Development Of Immune Assays For Oral Human Papillomavirus (HPV) In Patients With Human Immunodeficiency Virus (HIV) Infection

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Abstract

Opportunistic infections of the oral cavity afflict 50% of all HIV infected patients, and include oropharyngeal candidiasis (OPC), oral hairy leukoplakia (OHL) and oral warts caused by the mucosatropic human papillomavirus (HPV). The aggressive treatment of HIV with highly-active antiretroviral therapy (HAART) has significantly improved HIV patients' health and prognosis by lowering systemic HIV viral loads and restoring immune function primarily through increases in CD4+ T lymphocytes. This has resulted in a substantial decrease in the incidence of HIV-associated opportunistic oral diseases, including OPC and OHL. In stark contrast, the incidence of oral papillomas (warts) has reportedly increased since the widespread administration of HAART. It is felt that asymptomatic oral HPV infection occurs frequently but oral warts occur infrequently, presumably due to immunological control of the virus. The critical aspects of the immune response that prevent the progression from asymptomatic HPV infection to HPV disease are unknown, but previous studies have focused on HPV genotype-specific response against the major capsid protein, L1. The increased rate of HPV-related oral pathology seen in HIV+ patients' highlight the need for a more thorough understanding of the immune response to oral HPV infections. Furthermore, the accessibility of the oral cavity affords a unique opportunity to conduct rigorous analysis of HPV infection, immunity and pathogenesis. To initiate these studies, the development of the immunological assays specific for oral HPV genotypes such as HPV-32 are required. Ultimately, these assays will be used to investigate the role of immunity in the acquisition and subsequent clearing or progression of oral HPV infections, particularly in highly susceptible HIV+ patients.

INTRODUCTION

HIV has indirectly claimed the lives of over 3 million people to date, and over 40 million people worldwide are currently HIV infected (Joint United Nations Program on HIV/AIDS, 2002 statistics, http://www.unaids.org). HIV preferentially infects CD4+ T lymphocytes, resulting in a progressive depletion of these cells. This leads to immunodeficiency and subsequent opportunistic infections, including oral infections. Half of all HIV infected people experience an oral opportunistic infection within five years of HIV seroconversion[1]. These infections include oropharyngeal candidiasis (“oral thrush”, OPC), oral hairy leukoplakia (OHL, caused by Epstein-Barr virus), oral Kaposi's sarcoma (caused by human herpesvirus-8, HHV-8) and oral condylomas (caused by human papillomavirus, HPV). While opportunistic infections of the oral cavity are usually not life threatening, they do lead to significant morbidity and health care costs for HIV infected individuals.

The health of HIV-infected patients relies on the long-term control of HIV viral load and subsequent restoration of immunity. The most successful therapeutic approach to date has been a combination-drug approach known as highly-active antiretroviral therapy (HAART). HAART typically consists of a cocktail of three drugs, two of which are nucleoside analogue reverse transcriptase (RT) inhibitors, and one of which is either a non-nucleoside RT inhibitor or a protease inhibitor (PI). HAART has been responsible for decreasing HIV plasma viral loads, increasing CD4+ T lymphocyte counts, prolonging the progression to AIDS and decreasing the mortality from HIV[5]. HAART has also decreased the incidence of opportunistic infections, including those of the oral cavity. Since the introduction of HAART therapy, the incidences of OPC and OHL have significantly decreased [2]. In stark contrast, the reported incidence of oral warts in HIV+ individuals has significantly increased during the era of HAART[6]. Subsequently a report in Lancet by Greenspan et al. demonstrated a rise in the incidence of oral warts in HIV+ patients in San Francisco in

the 1990s[2]. Similarly, King et.al. reported an increased incidence of oral warts in an Atlanta HIV cohort[3]. The reasons for the unexpected rise in incidence of oral warts remain unknown; the spectrum of possibilities includes epidemiological (e.g., an epidemic of oral HPV infections, or unknown epidemiological confounder that explains the observation), chemical (e.g., a direct impact of treatment regimens on host or virus), viral (interactions between HIV and HPV and/or changes in their ecological niches), and immunological (e.g., immune restoration disease, or a lack of restoration of an essential component of HPV immune control) . Development of the immunological assays specific for oral HPV genotypes would provide the tools to examine the immunological aspect of this phenomenon, which may shed light on potential therapeutic or preventative modalities for oral HPV infection in HIV+ individuals.

