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New studies help put mucosal immunity on the radar

by Emily Bass

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The human body's mucosal surfaces — a vast immunological territory with a surface area equivalent to one and a half basketball courts — are its first immune barriers to the outside world. As such, they are thought to play a key role in susceptibility to HIV. And with over 80% of HIV infections transmitted sexually, meaning that they begin with virus crossing a mucosal surface in the genitals or rectum, immune responses at these borders could be a critical component of vaccine-induced protection.

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But despite its potential importance, there are relatively few studies on mucosal immunity against HIV, making it hard to fit into the big picture of protection. That's largely because analysis of mucosal immune activity (particularly cell-mediated responses) requires invasive procedures — unlike systemic immune responses, which are easily measured from blood samples. Not surprisingly, AIDS vaccine trials have not focused on delivery of vaccine to mucosal surfaces and rarely include mucosal sampling, so there is almost nothing known about whether current vaccine candidates stimulate local responses.

In recent months, however, new research has helped bring some key issues into focus. Novel assays are beginning to replace the invasive, variable sampling methods that have been a major bottleneck, as several speakers described at the recent AIDS vaccines conference in Keystone ("*AIDS Vaccines in the New Millennium*," 28 March - 3 April). A number of labs are comparing different immunization routes for their ability to induce mucosal responses, and there are even a few mucosal vaccine candidates in the pipeline. And overall, more and more animal studies are using mucosal (rather than intravenous) challenges. Taken together, these leads could help move mucosal research closer to the mainstream of AIDS vaccine work.

"More people are working in mucosal immunology," says George Lewis, director of vaccine development at the Institute of Human Virology (Baltimore). "The science that's getting done there is better than it used to be. We're seeing a shift in momentum," he adds, in a field that

doesn't change gears easily or quickly.

Mucosal responses and protection: making the link

The first evidence for an association between mucosal responses and protection in humans came from studies on the immune systems of women who were highly exposed to HIV, either through sex work or through an infected stable sex partner, but remained seronegative (exposed seronegatives, or ESN). Rupert Kaul (Oxford University) and colleagues and Mario Clerici's lab (University of Milan) each found that a high proportion of these women had secretory IgA in their genital secretions, compared to HIV-positive women or low risk controls. Since then, other groups have made similar observations in some, but not all, ESN cohorts. In a separate study by the Kenyan research group, many ESNs also showed HIV-specific CD8 T-cells in their genital tracts, and the ratio of mucosal to blood CTLs was generally high; HIV-infected women tended to show the reverse ratio.

In Keystone, Sarah Rowland-Jones (Oxford University) extended these findings, reporting that CD8 responses in ESN women who have remained seronegative for at least three years tended to have higher HIV-specific CD8 T-cell levels in the blood than ESN women whose period of exposure was less than three years. The team has not yet looked at these responses in the mucosa, but they now plan to follow a group of these sex workers prospectively and look at the effects of a break from sex work on both systemic and mucosal responses, as well as on HIV status.

Mucosal versus systemic immunization in macaques

At Keystone, Jay Berzofsky (National Cancer Institute, Bethesda) caused a buzz with data from a small study showing that mucosal, but not systemic, immunization protected macaques against an SIV challenge results that mimic his lab's earlier findings in mice. In this study, macaques were immunized intrarectally (ir) or subcutaneously (sc) with a peptide vaccine containing two epitopes from SIV-Gag and one from SIV-Pol.

Berzofsky reviewed data from three animals immunized ir, four sc and two control animals that were successfully infected via ir challenge with pathogenic SHIV-ku and then monitored for 200 days. (One control and one animal from the ir group did not become infected.) All infected animals showed similar viral peaks shortly after challenge. But following this peak, the three IR-immunized animals brought their viral loads down to undetectable levels and maintained high CD4 counts out to 200 days post-infection. In contrast, the four sc-immunized animals had significant viral loads and CD4 T-cell count declines. All seven immunized animals showed some degree of protection in comparison with the two controls, which had the most pronounced CD4 T-cell depletion.

At 200 days, animals were sacrificed, autopsied, and evaluated for HIV levels in the colon and jejunum. Both these organs are lined with gut-associated lymphoid tissue (GALT) where, early in infection, HIV establishes a large pool of replicating virus which then seeds the bloodstream. Berzofsky and colleagues hypothesized that mucosal immunization could enhance protection by boosting responses in these tissues, thereby reducing viral load at the "supply source."

Their results were consistent with this notion: little or no HIV was seen in the colon and

jejunum of ir-immunized macaques, while control and sc-immunized animals showed 10-100 times more virus. Most important, the three infected IR-immunized animals had significantly higher levels of HIV-specific CTLs in their colon than the sc-immunized animals, suggesting that CTL played a key role in controlling viral replication in these tissues. The data also agree with Berzofsky's earlier studies in mice showing a clear link between mucosal vaccination, the generation of mucosal CTLs, and protection against subsequent mucosal challenge. In addition, Michael Murphey-Corb's group at the University of Pittsburgh found a correlation between strong anti-SIV responses in the gut of macaques and protection against subsequent challenge with a heterologous primary isolate (SHIV/Delta-B670). The latest work from Berzofsky is the first to correlate a mucosal (vs. systemic) route of immunization with both improved local responses and improved protection in primates.

