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Genetic Variation of Adenoviruses Causing Conjunctivitis

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Abstract

Human adenovirus type 8 (HAdV-8) is the most common causative agent of a highly contagious eye disease known as epidemic keratoconjunctivitis (EKC). HAdV-8 strains have been classified into genome types HAdV-8A to 8K and HAdV/D1 to D12 according to restriction endonuclease analysis. This review focuses on the significance of HAdV-8 as an agent of EKC. Molecular analysis of HAdV-8 genome types HAdV-53 and HAdV-54 was performed to reveal potential genetic variation in the hexon and fiber, which might affect the antigenicity and tropism of the virus, respectively. On the basis of the published data, three patterns of HAdV-8 genome type distribution were observed worldwide: (1) genome types restricted to a microenvironment, (2) genome types distributed within a country, and (3) globally dispersed genome types. It showed that the HAdV-8 genome types were nearly identical to each other. HAdV-54 is very close to the HAdV-8P, B and E genomes, exceptin the hexon. In a restriction map, HAdV-8P, B, and E share a very high percentage of restriction sites with each other. Hypervariable regions (HVRs) of the hexon were conserved and were 100% identical among the genome types. The fiber knob of HAdV-8P, A, E, J and HAdV-53 were 100% identical. In phylogeny, HVRs of the hexon and fiber knob ofthe HAdV-8 genome types segregated into monophyletic clusters. Neutralizing antibodies against one genome type will provide protection against other genome types, and the selection of future vaccine strains would be simple due to the stable HVRs. Molecular analysis of whole genomes, particularly of the capsid proteins of the remaining genome types, would be useful to substantiate the observations.

Keyword: keratokonjunctivitis, Adenovirus type 8, Genome type, Molecular analysis.

Introduction

Epidemic keratoconjunctivitis or EKC is a highly contagious and severe form of eye disease caused by human adenoviruses (HAdVs). HAdV is a non-enveloped, double-stranded DNA virus belonging to the genus Mastadenovirus of the Adenoviridae family. Fifty-four types of human adenoviruses (HAdVs) have been divided into seven species, A (HAdV-A) through G (HAdV-G)[1-3]. Traditionally, the first 51 serotypes are distinguished on the basis of a serum neutralization assay and the remaining types are distinguished on the basis of their genome sequence and bioinformatics analysis[4]. All human adenoviruses have the same general structure. They are non-enveloped double-stranded DNA viruses that are 70–90 nm in diameter and display icosahedral symmetry. The capsid consists of 252 capsomeres, which form three major capsid proteins: the hexon, penton and fiber. The fiber projects from each of the 12 vertices or penton bases. The rest of the capsid consists of 240 hexon capsomeres.Within the core,the viral genome, which carries approximately 40 genes, is condensed in association with proteins V, VII, and X (or) and the five termini of the HAdV DNA are covalently linked to the terminal protein (TP)[2,5]. HAdV-8, HAdV-19 and HAdV-37 are common causative agents of EKC.Inadditionto these above-mentioned types, HAdV-3, HAdV4, HAdV-7, HAdV-9, HAdV-15, HAdV-53, and HAdV-54 have also been identified as causative agents of EKC[6-8]. However, HAV-8 is responsible for the highest number of EKC cases worldwide and is associated with severe clinical manifestations[9,10]. The virus is included in species D on the basis of various biological properties and genome sequence homology. The fiber protein of the virus is approximately 9–11 nm in length and binds to (2-3)-linked sialic acid (SA) and desialylated, branched hexasaccharide, GD1a glycan, as their receptor is oncorneal and conjunctival cells during the early stage of infection[11,12]. HAdV-8 also completely agglutinates rat erythrocytes. HAdV-8 does not exhibit oncogenic properties[5,13,14]. The prototype viral genome contains 34,980 base pairs and shows 94–99% DNA homology within the same species[15,16]. Most of the strains of HAdV-8, except the prototype (Trim), grow well in various epithelial cell lines, such as HEp-2, HeLa, and KB. Because Trim grows poorly in cell culture and yields an insufficient amount of DNA on extraction, it is known as a fastidious adenovirus. However, similar to other HAdVs, the A549 cell line is also very effective for the propagation of HAdV-8. The cytopathic effect (CPE) of HAdV-8 in a monolayer cell culture is characterized by the rounding and swelling of cells with nuclear enlargement, followed by cellular detachment from the culture surface into grape-like clusters[3,15-16]. The first strain of HAdV-8 was isolated in the United States in 1955 and was designated as the prototype (P), Trim[19,20]. Since its original isolation, HAdV-8 has been isolated from many

