich, a key member of the team which developed the Russian AIDS ELISA diagnostic in the late 1980s. (The diagnostic had to be field-tested in Uganda, because of the low HIV incidence in Russia at the time.)

Ultimately, the scientists won a half-victory. Since 1997 a Ministry of Science and Technologies program to develop new vaccines and diagnostics has included a funding stream for AIDS vaccine development. Originally, the AIDS vaccine development effort were centrally coordinated by the Biomedical Center. Since 2001 it has consisted of three independent teams—each of which received roughly \$200,000 in 2003. One, based at a former bioweapons facility in Novosibirsk, focuses on vector-based strategies, including salmonella constructs. The Biomedical Center is developing a DNA vaccine, and a group at Moscow's Russian Institute of Immunology (led by Igor Sidorovich and including Eduard Karamov) is developing a recombinant vaccine using the team's novel adjuvant, polyoxidonium (PO), which is widely used in a licensed Russian flu vaccine.

The decision to split the program into three separate efforts may make it harder for each groups to assemble all of the elements needed to bring a candidate to trial. Missing pieces at one or more of the sites include access to non-human primate facilities and GLP-compliant vivariums for small animal studies. Where improvements are being made, it is with foreign dollars—for example, BTEP funding is helping to renovate the current vivarium at the Biomedical Center. In spite of these obstacles, the teams project a symbolic readiness—a best effort under difficult circumstances, designed to

show their government, and the world, that there is AIDS vaccine research capacity in Russia.

Stumbling blocks and successes for trials

The combination of passionate local scientists and foreign dollars is promising—but there are still major hurdles to building a comprehensive AIDS research agenda. On a scientific level, one of the biggest stumbling blocks is the lack of sound epidemiological data about the crisis. “There is no history of cohort research in Russia,” says Shaboltsa.

In keeping with Soviet-era approaches, Russia has favored massive, mandatory-testing campaigns over targeted HIV surveillance. Since 1987 Russia has conducted over 200 million HIV tests—an average of 24 million a year—in a relatively random selection of the population, including job applicants, pregnant women, and hospital admissions. Given the many disincentives for IDUs to seek testing, this sampling method may not grasp prevalence in this group, nor can it provide accurate incidence data.

The ongoing HPTN trial at the 8 Plus Clinic will nail down some of this information. After screening nearly 1,000 IDUs—30% of whom proved to be already infected—the clinic is following 520 HIV-negative IDUs for 12 months, with biannual HIV tests. After six months the trial has achieved a retention rate of over 80%. While this is too low for vaccine efficacy trials, which last two to three years, it is a strong start with a population often perceived as difficult to engage in trials. The study’s community advisory board even includes former IDUs—a radical step for the Russian research world.

So far, the study’s findings confirm the catastrophic state of the epidemic. Unofficial interim data analyses show an HIV incidence rate of about 3%. Rough figures also show that the median

Russia’s Current Vaccine Manufacturing Capacity

The demise of the Soviet Union dealt a blow to vaccine producers, who had to adjust to the loss of state subsidies and purchase guarantees, and to the introduction of competition from foreign vaccine manufacturers. Today, Russian facilities—many of which were built between the 1950s and the 1980s—are seeking to catch up and to begin competing in the global market. A recent government law set a 2005 deadline for bringing all plants up to international GMP (Good Manufacturing Practice) standards for production practices, although Russian and international experts say that many many plants will not make this deadline. Currently the GMP status of Russian production facilities varies considerably even within the same organization, ranging from WHO-accredited, to nearly-compliant, to facilities which are at least a decade away from GMP status. Biomed (Moscow Region) and Immunopreparat have at least one production line accredited by WHO.

There are signs that the industry is adjusting to the new conditions. Russian vaccine manufacturers are beginning to apply new production technologies

and invest some of their returns into research and development. International groups including the WHO and the International Science and Technology Center (Moscow) are providing oversight in quality control training and funds for equipment upgrades. Furthermore, Russian producers are showing increased concern that there is a domestic vaccine available to the population for each illness.

Strengthening domestic production also has public health implications. While coverage with locally-produced measles and mumps vaccines exceeds 90%, rubella coverage “is low because there is no domestic vaccine,” says health ministry official Galina Lazikova.

These developments are being closely watched by some AIDS vaccine researchers. “Russia may well emerge as an important player in AIDS vaccine R&D. When an AIDS vaccine is finally licensed, Russia could play a key role in manufacturing for many parts of the world,” says Don Burke, head of the Center for Immunization Research at Johns Hopkins University.

EMILY LOCKE

MAJOR VACCINE PRODUCTION FACILITIES IN RUSSIA

| Company (location); State or private | Vaccines produced annually | Approximate # doses | Upcoming products |
|---|--|--|---|
| Biomed (Moscow region) Private | HBV, DIP, TET, DT, DTP | 10-15 million | |
| Biomed (Perm) State | DIP, TET, HBV, DT, TYP | Unknown | |
| Combiotech (Moscow) Private | HBV, DIP-TET-HBV, DIP-PER-TET-HBV, HBV (preservative free) | 5 million | HAV-HBV, DPT-HBV-HIB |
| Immunopreparat (Ufa) State | DIP, TET, DIP-TT, DPT, INF, RAB | Unknown | |
| Institute of Poliomyelitis and Viral Encephalitis (Moscow region) State | OPV, TBE, RAB, YEL | 100-150 million | |
| Moscow Enterprise of Bacterial Preparations/Institute for Viral Preparations (Moscow) State | MEA, MUM | Previously produced 100 million, expected to produce this amount after production unit is certified. | RUB, MMR |
| St. Petersburg Institute of Vaccines and Serums, Krasnoe Selo (St Petersburg) State | INF, DIP, TYP, | 5-6 million | |
| Vector (Koltsovo) State | HAV, MEA | 100,000 | HAV-HBV (collaborating with Combiotech) |
| Virion (Tomsk) State | HBV, TBE | Unknown | |

DIP: diphtheria; DTP: diphtheria-tetanus-pertussis; HAV: hepatitis A virus; HBV: hepatitis B virus; INF: influenza; MEA: measles; MUM: mumps; OPV: oral polio vaccine; PER: pertussis; RAB: rabies; RUB: rubella; TBE: tick borne encephalitis; TYP: typhoid; TT: tetanus toxoid; YEL: yellow fever.

Sources: <http://www.privivka.ru/vaccines/complete.htm>, data as of July 3 2003; and personal communication with institute representatives.

Emily Locke PhD, MPH (Johns Hopkins University) is based in St Petersburg, Russia, where she is researching Russian vaccine development and production capacity.

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age is 24 years; over 60% have secondary education; 40% are employed full time; 62% live with their parents; 11% own a home.

Another cohort initiative is underway in Moscow, where epidemiologists Chris Beyrer, Julie Stachowiak (Johns Hopkins University) and the NGO AIDSInfoshare (which Stachowiak co-founded) are building a sex worker cohort. After a series of thwarted attempts to engage the Moscow health system, Beyrer and Stachowski decided to train sex workers and AIDSInfoshare staff (many of whom have medical training) as lay epidemiologists. They have completed a qualitative phase and plan to launch a 500-woman cohort in late 2003.

Once cohorts are formed there are other hurdles, such as laws that constrain provision of treatment (including ART and

names—and so are unable to report individuals' HIV status or drug-use to the authorities.

These regulations also curtail the treatment that can be provided by research sites. Russia maintains a centralized surveillance and treatment system for diseases such as hepatitis B and C, which are endemic among drug users. As with HIV treatment, active drug users are ineligible for subsidized care, which is often minimal. 8 Plus project physician Natalya Khaldeeva would like to treat her patients—nearly all of whom have hepatitis B or C—and the site's funds might be able to pay for it, but this offering is not feasible under current laws.

The laws constraining treatment of active IDUs would also complicate large-scale vaccine trials, given the growing international consensus that volunteers who become infected should have access to ART. But, Beyrer says, research could help leverage change. "These trials are a long way off. We should proceed with all due speed. If it turned out that the law was the primary obstacle, then Russia might look at ways to deal with it," he says.

A lack of central coordination

Perhaps the greatest unknown is whether research findings will affect government policies. Without state implementation of key findings, research projects will only impact a fraction of the population. Until recently there was no government monitoring to learn about successful interventions or to identify the optimal use of international dollars flowing into research and programs. In June the government formed an AIDS Advisory Council to provide this guidance, but it remains to be seen whether this council will make policy recommendations which counter the existing government positions.

Observers are cautiously optimistic. The advisory council is "a very positive sign," says Pedro Chequer, head of the

UNAIDS program in Russia, adding that "It looks like the country is being divided between international donors, without government coordination."

As small as it is, the domestic AIDS vaccine effort has already suffered from the historic lack of coordination. The program has no provision for media outreach or public education, or for site preparedness activities. For now scientists shoulder these roles—with mixed results. In 2002, when the Moscow team from the state-funded AIDS vaccine development program submitted a trial application (which is still pending) for its candidate, team scientist Irina Nikolaeva wrote a press release. Nevertheless, the media carried erroneous reports that Russia had discovered an AIDS vaccine.

To address this, the WHO-UNAIDS HIV/AIDS Vaccine Initiative has proposed consensus-building activities to the major Russian stakeholders. A plan has yet to emerge—a delay which may reflect the fact that the different teams work, by all accounts, more or less independently of one another.

On the positive side, public statements by Vadim Pokrovksi, the head of Russia's Federal AIDS Centers, led to a recent flurry of media attention; Russian scientists also report growing support from individual politicians. Regionally, there is a growing AIDS activist movement, which recently held a summit on treatment access in Minsk.