"It's an important result," says Paul Johnson (New England Regional Primate Research Center, Cambridge), who also presented new data on induction of mucosal responses at the conference (see below). "It suggests that it may be better to induce mucosal versus systemic responses." For Berzofsky, the results are a step in the direction of complete protection. "Our hope is that with a stronger mucosal response we might do even better," he said at Keystone.

Can systemic immunization induce sufficient mucosal responses?

Berzofsky's study also raises a key question for the field: is mucosal immunization the best way to stimulate mucosal responses, as his data suggests, or is systemic immunization sufficient, at least with some vaccines?

Julie McElrath (University of Washington, Seattle) has conducted one of the few studies of human mucosal responses to HIV vaccines. Last year at the World AIDS Conference in Durban, her colleague Luwy Musey reported that systemic immunization with an HIV-canarypox vaccine (ALVAC vCP205) induced cervical or rectal CTLs in 4 of 7 tested volunteers who also had blood CTLs. Blood and mucosal CTL had the same epitope specificities, suggesting that systemic immunization induced responses in both compartments.

Based on these observations, and lack of evidence to the contrary, McElrath believes that systemic immunization will induce sufficient protection in the blood and at the mucosal sites. She believes that most CTLs in the blood are en route to the site of infection and that, regardless of immunization route, a large proportion of T-cells in vaccinees who subsequently become infected will home to the genital mucosa. "I am not yet convinced that we have data in humans saying we have to target mucosa,' she says. "I'm sure it doesn't hurt, but I don't think we need to do it."

Other researchers point to findings that the numbers and types of immune cells can differ dramatically in the systemic versus mucosal compartments, which operate largely independently of one another. While systemic responses can correlate with mucosal responses, there are also hints from the Kenyan ESNs and elsewhere that anti-HIV responses differ between the two compartments. For example, Deb Anderson of Harvard University (Cambridge) has studied CTL in the blood and semen of HIV-infected men and ALVAC trial volunteers, and found differences in cell-mediated responses in the two immune compartments. "This suggests that the peripheral immune response isn't reflective of the genital tract," she says.

Do these differences mean that systemic vaccination will leave mucosal sites unprotected or is exchange between the two compartments enough? Looking at other diseases, it's known that systemic vaccines against pertussis and influenza (both of which infect mucosal tissue) induce protective mucosal antibodies which appear to be derived from the blood. Yet conclusions drawn from vaccines for diseases that target the respiratory mucosa may be less relevant to HIV, a chronic, sexually-transmitted infection that targets immune cells. More antibody may be needed for a disease like HIV, say some researchers, who argue that until we know otherwise, it makes sense to pursue strategies which maximize responses at the site of infection.

"Nobody knows how much or what type of antibody you need in the serum or secretions to get mucosal protection from HIV," says Harvard's Marian Neutra, who is working on this problem. "The highest level of antibodies in tissues and secretions is attained when you immunize locally." At Keystone, Neutra presented recent data by colleague Pam Kozlowski comparing IgA levels induced in various mucosal tissues by a test vaccine (containing recombinant cholera toxin B) given to women via different immunization routes. The results showed large variations: high antibody levels in the rectum were seen after rectal immunization, but not when vaccines were given orally, vaginally or nasally. Vaginal and nasal immunization both induced good cervical responses.

More insight on immunization routes is coming from the work of immunologist Tom Lehner (Guy's, King's & St. Thomas' Hospital Medical Schools, London). Next year, he will launch an amfAR-funded trial of targeted iliac lymph node immunization (TILN) in men using a canarypox vaccine and gp140 boost. Lehner has pioneered TILN, which deposits vaccine in the vicinity of the local lymph nodes in the groin. In his earlier comparative studies with oral, nasal, rectal, vaginal, and systemic immunization, TILN induced the most consistent levels of mucosal IgA and IgG. These studies also suggest that the vaccine does not need to be applied at the mucosa as long as primed immune cells travel there after immunization.

Several researchers, including Tom Lehner, George Lewis and Paul Johnson, have suggested that the antigen itself may be a determining factor: perhaps a replicating vaccine vector such as live-attenuated virus or attenuated salmonella makes its way to the mucosa and induces responses at these sites, even if it is administered systemically. That's just what Johnson found when he compared immune responses in macaques immunized with live-attenuated SIV to those given a DNA-MVA vaccine: In four macaques given a live-attenuated SIV vaccine, between 36-84% of the total SIV-specific CD8 T-cells expressed alpha4beta7, the "homing" marker that identifies cells trafficking to the gut mucosa. In contrast, the range in three DNA-MVA vaccinated animals was 5-6%.

