CHAPTER 1

INTRODUCTION

In 1988, the World Health Assembly resolved to eradicate poliomyelitis globally by the year 2000 and this term was later postponed until 2005 (Centers for Disease Control and Prevention [CDC], 2003; World Health Organization [WHO], 2003). Substantial progress has been achieved towards this goal (CDC, 2003; WHO, 2003) and with the eliminated circulation of wild-type poliovirus (PV) in most parts of the world, attention has focussed on examining the potential for vaccine-derived polioviruses (VDPVs) to circulate where wild-type PV has disappeared.

The Sabin live attenuated oral poliovirus vaccine (OPV) has been effectively used in the reduction and control of poliomyelitis. The OPV has had a remarkable track record of success since the number of wild-type polio cases decreased from 350 000 to less than 500 and the number of polio endemic countries declined from more than 125 to 10 (Wood and Thorley, 2003). The last case of polio, caused by a wild-type PV in South Africa occurred in 1989 (CDC, 2003). Despite the advantages in using the attenuated OPV, one disadvantage is the potential risk of revertants of the PV vaccine strains, which may cause neurological complications in vaccine recipients and their susceptible contacts (Minor, 1992). During prolonged replication of the attenuated PV vaccine strains in humans different genomic modifications such as mutations, deletions, insertions and recombinations may occur, thus, leading to the almost invariable reversion of the OPV strains to increased neurovirulence (Bellmunt *et al.,* 1999; Hovi *et al.,* 2004).

As OPV-derived strains are excreted in nasopharyngeal secretions and stool after vaccination, this vaccine could become a source of dissemination of PVs and the potential cause of poliomyelitis (Bellmunt *et al.,* 1999). The choice of strategies for the termination of immunisation depends, among other things, on the circulation of VDPVs and their potential health implications (Dowdle *et al.,* 1999). Although the transmission of vaccine strains of PVs from person-to-person in family situations is well established, little is known about

circulation in the community, especially in settings of susceptible populations such as immunodeficient individuals (Dowdle *et al.*, 1999).

Rapid evolution is characteristic of both wild and vaccine-derived polioviruses. Nucleotide substitutions accumulate at a rate of approximately 1% per year and consist primarily of changes at synonymous codon positions, in other words, do not result in amino acid changes at those loci (Kew *et al.*, 1995; Martin *et al.*, 2000). The mutations initially appearing and fixed into the genomes of the Sabin PV vaccine strains upon administration of OPV are frequently associated with reversion of the attenuated phenotype and alteration of the neutralising antigenic sites of the OPV strains (Minor, 1992). Reversion of the OPV strains to increased neurovirulence is one key factor for the occurrence of vaccine-associated paralytic poliomyelitis (VAPP), which occurs at a rate of approximately 1 per 500 000 first doses of OPV in immunocompetent individuals, and at a 3 000 fold higher rate in immunodeficient patients (Sutter and Prevots, 1994).

Poliovirus excretion in immunocompetent hosts is usually short lived, seldom exceeding 2 months (Wood *et al.*, 2000). Hovi and colleagues (2004) have shown that excretion of wild-type PV by healthy children may continue for at least 6 months and is associated with the accumulation of single nucleotide substitutions during replication within an individual host. In the early 1960s and 1970s, several instances of prolonged excretion of VDPV for up to 2 years were reported, all among individuals suffering from B-cell deficiencies (Wood *et al.*, 2000). Bellmunt *et al.* (1999) reported the complete sequence of two PV isolates obtained from a patient with common variable immunodeficiency syndrome (CVID). The isolates were taken after the onset of paralysis and after 5.5 years of continuous virus excretion demonstrating evolutionary changes toward a “wild-type-like” genotype (Bellmunt *et al.*, 1999). Combined anamnestic and evolutionary data indicated about 10 years of persistent enteral PV replication (Bellmunt *et al.*, 1999). Similar molecular analyses from other examples of long-term polio excretion among individuals with primary immunodeficiencies have been recently reported (Kew *et al.*, 1998; Martin *et al.*, 2000; Minor, 2001; Buttinelli *et al.*, 2003).