Whether these elements will coalesce into a comprehensive response remains to be seen. "If we look at the democracy which has emerged in Russia, it's good for people and even better for viruses," says Igor Sidorovich. "Perestroika led to the destruction of state systems, which are only now being rebuilt. Let's hope Russia is open not only to infection but to valuable technology and ideas. This is why our team has remained here. We hope we survive." ♦

RUSSIA'S HEALTH CRISIS

- **Estimated 2-4 million intravenous drug users (IDUs)—up to 2.7% of the population. In some regions, more than 60% of IDUs are HIV-positive**
- **22-fold increase in HIV prevalence among pregnant women from 1998 to 2002**
- **More than half of registered HIV-positive individuals are co-infected with hepatitis C virus**
- **Roughly 40% of HIV-positive people are co-infected with TB**
- **1 in 10 prisoners have TB, 1/3 of them multi-drug resistant TB**
- **Since 1965, the average Russian life expectancy has dropped from more than 67 years to 65 years**

hepatitis medications) to active drug users and other marginalized groups. The 8 Plus Clinic has had to walk a fine line between complying with state regulations and addressing volunteers' well-founded fears about the legal repercussions of seeking care. In the ongoing trial, for example, volunteers are known solely by their numerical identifiers. Doctors never learn their patients'

Keystone Symposium: Updates on Trials, New Candidates and Immune Basis of Protection

BY MARK BOAZ

Continuing our report of April's Keystone Symposium on HIV Vaccine Development, one of the field's premiere research conferences, we cover an array of talks ranging from animal models to ongoing clinical trials (see also *IAVI Report* Apr-May 2003, p.1). Although there were no major surprises or new findings announced, the meeting gave a good overview of the broad efforts underway on many different fronts.

Update on human clinical trials

Updates from ongoing vaccine trials and from monkey studies of candidates due to enter Phase I studies soon, featured heavily on the conference agenda. Since last year's Keystone conference, four completely new vaccines have entered Phase I trials and several other Phase I and I/II studies were launched. (See database at www.iavi.org/trialsdb).

DNA candidates from the Vaccine Research Center

Barney Graham, who directs clinical studies at the NIH Vaccine Research Center (VRC; Bethesda), discussed early results from Phase I trials of the Center's new DNA vaccines. These constructs are the first step in a multi-clade, prime-boost strategy that will use adenovirus-based vaccines as a boost and incorporate *env* from three different subtypes.

The first VRC trial, started in late 2001, tested DNA containing HIV *gag* and *pol* from subtype B at three doses (0.5 mg, 1.5 mg or 4 mg). Immune responses were determined by measuring interferon-(IFN)-gamma-producing cells using intracytoplasmic cytokine staining (ICS) assays. Each group had seven volunteers, two of whom received placebo. Graham presented unblinded data on responses after the second of three vaccinations.

CD4 T-cell responses were present in 4/7 individuals in the lowest-dose group, mainly to Gag but also to Pol, and Gag-specific CD8 cells were found in two of these volunteers. The 1.5 mg dose led to CD4 and CD8 responses in 2/7 volunteers. No data is available yet for the 4 mg group.

Graham also presented early results from the more recently launched trial of multi-clade DNA containing *gag*, *pol*, and *nef* from subtype B and *env* from subtypes A, B and C. Groups again consisted of seven volunteers with two receiving placebo. After two of three scheduled vaccinations, 3/7 volunteers in the 2 mg dose group had HIV-specific CD8 cells, and two of these three volunteers also had CD4 T-cell responses.

An important question for vaccines with multiple antigens is whether responses are reduced compared to single antigen vaccines. Another is whether using 3 different Env antigens induces a broader

response than a single Env protein. Graham said that macaque data showed no reduction in Gag responses when Env antigens are incorporated, and that data on the breadth of Env responses may be available by the September AIDS Vaccine 2003 meeting in New York.

High-dose canarypox study

Juliana McElrath (University of Washington, Seattle) presented data from HVTN 039, which tested a well-studied canarypox-based vaccine (ALVAC vCP1452) given at the most commonly-used dose and at one higher dose ($10^{7.26}$ and 10^8 TCID₅₀). McElrath said that the immune responses to both doses, assessed using the Elispot assay to detect IFN-gamma-producing cells, were indistinguishable but low (between 40 and 70 spot-forming cells per million white blood cells), leading McElrath to conclude that use of the higher dose was not warranted. Some cross clade responses were seen to Gag, with three of the seven people who responded to subtype B Gag also responding to subtype C.

Preclinical studies in monkeys

Bob Johnston from the University of North Carolina gave an update on the development of HIV vaccines using vectors of Venezuelan Equine Encephalitis (VEE) virus. One potential advantage of this approach is that VEE targets immature dendritic cells, which play a key role in presenting antigens to the immune system. Together with the biotech company AlphaVax, Johnston's team has engineered a replication-incompetent vaccine vector encoding HIV clade C *gag*, which recently received approval from South Africa's regulatory authorities for a Phase I study. The trial will take place in Johannesburg and four US sites of the US HIV Vaccine Trials Network (see *Vaccine Briefs*, p. 20).

Johnston described results showing partial protection against disease in two studies of vaccinated macaques. In one, 2/6 macaques vaccinated with VEE plus *env* (gp160 and gp140) completely controlled an intrarectal challenge with SIVE660, one of the pathogenic SIV strains; the remaining four animals showed partial control. In contrast, only 1/6 unvaccinated monkeys partially controlled the challenge, while the rest showed no control. Both CD8 and neutralizing antibody responses were associated with protection.

In the second study, eight macaques were vaccinated with VEE carrying *env* (gp160), *gag* and *pol*, and then challenged intravenously with SHIV89.6P. Vaccinated monkeys showed some protection from disease compared to controls, with peak viral load lowered about 10-fold and a 100-fold reduction in set-

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point. The correlates of protection were less clear in this study, since pre-challenge CD8 T-cell responses were only detectable in 2/8 monkeys and no neutralizing antibodies were found—although Johnston said this may reflect differences between the different challenge viruses used in these studies.

Adeno-Associated Virus (AAV)

Phil Johnson (Children's Research Institute, Cincinnati) reviewed some new data on an HIV vaccine using vectors of adeno-associated virus (AAV), focusing on recent safety studies. A version of this vaccine containing *gag* from clade C is slated for human trials starting in late 2003.

Johnson presented data from animal toxicology and biodistribution studies, which are required for regulatory approval of clinical trials. No local or systemic reactions to the vaccine were seen in a 150-rabbit toxicology study, and analysis showed only trace amounts of vaccine present in about 15% of the tissues sampled at 180 days—mostly in the muscle at the injection site or in other highly perfused tissues. Importantly, no trace of vaccine was found in the gonads, indicating that vector is not transmitted into the genome of offspring. Overall, this safety profile is similar to that seen with DNA vaccines. Two immunized macaques sacrificed at 5 months also showed very low persistence of vector.

These studies also confirmed earlier findings that the AAV vector does not integrate into cellular DNA (*J. Virol* 2003;77:3495). Integration of the unmodified AAV has been seen in cultured cells and initially raised safety concerns about this approach.

Viral escape: Can it generate "immune-resistant" strains?

David Watkins described a recent monkey study which looked at some possible consequences of viral escape in a vaccinated population.

In infected macaques and in some vaccination studies, SIV and SHIV undergo mutations which enable them to evade the CD8 responses that normally control viremia. Escape also occurs during HIV infection, raising the possibility that T-cell-based vaccines—which allow infection but prevent replication—may lead to the selection of mutated viral strains lacking crucial CD8 epitopes, and which might then spread through populations unchecked by immune control.

To test this scenario, graduate student Tom Friedrich created a strain of SIVmac239 with mutations in each of the three most important epitopes (in the Gag, Tat and Nef proteins) targeted by CD8 T-cells in monkeys of a specific genetic background (MamuA*01 and B17). Four rhesus macaque monkeys with this background, and 2 without (so they target different epitopes) were then infected with the mutant escape strain, which the researchers called 3xSIV.

Watkins said that, as expected, the MamuA*01/B17-

positive animals generally failed to control replication of the escaped strain and showed no CD8 responses to the mutated epitopes. But surprisingly, in both of the monkeys negative for MamuA*01 and B17—where there is no immune pressure against these epitopes—the Gag and Nef epitopes reverted back towards their original sequence, suggesting that the escape mutations reduce viral fitness. Watkins called this surprising finding a “saving grace” for CTL-based HIV vaccines, since it suggests that even when escape occurs, the escaped strains are unlikely to sweep through human populations, which are genetically diverse; rather, important CD8 epitopes will probably be regenerated, thanks to the higher fitness they confer.

Correlates of protection

Identifying the specific immune responses which control HIV infection has long been a central issue for HIV vaccines, since this knowledge would greatly simplify the tasks of designing and testing candidates. In Banff, several speakers reported on studies that tackled this problem from different angles.

Jeff Lifson (National Cancer Institute) gave an interesting talk describing examples of control of SIV infection, whether achieved through early antiretroviral treatment (ART), vaccination, or spontaneously, and the associated immune responses. One SIV-naïve macaque inoculated with the virulent SIVmac239 controlled viral replication following the initial peak to less than 20 copies/ml of plasma. CD8 responses seemed important in this control as these increased progressively over time and the viral load rebounded upon depletion of CD8+ cells. Of note, Elispot responses in this animal during control of SIVmac239 infection were lower than levels seen in other animals that were unable to control this same virus in other vaccine studies. Neutralizing antibodies were low through the period of initial control, but were present at high levels as the animal re-established viral control after the depletion of CD8+ lymphocytes. Conversely, a vaccinated animal controlling virus to <20 copies had detectable low-level Elispot responses, yet upon CD8 depletion, no rebound of virus was seen. A greater role may have been played in this animal by antibodies, which were detectable all the way through.

In a different setting, a macaque which controlled SIVmacE660 infection following limited early ART also had modest levels of Elispot responses, but was able to control a heterologous challenge with SIVmac239. Lifson concluded that qualitative characteristics rather than the level of the immune response were likely important in these animals, since low-level responses were associated with control of viremia. Early control probably also limited virus diversification, thereby facilitating sustained immune control.

