Mucosal Immunology and Models of Mucosal HIV Infection

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The mucosa associated lymphoid tissue regulates and coordinates immune responses against mucosal pathogens. Mucosal tissues are the major targets exposed to HIV during transmission. In this paper we describe in vitro *models of HIV mucosal infection using human explants to investigate target cells within this tissue.*

Key words: mucosal immunology - HIV - immunity to HIV - mucosa associated lymphoid tissue

COMMON MUCOSAL-ASSOCIATED LYMPHOID TISSUE

The concept of a common mucosal-associated system regulating and coordinating immune responses at mucosal surfaces has been an important advance in our understanding of protection against mucosal pathogens. This system, called the mucosa associated lymphoid tissue (MALT), is based on primed T and B lymphocytes that migrate from the site of antigen presentation via the lymphatics and blood to selectively "home" to lymphoid tissue at distant sites in gastrointestinal, respiratory, genitourinary and other mucosa-associated regions.

The majority of human pathogens are encountered at a mucosal surface, and therefore, in addition to providing protection against naturally acquired pathogens, MALT has great attraction for the development of mucosally protective vaccines. The gut in particular, with its great surface area and huge population of immune cells (gut-associated lymphoid tissue) and specific antigen sampling M cells overlying Peyer's patches, provides an attractive target for immunization. Novel vaccines to protect inaccessible human mucosal surfaces and secretions (such as the genital tract or breast milk) might therefore be delivered to the gut or nasal tract and protection disseminated throughout MALT. Although evidence exists, however, for the trafficking of primed B (Lewis 1991) and T (Castello-Branco 1994) cells after oral antigen delivery, the characterization of a common mucosal immune system in humans is incomplete.

When compared with trafficking B cells induced by systemic immunization, mucosally derived B cells expressed more advanced markers of B cell maturation, were phenotypically more homogeneous, and lacked the adhesion molecule L-selectin which is associated with homing to peripheral lymph nodes (Quiding 1995b). This study demonstrated for the first time in humans clear phenotypic differences between mucosal and systemic B-cell responses, and further studies, particularly with reagents to detect mucosa-specific adhesion molecules, may begin to unravel the complex mechanisms regulating the differential homing seen after immunization of various mucosal sites.

A key addressin appears to be the integrin $\alpha 4\beta 7$, which is expressed on mucosal T and B cells and is the dominant receptor for the glycoprotein mucosal vascular addressin (MAdCAM-1). Pals et al. (1994) found that malignant lymphomatous polyposis tumors (a gastrointestinal variant of mantle cell lymphoma characterized by multiple lymphomatous polyps in the gastrointestinal tract) express $\alpha 4\beta 7$ integrin, in contrast with control cases of lymph node mantle cell lymphoma. This suggests that $\alpha 4\beta 7$ may act as a mucosal addressin for these human gastrointestinal tumours. Boll et al. (1995) studied the phenotypic nature of gastrointestinal T cells in the rodent model, and found differential expression of $\alpha 4\beta 7$-related integrins by large and small intestinal lymphocytes, suggesting that either the specific homing of T cell subsets to different parts of the gut, or the modulation of phenotypic differential characteristics by the regional microenvironment of the gut may be regulated by expression of adhesion molecules. Quiding et al. (1995a) also found sub-compartmentalization within MALT in a study of human volunteers receiving nasal or intratonsillar immunization. Although nasal immunization resulted in a more disseminated response, response to tonsillar immunization was localized to the upper aerodigestive tract. The fine detail of MALT compartmental-

ization in humans will need to be explored if these novel routes of immunization are to be exploited effectively.

HIV AND THE MUCOSA

Mucosal tissues are the major targets exposed to the HIV during transmission. In the majority of subjects the initial acquisition of HIV involves passage of virus across a mucosal surface. The sexual route is the most important route of transmission in: (1) homosexuals where lymphoid cells are likely to be the prime target and (2) heterosexuals where the genital tract provides the virus access to lymphoid cells. In children, the upper gastrointestinal tract mucosa seems to participate in acquisition of infection in vertical transmission through swallowing of (1) HIV-infected amniotic fluid *in utero*, (2) HIV-infected blood and cervical secretions intrapartum, and (3) HIV-infected breast milk post-partum.