The spectrum of possibilities for behaviour of PVs in immunodeficient individuals was illustrated by the accidental discovery in Europe of an immunodeficient man who was

carrying highly evolved VDPV type 2 strain (Minor, 2001; MacLennan *et al.,* 2004). This individual is known to have been excreting VDPV type 2 for an estimated 20 years and is still excreting at present without showing any clinical symptoms (MacLennan *et al.,* 2004).

Derivatives of the Sabin live attenuated vaccine strains present in OPV have been classified into two broad categories for programmatic reasons (WHO, 2004). "Oral poliovirus vaccine-like viruses" represent the vast majority of vaccine related isolates and have close sequence relationships (>99% VP1 sequence identity) to the original Sabin PV vaccine strains (WHO, 2004). "Vaccine-derived polioviruses" are those strains showing ≤99% VP1 sequence identity to the parental Sabin PV vaccine strains and are uncommon (WHO, 2004). The sequence drift shown in VDPVs is indicative of prolonged replication of the vaccine strain either in one individual or in the community (circulating VDPVs) (WHO, 2004).

Shulman and colleagues (2000) have isolated an unusual, highly diverged derivative of the Sabin PV type 2 strain from environmental samples during routine screening for wild-type PV in Israel. The extensive genetic divergence of the isolate from its parental Sabin PV type 2 vaccine strain suggested that the virus had replicated in one or more individuals for approximately 6 years (Shulman *et al.,* 2000). More recently, a highly evolved VDPV type 3 harbouring a 13% sequence drift from Sabin PV type 3 vaccine strain has been isolated from sewage in Estonia (Blomqvist *et al.,* 2004). The presence in the environment of highly evolved, neurovirulent VDPV strains in the absence of polio cases would have important implications for strategies to interrupt immunisation with OPV following global polio eradication.

Concerns about the potential risks constituted by VDPV strains are supported by recent outbreaks of acute flaccid paralysis (AFP) caused by circulating VDPVs that have been reported in four different regions of the world, namely the Middle East (Egypt), the Americas (Hispaniola: Dominican Republic and Haiti), the Western Pacific (Philippines), and Africa (Madagascar) (Kew *et al.,* 2004). In all four cases, the outbreaks occurred in pockets of unvaccinated or incompletely vaccinated individuals (Kew *et al.,* 2004). During the Hispaniola outbreak, VDPV type 1 was spread in the poorly vaccinated population and was able to cause more than 20 paralytic cases (Kew *et al.,* 2004). In Madagascar, five cases of AFP associated with VDPV type 2 were reported and partial genomic sequencing indicated that two of the PV

strains had been circulating for approximately 1 and 2.5 years, respectively (Rousset *et al.,* 2003).

These findings suggest, that the final step of PV eradication will require details on the possibility of persistent infections and excretion of VDPVs for long periods by immunodeficient patients, and the survival in the environment of these strains to the extent that they may infect non-immune individuals after termination of PV vaccination (Fine and Carneiro, 1999). Research is required to provide information in the following priority areas: the extent and duration of circulation of VDPVs in populations; risk factors for prolonged replication of PV among immunodeficient individuals; assessment of the prevalence and behaviour of VDPV strains in the environment (notably water resources) and in different population settings (Wood *et al.,* 2000).

There is little information on secondary immunodeficiency as a risk factor for VAPP or prolonged PV excretion (Wood *et al.,* 2000). The likelihood of prolonged PV excretion in cohorts of infected with human immunodeficiency virus (HIV) children is being investigated in several developing countries (Wood *et al.,* 2000; Haisey *et al.,* 2004). The current situation in South Africa offers opportunities well suited for research along these lines, since OPV immunisation is compulsory in the country. The purpose of this study was to isolate OPV strains from stool specimens of immunodeficient children as well as from environmental water samples (sewage and river water), and to investigate the genetic features of these PV isolates using advanced molecular techniques.

The objectives of this study were as follows:

1. To investigate the circulation of OPV strains in the environment (sewage and river water).
2. To isolate OPV strains from stool specimens of immunodeficient patients at Kalafong Hospital, such as HIV-positive children (including those with an acquired immunodeficiency syndrome [AIDS] indicator condition, according to the CDC classification).
3. To determine the occurrence of genomic mutations in the OPV isolates.
4. To determine the prevalence of VDPV strains in the environment and in stool specimens of immunodeficient patients.

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