

do clades matter for aids vaccines?

PATRICIA KAHN

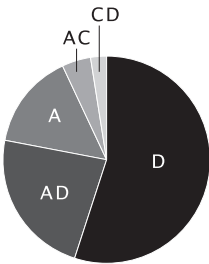
ALTHOUGH WE TALK ABOUT (and treat) HIV as one *virus*, this doesn't mean that everyone is infected with an identical version of HIV. In fact, analyzing its genetic makeup reveals that there are many, many different versions—a phenomenon called *genetic diversity* (or *genetic variation*). And this diversity is continually increasing, since HIV is always changing at the genetic level and creating new versions of itself.

The notion of viral genetic diversity is nothing new: Most viruses which cause disease in humans exist as distinct *strains*. But the amount of diversity with HIV dwarfs that seen for any other virus. To describe this variation, researchers classify HIV strains into one of three groups, based on their degree of genetic similarity: group M, the main one behind the global epidemic, group N and group O. The M viruses are further divided into *subtypes* (or *clades*), named by the letters A through K.

Another key point about HIV diversity is that the various clades aren't distributed uniformly around the world; instead, different clades predominate in particular regions. For example, the epidemic in southern Africa is essentially all clade C viruses

(which are also common in India and China), while North America, Europe and Australia have mostly clade B strains. Other regions have several clades in circulation (see figures 2.3–2.5), with the most extreme examples found in western and central Africa—where just about every known clade is seen. (For a map of global distribution patterns, see resources at the end of this chapter.)

Figure 2.3 UGANDA



Proportion of different HIV clades and recombinants in circulation, based on analyzing 47 HIV genomes from infected people in the Rakai district. Over half were infected with clade D viruses; the second most common strains are recombinants between A and D.

Source: Francine McCutchan

What does any of this have to do with vaccines? This enormous diversity potentially presents a huge problem: Can a single, “universal” vaccine protect against the full range of HIV strains? Or will we need different vaccines, each tailored to the most common strains in a given region? Even worse, with new HIV variants continuously being generated, is it possible that new vaccine formulations might be needed every few years, as they are for influenza?

The answers to these questions will have a big impact on how fast, and at what cost, a successful AIDS vaccine can be developed and distributed globally. Manufacturing even one vaccine formulation and getting it to people quickly once it's licensed will be far more complicated and expensive than anything the public health field has ever attempted. Doing this with several vaccines, or having to repeat it every few years, would make the task even harder. And for regions with more than one HIV clade, the problem goes deeper: Unless an AIDS vaccine protects against all (or most) clades in circulation, it may simply shift the

local epidemic over time towards whatever strains the vaccine can't protect against. For these regions, success in curbing AIDS through vaccination will be especially dependent on having products that induce the broadest possible protection.

That's the bad news. Yet there is room for some optimism. Over 90% of all HIV infections worldwide are caused by four clades (A through D) plus two “mosaic” viruses (see below) that

both contain about 70% clade A sequences—a more manageable focus for vaccine developers.

generating diversity

Before describing how the field is tackling HIV diversity, let's start with a primer on how diversity and clades arise, and what they do (and don't) mean.

The bottom line is that diversity stems from the tendency of HIV to make mistakes when it copies its genetic material while multiplying inside an infected cell, and from the fact that it can produce billions of new virus particles a day. That's a lot of mistakes, hence a lot of new variation.

Let's look closer at how this works. The genetic material, or *genome*, of HIV is made from four different building blocks linked together like beads in a chain, about 10,000 units long. As with all living things, the genetic information is contained in the precise sequence of these four units—information which tells the cell how to build *proteins* that each carry out a specific job. When HIV copies its genome, it sometimes incorporates the wrong unit somewhere in the sequence. The result: a genetic change, or *mutation*.