HIV-ASSOCIATED ORAL PAPILLOMAS: PATHOLOGY AND ETIOLOGY

Papillomas can occur at virtually all oral mucosal surfaces[7]. While the majority of papillomas occur on the labial mucosa, they have also been reported on the buccal mucosa, the tongue, the palate and the gingiva. Papillomaviruses are a large family of DNA viruses capable of infecting a variety of epithelial surfaces, including both mucosal and cutaneous epithelium. Over 100 distinct genotypes of HPV have been identified (based on the genetic sequence of the L1, E6 and E7 ORFs), and at least 30 of these have been detected in the oral cavity. The gross appearance of oral warts varies greatly and often reflects the specific HPV genotype causing the lesion[7, 8]. For instance, HPV genotypes 6 and 11, the most common causes of genital warts, tend to cause soft cauliflower-like lesions (condyloma accuminatum) in the oral cavity. These infections can be chronic and cause recurrent respiratory papillomatosis (RRP). HPV genotypes 1, 2, and 7, which are associated with cutaneous warts, are often the cause of firm oral common warts (verruca vulgaris). HPV genotypes 13 and 32, which have been described exclusively in the oral cavity, are found in oral focal epithelial hyperplasia (FEH), a rare dysplastic lesion characterized by multiple small, flat papules that affects Eskimos, American Indians and HIV positive individuals. Unusual manifestations of HPV in the HIV positive patient frequently occur[7], and clinical appearance alone is not sufficient for assessing the HPV genotype present in an oral lesion. Therefore, identification of HPV genotypes requires molecular confirmation of the HPV DNA sequence present in the oral lesions of HIV positive individuals.

Greenspan et al. were the first to apply molecular techniques to describe the HPV genotypes found in oral warts in HIV infected individuals[7]. Southern blot analysis of wart biopsies demonstrated predominantly HPV-7; HPV types 13, 18, and 32 were also detected in one specimen each. The authors noted that the HPV genotypes identified in the lesions were predominately non-genital genotypes of HPV. Unfortunately, the analysis was limited to HPV types 1-32, and no HPV type was identified in 7 out of the 17 lesions examined. More sensitive methods such as PCR and DNA sequencing might have identified additional HPV genotypes. These methods were applied by Volter et. al. in an examination of 67 oral wart biopsies[9]. Southern blot hybridization was combined with PCR and DNA sequencing, and HPV genotype was identified in 67% (45/67) of the lesions biopsies. The predominant genotype identified was HPV-32, followed by HPV-7. A mixture of cutaneous and mucosal genotypes was detected, including the cutaneous type 2, genital types 6, 16 and 18, and oral type 13. This is the largest study to date examining the HPV genotypes in HIV-associated oral warts.

HUMORAL IMMUNITY TO HPV

Asymptomatic infection with HPV frequently occurs and the development of virus-associated lesions is a relatively rare event, presumably due to immunological control of the virus. This is exemplified by the increase in HPV-associated lesions in immunosuppressed individuals such as those infected with HIV. The specific immunological responses that are important for protection against HPV related pathology are largely unknown. This is in part due to the general inability to grow HPV in the laboratory, which makes development of HPV immune assays challenging. The deficiency in understanding of HPV-specific immunity is also due to the lack of an adequate small animal model for HPV. Studies of the humoral response to HPV have predominantly focused on human antibodies to the major capsid (L1) protein, which makes up 80% of the total protein produced by the virus[10].

HPV capsids (also known as virus-like particles, or VLPs) of various types of HPV have been produced by expression of the L1 protein in vaccinia, yeast or baculovirus expression systems[11,12,13,14]. These viral capsids appear identical to natural viral capsids[15] and have been used extensively in serological assays. The antibody response is conformational and genotype-specific, making intact HPV capsids ideal for use in enzyme-linked immunosorbent assay (ELISA)

systems for population screening.

Some of the first HPV types for which corresponding capsids were produced were low-oncogenic risk genotypes (HPV 6 and 11)[12, 16] and cutaneous genotypes (HPV 1)[11]. The majority of the early studies utilizing these capsids in serological assays were small case-control studies of persons known to be HPV infected, either by DNA detection or presence of warty lesions. All of these early studies reported significantly higher seroprevalence rates for HPV 6 and HPV 11 in people with genital warts than in people with no history of warts[4,17,18]. The HPV-specificity of the detected capsid antibody responses was further supported by the correlation between seroreactivity for a specific HPV genotype and detection of the same genotype in the cervix[4, 19]. Overall, the IgG serum response to HPV correlates with the detection of HPV DNA or HPV related disease. The serological response to oral-specific HPV genotypes (13, 32) has not been examined. This is well illustrated in Figure 1.