community and nosocomial outbreaks of EKC and is associated with morbidity and economic damage[15-16]. On the basis of restriction endonuclease analysis (REA), globally isolated field strains are categorized as genome type or genomic variant. Currently, genome types HAdV-8A to 8K and HAdV-8/D1 to D12 are incorporated in the Asian and European classification, respectively[17-18]. Some of these genome types (e.g., HAdV-8J) were isolated from sporadic cases of EKC and disappeared without causing any further outbreak, while others (e.g., HAdV-8E) have been frequently isolated from outbreaks and are distributed worldwide[10]. Furthermore, because specific genome types, such as HAdV-8H, showed more severe clinical symptoms compared to other types [17-18-19], it is reasonable to speculate that the outbreak-causing potentiality as well as the severity of clinical manifestation among the different genome types may be related to variation in tropism or in the major neutralization epitope of the virus. Considering the clinical effect of EKC, elucidation of the genetic makeup of HAdV-8 using molecular methods is very useful for future epidemiological study and selection of a vaccine strain. In this review, we focused on the significance of HAdV-8 as an agent of EKC. On the basis of published data, different genome types of HAdV-8 and recently emerged HAdV-53 and HAdV-54 have been analyzed to reveal potential genetic variation in the hexon and fiber, which might affect the antigenicity and tropism of the virus, respectively

Epidemic of keratoconjunctivitis

Adenoviral keratoconjunctivitis (AK) usually occurs as epidemics; serotypes 8 and 19 cause most outbreaks. Infection is usually transmitted through fomites or contaminated body fluids. The virus has been demonstrated in tears for up to 3 weeks after infection.[1] The cornea is usually involved 2 or 3 days after the onset of symptoms and most common presentation is multifocal subepithelial infiltrates (SEIs), which are considered pathognomonic of adenoviral infection.[2] In subcontinent countries, SEIs may be observed in about 50% of AK cases. These focal lesions may represent a cellular immune reaction against viral antigens deposited in the corneal stroma under the Bowman membrane.[3] Histopathologically, SEIs show disruption of collagen in the Bowman layer along with infiltration of lymphocytes, histiocytes, and fibroblasts; these are usually bilateral and often asymmetric and have the potential to cause significant ocular morbidity, reduced vision, photophobia, glare, halos, and foreign body sensation and can persist for months or years after the initial infection.[4,5] Although, AK is a self-limiting disease, most affected individuals seek treatment due to diminution of vision from persistent SEIs, pseudomembranes and iridocyclitis.[6,7] Various

modalities have been triedas treatment options for AK including palliative therapy, such as cool compresses, artificial tears, and topical steroids; it is believed that steroids, by suppressing conjunctival and corneal inflammation, provide symptomatic relief, but they do not shorten the course of the disease. The use of long-term topical steroids may be associated with side effects such as cataract and glaucoma, and topical administration of corticosteroids may also cause prolonged viral seeding

Identification and molecular characterization of adenovirus

The exact incidence of AdV associated keratoconjunctivitis is poorly known. Though the incidence of EKC at international level are unknown, some reports mentioned that EKC incidence occurred in summer months, or spring and winter seasons[8]. EKC is transmitted from person to person through direct contact or fomites. Virus (AdV) spread easily from infectious ocular secretions such as tear fluids, hands of health-care personnel, medical devices, products (eye drops) contaminated by virus have been implicated in the transmission in the environment[10]. In India, an outbreak of keratoconjunctivitis was reported earlier in Chennai (Tamil Nadu) during August–October 2010, and in this study, HAdV-6 and HAdV-2 were found associated with EKC based on PCR and phylogenetic analysis[10]. In the present retrospective observational study, keratoconjunctivitis cases were reported from Pune, (Maharashtra) Western India during the months of November–December 2013 and January, October– November 2014. This study is not related to any ongoing or systematic hospital based surveillance. Investigation was conducted to identify the viral etiological agents such as adenoviruses, enteroviruses associated. The study also aimed to characterize their genotype distribution in EKC. The present study identified adenovirus as the etiological agent with HAdV-8, as major and HAdV-3, 4, 37 are minor types associated to cause EKC. None of these cases were found positive for enterovirus. To the best of our knowledge this is the first report on AdV associated keratoconjunctivitis with multiple AdV types reported to cause EKC in Pune, (Maharashtra) Western India.