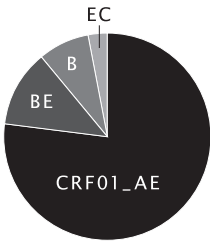
HIV genes vary in how often they mutate. The champion is the *envelope* gene (*env*), which encodes the main surface protein of the virus: *env* genes in viruses taken from a single infected person vary by as much as 10%, and among different clades they vary by up to 35%. (This gives the virus an advantage during infection, since it's difficult for the immune system to keep up with constant change—so some new variants *escape immune* recognition.) Other HIV proteins, like the *Gag* protein that forms part of the virus' internal core, show less than 10% variation from one clade to another; these are said to be more genetically *conserved*.

Beyond mutation, HIV undergoes another level of change: if a person is infected with two different strains, these can exchange whole segments of their genomes—creating a “mosaic” virus called a *recombinant*. Sometimes recombinants spread to other people and become common circulating strains (called

circulating recombinant forms, or CRFs). For example, nearly 80% of infections in Thailand, and 50% in Cameroon, involve CRFs (see figures 2.4 and 2.5).

Now comes the rub in terms of figuring out if and how all this diversity will affect vaccine protection: clades don't correspond to what the immune system recognizes. Some genetic mutations are "silent," in that they don't cause any change in the protein they encode. Others *do* cause changes in the protein, but not all changes are noticed by the immune system. (The immune system doesn't recognize entire proteins but only certain portions, called *epitopes*—and only certain changes within epitopes.) In other words, some mutations stay under the radar screen of the immune system, while others wipe out its ability to recognize particular epitopes—which is what matters for vaccines.

Figure 2.4 THAILAND



Proportion of different HIV clades and recombinants in circulation, based on analyzing 70 complete HIV genomes from newly-infected people in northern and southern provinces. The vast majority of strains in both regions were recombinants, although the regions had different proportions of the types shown. Over 75% of all infections involved a group of recombinants called Circulating Recombinant Form (CRF)01_AE.

Source: Francine McCutchan

immunity and cross-clade recognition

So where do vaccine developers start? Beyond the challenge of making a vaccine that induces *any* protection, tackling HIV diversity involves working from two different angles. One is to figure out whether *immune responses* to one strain of HIV recognize a wide range of other strains (that is, whether they *cross-react*) or only very closely related ones. Second is to find vaccine designs that induce responses to the broadest possible range of strains.

To measure the degree of cross-reaction among HIV clades, researchers are studying immune responses both in HIV-infected people and in uninfected people given an HIV vaccine. For example, these studies might test how well blood cells from people *immunized* with a vaccine made from pieces of a clade B virus

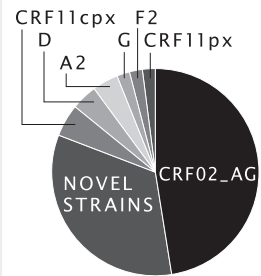
recognize very similar strains compared with more divergent ones. (This type of laboratory test is illustrated in chapter 12.)

So far, the results are reasonably encouraging for vaccines that induce *cellular immunity*: *T-cell* responses often cross-react to HIV strains within and across clades, although they may recognize fewer epitopes, or respond less strongly, than to the original strain. (It's important to note that responses don't fall into neat categories based on clade. There's also some variation from person to person, since people's genetic makeup helps determine what epitopes their immune system can recognize.)

But cross-reaction in a laboratory test doesn't tell us for sure that protection will work across clades, since we don't know if these tests measure the immune responses (or epitopes) that matter most for protection. So answers about cross-protection will need to come from *clinical trials* that compare how well a vaccine protects people against closely related HIV strains versus more distant ones.

For *neutralizing antibodies (NABs)*, the picture looks bleaker: no vaccine tested in people so far generates NABs to anything beyond the strain that induced them and a few closely related strains. Yet studies of NABs in HIV-infected people show that broadly cross-neutralizing antibodies *do* exist—findings that have re-kindled efforts to devise strategies for generating them by vaccination (see below and chapter 7 on vaccine approaches).

Figure 2.5 CAMEROON



Proportion of different HIV clades and recombinants in circulation, based on 30 complete HIV genomes. Samples were drawn from HIV-infected people in rural villages, blood banks, hospitalized adults and STD clinics.

Source: Francine McCutchan

designing for diversity

Until recently, few vaccine candidates were designed to test specific approaches to HIV diversity, since researchers were focused on finding strategies for inducing *any* strong responses. But as more products are developed, several approaches are emerging.