In a talk on CTL-based vaccines, Andrew McMichael (Oxford University) emphasized the importance of looking at memory cells in vaccinated

“Viral escape in monkeys is found to lower fitness—a “saving grace” for CTL-based vaccines?”

individuals, rather than at only the more short-term post-vaccination responses. He pointed out that CD8 memory responses in EBV-infected people do not strictly reflect the responses seen shortly after infection due to a change in immunodominance: whilst directed at a similar range of epitopes, cells that showed smaller responses after the initial infection come to dominate the memory response, whereas those that were initially dominant decrease proportionally (*J. Immunol* 2002;168:3309). Since vaccinees are most likely to be exposed to virus when their immune responses are in the memory phase, he suggested it would be important to study these responses. McMichael described several different assays that can be used to measure memory responses, including Elispot and ICS to detect cytokines and cell markers, and killing assays using cultured cells. He said the ICS tetramer assay and ICS cell proliferation assay (the CFSE assay) would probably be the best ways to quantitate long-term memory.

Measurement of immune responses

Current measures used by researchers to determine the level of HIV specific CD4 and CD8 T-cell responses focus mainly on production of IFN-gamma, a cytokine with antiviral activity that is relatively easy to detect by Elispot or ICS assays. However, some studies presented at Keystone add to the growing evidence indicating that this measurement is not sufficient to identify all responding cells.

For example, data presented by Michael Betts (VRC, Bethesda, Maryland) showed that not all cells which kill virally-infected cells produce IFN-gamma. To do this, he used flow cytometry to examine CD8 T-cells which degranulate (by measuring the CD8 cell surface degranulation markers CD107a and b), the main mechanism by which they kill virally infected cells, in addition to IFN-gamma. These results suggest that both the quality and quantity of HIV-specific CD8 T-cell responses determined by IFN-gamma measurement may be underestimated.

The magnitude of CD4 T-cell immune responses also appears to be underestimated by the measurement of IFN-gamma alone. Stephen De la Rosa (VRC) illustrated this point with data on 9 individuals who received booster shots of either tetanus toxoid (TT) or Hepatitis B vaccine. For the HepB-specific CD4 T-cells, and to a lesser extent the TT-specific cells, IL-2 and MIP-1 beta production were the predominant cytokine responses, with IFN-gamma-producing cells accounting for as little as 25% of the responding CD4 cells.

Other data suggested that the methods used to stimulate HIV-specific cells so they can be measured are also part of the problem. Normally, researchers stimulate cells with consensus or reference virus strains (or peptides) that are representative of circulating viruses. However, a poster by Marylyn Addo and Marcus Altfeld from Bruce Walker's group (Massachusetts General Hospital, Boston) suggests

that this method may underestimate response levels, especially to the more variable HIV proteins.

The researchers analyzed CD8 responses in 6 acutely infected patients using sequences based on consensus virus or patients own (autologous) virus to the p24, Tat and Vpr proteins. The breadth and sum of CD8 responses to Tat and Vpr were underestimated when consensus sequence was used rather than autologous sequence, with 8/24 peptides not being recognized with consensus sequence and an average magnitude of 179 SFC versus 514 SFC. In contrast, a slight underestimation of CD8 responses to p24 were seen when using consensus sequence compared to autologous sequences, with 4/18 peptide responses missed using consensus and a magnitude of 328 SFC versus 464 SFC. The responses to consensus and autologous sequences differed more to Tat and Vpr than p24 because the virus was more different in these regions—approximately 10% in Tat and Vpr compared to only 1.8% in p24.

Altfeld said that underestimation of responses to the more variable HIV proteins may be a problem that plagues research if autologous sequence is not used for stimulation. (This was recently published in *J. Virol* 2003;77;7330.)

HIV transmission and envelope protein selection

From the swarm of different virus strains present in infected individuals, only a small subset is transmitted sexually. This partly reflects the selection of viruses that use CCR5 as a co-receptor to enter cells, but other viral properties also appear to play a role. Since it is these newly-transmitted strains which vaccinees must initially combat, a fuller characterization could be enormously useful for vaccine design.

Cynthia Derdeyn (University of Alabama) gave a thought-provoking presentation on an analysis of Env proteins in eight transmission pairs, drawn from a serodiscordant couples cohort in Zambia. In addition to sequencing *env* genes from 8 newly infected people and their partners, the researchers also measured the sensitivity of transmitted virus to neutralization by antibodies present in the donor's plasma.

Derdeyn reported that the transmitted *env* sequences were all homogenous in the V1-V4 region, regardless of the complexity of viral strains present in the donor. Interestingly, they also showed a compact variable loop structure, suggesting that variants with larger V1-V2 and V4 loops were selected against during transmission, as were heavily glycosylated strains. Recipient strains also proved to be roughly seven times more sensitive to neutralization with donor plasma than the donor's viral population at large, indicating that strains which escaped neutralization are *not* the source of transmitted virus.

Further understanding of exactly what is transmitted should come from analysis of newly-infected individuals in the VaxGen trial, where over 300 full envelope genes have been sequenced. ♦

AIDS Vaccines, From Monkeys to People

AN INTERVIEW WITH

For many years, John Shiver, Merck's Executive Director of Viral Vaccine Research, has been a regular speaker at major conferences on HIV vaccines. With 11 clinical trials in progress and an extensive pre-clinical program for testing candidate vaccines in monkeys, Merck's program ranks among the major HIV vaccine development efforts.

Shiver, who earned a Ph.D. in chemistry and then trained in immunology at the National Institutes of Health, has been at

Merck since 1991. He now oversees not only the company's HIV vaccine research activities but also its work on vaccines against other viral pathogens.

The previous IAVI Report [Feb-Apr 2003] reviewed Merck's clinical program on HIV-1 vaccines. Here, Shiver speaks with IAVI Report editor Patricia Kahn about the company's newly-launched international trial and on lessons learned along the way about using the rhesus macaque model for AIDS vaccine development.

John Shiver



Can you tell us about the international trial just launched with one of your vaccines?

The trial will enroll 435 volunteers at sites in the US, Puerto Rico, Brazil, Peru, Haiti, Thailand, South Africa and Malawi, which are part of the HIV Vaccine Trials Network. We're testing a "proof-of-concept" vaccine containing the HIV-1 *gag* gene in an adenovirus vector. The vaccine is already being studied in several hundred North American volunteers, so we know it looks safe and can induce good immune responses. This trial will gather more safety and immunogenicity data in people of diverse genetic backgrounds.

What new information do you expect to get by scaling up the numbers and diversity of volunteers?

The key question is whether people of diverse genetic backgrounds respond equally well to the vaccine—even though they may target different epitopes. We'll also analyze how well the vaccine-induced responses in these populations recognize HIV-1 antigens based on different clades, as we're doing in the North American volunteers. [For further discussion of vaccines and HIV clades, see article, p.1.] And we'll continue exploring the effect of pre-existing immunity to the adenovirus vector.

Your newer studies all involve adeno-based vaccines. Are these now your main focus?

We already know a lot about our adeno-based candidates, and see many advantages: immunological, convenience of construction, and so on. But we're taking a broad approach to viral vectors, exemplified by two studies we presented at the recent Keystone meeting. One is the testing of three different poxviruses in prime-boost combinations with adeno. The other is our work on different adenovirus serotypes, which we'll also test in various prime-boost combinations.

Our goal is to collect a set of vaccine tools and analyze each one alone and in combination. Right now, pre-clinical data suggest that prime-boost combinations of adenoviruses and poxviruses, or of different adenovirus serotypes, are our most promising vaccine

tools for inducing cellular immunity. The next step—finding the best combinations—is unpredictable: you still have to rely basically on intuition and the empiricism of seeing what happens.

So it will take a while to figure out what our best options are. But we'll make these decisions based on a substantial body of clinical data.

You mentioned genetic diversity of human populations as one rationale for the international trial. At Keystone you presented some monkey data relevant to genetic differences and vaccine protection. Can you describe these findings?

We're trying to develop a broad perspective about our challenge studies, by looking at choices like which monkeys or challenge viruses to use, and then seeing what differences result from this choice.

Our earlier studies on adeno-based vaccines used animals harboring the MamuA*01 MHC class I allele [a genetic marker associated with greater natural ability to control SIV and SHIV replication] and challenged with SHIV89.6P. These animals have controlled viremia to undetectable levels, with detectability being about 50 virus copies per ml of plasma, and stayed there so far for 2-3 years. When we induced stronger immune responses with a really good DNA prime plus adjuvant, and an adenovirus boost, it didn't improve on this—we couldn't crimp the primary viremia further, and chronic viral load can't go below undetectable. All animals showed a strongly dominant response to the Gag CM9 epitope, although they also responded to other epitopes.

The new experiments tested different adeno-based vaccines against the same challenge strain, in monkeys with genetic backgrounds that don't give immunodominant responses to Gag [MamuA*01-negative]. We found that vaccination lowered viral set point 100-fold or more compared with unvaccinated animals. That's a significant drop. But the load was still a few thousand copies, which is a lot more than 50.

This tells us that strong immunodominance for one Gag epitope confers a big advantage against SHIV challenge. But even in animals without this advantage, two or three orders of magnitude is still a lot of control—actually, an outcome many people would probably be satisfied to see in a human study. Think about it:

if we start with a setpoint of 30,000, which is what's found in untreated people in the MACS cohort [a long-term, prospective US-based cohort of HIV-positive men] and drop it by two logs, we're in the hundreds. That should mean a much better clinical prognosis.

These results also tell us that SHIV is a more stringent challenge in MamuA*01-negative monkeys than in positive animals—another indication that genetic background is important.

What else have you gleaned about genetic background and control of viremia?

We've seen that even without a vaccine, MamuA*01-negative animals tend to have a worse clinical outcome after challenge than the A*01-positives. They advance to AIDS much more rapidly.

Another observation is that unvaccinated animals occasionally control virus spontaneously. I've seen this with SIV, and even more with SHIV—we see about 1 in 5 or 6 animals which do that, regardless of their MHC background. I'm not sure why. But it shows that there's a lot going on in individuals that determines disease course, whether they're monkeys or people.

That's why human studies need to look at a lot of diversity in volunteers, in terms of their genetic backgrounds, to really get a sense of how effective a vaccine will be. And it's why international studies are so crucial—to start getting a handle on this.

