Chapter 28  Polymerase Chain Reaction for Detection of Hantaviruses*

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This work is dedicated to the memory of the great virologist Professor Dr. Friedrich Deinhardt (1926–1992)

Summary

The diagnosis of hantaviruses as the etiologic agent of hemorrhagic fever with renal syndrome (HFRS) so far has relied on immunofluorescence assays which require cells infected with pathogenic viruses. In this chapter we describe the use of gene amplification by the polymerase chain reaction (PCR) to diagnose hantavirus infections rapidly. In combination with direct nucleotide sequence analyses or differential oligonucleotide hybridization, PCR can also be used to identify different hantavirus strains and even to detect previously unknown hantaviruses.

Introduction

Hantaviruses are a genus of human pathogenic viruses which cause a variety of clinically similar diseases collectively termed hemorrhagic fever with renal syndrome (HFRS). Hantavirus infections were first recognized during the Korean war between 1951 and 1954 when 3000 soldiers of the United Nations suffered from HFRS. Mortality was reported to be 5%–10% (Smadel 1953; Earle 1954). Hantaviruses are transmitted to humans by direct or indirect contact with subclinically infected rodents which serve as their natural reservoir. Today, many strains of hantaviruses have been isolated in virtually every part of the world. Five serologically distinct groups have been established: Hantaan (Apodemus) (Lee et al. 1978), Seoul (Rattus) (Sugiyama et al. 1984),

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Puuma (Hällnäs B1; CG18–20, Clethrionomys) (Yanagihara et al. 1984), Prospect Hill (Microtus) (Lee 1989), and Leaky (Mus) (Baek et al. 1989). These serotypes are more closely associated with the rodent genus (given in parentheses) than with the geographical areas of their isolation. The Asian strains Hantaan and Seoul cause severe clinical symptoms in humans with mortality rates of up to 10%. European Puuma or Hällnäs B1 infections cause much milder symptoms with less than 1% mortality. In contrast, the American Prospect Hill and Leaky serotypes so far have not been associated with any known diseases in humans. Clinically, the infections are characterized by acute, sudden onset of fever with chills, conjunctival injection, prostration, anorexia, vomiting, abdominal pain, hemorrhagic manifestations, followed by proteinuria and hypotension. Renal disorders vary from mild symptoms to acute renal failure and may persist for several weeks.

In China, Hantaan virus infections account for 100,000–150,000 hospitalizations per year. Recent epidemiologic studies in several areas of Europe demonstrated antibody titers against the Puuma/Hällnäs B1 serotype in up to 5% of the local population (Lee 1989). The properties of genomic organization and coding capacities of hantaviruses are summarized in Table 1. The hantaviral large L RNA segment codes for the viral RNA-dependent RNA polymerase (250 kDa). The medium-size M RNA segments of hantaviruses encode a precursor polypeptide of approximately 126 kDa, which is cleaved into glycoproteins G1 (64 kDa) and G2 (54 kDa) of the viral envelope. Finally, unlike all the other genera of the Bunyaviridae family which utilize overlapping reading frames or ambisense coding strategies to encode a nucleocapsid protein N and nonstructural protein NSs (Ihara et al. 1984; Elliott and McGregor 1989; Simons et al. 1990), the Hantavirus S RNA segment apparently only codes for the nucleocapsid protein. This nucleocapsid protein is the major antigenic determinant detected by sera of hantavirus-infected patients (Sheshberadaran et al. 1988; Zöller et al. 1989; Gött et al. 1991).

Detection of Hantavirus Infections by Immunological Assays

Detection of hantavirus infections in humans relies exclusively on immunological assays. Many different assays have been developed and successfully applied.

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1 Recent nucleotide sequence analyses of a variety of strains have shown that the isolate labeled nephropathia epidemica virus (NEV) Hällnäs B1 (provided by Dr. Pilaski, Düsseldorf, FRG, and sequenced in our laboratory; Giebel et al. 1989; Stöhwasser et al. 1990, 1991) seems to be identical with the NEV strain CG18–20 (obtained from Dr. B. Niklasson, Stockholm, Sweden). The nucleotide sequence analysis of the cDNAs of the NEV Hällnäs B1 and CG18–20 strains from different laboratories is essential for final clarification of this discrepancy.