Among the T-cell-based vaccines in clinical trials, a common strategy is to use the most conserved regions of HIV, usually Gag, followed by *Pol* and sometimes *Nef*. In a variation on this theme, two companies are developing candidates containing highly conserved, widely recognized epitopes (rather than whole genes or proteins) from different parts of HIV.

Vaccines aimed at the NAb response are a harder problem, since the protein they target (Env) is so variable. One approach is to make vaccines with Env from several clades—often called *cocktail vaccines*. This strategy was first used by VaxGen to make the vaccines tested in its already-completed *Phase III* trials; for example, the study in Thailand mixed Env proteins from the two most common clades circulating in the country. Several newer candidates are also taking this route; for example, the Vaccine Research Center in the US has made cocktails with *env* genes from clades A, B and C.

Another approach (not yet in the clinic) uses hypothetical HIV sequences rather than real ones, and designs them to recognize the broadest possible set of Env proteins (at least in theory). These vaccines are made by first comparing the sequences of many HIV genomes from different clades using computers and then creating the sequence that best matches the most strains. Researchers are also analyzing the structure of the Env protein down to the finest level of detail—which may help them manipulate it in ways that unmask broadly neutralizing epitopes tucked inside the protein, or perhaps to engineer epitopes that “fit” the few broad NABs which have been isolated from infected people. (These approaches are described in chapter 7.)

testing for vaccine protection across clades

Having a wider range of vaccine designs, along with data on cross-reactivity, puts the field in a better position to address questions of cross-clade protection in *efficacy* studies.

So does the growing number of candidates based on different clades. Until a few years ago, vaccine developers

focused almost exclusively on clade B, which dominates the epidemic in industrialized countries but causes only about 12% of infections globally. (Two important exceptions are the products used in both Phase III trials conducted in Thailand.) But products from other clades account for most of the newer products, including several based on clade C—which is behind over half of all infections worldwide—and on A and D, both common in Africa.

How does all this help study cross-protection? There is broad agreement in the field that vaccine efficacy studies should start in a population where “matching” HIV strains are common, since this will measure whether the vaccine has any ability to protect, while minimizing any possible effects of strain diversity. Figuring out whether it also protects against strains from mismatched clades could be done either in parallel or after initial proof of efficacy, either by adding trial sites in regions where other clades predominate or by doing a single trial where several clades (the matched one plus some mismatches) are in circulation. Having candidates of different clades means that these trials can take place in diverse settings throughout highly affected developing countries.

Cross-reaction data also comes in here. Knowing what clades have at least some strains recognized by the vaccine strain gives clinical trial planners a rational basis for choosing mismatched populations—that is, populations with cross-reacting strains in circulation.

These developments have also helped reduce the political dimension that sometimes attached to the clade issue, rooted in the years when most candidates were based on clade B. This left some countries reluctant to carry out even safety trials of non-matched clades, and created demand for country-level “tailoring” of candidates for *Phase I* studies.

But attitudes have shifted, and there is wide recognition that broader, not narrower, vaccines are desperately needed. For example, in 2003 the African AIDS Vaccine Programme came out strongly in favor of planning efficacy trials to give clear answers about cross-protection. And it endorsed the

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notion of unmatched trials in Phases I and *II*, and in efficacy studies as long as there is evidence for cross-reactivity between the vaccine strain and local ones. With several Phase I trials of unmatched vaccines now going on in different parts of the world, hopefully the ground is getting prepared for the day when we have promising vaccines ready to be put to this test.

resources

www.iavireport.org/specials/specials.asp

Map showing “Global distribution of HIV-1 subtypes and recombinants,” which summarizes current understanding of the global distribution of HIV-1 strains (August 2003). Available from the International AIDS Vaccine Initiative, *IAVI Report* online special features.

<http://hiv-web.lanl.gov/content/hiv-db/mainpage.html>

Los Alamos National Laboratory. Databases of HIV and SIV sequences, and defined HIV epitopes, with software tools for analyzing and comparing them, plus research and review articles.