You've also looked at different challenge viruses. What have you learned?

At Keystone, we showed data on SIVmac239, which is more pathogenic than SHIV, in both MamuA*01-positive and -negative animals challenged intrarectally. The question was whether vaccines that controlled SHIV89.6P would give at least partial control against this challenge.

Only one group did—the one given a DNA-prime, adeno boost, which also gave the best T-cell responses. The relative control of viremia isn't necessarily due to these specific vaccines, but might happen with any vaccines that get T-cell responses to this higher level. In the MamuA*01-positive animals, DNA/adeno reduced SIV loads 10-30-fold below the control levels, at least out to 260 days. We still need to follow this longer—it's a young study.

That's more than what many studies find with SIV, although still far from full protection. But is a challenge virus like SIVmac239 perhaps too stringent?

I don't know. Well, yes, I know one thing. Both SHIV and SIV give extremely high viremia in macaques—between 100,000 and over one million viral copies of virus per cc of plasma within a few weeks of challenge. They maintain this level for months, until AIDS-associated pathogenesis sets in. Clearly, that's much more viremia, and faster pathogenesis, than HIV-1 causes in people, where median viral setpoint is about 30,000.

One consequence is that, so far, it takes two different types of vaccines to control SIV viremia in monkeys. But we *are* seeing successes in monkeys, so there's a strong rationale for moving these approaches into people.

To me the value of these challenge systems—even if the disease they cause is exaggerated compared to humans—is that they help us discriminate among candidates. If no vaccines impacted viremia or disease at all, we couldn't learn anything about what to test in humans. But we do have vaccines that control SHIV or SIV. So our foot is in the door. The task is to use animals for defining which combinations of vectors and antigens open the door further, and then to translate this experience into human trials.

But I wouldn't say that a particular vaccine approach is the one thing to move into people because it's so great in monkeys. Instead, you use the animals to make a short list of what to take forward into people.

Given all these variables, what challenge model will you use from now on?

I'll use both SIV and SHIV, and probably stick to MamuA*01-negative animals for SHIV. I don't think there's any further room to mine for control of SHIV with these vaccines in MamuA*01-positives.

You mentioned that your successfully vaccinated monkeys are still protected 2-3 years after challenge, with no sign of viral escape. That's especially surprising since your vaccines carry only one HIV gene. Why does virus escape in some studies but not others?

This is an important point. Our approach, which is based on our experience with anti-retroviral drugs, is that you need strong immune pressure against the virus. You need it as quickly possible, and it needs to be fairly diverse. Even though our adenovirus carries only *gag*, it's very potent in monkeys—the magnitude of T-cell responses is strong prior to challenge, and it tends to recognize multiple Gag epitopes.

I collaborated on the DNA vaccine studies presented at Keystone by Dan Barouch and Norman Letvin [*reporting viral escape for several animals in two challenge studies*]. Those are early vaccines developed in my group, but that elicit relatively weak immune responses. And where responses are weak, they also tend to be narrow. Less immune pressure on the virus means greater potential for escape.

That doesn't mean escape can't happen where responses are stronger and broader. But it stands to reason—and our data bear it out—that better containment of HIV is much more likely when there's a stronger immune response from the start.

By now Merck has tested certain approaches in both monkeys and humans. How well did monkeys predict the human results?

continued on 12 ►

With adenovirus, many of our findings are very similar. You get the same magnitude of responses in people and in monkeys, the same proportions of CD8 and CD4 contributions, the same durability of responses and diversity of epitopes. In terms of dose-response, responses to adenoviruses in people exceed what we saw in monkeys, at least in people without pre-existing antibodies to Ad5.

With DNA, immunogenicity decreased as we moved from mice into bigger rodents like rats or guinea pigs, and then into monkeys. It took more immunizations and more DNA, and the responses became lower. But we still came up with a dose and regimen in monkeys that consistently primed T-cell responses. Even with DNA in saline, nearly every monkey makes a sustained T-cell response, and the CRL1005 adjuvant increased responses by about 5-fold. But in people, that trend falls off: our best DNA approach gives responses in only 40% of the volunteers; they're not very durable, and don't synergize well with adenovirus vaccine. The synergy between vaccines was there, though, from the animal work.

These results also suggest another conclusion: cytokines and adjuvants generally don't translate well from monkeys into humans.

So would you say that non-human primate models can help identify the best approaches, but not the best dosage or immunization regimens?

In general, yes, but may depend on the animal species and the specific vaccine. For example, we've done some testing of DNA vaccines in baboons, and found that their dose response, and the impact of adjuvants, were close to what we saw in people.

So you could optimize your immunization regimens in baboons after macaque studies identify the best overall candidates?

The thought frequently occurs to me that, yes, I'd like to see responses in baboons with a particular regimen. But these are large animals—30 or 40 kilos, not 3 or 4 kilos like macaques, so they're much more difficult and expensive to work with. And our list of candidates is short enough to be manageable for clinical testing. Besides, you can only do so much, and clinical studies are our top priority right now.

What are your criteria for deciding which approaches to move into Phase I testing?

There are lots of things. We're working to generate complete datasets, characterizing and comparing vaccines in terms of T-cell responses—overall magnitude, CD4 versus CD8 contributions, how many epitopes they recognize, and of course their ability to control viremia after challenge. When you do the science right, the answers are actually pretty clear as to which vaccines look better.

Beyond how well the vaccine performs, a particular vaccine may be more attractive for development based on pragmatic things. Can we demonstrate pre-

clinical safety and create the right type of information for FDA review? How well can we make the vaccine?

In evaluating T-cell responses, most groups rely on assays that detect interferon-gamma. Are you satisfied that this detects the right cells?

I would not accept any single immunological parameter as the gatekeeper for clinical trials. We assess immune function based on many types of data. Besides interferon-gamma, we measure cytokines like IL-2 and TNF-alpha. We look at CD4 and CD8 contributions. We have a highly validated cytotoxicity assay, which correlates strongly with interferon-gamma responses for CD8 cells. Tetramer assays are great because they provide a reference point, an upper limit on the frequencies of antigen-specific cells. And we look at cellular phenotypes: Do the responding cells look more like activated or resting cells? Are they en route to a lymph node, or emerging from one?

It's not clear what all these data mean. But they help you stay broad-minded about decisions, and to remember that you don't really know what you're doing.

That said, when I look for a common thread in our challenge studies, I have to say that it's the magnitude and diversity of CD8 T-cell responses.

We can't define a cutoff where you know that above this level, every animal will control virus. The numbers are too limited. But at this point, it looks like the stronger the response and the more epitopes it targets, the more likely an animal will control virus, at least for some time. Maybe we can still improve our predictions by looking at subsets of these determinants. But I think we're looking in the right direction.

Why is it so hard to find definitive correlates of protection in monkeys?

I think there *are* indications of correlates, in terms of trends. But it's hard to nail down more precisely without lots of data, which in turn means lots of monkeys. And you'll never have enough monkeys with the types of determinants we're looking at. The correlate will probably be something like a probability of controlling viremia as a composite of immune response strength, diversity, CD4-CD8 contributions and ability to establish memory. Given the complexities of multi-component correlates, you won't resolve this without a Phase III study in people. It's not like hepatitis B vaccine, where if you get to a specific antibody titer, you're considered protected.

Is Merck working on neutralizing antibodies?

We've had our share of negative data. But we kept an active effort in this area, and showed some results in posters at Keystone and in publications over the last year. These efforts are mostly directed at gp41 and gp120-CD4 complexes. So far we haven't been able to define an interesting immunogen based on these proteins, but there are still some approaches to try.

MACAQUE STUDIES TO OPTIMIZE VACCINE STRATEGIES

Early this year IAVI launched a pre-clinical program aimed at optimizing vaccine design, dosage and immunization regimen and prime-boost combinations with its current crop of candidates. So far it has launched the first vaccination studies in macaques and established a core lab at the pharmaceutical company Becton Dickinson (San Diego), where the analyses of immune responses in these studies will be carried out.

Ongoing studies are working out the best dosing regimes with DNA/MVA and the adeno-associated virus (AAV), and testing whether responses to AAV can be boosted with either AAV (using a different subtype than the prime) or with MVA. Future experiments will analyze which HIV genes are necessary for protective immunity, compare different viral vectors head-to-head, and examine the ability of adjuvants to enhance the immunogenicity of DNA vaccines. The findings of these studies will be published, and should help guide the design of future vaccines.

These efforts are being led by Alan Schultz at IAVI and Shiu-Lok Hu from the University of Washington. Collaborating non-human primate centers include the Oregon Health and Science University, Children's Research Institute, and the University of Pittsburgh—with possible future collaborations to include the Tulane National Primate Research Center and the Air Force Research Institute for Medical Science in Bangkok.

NEUTRALIZING ANTIBODY CONSORTIUM UPDATE

In 2002, IAVI, the National Institutes of Health Vaccine Research Center (VRC), and a number of leading academic and industry laboratories joined forces to create the Neutralizing Antibody Consortium (NAC) (see *IAVI Report*, May-Jun 2002). In the year since its inception, NAC members have made steady progress towards the design of immunogens that elicit broadly neutralizing antibodies to HIV.

Members of the consortium are taking a systematic approach to developing effective immunogens. The first stage involves gathering

information on key aspects of the structures involved in neutralization. This is done through crystallization of HIV envelope proteins and monoclonal antibodies, which is now being executed by a robotic system from Syrrx (San Diego, California), at the Scripps Research Institute (La Jolla, California) in collaboration with the Joint Center for Structural Genomics (a California-based consortium). The automated high-throughput platform can produce crystals in as little as two weeks, in contrast to the usual manual procedures, which generally take months and are far more costly. This system has now been used successfully to determine the structure of several broadly neutralizing monoclonal antibodies, including IgG1 b12, which recognizes the CD4 binding site, and 2G12, a highly unusual antibody that recognizes a cluster of carbohydrates on gp120, among others directed at gp41 or the V3 loop.