For non-replicating antigens, the route of administration may be far more important, according to this line of thinking. That's why induction of mucosal responses with a peptide antigen, such as the one Berzofsky used, may require a mucosal route otherwise these key barriers will never "see" the antigen. This could also hold true for vaccines like MVA, which have limited replicative capacity.

Homing in on new markers of mucosal protection

Until recently, all discussions of mucosal immunity have led to the same impasse: the extreme

difficulty of gathering data from human subjects. Cytobrush technique, the standard assay for gathering cells from the female genital tract, gathers fewer than one million cells, and samples are easily contaminated by blood. Paul Johnson's lab has also developed a new "pinch biopsy" technique that allows for relatively atraumatic, ongoing sampling of vaginal and rectal tissue. While useful for animal models, however, this procedure is limited by huge variability within the mucosal tissue, making sampling highly non-reproducible: the composition of a biopsy sample can be dramatically different from tissue just a few millimeters away.

At least a partial solution now appears to have been found: several researchers, including Johnson, are pioneering the use of homing markers as a way of measuring mucosal responses in the blood. Well-known to basic immunologists, homing markers are cell surface molecules that indicate the destination of cells in the blood stream. One such marker is alpha4beta7, an integrin that appears on virtually all cells trafficking to the gut-making it possible to monitor these cells from blood samples rather than from the mucosa itself. Although the exact specificities of other potential markers have yet to be fully defined, these molecules could become powerful tools for applied vaccine research.

Their usefulness in the clinical assessment of vaccines will soon be put to the test. Phase I clinical trials are expected to start next year for a mucosal (oral) vaccine candidate based on attenuated Salmonella, an enteric bacteria, as a vector for HIV-DNA. The study will be carried out in the US by the IHV (George Lewis' home institute) and in Uganda as a collaboration between the IHV, the Uganda Virus Research Institute in Entebbe, and IAVI. The HIV-DNA to be incorporated into the vector is now in trials as a systemic naked DNA vaccine in Oxford and Nairobi and in an MVA viral vector, which will allow comparison of these different immunization routes.

Besides alpha4beta7, the study may also analyze CXCR3, a chemokine receptor found by Paul Johnson on a high percentage of cells trafficking to the female genital tract in macaques. Another candidate is CCR7, a marker of immune memory cells that localize in the lymph nodes. Cells without CCR7 appear to home to peripheral sites, including the mucosa. When combined with tetramer staining, these markers should allow for more precise quantitation of cells, both in blood and in the small samples obtained by cytobrush or biopsy.

Future directions

These new assays should make it easier to monitor mucosal immune responses in large-scale vaccine trials, taking the field into uncharted territory. With the exception of Sabin's oral polio vaccine and the nasal adenovirus vaccines used to protect military recruits from colds, almost all licensed human vaccines are thought to work via systemic immunization. The new possibilities in mucosal research may not change this focus which has yielded many successful vaccines thus far but should provide a much clearer picture of how these vaccines work at mucosal sites.

At the more basic research level, several other promising avenues of research could lead to better targeting of the mucosa. For example, efforts to fill in the picture of early events in HIV infection are pointing to steps where intervention might contain the virus locally, before it spreads through the body. Dendritic cells in the mucosa appear to ferry HIV to the local lymph nodes, and from there it quickly spreads to other sites, including the gut. Over the past

year, this understanding has led to an intense focus on DC-SIGN, the receptor which plays a key role in carrying HIV to the lymph nodes; efforts to understand and inhibit this activity are ongoing.

Other efforts are focused on developing strong mucosal adjuvants. Immunologist Tom Lehner is studying 70kD heat shock protein (HSP70), which appears to upregulate expression of some protective chemokines, while Ken Rosenthal (McMasters University, Ontario) is testing CpG, an adjuvant made from fragments of synthetic bacterial DNA, which he showed can enhance genital immune responses, including antibodies and killer T-cells, in mice given an intranasal herpes vaccine. And at IHV, George Lewis and David Hone have developed an altered form of cholera toxin, one of the most widely-used mucosal adjuvants, that is considerably less toxic than the current formulation, which causes diarrhea.

Useful data on immunization routes could also come from studies that challenge monkeys in a manner more akin to the actual conditions of sexual transmission multiple low-dose exposures over time rather than single, high-dose i.v. challenges, says Julie McElrath. Another possible strategy is a prime-boost local-systemic combination.

Once again, the polio vaccine story offers important lessons, says Tom Lehner, who points out that Salk and Sabin reached the same goal with two different vaccines one systemic, the other mucosal. "In the final analysis, it may be a situation like polio, where you have two different vaccines and they both work," he says. But with a virus that has so far eluded a vaccine, it is important to look at all strategies including mucosal immunization, he says. "HIV goes for some essential parts of the immune system. Whatever we have learned previously may not apply."