HAdV-8 & EKC

EKC was first described in 1889[18]. However, after a period of 40 years (1930), a viral etiology was suggested[19]. During the Second World War, a very large outbreak of keratoconjunctivitis occurred in naval shipyards in Hawaii. Subsequently, this outbreak spread to the West Coast of the

United States. Due to its epidemic nature,the illness was called “epidemic keratoconjunctivitis” (EKC) [10]. Because most of the sufferers were shipyard workers, the disease was also named “shipyard eye”[20]. However, the causative agent of EKC was unknown until 1955, when the conjunctival swab from a seaman (Trim) suffering from keratoconjunctivitis was cultured in HeLa cells and the agent was identified as HAdV-8 [11,12]. EKC is characterized by severe bilateral conjunctivitis with substantial corneal involvement. Other clinical presentations, such as edema of the eyelids, photophobia, and lacrimation may also be present. As the disease progresses, the appearance of corneal opacity emerges due to subepithelial infiltrates, which is observed in up to 50% of cases. Indeed, this infiltrate is a cellular immune response against HAd-V antigens. It consists of dendritic cells, lymphocytes, histiocytes and fibroblasts, which are trapped in the corneal stroma under Bowman’s membrane[13,14]. This infiltration is frequent in HAdV-8 infection[15]. When the opacity is located in the pupillary area, blurring of vision develops, which may persist for months and occasionally, for years. Although, EKC does not progress to blindness, ocular infections with the above-mentioned clinical features eventually result in significant morbidity and economic damage. As a naked virus, HAdV-8 is remarkably resistant to environmental conditions and remains infectious at room temperature for prolonged periods,thereby facilitating transmission fromhand-to-eye, via fomites, ophthalmic instruments and even ocular drops[16,17].

Nosocomial EKC often occurs, resulting in severe outbreaks in ophthalmology outpatients and inpatients, pediatric units and even neonatal care units. The outbreak may necessitate the restriction of clinical practices, such as the postponement of eye surgery, early release of inpatients from the hospital and even closure of wards[11-16]. Studies worldwide show that HAdV-8 is the most important agent of EKC and accounts for up to 80% of the cases[16,17,20]. Until recently, there has been no effective antiviral drug against HAdVs. The mainstay of patient management in EKC includes accurate diagnosis and symptomatic treatment, with artificialteardrops and (in some cases) antibiotics to prevent or treat superinfection. To reduce viral load and to provide symptomatic relief, local administration of povidone iodine and ganciclovir gel could be useful. Povidone-iodine in eye drops or gel form is well tolerated and results in a slightly shorter duration of illness and a somewhat lower frequency of subepithelial infiltrates formation. Chronic subepithelial infiltrates that do not resolve spontaneously over time can be treated with topical steroids because the drug has immunosuppressive and anti-inflammatory effects, although a recurrence rate of 30% has been reported after cessation of the drug[4-5]. Recently, multivalent sialic acid constructs based on 10,12-pentacosadiynoic acid (PDA) have been synthesized, and these constructs aggregate virus particles

and thereby prevent them from binding to ocular cells. Such formulations may be useful for the topical treatment of adenovirus-caused EKC[6].

Seroprevalence of HAdV-8

The major neutralization epitopes specific to each HAdV type are located in hexon protein loops 1 and 2, which have been utilized to type HAdVusing serological methods[17]. Antibodies againstthese epitopes neutralize the infectivity of HAdV[18]. The prevalence of antibodies against HAdV-8 is variable geographically. Studies among select populations in the United States, Italy and Britain showed only 5% positivity for anti-HAdV-8; whereas in Japan and Taiwan it was remarkably high, which accounted for approximately 30% and 60%, respectively[19,20]. The rise in the antibody titer appears to be quite slow against HAdV-8 and is detectable 14 days after the clinical onset of EKC[11]. In a preliminary serosurvey conducted by the World Health Organization, the lowest frequency of anti-HAdV-8 was found in Liverpool and from Eskimos in Alaska (4.5% and 12%, respectively). Frequencies of 30% and 31% were obtained with the sera from Hottentots and the Tunisian military recruits.