The next step is the modification or enhancement of envelope proteins to expose important regions for neutralization. One design approach is to mimic the native trimeric form of envelope spikes that occur on the virus surface. One such candidate immunogen (gp140 GCN4) previously developed by the group of Joseph Sodroski (Harvard University, Cambridge), an NAC member, has been shown to induce neutralizing antibodies in rabbits and is being modified further to increase its immunogenicity. Sera from rabbits immunized with these novel immunogens will be tested against a broad range of viruses, using ViroLogic's high-throughput pseudovirion assay. Another ongoing study is comparing immune responses induced by DNA vaccines encoding *env* with responses to the envelope protein itself, since vaccines using DNA are potentially cheaper and easier to manufacture.

The consortium has also established a repository to store and distribute key reagents, such as the various envelope proteins and monoclonal antibodies used in the crystallization studies. Looking ahead, NAC members plan continued structural studies and production of new immunogens, along with head-to-head immunogenicity studies in animals.



How important are neutralizing antibodies for making a highly effective HIV vaccine?

There's no doubt in my mind that a good antibody-inducing component will ultimately yield a better vaccine. But I also think we can have a useful vaccine if a good antibody component isn't found.

By 'good antibodies,' I mean antibodies that neutralize most wild-type primary isolates of HIV. Adding recombinant gp120 to a viral vector in one arm of a trial, or putting an envelope gene into a viral vector, is not a solution, because the responses they induce aren't functional, in the sense that they don't meet the goal of what antibodies need to do.

Do you think sterilizing immunity is achievable?

I think neutralizing antibodies might increase the probability of getting sterilizing immunity, of not having productive infections, and shifting average viremia lower. How much depends on how good the T-cell part of the vaccine is.

Do you have a timetable for deciding which

candidates to move into efficacy trials?

Our goal now is to finish defining our basic vaccines, through the one we consider most promising—the trivalent vaccine with *gag*, *pol* and *nef*, which is now going into people. We're also expanding into populations similar to those where efficacy studies will be done. You'll see more and more sites opening up that are relevant to efficacy questions, whether it's for studies with the HVTN or new sites we're opening up with the trivalent vaccine.

We're planning very aggressively. I can't give you a time frame because it's not defined yet. But we're planning for diverse efficacy studies with a clade B vaccine. While those efficacy studies are underway, we'll continue developing our other potential vaccine components, so that we'll have a short list of next best choices by the time the first efficacy data come in.

Even a vaccine that works will probably have shortcomings. We'll see what these are, and with an understanding of our next best tools, we'll marry the two together and test efficacy again. That's our basic strategy. ♦

tion across Africa that the question of diversity needs to be rigorously tested through well-designed clinical trials—including some in unmatched settings.” While the clade issue is most relevant for efficacy trials, at this point there’s progress at the Phase I level: South Africa—with an overwhelmingly clade C epidemic—has just approved a Phase I trial of a vaccine based on clade A, shortly after giving the green light to its first HIV vaccine study, which uses a clade C-based candidate (see p. 20). (Africa’s first HIV vaccine trial, a Phase I study in Uganda in 1998-2000, also used a vaccine from an unmatched clade—in this case, clade B, which is all that was available at the time.)

But at the same time, the picture of global diversity—and the task of tracking it—are getting more complicated. That’s partly because new HIV variants and recombinants are continuously generated, and established ones move into new geographic areas. But it also reflects the growing use of more sophisticated technologies to characterize HIV strains, which are uncovering some unexpected layers of complexity.

Describing global diversity

At first glance, it might seem that classifying and tracking HIV variation around the world is a straightforward, albeit mammoth, undertaking. But that hasn’t turned out to be so.

The original definition of clades was based on short sequences, mostly within the HIV envelope, and has expanded to include nine clades (designated A through K, with no E or D). As the amount of sequence data grew over time—all captured in the NIH-supported database now run by Bette Korber at the Los

Alamos National Laboratory (LANL) in New Mexico (www.hiv-web.lanl.gov)—it became clear that each HIV gene shows a different, and characteristic, degree of variation. Env heads the list, with up to 35% sequence diversity between clades, 20% within one clade and even 10% in a single infected person. At the other end of the spectrum, the Gag and Pol proteins show only 10-15% sequence divergence across clades.

But over the past few years, as more HIV labs have established high-throughput sequencing technologies, the field has accumulated hundreds of full-length HIV sequences from around the world—allowing large-scale comparison of whole genomes rather than just selected regions. The result: “a very rapid change in the epidemiological picture,” says McCutchan, whose updated map of global HIV diversity (included in this issue as a special poster and available at www.iavi.org/iavireport) illustrates the 10 major epidemiological patterns.

One big change is a growing awareness of the role recombinants play in the

epidemic, especially in regions where several clades co-circulate. Some of them, like the B/F recombinant found in parts of South America, were previously thought to be pure subtypes. (A few were already known to be important circulating strains, especially the A/G recombinant called CRF02_AG, for Circulating Recombinant Form, common in west and central Africa, and CRF01_AE, originally called clade E, in Southeast Asia.) Even more surprising, says McCutchan, is the high number of unique recombinants (those found so far only in a single person). For example, in Tanzania, where McCutchan and collaborator Michael Hoelscher (University of Munich and director of Tanzania’s Mbeya Medical Research Program, MMRP) have dozens of full-length sequences from low-risk populations, at least 40% are turning out to be unique recombinants (see figure 3, p.17). Similarly, full-length sequencing of HIV from low-risk adults in Uganda and Thailand, countries that each have two important co-circulating strains, found about 30% and 13% recombinants, respectively (see Figs. 1 and 2).

Since recombination can only occur if two viral strains have co-infected a single cell, these findings hinted at yet another unexpected layer of diversity: that double infections may be far more common than previously recognized. Consistent with this notion, a prospective study of 600 female bar workers in southwestern Tanzania suggests so far that, astonishingly, a substantial proportion of these high-risk women show preliminary evidence of double infection with HIV from two different clades (see article, p.17).

But characterizing these dual infections and pinpointing when each one occurred is proving to be technically daunting, even with the project’s large collection of blood samples from individuals at different time points. The difficulty is that the relative proportions of the two strains fluctuate widely over time, according to Hoelscher—one strain may remain barely detectable for months, then suddenly emerge to dominate the viral population in the blood. With superinfection now a hot issue in the HIV field (see *IAVI Report*, Jul-Sep 2002), resolving whether the infections happened more or less simultaneously, or if one occurred after the other was well-established (true superinfection), is a high priority for these researchers.

The issue has enormous implications for mapping diversity, in terms of identifying the proportions of different strains circulating in a population, and for genotyping HIV in samples used for studies of cross-clade immunity. And even though dual infections may prove less common outside highly-exposed groups like the Tanzanian cohort, HIV varies up to 10% even in singly-infected people. “I think we’ll find that sampling from a single time point, and sequencing only one full clone, has pretty poor power to reveal what’s going on,” says McCutchan. “We’ve uncovered a gigantic can of worms. It will take new technologies to sort this out.”

Yet there’s a bright spot in the data on HIV variation, she adds. Looking at the global numbers, it

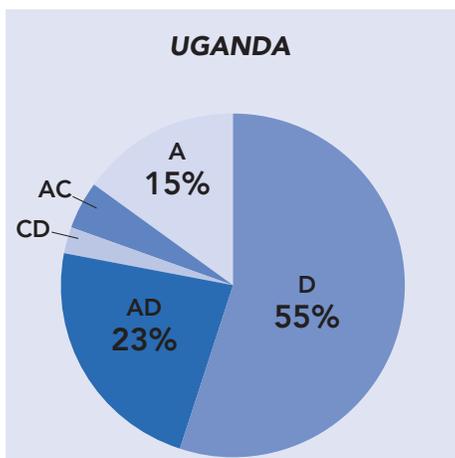


Fig. 1: Proportion of different HIV subtypes and recombinants in circulation, based on 47 full-length HIV sequences. Samples represent incident infections from a community-based cohort study in the Rakai district, 1999-present.

Source: Francine McCutchan

emerges that four clades (A through D) plus two CRFs (01 and 02, both of which are about 70% clade A) account for over 90% of all infections worldwide. From this perspective, diversity can be boiled down to 4 key clades, plus small contributions from the non-A segments of these two CRFs—a more manageable focus for vaccine developers.

But the missing link remains to connect this increasingly detailed understanding of genetic diversity with a picture of immune properties. The usual way of classifying viruses immunologically is based on serotype—a group of antigenically-related strains recognized by a specific reference antibody, usually a neutralizing antibody. By this criterion, “there’s absolutely no sense of serotypes for HIV,” says antibody expert David Montefiori (Duke University, Durham). “Although antibody *binding* tracks somewhat based on clade, we don’t know if neutralizing serotypes even exist and can be defined.” He also points out that it’s extremely difficult to pinpoint NAB epitopes, which are often based on 3D shape and map to discontinuous sequences.

On a more promising note, mapping T-cell epitopes (which are based strictly on sequence) and defining their patterns of cross-clade recognition is easier, and work in this area is ongoing

Vaccines and cross-clade responses

If there’s been one set of findings to cheer in the recent past, it’s the emerging clinical data suggesting that immune responses to T-cell-based vaccines frequently recognize at least some HIV strains of other clades—albeit possibly fewer epitopes and/or at a lower magnitude.

For example, trials of Merck’s adenovirus-based candidate, which carries a clade B-derived gag gene, found that 10 out of 13 volunteers who responded to vaccine also recognized peptides from clades A and C Gag. Consistent with these findings, an international study of people infected with HIV of different clades detected similar frequencies of cross-clade T-cell responses to Gag and to the relatively conserved proteins Pol and Nef, but far fewer responders to the more diverse Rev and Tat proteins. Cross-clade CTL responses are also seen with canarypox-based vaccines, as first shown in studies of Ugandan volunteers given clade B-based vaccines and who often responded to some A and D peptides (*J Inf Dis* 182:1350;2000). Overall, says Larry Corey, who directs the NIH-sponsored HIV Vaccine Trials Network, “clades aren’t making the kind of difference people thought they would.” He also predicts that “cross-clade responses will be more the norm than the exception.”