CELLULAR IMMUNITY TO HPV

The importance of cellular immunity in the prevention of HPV disease can be inferred from the clinical observations of increased HPV related pathologies (cervical abnormalities or warts) in immunosuppressed populations (HIV+, transplant patients)[20,21]. Further evidence of a protective role for T cell responses to HPV is provided by the presence of a CD4+ cellular infiltration in biopsies of warts [22, 23]. Due to the difficulties in establishing productive culture systems for HPV, most research on the cellular immune response to HPV was conducted using synthetic peptides of the L1, E6, and E7 proteins[24,25,26,27,28,29,30]. Initial studies showed that cellular responses to HPV E6 and E7 peptides do not correlate with regression of disease, possibly due to the low expression of these proteins until the advanced stages of disease, when cellular transformation has already occurred[31,32,33,34]. It is encouraging to note that two recent studies have provided evidence of a protective role for these responses earlier in the infection process [29, 30]. These studies have focused on HPV-16, the most relevant virus for understanding cervical cancer, and the generalizability of the data to low-oncogenic risk viruses, such as those found in HIV+ oral warts, is unclear. Cellular responses to HPV L1 peptides have also been examined[26,27,28]. These responses were strongest in people infected with that same type of virus, and in those with HPV-related cervical intraepithelial neoplasia grade III[26]. In normal, healthy people, the L1-specific CD4+ lymphoproliferative response consisted primarily of cells of memory phenotype2[7]. The population responded to a large range of peptides and these responses were HLA-restricted.

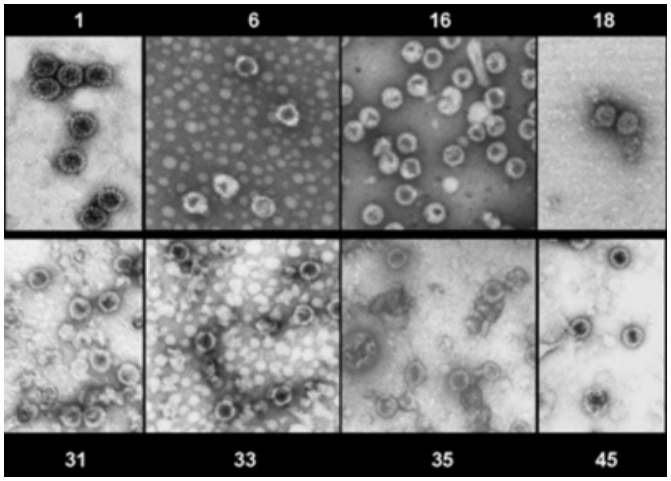
In 1988, Cubie and Norval[24] reported cellular immune responses to HPV virions, becoming the first to show CMI against full-length HPV L1 protein. Very few other studies have examined lymphoproliferative responses to intact viral capsids. Williams et.al, using HPV-11 capsids[27], found that ~50% of a population of healthy individual's demonstrated proliferative responses to these capsids, indicating that HPV capsids can serve as an effective antigen for LPAs. In a recent study by Gelder et al, the LPA responses to HPV 11 VLPs were studied in a group of people afflicted with RRP and controls[35]. This study showed a correlation between a strong LPA response to VLPs and an improved clinical course for the juvenile cases. This response was not as clearly seen when utilizing groups of peptides as antigen. Capsids may prove a better antigen for HPV-specific CMI than synthetic peptides because they should be taken up and properly processed according to the individual, making the detected response less HLA-restricted than that of peptide sequences. The CMI response to oral-specific HPV genotypes has not been examined.

SUMMARY

HIV infected individuals remain susceptible to oral warts despite widespread use of HAART, and the majority of these lesions contain HPV-32. Asymptomatic infections with HPV are common, while progression to HPV-related disease is rare. The aspects of the immune response which are critical for preventing the progression from asymptomatic HPV infection to HPV disease are unknown and the nature of the immune response to oral-specific HPV genotypes has never been examined. The increased rate of HPV-related oral pathology seen in HIV+ patients highlights the need for a more thorough understanding of the role of immunity in the natural history of oral HPV infections. This requires the development of the immunological assays specific for oral HPV genotypes such as HPV-32. These assays will be tested for optimal assay conditions, specificity, and reproducibility. Ultimately these assays will be used to investigate the role of immunity in the acquisition and subsequent clearing or progression of oral HPV infections, particularly in the highly susceptible HIV+ patient, with the hope of elucidating potential therapeutic or preventative strategies for HPV related disease.

Figure 1

Figure 1: Conformationally intact capsid particles that closely resemble native virions produced for HPV genotypes 1, 6,11,16,18, and 31,33,35,45.



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