Finally, the highest frequencies were found in sera from the inhabitants of Sarawak, Arctic Indians in Alaska and Micronesians on the island of Rongelap (45%, 52% and 55%, respectively)[12]. A higher percentage of seropositivity indicates that HAdV-8 infection is common in these countries. The efficacy of HAdV-8 vaccination, i.e., the antibody titer and their protective ability against EKC, was evaluated on human volunteers. Two or three doses of injections of viable HAdV-8 showed the development of a good titer of type-specific neutralizing antibodies, and the volunteers did not develop EKC after an ocular challenge with HAdV-8. This in vivo trial indicates that vaccination could be an effective measure to prevent EKC, particularly among populations with a lower sero-prevalence[13]. Military recruits are more susceptible to HAdV-4 and HAdV-7- associated severe outbreaks of acute respiratory disease (ARD)than adult civilians [14–16]. Previous studies showed that one in every six recruits in affected camps required hospitalization[17]. It is more likely that environmental conditions, such as cold weather (winter) and the direct inhalation of the virus into the lungs during training, crowded sleeping conditions or fatigue associated with basic training, were contributing factors[3]. Thus, an entericcoated live vaccine was developed, which significantly reduced the disease burden [18]. However, vaccines against medically important HAdVs for use in the general population have yet to be developed.

Genome types of HAdV-8

REA analysis is a sensitive method for the comparison of closely related genomes, second only to nucleotide sequence analysis[19-20]. The appearance of a new genome type is a mutational event in the genome that is not always associated with serological changes[12]. Genome typing studies of HAdV-8 began in Europe in 1983[18], but an organized classification has been described by Adrian et al. in 1990[34]. In that classification, a numerical code of enzymes was used to denominate HAdV-8 genome types. Six restriction endonuclease (RE) patterns in alphabetical order (BamHI, BglII, HindIII, KpnI, SalI, and SmaI) were used. Subsequently, BglI, BstEII, and SacI were included in successive studies. The cleavage pattern of the prototype for all REs was labeled as enzyme code 1. Patterns deviating from those ofthe prototype were named 2, 3, and so on depending on the chronological order of the respective isolates. Thus far, strains HAdV-8/D1 to HAdV-8/D12 have been described[14-16]. In Asia, HAdV-8 genome typing studies began in 1983 and four REs, BamHI, HindIII, PstI, and SalI were applied in the analysis of the HAdV-8 prototype and field strains[17]. Many collaborative studies among Asian countries occurred subsequently with increasing numbers of REs. Currently, BamHI, HindIII, PstI, SacI, SalI, and SmaI are in use as a standard for this classification system. The letter 'P' is assigned to the prototype strain (Trim), and A, B, C, D, E, and so on are assigned to the remaining strains. Thus far, strains HAdV-8A to HAdV-8K have been described[17-20]. On the basis of the available published data, three patterns of the HAdV-8 genome type distribution were observed worldwide: (1) genome types restricted to a microenvironment, (2) genome types distributed within a country, and (3) globally dispersed genome types (Table 1). However, it cannot be ruled out that a specific genome type might have global distribution but is not yet detected due to insufficient screening.

Study of the genetic variability of HAdV-8 is valuable in epidemiology for several reasons: first, understanding the geographical distribution of pathogenic HAdV-8 strains can provide insight toward disease prevention and control; second, mapping the genetic similarities or differences among HAdV-8 strains is extremely important for the identification of candidate determinants of virulence and fitness; and third, examination of circulation patterns and the occurrences of different genome types can help to identify whether a virus is sufficient to circulate frequently across a geographic area[10,13,14]. Despite the clinical and epidemiological significance, genome-typing studies

of HAdV-8 is limited by its prolonged and labor-intensive procedures. Recently, a cost-effective REA method has been developed that is significantly quicker compared to conventional methods[15].

