

## ESSAY

## SCIENCE &amp; SCILIFELAB PRIZE

# HIV-specific B cell response in patients with broadly neutralizing serum activity

Antibody characterization from single B cells led to identification of monoclonal antibodies with broad and potent activity against HIV

By Johannes F. Scheid\*

Since its discovery in 1981, HIV has killed over 25 million people; more than 33 million humans are now infected worldwide. Despite extensive efforts, a vaccine against HIV remains elusive. The reason for this lies in HIV's remarkable mutability.

Soon after HIV infection, the immune system attacks the virus, which then escapes immune recognition by mutating its surface envelope protein, gp140. The escaped viral variants are targeted again by the immune system, and what follows is a race between virus and the immune system in which the virus continually diversifies and remains one step ahead in most patients. It is surprising, that 5 to 10% of HIV patients eventually develop high titers of "broadly neutralizing" serum antibodies that neutralize a diverse panel of HIV isolates (see the figure, part A). Until recently, very little

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was known about the composition of these broadly neutralizing serum responses, which is why I set out to characterize them during my Ph.D. thesis.

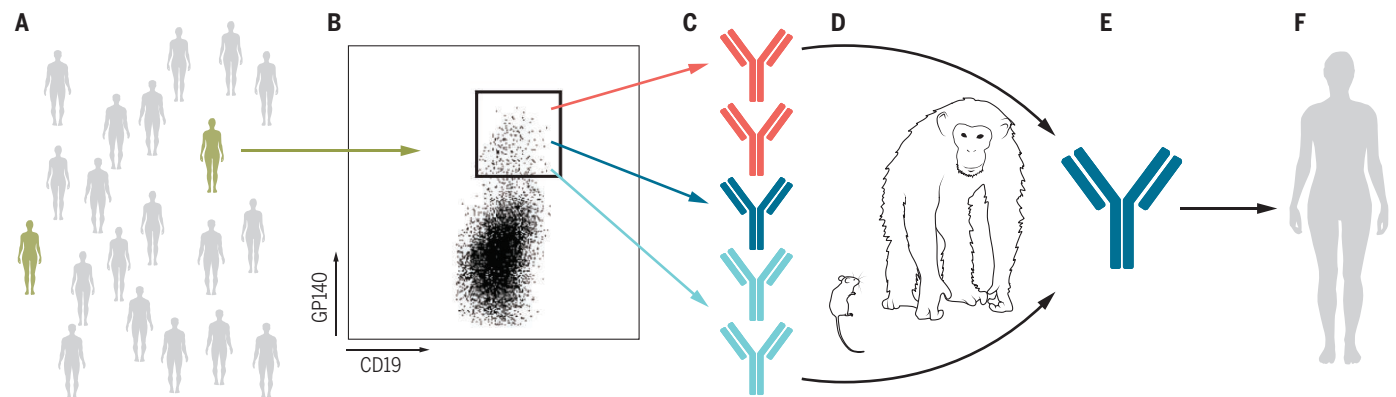
For this, I developed a technique that allowed me to isolate single HIV-specific memory B cells (1). Memory B cells originate from germinal center reactions and carry their affinity matured antibodies on the cell surface (2). I exploited this feature using a fluorescently labeled version of gp140 and flow cytometry in order to identify a population of gp140-binding

B cells (see the figure, part B). Once we identified this population, we used single-cell sorting and polymerase chain reaction (PCR) to amplify the immunoglobulin genes of single gp140-specific B cells (see the figure, part C) (3, 4).

We applied this technique to six different HIV-infected patients with broadly neutralizing sera. From these patients, we cloned 432 monoclonal antibodies specific for gp140; this allowed us to gain insight into the molecular nature of these individuals' HIV antibody responses (3). We found that each response comprised 22 to 50 differently expanded B cell clones. The majority

of these families of antibodies displayed extraordinary levels of somatic hypermutation. Cloning each antibody's matching heavy- and light-chain gene into expression vectors allowed us to reproduce the antibodies and to characterize their epitopes, affinity, and neutralizing activity. We found that 70% of the antibodies bound to the gp120 portion of the gp140 trimer and 30% bound to gp41 (3, 5). The gp120-specific antibodies bound with nanomolar affinities to sub-epitopes, including the CD4-binding site (CD4bs), gp120 core, the variable loops, or the CD4-induced site on gp140 (3, 6). The proportion of clones targeting these epitopes varied among different patients, in contrast to previous results, which reflected immunodominance of certain epitopes. When we tested them for neutralizing activity, we were able to reconstitute the breadth of serum neutralizing activity in two out of the four patients in this study but only with high concentrations of pooled antibodies. This indicated to us that we were missing an important part of the activity in the patients' sera.