But the case is far from proven. It’s unknown whether cross-reactivity will translate into cross-protection, says Jaap Goudsmit, chief scientific officer at Crucell, a Dutch biotechnology company; without more detailed data on which epitopes are and aren’t recognized across clades, and which ones matter for protection, he’s reserving judgment. Lower levels of responses

across clade are another potential factor—although new data showing that a candidate Ebola vaccine protects monkeys even after one dose, which induces a weaker response than a full prime-boost regimen (*Nature* 424; 681: 2003), indicate that less-than-optimal responses can suffice for a successful vaccine. And 90% sequence conservation between, say, the vaccine and an infecting Gag protein still corresponds to an average of one amino acid difference per epitope, write Tomas Hanke and Andrew McMichael—which, despite some “wobble” (tolerance for mismatch), could abrogate a significant number of responses across clades (*Vaccine* 20:1918;2001). Some loss was seen in the Ugandan canarypox vaccine study, where cross-clade responses were sometimes as strong as those to vaccine strain (as measured by peptide titrations in Elispot assays), and sometimes weaker.

In any case, studies of cross-clade recognition—including some with new non-clade B candidates—are yielding a growing body of valuable information. And as Peggy Johnston (Assistant Director for AIDS Vaccines, National Institutes of Allergy and Infectious Diseases) points out, “this will allow the field to make decisions based on data, not conjecture,” she says—decisions such as what vaccine strains are most promising for testing in regions with particular non-matching clades in circulation.

Merck’s John Shiver, who heads the company’s vaccine research program (see article, p.10), points to another potentially important (and under-recognized) component of cross-clade protection: immune responses to HIV antigens not present in the vaccine. This notion emerged from Merck’s studies of its gag-only candidates in monkeys, which show “terrific” responses to Nef after challenge with either SIV or SHIV—responses rarely seen post-challenge in unvaccinated animals. “This suggests that vaccines may only need to generate enough of an immune response so that after infection, you can make lots of natural responses to the infecting virus, whatever clade it is,” he says. “The vaccine may not have to generate all the heterologous [cross-clade] coverage.”

For vaccines that target the antibody-producing B-cells, the picture remains much bleaker. So far, no vaccine tested in humans has generated neutralizing antibodies (NAb) to anything beyond the vaccine strain and a few closely related isolates, and there are few ongoing clinical trials involving candidates that even target this arm of the immune system.

Yet data on HIV-infected people show that cross-neutralizing antibodies do exist—findings that have re-kindled efforts to find strategies for generating them via vaccination.

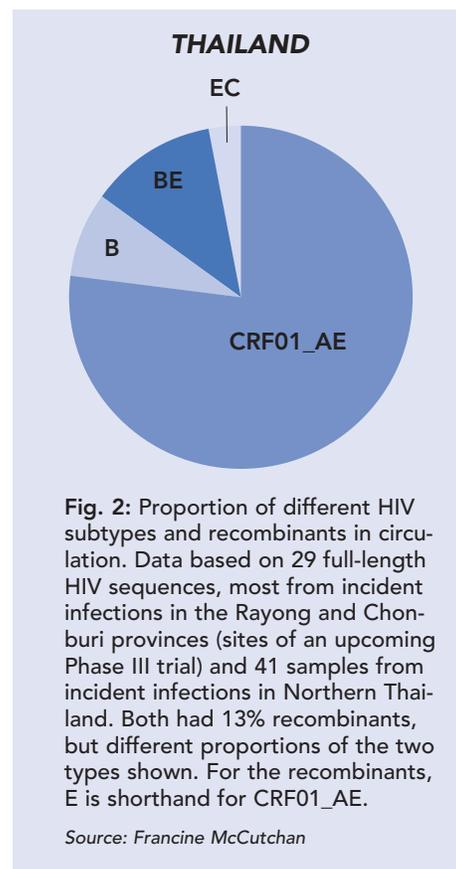


Fig. 2: Proportion of different HIV subtypes and recombinants in circulation. Data based on 29 full-length HIV sequences, most from incident infections in the Rayong and Chonburi provinces (sites of an upcoming Phase III trial) and 41 samples from incident infections in Northern Thailand. Both had 13% recombinants, but different proportions of the two types shown. For the recombinants, E is shorthand for CRF01_AE.

Source: Francine McCutchan

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Designing Vaccines for Breadth

All this leaves vaccine developers still operating largely in the dark in terms of how to design for maximal breadth.

For T-cell-based vaccines, a common starting point is to use the most conserved regions of HIV—first and foremost the gag gene (or protein), followed by *pol* and sometimes *nef*. These are usually derived from a primary HIV isolate, sometimes selected for a particular biological property such as use of the CCR5 receptor, and/or origin in the geographic region where the vaccine will be used. In a variation on this theme, the San Diego-based company Epimmune developed a candidate containing highly conserved epitopes (rather than whole genes) from across the genome, selecting further for those recognized by the most common HLA genotypes. This candidate recently entered Phase I clinical trials in the US and Botswana.

More recently, researchers have begun looking at artificial sequences derived by computer analysis, rather than actual circulating viruses, as sources of vaccine strains. These are often consensus sequences, made by analyzing a set of sequences (say, primary isolates of clade C) and choosing the nucleotide found most commonly at each position. Or they may be ancestral sequences, which represent the most likely common ancestor to a group of isolates. The rationale for these approaches—articulated in depth by Bette Korber (*Science* 296: 2354; 2002)—is that they minimize the genetic distance between the vaccine and the pool of circulating strains; in contrast, a primary isolate might be an “outlier,” genetically speaking, relative to many other isolates of the same subtype.

These sequences have not yet been incorporated into vaccines, although similarity to a clade consensus sequence is sometimes used to help select primary isolates for vaccine strains. But several groups are developing Env immunogens from consensus or ancestral sequences, and two teams reported at the 2003 Retrovirus conference that they seem to fold and function like real Env proteins, and to show potential as cross-clade immunogens. Nancy Haigwood and Jim Mullins (University of Washington, Seattle) made two ‘proof-of-concept’ ancestral clade B Env proteins, which so far (as a DNA vaccine in rabbits) induce “reproducible but fairly low titers” of NAbs that cross-neutralize a clade C isolate, says Haigwood (poster 409). And researchers from Duke University, LANL and the University of Alabama reported on an immunogen made from a consensus Env sequence of all major clades, and which was recognized by several clade B and C antisera (from infected patients)—unlike Env from either clade, which reacted best with same-clade sera (poster 410). Both posters are available at www.retroconference.org/2003.

Other strategies for generating broad NAbs are based on modifying the shape of the Env antigen, rather than focusing on sequence (*IAVI Report*, Dec. 2002-Jan. 2003, p.1). These include development of native, trimeric structures; modification of Env to remove the more variable regions (exemplified by

Chiron’s gp140 immunogen, which should enter clinical trials this year); generating immunogens that bind to well-characterized, neutralizing broad Nabs, or which expose normally-hidden neutralizing epitopes (fusion intermediates).

And for both B- and T-cell-based vaccines, several groups are combining immunogens from different clades to create “cocktail” vaccines (for example, the A/B/C candidate described in the article on p.7). In the future, cocktails could also contain mixtures of primary isolates (or shape-based immunogens) plus consensus sequences.

Vaccines and cross-clade responses

Last but far from least of the hurdles in developing a broad HIV vaccine will be the challenge of determining just how broadly protective a vaccine actually is. Intended to help mobilize support for the steps this will require, the new African AIDS Vaccine Programme document emphasizes that clade-mismatched trials are a crucial part of the solution, and that politics must recognize this reality.

The document also proposes some guidelines for decision-makers. For Phase I/II testing, it advocates moving ahead with good candidates, regardless of the subtypes involved, for the sake of other benefits—such as building capacity for running trials, and for conducting scientific and ethics reviews; establishing dialogs among scientists, policy makers and communities; and gathering data on a vaccine’s ability to induce cross-clade responses in diverse populations. It also recommends that decisions about efficacy trials should be based on evidence of cross-reactivity between a candidate vaccine and the unmatched clades and/or CRFs circulating in the trial population, along with a good safety record from Phase I/II studies.

From a scientific perspective, this testing is likely to involve several different trial scenarios, says veteran vaccine developer Don Burke, who directs the Center for Immunization Research at Johns Hopkins University. One is to compare the efficacy of vaccines based on a single clade in matched versus unmatched settings—the strategy Merck is likely to pursue with its clade B-based candidates. Another is to ask whether clade-matched vaccines work better than unmatched ones—studies that could be done with two different vaccines in one setting, or by testing one vaccine in a region with multiple circulating clades, powering the trial to detect efficacy in at least one of them. In all cases, getting an answer will require careful analysis of breakthrough infections.

Trial design, along with logistics, gets more complicated for multi-clade candidates and/or sites with multiple clades in circulation (see articles, pp. 17, 18), since both these variables raise the number of volunteers needed to identify statistically significant trends in vaccine cross-protection. But with the politics gradually aligning more closely with the science, and a growing roster of potential trial sites in the picture, there should at least be a few less obstacles in the way. ♦

Studying HIV Diversity in Multi-Clade Regions

In the southernmost countries of Africa, where the AIDS epidemic is due almost exclusively to clade C HIV strains, the highly politicized issue of clades and vaccines has usually boiled down to decisions on whether to conduct trials only of candidates that closely match strains circulating locally; or to consider studies involving unmatched clades as well. But in other parts of Africa, HIV diversity is far higher—shifting the focus of the debate from the issue of getting a close match onto understanding how to induce the broadest possible protection.

Here, we profile two clinical research sites—one in the highlands of Tanzania, the other in Cameroon's capital city. Both are in countries with an especially broad diversity of circulating HIV strains. And both have made tackling this diversity a cornerstone of their vaccine program.

Since the early days of the AIDS epidemic, the Mbeya region in southwestern Tanzania has been among the country's hardest-hit areas. One glance at the map explains why: it's located at two crossroads of the Trans-African highway—making Mbeya a hub for HIV spread along this major trucking route, which connects the capital city of Dar es Salaam with Malawi and Zambia, and then further into southernmost Africa. The region is also a bridge between the eastern and southern African epidemics, with all three of the most globally important clades (A, C and D) in circulation—plus at least 40% unique recombinants (see figure 3), the highest reported proportion anywhere in the world.