In order to define more clearly the possible effect of HIV on intestinal mucosa, we developed *in vitro* models of HIV infection using human foetal explants. In addition we developed a model of cervical explant culture to investigate target cells within this tissue.

IN VITRO STUDIES OF INFECTION OF HUMAN MUCOSAL TISSUE BY HIV

Intestine - The large intestine and rectum in male homosexuals, and the upper intestine in children being breastfed by HIV-infected mothers, are potential mucosal surfaces for HIV infection. However, debate exists as to the intestinal mucosa cells that are targets for infection with HIV. HIV-infected cells detected within intestinal mucosa in late stages of disease may represent trafficking of lymphocytes or macrophages from distant sites of the reticuloendothelial system. Results of an initial study of intestinal mucosal biopsy samples (Nelson et al. 1988) from patients with AIDS suggested that HIV infection of epithelial argentacromaffin was etiologic in HIV enteropathy. However, other studies have failed to detect HIV genome or proteins within epithelial cells but within small numbers of cells resembling macrophages in the lamina propria, and using immunocytochemistry we have detected HIV p24 antigen only within reticuloendothelial cells of European and African small intestinal biposy samples (unpublished observations).

In order to shed light on possible target cells for HIV in the intestine, we have developed *in vitro* mucosal explant culture systems using human foetal intestine. Adult intestinal biopsy samples disintegrate within 24 hr of *in vitro* culture and are therefore not suitable for detailed investigation over several days. In contrast, human foetal mucosal explants of small and large intestine survive in *in vitro* culture for up to two weeks. Using this system, we found that epithelial cells are not targets for HIV infection *per se*, but that CD4+ cells of both macrophage and lymphocyte phenotype were productively infected with virus (Fleming et al. 1992). Although atypical of the *in vivo* situation, this simple clearly excludes productive infection of enterocytes within the limits of detection used. We have analyzed HIV-infected mucosal explants using an antibody to a nuclear protein (PCNA) present in the mitotic spindle of actively replicating epithelial cells and have shown that there is an increase in epithelial cells expressing this protein, indicating that HIV infection induces epithelial cell proliferation (Batman et al. 1995). Such effects are clearly indirect since HIV cannot be detected within epithelial cells in this system, and we are investigating the possibility of cytokine production by HIV-infected immune cells.

Cervix - The mucosal route of infection in Africa is undoubtedly via heterosexual intercourse, and the paradox of the apparently relative infrequency of this route of transmission in the developed world remains a fundamental enigma. Seminal and vaginal secretions contain HIV, and it is therefore not unreasonable to assume that cells within the reproductive tract of men and women are targets for infection. Cervical biopsy samples from HIV-infected women have been shown to contain HIV-infected macrophages, but again this may represent trafficking from distant sites. We have recently investigated this using a simple *in vitro* culture system of normal ectocervix dissected from hysterectomy specimens. Such explants can be maintained in culture for up to 12 days. We find that cervical macrophages are the primary targets of HIV when macrophage-tropic strains of the virus are used. Surprisingly, using this explant system which contains both Langerhans cells (CD1a) and lymphocytes (CD3) neither of these cell types became infected with HIV. We are now using the system to investigate factors regulating HIV gene transcription in cervical cells, as well as agents-such as cytokines-that may be locally active in women with vaginal or cervical infection. Such sexual transmitted infections of the female genital tract are likely to influence susceptibility to HIV infection by attracting a population of CD4 bearing cells as part of the immune response and the production of ulcers in the epithelium exposing such cells to HIV.

CONCLUSION

We have shown that gut and cervical immune cells are potential targets for direct HIV infection

and have begun to study control mechanisms involved in subsequent mucosal immunopathological events with *in vitro* systems. We have demonstrated that humans respond to oral immunogens until late HIV disease with antibody and circulating B-cell responses. These findings raise the possibility of contriving immunity against HIV-related pathogens with novel mucosal vaccines. A better understanding of *in vitro* and *in vivo* interactions between HIV and the mucosal immune system will also be fundamental in vaccines against mucosal transmission of HIV. Ideally, the integration of cellular and molecular studies with an *in vivo* patient-centered approach will lead to the final understanding and control of these interactions, and it is this fundamental principle that directs our current and future studies.

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