Mucosal specimen collection, processing and assay - Experience from a resource-limited setting in Kenya (P04.16)

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ABSTRACT

In sub-Saharan Africa sexual intercourse remains the primary route of HIV-1 infection. Induction of mucosal immune responses will likely be necessary for an effective preventative HIV vaccine. KAVI embarked on building capacity for mucosal immunological work and disseminating this to other African research sites. With support from the International AIDS Vaccine Initiative (IAVI), KAVI clinical and laboratory teams designed SOPs for collection, processing and assay of cells and secretions from gastrointestinal, genital and upper respiratory mucosal surfaces over the course of 4 years and multiple studies. Specimens analyzed for humoral immune responses included saliva, nasal turbinate, nasopharyngeal, cervical-vaginal and rectal secretions. Cervical-vaginal and rectal cytobrush samples, and rectal and sigmoid biopsies were stained and analyzed by flow cytometry. Participants were free to opt out of any collection. Reasons for refusal and other acceptability/tolerability data were collected. Depending on acceptability/tolerability and assay results, collection methods were dropped or SOPs improved as needed. A curriculum was developed for training other African research centers involved in HIV research on mucosal sample collection and processing. Four mucosal studies were conducted at KAVI, one involving participants from three Phase 1 preventative HIV vaccine trials. Repeated mucosal sampling in both high and low risk participants was generally well accepted/tolerated (AIDS Vaccine 2010 and 2012, P10.07 and P122 respectively). Cellular and humoral immune responses to HIV were detectable in various mucosal compartments including relatively easier sampling sites like the mouth and nose. One centre in Rwanda received training and subsequently conducted a mucosal study; training of more centers is ongoing. In conclusion, mucosal sample collection and processing from various mucosal compartments and by various sampling techniques is possible in a resource-limited setting. HIV-relevant immunological responses are detectable in both genital and non-genital mucosal compartments. South-to-south collaborations for technology transfer in mucosal immunological studies is feasible and should be encouraged.

BACKGROUND

Induction of mucosal immune responses will likely be necessary for an effective HIV vaccine. Accordingly, understanding mucosal immune function is essential. It is thus necessary that research sites build capacity in consenting, sample collection and processing, and laboratory assay techniques for mucosal immune studies. With support from IAVI, KAVI embarked on building clinical and laboratory capacity for mucosal immunological work internally, and subsequently designed and implemented a mucosal sampling, processing and analysis training program for African research sites.

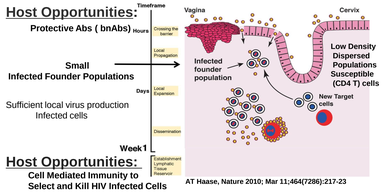

Figure 1. The short window of opportunity to control HIV

METHODS

With support from IAVI, the KAVI clinical and laboratory teams designed Standard Operating Procedures (SOPs) for mucosal sample collection, processing and assay of mucosal specimens over the course of 4 years and several studies (Table 1). The specimens included cells and secretions from gastrointestinal, genital and upper respiratory mucosal surfaces. Participants were consented and given the opportunity to opt out of any mucosal sampling procedure. Reasons for refusal and other acceptability/tolerability data were collected. Depending on acceptability/tolerability and assay results, collection methods were dropped or SOPs improved as needed. Subsequently, a curriculum was developed for training other African sites involved in HIV research on mucosal sample collection and processing.

ESN (Protocol J)	Gut Biopsies (Protocol I)	Vaccine-related (Protocol M and S001)	Sample Collection (Protocol L)
Evaluates immunologic markers of exposure in Exposed Seronegative compared with low risk HIV-uninfected and HIV-infected volunteers	Gut biopsies in patients undergoing colonoscopy for clinical conditions	Mucosal collection within IAVI vaccine trials	Allows sample collection to prepare for upcoming vaccine trials (e.g., test collection methods, develop assays)

Table 1: Mucosal studies at KAVI

Mucosal secretions: Mucosal secretions were collected by the methods shown in Table 2. The Merocel sponges and swabs were placed into spin-X tubes containing extraction buffer. Semen was collected into a sterile plastic container with transport media. The mucosal secretions were stored at -80ºC and later analysed for anti-HIV p24/gp140 IgG and IgA antibodies by ELISA.
Mucosal cells: Biopsies were digested to release mucosal mononuclear cells and all populations were assessed using multiparametric flow cytometry (Kaltsidis *et al*.).