Today Mbeya is also home to a thriving HIV/AIDS clinical research effort that's deeply involved in studying this diversity, and is embedded in a regional program of HIV surveillance, prevention and care. Under the umbrella of the Mbeya Medical Research Program (MMRP), a team of Tanzanians working with University of Munich scientists, plus collaborators and funders from the US, Europe and Africa, is conducting two longitudinal studies with a total of nearly 4,000 participants. As the first of these studies yields data showing that double HIV infections are surprisingly common in the region, together they are building capacity and laying groundwork for vaccine trials, which are likely to involve multi-clade "cocktail" candidates.

The MMRP has its roots in an intervention program launched 15 years ago at the Mbeya Referral Hospital, through Tanzania's Ministry of Health, the German Technical Cooperation and the University of Munich. It gradually expanded to include broader surveillance and prevention activities, with research entering the picture in 1995 when Michael Hoelscher (University of Munich), now the site's scientific director, began subtyping HIV in blood samples collected at antenatal clinics. By sequencing short regions of the envelope gene, he found substantial proportions of clades A, C and D. But when he did full genome sequencing in Francine McCutchan's lab at the US Military HIV Research Program (Maryland), it quickly became clear that about half these samples were actually unique recombinants—suggesting that double infection, which had been documented in only a few cases worldwide, might actually be relatively frequent in the region. And that, in turn, led Hoelscher

and the US military program, with support from the European Commission, to launch a systematic study of double infection—basing their search on high-throughput methods they developed for detecting HIV from two different clades in a single individual.

Dual infections in highly-exposed women

Looking for a population with very high exposure to HIV, the researchers focused on women working in bars, local brew shops and guesthouses along the trucking route, many of whom live partly from commercial sex work or casual partnerships. Before launching a formal study, Oliver Hoffman—a University of Munich physician who leads the team's cohort development—got to know the community through a baseline survey of 1,500 women, probing their level of general knowledge about HIV/AIDS, their risk behaviors, and in particular their movements within the region—key information for a study involving 4 years of frequent follow-up in a highly mobile group. The study got underway in September 2000, recruiting a total of 600 women irrespective of HIV status—and resulting in a cohort with the staggering prevalence of 68%, says MMRP coordinator Leonard Maboko.

Now nearing the end of its third year and with one more to go, the study (dubbed HISIS, for **HIV SuperInfection Study**), has a well-practiced rhythm. Once every three months a mobile unit pulls into each of the 20 villages serving as study nodes. The day before, volunteers and study staff meet for an informal session on an AIDS-related topic, such as living with HIV, or nutrition. By 8 a.m. the next day, the mobile unit has set up a clinic with everything from examination beds and tables to blood-drawing equipment, and each woman goes through a full round of visits—interviews on HIV exposure and risk behaviors, counseling sessions on prevention strategies, medical exams, blood draws and vaginal swabs and lavage. Acute diseases, including STIs and opportunistic infections, are treated on the spot, and trial staff provide medi-

Tanzania: At the Crossroads of Africa's Major Clades

BY PATRICIA KAHN

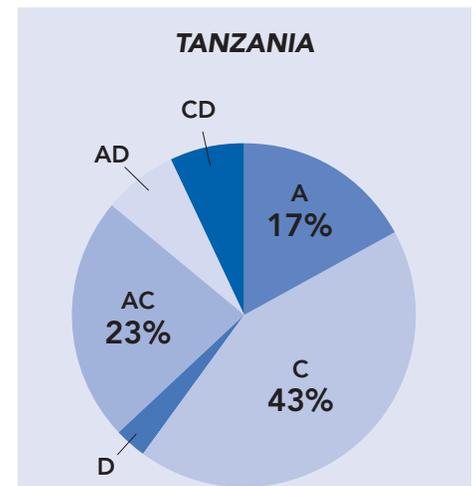


Fig. 3: Proportion of different HIV subtypes and recombinants in circulation, based on 30 full-length HIV sequences. Samples were drawn from low-risk adults and blood banks in the Mbeya region.

Source: Francine McCutchan

continued on 18 ►

cines and condoms. A village contact person, who keeps monthly tabs on volunteers between clinic visits, searches out those who don't appear. By noon, the field work is done.

So far it's clear that many of the women are doubly infected, says Hoelscher, although immense technical difficulties in tracking the two strains over time mean that it's not yet known whether most of them are true superinfections (i.e., where a second infection happened after the first one was established) or occurred simultaneously (see article, p. 1). But there are a few cases of "incident superinfections"—those which happened during the course of the study, where the researchers are more certain of the timing.

Once they can definitively characterize enough infections, there are several key issues to tackle, such as comparing cross-clade immune responses among doubly-infected women, women with single infections, and the few who remain uninfected despite intense HIV exposure. The goal: insight into correlates of protection against infection and—for those already infected—against HIV of a second clade. At the same time, the Mbeya site is establishing the immunological methods and laboratory capacity it will need for vaccine trials.

Towards vaccine trials: cohorts, and a new lab

With HISIS in full gear, last year brought two other big steps for the MMRP. First was the launch of

the CODE (**CO**hort **DE**velopment) study, a longitudinal study of 3,100 uninfected and HIV-infected people from a rural and an urban population, to analyze a variety of parameters important for trials—such as HIV incidence, major risk factors, knowledge and attitudes about vaccines, the course of HIV infection (including CD4 and viral load setpoints) and overall disease profile of the population. It will also compare strategies for enrollment and follow-up. Like HISIS, the project offers free basic medical care, including treatment for opportunistic infections, malaria and TB. Enrollment was complete within six months, using three different methods, and resulted in cohorts with an HIV prevalence at uptake of 19.6% in the urban group and 14.4% in the rural one.

A second milestone was the opening of a new 12-room laboratory building next to the Mbeya Referral Hospital, which processes up to 60 samples a day (from HISIS and CODE) and where immunological methods like Elispot and flow cytometry are now up and running.

As they work towards vaccine trials, MMRP also aims to establish anti-retroviral therapy (ART) treatment, not just for trial participants but for the broader community. Plans call for an ART feasibility study next year to examine different regimens, along with supply and monitoring systems. The hope is to move quickly from Phase I studies in 2004 or 2005 (with several European and multi-clade candidates in discussion) into larger trials. ♦

Cameroon: "Final Common Pathway" for a Global Vaccine

BY PATRICIA KAHN

With its richly diverse population of some 200 ethnic groups speaking 80 different languages, Cameroon is often seen as "Africa in miniature." Sadly, that description also applies to its AIDS epidemic: the country is home to virtually every known HIV subtype, plus the CRF02_AG recombinant—second only to clade C in numbers of infections worldwide, according to UNAIDS—and a vast array of unique recombinants.

That makes Cameroon among the most complex places in the world for an HIV vaccine—as well as one of the few where vaccine efficacy can be tested against a global range of subtypes and recombinants. And that's just what the country is gearing up to do, in partnership with the Johns Hopkins School of Public Health and the US Military HIV Research Program, as it builds the small Center for Military Health Research (CRESAR) in the capital city of Yaoundé into the hub of a national HIV vaccine program. "Cameroon can be a final common pathway for testing a global vaccine," says Debbie Bix, who leads the US Military HIV program. "If a vaccine shows efficacy here, it's very likely to work around the world."

Whether the country can play that role will hinge largely on success in identifying populations with high enough incidence to support vaccine trials, says Nathan Wolfe (Johns Hopkins), who heads

the site alongside CRESAR director Col. Eitel Mpoudi-Ngole. Cameroon, like the rest of west and central Africa, has a less severe HIV epidemic than other sub-Saharan countries (although solid data on HIV prevalence and incidence is scarce). So the feasibility of vaccine efficacy studies will become clear once the Yaoundé team collects the needed data on incidence (the rate of new infections)—a key determinant of how many volunteers are needed to measure efficacy.

The site's beginnings

Cameroon's vaccine program stems from a collaboration started in 1997 between Don Burke, Bix' predecessor (now director of the Center for Immunization Research at Hopkins) and Mpoudi, then the well-known head of the national AIDS control program, whose openness and leadership on AIDS earned him the nickname Col. SIDA (the French acronym for AIDS). With evidence emerging that equatorial Africa has the highest HIV diversity worldwide, the project's initial focus was on understanding how this diversity is generated, and on expanding surveillance and prevention efforts, especially in rural villages and in Yaoundé's military garrisons.

As work progressed on establishing VCT, training staff and building laboratory capacity, it became

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IAVI is a scientific organization founded in 1996 whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. IAVI focuses on four key areas: Accelerating scientific progress; education and advocacy; ensuring vaccine access and creating a more supportive environment for industrial involvement in HIV vaccine development.

IAVI is a UNAIDS Collaborating Centre. Its major financial supporters include the Bill & Melinda Gates Foundation; the Rockefeller Sloan and Starr foundations; the World Bank; BD (Becton, Dickinson & Co.); and the governments of Canada, The Netherlands, United Kingdom, United States, Ireland, Denmark, Norway and Sweden. IAVI also has received support from the Vincent P. Belotsky Jr. Foundation, Cruisaid, the Elton John AIDS Foundation, James B. Pendleton Charitable Trust, Until There's a Cure Foundation, and other generous corporate, foundation and individual donors worldwide.