To address this, I turned to a characteristic feature of the HIV antibodies we had found; their high levels of mutation. I knew the mutations were central to antibody function



**Isolation and testing of broadly neutralizing antibodies against HIV.** (A) Five to 10% of HIV-positive individuals eventually develop broad and potent serum neutralizing activity. (B) Fluorescence-activated cell sorting plot of a single-cell sort of gp140-specific B cells. (C) Single-cell antibody cloning of differently expanded B cell clones shown in red, dark blue, and light blue, respectively. (D) In vivo testing of the therapeutic and protective effect of antibodies in humanized mice and nonhuman primates. (E) Selection of highly potent and broad monoclonal antibodies. (F) Clinical testing in phase-I human trials.



## CATEGORY WINNER: TRANSLATIONAL MEDICINE

### Johannes F. Scheid

Growing up in New York and Germany in a family of scientists, Johannes Scheid was fascinated early in life by the career of a physician scientist. During medical school at the Charité—University, Berlin, he decided to pursue a Ph.D. at The Rockefeller University. His Ph.D. was based on the observation that some HIV patients develop very potent antibodies against HIV. The Ph.D. was awarded the 2012 Harold Weintraub award. After completing medical school

and his Ph.D., he worked for 1 year as an instructor in clinical investigation at The Rockefeller University and now is continuing his clinical training at the Massachusetts General Hospital in Boston.

because they lost their binding and neutralizing activity when reverted to their germline sequences (3, 7). This high frequency of mutations could, however, impede our antibody identification if mutations occurred in PCR primer-binding sites. I therefore opted to move the 5' primer further upstream and away from potentially mutated regions. In side-by-side comparisons, we found that the redesigned PCR primer set recovered highly mutated clones not identified with the original primers. We then decided to combine this improved amplification strategy with single-cell sorting, in which a modified version of gp120 (2CC core) was used as a target antigen. The 2CC core was shown to preferentially bind to neutralizing antibodies directed at the CD4bs and CD4-induced site on gp140 (8). Similar to our previous findings, a majority of the 576 new antibodies we cloned from two new and two of the previously studied patients were members of differently expanded B cell clones (9). However, many of the clones now carried even more mutations than we had previously observed. Moreover, six highly mutated B cell clones showed potent and broad neutralizing activity directed to the CD4bs and a new epitope that bridges gp120 and gp41 (9–11). The best performing of the new antibodies, 3BNC117, showed an average 80% inhibitory concentration on 95 different HIV strains of 1.4  $\mu$ g/

ml; this was broader and more potent than any of the previously described antibodies against HIV (9).

The exceptional potency and breadth of 3BNC117 set off a number of collaborative studies to investigate its *in vivo* therapeutic effect (see the figure, parts D and E). These revealed that 3BNC117 alone or in combination with other neutralizing antibodies could suppress plasma viremia to levels below detection in mice and nonhuman primates (12, 13). Moreover, intravenous administration of 3BNC117 protected rhesus macaques from challenge with simian HIV (13). In a phase-I human clinical trial conducted at The Rockefeller University Hospital, eight out of eight viremic individuals off antiretroviral therapy who received one dose of 3BNC117 at 30 mg/kg body weight showed rapid decreases in their viral loads, from 0.8 to 2.5  $\log_{10}$  depending on the individual (14). Further clinical trials with 3BNC117 are under way (see the figure, part F).

To determine whether broad and potent CD4bs antibodies share common sequence features, we aligned the 10 best CD4bs antibodies from our study (9). This revealed a conserved consensus sequence that covered 68 immunoglobulin heavy chain variable region (Igh-V) residues. In addition, all 10 of these antibodies arose from only two closely related germline *Igh-V* genes. Given

that these antibodies were isolated from different individuals infected with different HIV strains, this key observation suggests that the path to this class of antibodies is restricted—an important consideration for ongoing efforts to elicit this class of antibodies by vaccination (9, 15, 16).

This project has started an exciting journey from a bedside observation that certain individuals develop broad serum activity against HIV, to the bench, where we developed a technique to characterize this type of activity, and back to the bedside, where one of these antibodies is now being infused in clinical trials (see the figure). The single-B cell isolation approach has now been adapted and modified by other groups working on HIV (17, 18). By investigating some exceptional antibody responses against this constantly evading virus, our colleagues and we hope to broaden our therapeutic armament against HIV and design an efficient HIV vaccine. ■

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