	Protocol				
Sampling Method	**J**	**M**	**L**	**I**	**S001**
Merocel sponge (rectal and cervical)	X	X			X
Digene cytobrush (rectal and cervical)	X				
Semen	X	X			
Rectal biopsies	X				
Softcup	X	X			X
Aspirator	X				
Saliva (parotid gland – Salimetrics Oral Swab, oral fluids/transudate – straw)	X	X	X		X
Nasal flocked swab			X		X
Synthetic Absorptive Matrix strip (nasal secretions)			X		
Gut biopsies				X	X

Table 2. Sample collection methods used in each protocol

RESULTS

Since 2008 KAVI has conducted five distinct mucosal studies, including two where participants from four ongoing HIV vaccine trials were sampled. To date, 196 participants have consented and joined the mucosal studies; 97 of these (49%) were participants enrolled in HIV vaccine trials. Of 34 volunteers undergoing colorectal biopsies at KAVI only 6 were HIV seropositive. While backgrounds remained low in the seronegative volunteers TNFa, IFNg and/or IL-2 T-cel responses against Gag (p24) and Nef peptides could be detected in 3 of the 6 seropositive volunteers (data not shown).

Repeated mucosal sampling in both high and low risk participants was generally well accepted and tolerated (AIDS Vaccine 2010 and 2012, P10.07 and P122 respectively). KAVI has established which mucosal specimen collection methods are least tolerated and optimized sampling to make it more tolerable to the volunteer. Rectal cytobrush collection, in addition to eliciting some discomfort in some participants, did not yield enough cells for functional assays and was subsequently removed from the studies. Similarly, nasopharyngeal collection of secretions was uncomfortable compared to mid-turbinate collection; leading to mid-turbinate being adopted for mucosal collection (Please see Poster P08.27 LB for additional information on this sample collection). Nasal samples collected using a flocked swab gave a better antibody yield compared to nasal samples collected by Synthetic Absorptive Matrix strips; the latter collection method was subsequently withdrawn (see Figure 2).

Humoral responses to HIV could be detected in several mucosal compartments, in a reproducible manner, including in compartments relatively easy to access, such as the mouth and nasal cavity.

KAVI was able to compare different locations and devices for sampling, such as the nasopharyngeal tract and mid-turbinate region of the nostril.

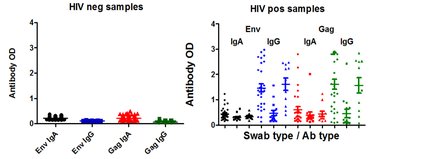

Figure 2. Comparison of nasal sampling methods to determine antibody yield. See P08.27 LB for further details.

KAVI has developed both clinical and laboratory capacity – including trained personnel and specific equipment – for mucosal immunological studies. KAVI subsequently transferred knowledge and skills in mucosal sample collection, processing and assay to personnel at the following African research sites: Projet San Francisco in Kigali Rwanda; KEMRI-CGMRC Kilifi, Kenya; Walter Reed Kericho, Kenya; and Uganda Virus Research Institute (UVRI-IAVI) Entebbe, Uganda.

DISCUSSION

We have established which mucosal sample collection devices are acceptable to volunteers for repeated mucosal specimen collection, and yield the best mucosal immunological data. These methods have subsequently been employed in HIV vaccine clinical trials to assess vaccine induced mucosal immunological responses. While rectal sampling among lower risk participants showed the lowest uptake rate, we observed a progressive improvement in uptake over time, likely to be attributable to staff experience. There is need for further research to better understand the reasons for uptake and refusal of rectal sampling in this group, and to understand how this type of sampling can be made more acceptable to study participants. KAVI has developed clinical and laboratory capacity - both personnel and equipment – for mucosal immunological studies. KAVI is conducting successful mucosal studies technology transfer to African research sites. This South-South technology transfer may have unique advantages; it is thus necessary to appraise this to determine benefits and areas for improvement.

CONCLUSION

Mucosal sample collection and processing from various mucosal compartments and by various sampling techniques is possible in a resource-limited setting. HIV-relevant immunological responses are detectable in both genital and non-genital mucosal compartments. There is the hope that immunological responses to candidate HIV vaccines would also be detectable at mucosal surfaces. South-to-South collaborations for technology transfer in mucosal immunological studies are feasible and should be encouraged. As the field of HIV vaccine development evolves, techniques for mucosal immunological studies are likely to evolve too, hence the need for continued North-South, North-North and South-South consultations and collaborations.

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