Over the last few years, HAdV-53 and HAdV-54 have emerged as agents of EKC[6,16,17]. HAdV-53 is an intertype recombinant strain (HAdV-22/37/H8) with global distribution and is serologically related to HAdV-22. It possesses the fiber of HAdV-8. It was originally isolated from an EKC case and was diagnosed as HAdV-22[19]. Since its emergence in 2000, HAdV-54 has become a frequent agent of EKC in Japan but has not yet been reported in any other country. Before 2008, HAdV-54 was misidentified as a variant strain of HAdV-8 due to cross neutralization with HAdV-8[13,18] antisera. Because of the emergence of many recombinant HAdVs, neutralization tests have been ambiguous. As a result, the identification of HAdV by partial sequences of hexon, penton and fiber (molecular typing) or by whole genome sequences (genotyping) is in use[19]. In this context, the traditional HAdV-8 genome typing method was updated for correct identification of genome type using a simple step-by-step procedure

Analysis of HAdV-8 genome types

Conventionally,the percentage of pairwise co-migrating restriction fragments (PCRF) is used to calculate the homology between HAdV-8 genome types[10,11]. Here we applied bioinformatics software Simplot[12] and zPicture tools [13] to determine the similarity between the genome types, and we compared them to HAdV-54 because it is related to HAdV-8. The Simplot analysis showed a high degree of identities over the entire genome of HAdV8P, B, and E. The identities of the hexon and fiber gene of HAdV-8P, B and E are 100%. HAdV-54 has a high similarity (96–98%) with HAdV8P in the fiber knob. However, it shows a variable range (45–90%) of identity in the hexon gene. The HAdV-8 genome types are similar to each other. HAdV-54 showed high percent of identities to HAdV-8P, B and E genome, except in the hexon. In a restriction map, HAdV-8P, B, and E share a very high percentage of restriction sites. Only one restriction site loss was found in HAdV-8B for each enzyme HindIII and SalI. Although HAdV-54 shares a high percentage of restriction sites with HAdV8 genome types, it is lower than those shared among the genome types. It has a higher number of restriction sites for all enzymes except SalI and SmaI.

Alignment of hexon and fiber

Type-specific antigenic determinants are located in loop 1 and loop 2 of HVRs and play a large role in the induction of the host immune response[14]. In the case of HAdV-8, HVR (including the intervening regions) is 951 nt (nucleotides) and 317 AA (amino acids) long. In this region, the identities of the nt and AA of HAdV8P, A, B, E, and J are 100%, indicating that HVRs of HAdV-8 are highly conserved. In contrast, in the case of HAdV-53 and HAdV-54, the identities are very low in all HVRs. The knob of the fiber protein of HAdV-8 is 362 amino acids (AA) long and contains a 43 AA tail, eight incomplete motif shafts (139 AA) and 180 AA globular knob[14]. The fiber knob is responsible for the attachment of HAdV-8 with the cornea and conjunctival cells[11,12,15]. The knob portion of HAdV-8P, A, B, E, J, and HAdV-53 is 540 nt and 180 AA long. HAdV-8P, A, E, and J are 100% similar at the nt and AA level. HAdV-8B is 99.6% similar to other genome types (P, A, E, J) at the AA level due to one AA substitution. HAdV-53 has the identical fiber of HAdV-8P, whereas HAdV-54 showed a 98% identity with HAdV-8P due to four AA differences across the fiber knob.

Phylogenetic analysis of the hexon and fiber

Genetic relationships (evolutionary) between HAdV-8 genome types and other HAdVs associated with ocular infection were measured using phylogenetic analysis of the HVRs of the hexon and knob region of the fiber[12,20]. A phylogram of HVRs of the hexon revealed that all of the HAdV-8 genome types, including the prototypes, are segregated into monophyletic clusters with other major EKC types of species D. HAdV-53 is close to HAdV-22. Although HAdV-54 was initially identified as HAdV-8 in a neutralization assay, in phylogeny, it is more similar to HAdV-9. A phylogram of the fiber knob showed that all HAdV-8 genome types and HAdV-53 were segregated into monophyletic clusters with other major EKC type of species D. Comparing the data of the alignment of the fiber knob, it is apparent that HAdV-53 has similar tropism for conjunctival and corneal cells.

Conclusions

In the HVRs of the hexon, the remarkable low identity of HAdV-54 with HAdV-8 indicates that anti-HAdV-8 may not be protective against HAdV-54. Despite the existence of 22 genome types of HAdV-8, their hexons and fibers are conserved. It is apparent that: (1) the genome types of

HAdV-8, including HAdV-53 have similar tropism for conjunctival and corneal cells; (2) antibodies against one genome type will provide protection against other genome types; and (3) a future vaccine made from one genome type could work against other genome types due to their stable HVRs. Finally, molecular analysis of whole genomes, specifically of the capsid proteins of the remaining genome types, would be useful to substantiate our observations.

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