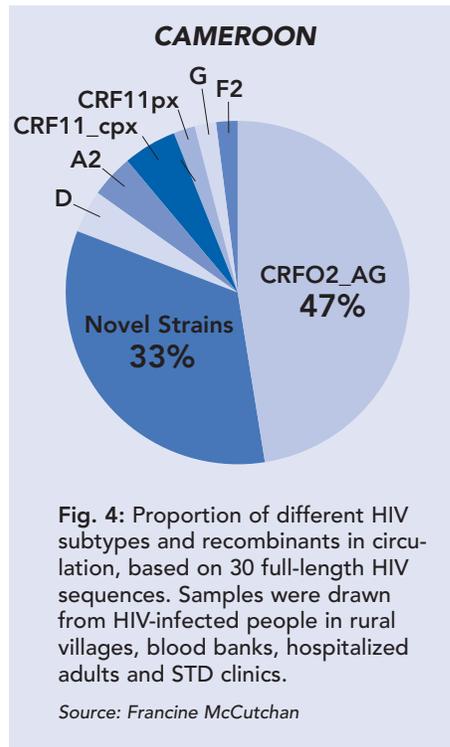


Fig. 4: Proportion of different HIV subtypes and recombinants in circulation, based on 30 full-length HIV sequences. Samples were drawn from HIV-infected people in rural villages, blood banks, hospitalized adults and STD clinics.

clear that Cameroon offered many advantages for vaccine work. Chief among them were strong political support from one of the few stable governments in a troubled region, and relatively good infrastructure. Another was the finding that about half of all infections in the country involved the CRF02_AG recombinant—an important strain in west/central Africa (but rare elsewhere), and one that a vaccine for the region must protect against. At the same time, new partners added support to the growing effort: besides the US Military Program, these included the US NIH Fogarty International Center, Centers for Disease Control and Naval Health Research Center, and Cameroon's Ministry of Health. And in 2003, a collaboration with Merck on cross-clade immunity in HIV-infected people provided a first foray into vaccine-related work.

Cohort pre-development

As the team now moves into finding potential groups for vaccine studies, the military remains a major focus. Its 60,000 members and their families offer a stable population and an established system of free health care for active military personnel. Surveillance work so far, which has involved about 2,900 enlisted active-duty forces in Yaoundé, found about 10% HIV prevalence. Over the coming two years a new longitudinal study of 1,000 uninfected people will gather incidence data, along with information on knowledge and attitudes about vaccines. And in a sign of the growing priority of HIV for African militaries, the Yaoundé team is collaborating with three neighboring countries—Chad, Congo-Brazzaville and Gabon—to establish VCT, surveillance and staff training.

Similar studies are also beginning at several agricultural plantations. HIV risk in these populations, says Ubaldo Tamoufe (program officer and coordinator of the cohort work) comes from “having thousands of low-income workers in isolated areas, usually far from their home villages,” and from the commercial sex trade that flourishes around the bi-monthly paydays. One study will take place at a rubber plantation of 6,000 workers and their 21,000 family members in the Southern Province, where data from antenatal clinics suggest about 10% HIV prevalence in adults. Another project involves the Cameroon Development Corporation, a group of

agricultural plantations with 25,000 workers and family members. Both collaborations offer the advantages of stable, mixed-gender populations and established health care infrastructures.

A third potential group is high-risk women, where Cameroon can build on its experience from two Phase III microbicide trials in the mid- to late 1990's—the source of some of the little prevalence and incidence data in Cameroon, says Tamoufe, who served as study coordinator of both trials. In the first one, which enrolled 1,292 sex workers, HIV prevalence at uptake screening was 16.8%, and the trial found an incidence of 6.6 infections per 100 person-years. The second

study, involving 1,251 high-risk women (excluding sex workers), found 11% prevalence at uptake and an incidence of 1.3 infections per 100 person-years. Those trials also offered some valuable lessons on retaining volunteers, Tamoufe adds, which paid off in far less loss to follow-up in the second one (1.5% compared with 20% over two years). Plans are now underway to recruit 1,000 HIV-negative sex workers from around Yaoundé for a two-year study similar to those in the military and plantation populations. The Yaoundé team will also expand laboratory capacity for evaluating immune responses to vaccines.

While no specific candidate is “on deck” for the site, the researchers envision a few possible scenarios. One is to test vaccines against the CRF02_AG recombinant. Another is to evaluate candidates that have already shown efficacy in clade-matched settings, pitting them against the full onslaught of Cameroon's HIV's diversity.

Studies on emerging diseases

Alongside its HIV work, the Yaoundé group uses its field experience in Cameroon's remote regions for another line of research: exploring the origins of HIV and other viral diseases that enter human populations from non-human primates (NHP). Central Africa has a huge diversity of wild NHP, along with forest-dwelling populations who hunt, butcher and eat them. But jump of a retrovirus into humans in a natural setting has never been documented—until the Yaoundé group's recent demonstration of crossover by the simian foamy virus (SFV), based on both antibodies to viral proteins (in nearly 1% of 1,100 people sampled) and the presence of SFV DNA sequences. ♦

vaccine BRIEFS

AUSTRALIAN CONSORTIUM LAUNCHES DNA-FOWLPOX TRIAL

On 17 June the first volunteer was vaccinated in an Australian Phase I/IIa trial of a prime-boost strategy that combines DNA- and fowlpox-based HIV vaccines. The trial, which will enroll 24 volunteers, is sponsored by Australia's National Center in HIV Epidemiology and Clinical Research and will be run at St Vincent's Hospital (Sydney.)

Fowlpox belongs to the same bird virus family as modified vaccinia Ankara virus (MVA) and canarypox, which have also been developed as HIV vaccine platforms. Volunteers will receive two 1 mg injections (0 and 4 weeks) of a DNA vaccine containing *gag*, *rev*, *tat*, *vpu* and truncated *env* genes from HIV-1 clade B, followed by an HIV-fowlpox boost with similar genes at week 8. It is the first study to test a DNA-fowlpox vaccine regimen in HIV-negative volunteers. A therapeutic trial of fowlpox vaccine also containing IFN-gamma, an immune-modulating cytokine, was conducted in HIV-positive individuals in 2002. Because participants in this study were on antiretroviral therapy, which reduces viral load, it was difficult to determine the benefits of the vaccine-cytokine combination, although a follow-up study is now underway to assess control of viral load off therapy. Another preventive trial of similar vaccines containing HIV genes from the CFR_01AE recombinant, Thailand's main circulating strain, is planned for Thailand later this year.

NEW PROPOSAL FOR A GLOBAL AIDS VACCINE ENTERPRISE

In an article titled, "The Need for a Global Vaccine Enterprise" (*Science* 300:2039;2003), 24 of the world's leading vaccine researchers and advocates called for a major effort to expand and restructure the search for an AIDS vaccine. Richard Klausner, Executive Director of the Bill & Melinda Gates Foundation global health program, was lead author on the paper.

The proposal calls for a coordinated effort similar to the Human Genome Project, which divided and assigned roles to a diverse group of scientists to meet its goal. Similarly, the new vaccine enterprise would map out the entire "grid" of potential vaccine approaches. It would then assign tasks, allocate funds and ensure that participating teams of researchers collectively covered the entire grid. To accomplish this, the enterprise would establish of new Vaccine Development Centers (VDCs), which could be actual institutes or virtual collaborations. The paper pointed out that VDCs could include efforts sponsored by existing funders, such as the National Institutes of Health, IAVI and the European Union—all of whom were signatories to the article—could participate in the enterprise.

The VDCs would be part of an interconnected network that also includes manufacturing facilities, central laboratories, and clinical trial sites capable of enrolling a projected figure of 35,000 volunteers into clinical studies each year. The paper did not specify funding requirements or sources for this massive endeavor. In August, major vaccine stakeholders gathered at a Washington, D.C. meeting hosted by the Bill & Melinda Gates Foundation and formed six working groups (product discovery, product development, standardization of assays, manufacturing, clinical trials capacity, and international regulatory and licensing issues) which will contribute to strategic framework.

AFRICAN AIDS VACCINE PROGRAMME MEETS IN ETHIOPIA

On 13-16 June nearly 200 scientists, trial investigators, national authorities, and community representatives from Africa and around the world gathered for the second meeting of the African AIDS Vaccine Programme, "Strategies for the Development of HIV Vaccine Trial Sites in Africa: Challenges and Opportunities." The meeting's focus reflected "anticipation of a new wave of candidate HIV vaccines based on strains prevalent in Africa," said Jose Esparza, head of the WHO-UNAIDS vaccine program. AAVP's primary funder. The meeting included intensive discussion of ethical issues, such as standard of care for trial participants and communities, and enrollment of women and adolescents, there were also updates on AAVP activities. In 2002, AAVP completed an assessment of the needs and capacities of African ethical review committees, developed a consensus document on clade (see article, p.1) and established a working group on community issues. Looking ahead, AAVP plans to develop a template for national vaccine plans.

FIRST HIV VACCINE TRIALS GET GREEN LIGHT IN SOUTH AFRICA

On 6 June 2003, the South African Medicines Control Council approved the country's first HIV vaccine trial, and the first clinical trial of a vaccine based on clade C. The Phase I study (HVTN 040) will test the safety and immunogenicity of a candidate based on a Venezuelan equine encephalitis (VEE) vector containing HIV-*gag* from a South African clade C isolate. Clade C strains cause nearly all infections in South Africa, and about 47% worldwide.

The vaccine was developed by Bob Johnston (University of North Carolina, Chapel Hill) and AlphaVax, a biotechnology company (Research Triangle Park, North Carolina). The VEE vector is derived from a vaccine designed to protect humans against a South American virus that often causes disease in horses. It targets mainly dendritic cells, which play a key role in inducing immune responses.

In August, the MCC approved a second Phase I trial (IAVI 011). This IAVI-sponsored study will test the safety and immunogenicity of HIVA.MVA, a candidate based on modified vaccinia Ankara virus (MVA) and containing a clade A *gag* consensus sequence plus a string of CTL epitopes from the *gag*, *pol*, *nef* and *env* genes. It was designed by scientists at the University of Nairobi and University of Oxford.

The two trials will be conducted separately, but at the same South African sites: one in Soweto, the other in Durban.

HVTN 040 will evaluate three different vaccine doses in a total of 96 volunteers. Testing will begin in the US and proceed in South Africa once safety is established.

IAVI 011 will take place at two European sites along with Durban and Soweto, and enroll 111 volunteers. HIVA.MVA is being studied alone and as part of a prime-boost combination with a DNA vaccine in Kenya, Uganda and the UK.