Characterization of the structural proteins of hemorrhagic enteritis virus

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Summary. The structural proteins of hemorrhagic enteritis (HEV), a turkey adenovirus, were analyzed by polyacrylamide gel electrophoresis (PAGE) and Western blotting using polyclonal, monoclonal and monoclonal antibodies for detection. In purified HEV preparations, eleven polypeptides with apparent molecular weights ranging from 96,000 to 9,500 (96k to 9.5k), were specifically recognized by convalescent turkey serum. Six of these polypeptides were further characterized by PAGE, Western blotting, ELISA, sucrose gradient centrifugation and electron microscopy. The 96k polypeptide was identified as the hexon polypeptide which is a monomer of the major outer capsid or hexon protein. The 51/52k and 29k polypeptides, identified as the penton base and fiber polypeptides respectively, were the components of the vertex or penton protein. The 57k polypeptide was identified as a homologue of the human adenovirus type 2 (Ad 2) IIIa protein with which it shares a common epitope. Two core proteins with molecular weights of 12.5 and 9.5k were present in purified HEV nucleoprotein cores. The proteins of two HEV isolates, one apathogenic (HEV-A) and one virulent (HEV-V), resembled each other in most respects. However, differences between HEV-A and HEV-V were found in electrophoretic migration of the penton base protein both under native and denatured conditions, and in the electrophoretic migration of the 43/44k polypeptide. Moreover, homologous antiserum against the fiber protein reacted stronger than heterologous antiserum in an ELISA. Single fibers were detected by electron microscopy attached to the penton base proteins of HEV virions and in isolated pentons. The feature of having single fibers is shared with the mammalian adenoviruses and the avian egg drop syndrome 1976 virus (EDS 76 V), but not with the fowl adenoviruses which have double fibers attached to their penton base proteins.

Introduction

The family Adenoviridae is divided into the mammalian adenoviruses (genus Mastadenovirus) and the avian adenoviruses (genus Aviadenovirus). This division
is based upon a difference in host range and the absence of an antigenic relationship between mammalian and avian adenoviruses [31]. Within the genus *Aviadenovirus* there are at least two groups; the fowl adenoviruses [45] and a second group comprised of hemorrhagic enteritis virus (HEV) of turkeys [5, 20, 38], marble spleen disease virus (MSDV) of pheasants [18, 19] and splenomegaly virus (SV) of chickens [10, 11]. It has been suggested that these be referred to as group I and group II avian adenovirus, respectively [7]. A major difference between fowl adenoviruses and mammalian adenoviruses is the composition of the penton protein which consists of a penton base and two fibers in the case of fowl adenoviruses and a penton base and one fiber in the case of mammalian adenoviruses [15, 26]. The fowl adenoviruses are distantly related to the human adenoviruses with which they share a limited amount of DNA sequence homology [1].

HEV causes an acute infectious disease in turkeys [8, 17]. It is classified as an adenovirus on the basis of its morphology, mode of replication, and physical-chemical properties [5, 20, 32, 38]. HEV, MSDV and SV are serologically identical viruses [7–9, 19, 39, 42]. To date, no serologic relationship has been found between these viruses and the fowl adenoviruses [10, 11, 21, 36]. However, the lack of suitable cell culture system for HEV propagation has hampered a thorough investigation of its properties [40].

Our overall study of HEV involved developing a vaccine for turkeys [42] and defining the role of viral components in eliciting protective immunity. Therefore, the identification and characterization of the structural proteins of HEV was required. Until recently, none of the structural proteins of HEV, with the exection of the hexon, has been well characterized [30, 42]. The best studied adenoviruses in both genera are the human adenoviruses type 2 (Ad 2) and type 5 (Ad 5), and chick embryo lethal orphan (CELO) virus (fowl adenovirus type 1, FAV 1). These viruses have been shown to consist of outer capsid proteins (hexons and pentons), proteins associated with the capsid, and core proteins associated with double-stranded DNA.

In the present study, the structural proteins of an apathogenic (HEV-A) and a virulent (HEV-V) strain of HEV were analyzed using polyacrylamide gel electrophoresis (PAGE) under non-denaturing and denaturing conditions, and Western blotting using polyspecific, monospecific, and monoclonal antibodies. Furthermore, the hexon and penton proteins of both HEV strains were purified by immunoaffinity chromatography and characterized by sucrose gradient sedimentation, PAGE, Western blotting, and electron microscopy. The data presented in this report are discussed and compared with those of human and fowl adenoviruses.

**Materials and methods**

*Viruses and virus propagation*

The characteristics of HEV-A and HEV-V and their propagation in young turkeys are described elsewhere [39]. Ad 2 was obtained from the American Type Culture Collection and propagated in HEp-2 cells.