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Biology of Parainfluenza Viruses

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INTRODUCTION

Parainfluenza viruses 1 through 4 (PIV1 through PIV4) are important human pathogens that cause upper and lower respiratory tract infections, especially in infants and small children. Together with respiratory syncytial virus, PIVs are the most frequent pathogens isolated in children with lower respiratory infections. In addition to the viruses infecting humans, PIVs include viruses that cause diseases in animals, such as Sendai virus of mice, which has been used as a model for studying the course of PIV infection. Recent work has revealed interesting aspects of the molecular biology of PIVs, and these findings can now be used in, for instance, development of new vaccine candidates.

MOLECULAR BIOLOGY

Structure

PIVs are typical members of the family *Paramyxoviridae* and belong to the genus *Paramyxovirus* (Table 1). A number of extensive reviews have recently been published concerning their structure and replication (12, 30, 31, 60). These RNA viruses are pleomorphic enveloped particles that are 150 to 300 nm in diameter. A structural model of the virion is shown in Fig. 1. The virion consists of a filamentous, herringbone-like nucleocapsid core surrounded by a lipid envelope with virus-specific glycoprotein spikes. The nucleocapsid is composed of the genome, a single-stranded RNA molecule of negative-sense polarity, which is tightly coated with the nucleocapsid protein (NP). The nucleocapsid structure also contains two other proteins, the phosphoprotein (P protein) and the large protein (L protein), which occur discontinuously as clusters. The NP is the most abundant protein in the virion, and it is believed to be responsible, together with the P and L proteins, for RNA-dependent RNA polymerase activity (47).

The viral envelope contains two virus-specific glycoproteins: the hemagglutinin-neuraminidase (HN) and the fusion (F) proteins. The F protein is synthesized as a biologically inactive precursor form (F_0), which is cleaved posttranslationally with cellular proteases to produce an active form of two disulfide-linked subunits, F_1 and F_2. The fatty acid composition of the envelope is identical to that of the host cell. The matrix (M) protein is highly hydrophobic in character, and its function in the virion is to mediate interaction between the glycoproteins and the nucleocapsid. PIVs also contain cellular actin as a structural component; however, its function in virus structure and replication is not fully understood (6, 39, 102, 111).

Genome

The PIV genome is a linear, nonsegmented, negative-sense RNA molecule containing an average of 15,000 nucleotides (nt) (15,463 nt in PIV3 [31]; 15,285 nt in Sendai virus [93]; 15,156 nt in Newcastle disease virus (NDV) [116]) and consists of six or seven genes that encode eight or nine proteins, some of which are not detected in the virion but participate in the virus replication cycle in the cell. The genomic RNA is never found in naked form in the cells but is always tightly bound to NP.

The organization of the parainfluenza virus genome is 3′-NP-P/C/V-M-F-(SH)-HN-L-5′. The presence or absence of the SH gene between the two glycoprotein genes divides parainfluenza viruses into two groups. The first group (including PIV3, Sendai virus, and NDV) does not contain the SH gene, whereas the second group (e.g., the animal parainfluenza virus, simian virus 5, and mumps virus, which is also a member of the family *Paramyxoviridae*) does. This SH gene encodes a small integral membrane protein, which is highly hydrophobic and is found only in infected cells. Its function is still unclear. Figure 2 shows a schematic representation of the PIV genome and its replication cycle.

Extensive sequence analysis of PIV3, Sendai virus, and NDV genomes carried out during the past few years (10, 18, 26, 32–35, 42, 55, 64, 65, 68, 70, 81, 90, 93, 96–99, 116) has shown that the 3′ and 5′ ends of these genomes contain the short, extracistronic noncoding regions, approximately 50 and 40 nt long, respectively. The first 20 nt at the 3′ end are highly conserved among paramyxoviruses (23, 35, 92, 93, 116), suggesting their biological significance in initiation of mRNA transcription and genome replication. The adjacent genes in the genome are separated by intercistronic regions, which contain a transcriptional control sequence found at the beginning of every gene (gene start), a stop sequence (gene end) at

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TABLE 1. Classification of the family *Paramyxoviridae*

Genus	Human viruses	Animal viruses[a]
Paramyxovirus	PIV1	Sendai virus (murine)
	PIV2	Simian virus 5 (canine)
	PIV3	Bovine parainfluenza virus
	PIV4	
		Newcastle disease virus (avian)
	Mumps virus	
Morbillivirus	Measles virus	
		Canine distemper virus
		Phocine distemper virus
		Peste-des-petits-ruminats virus (caprine, ovine)
		Rinderpest virus (bovine, caprine, ovine, porcine)
Pneumovirus	Respiratory syncytial virus	Bovine respiratory syncytial virus
		Pneumonia virus of mice (rodent)
		Turkey rhinotracheitis virus (avian)

[a] Animal viruses most closely related to a human pathogen are shown on the same line.

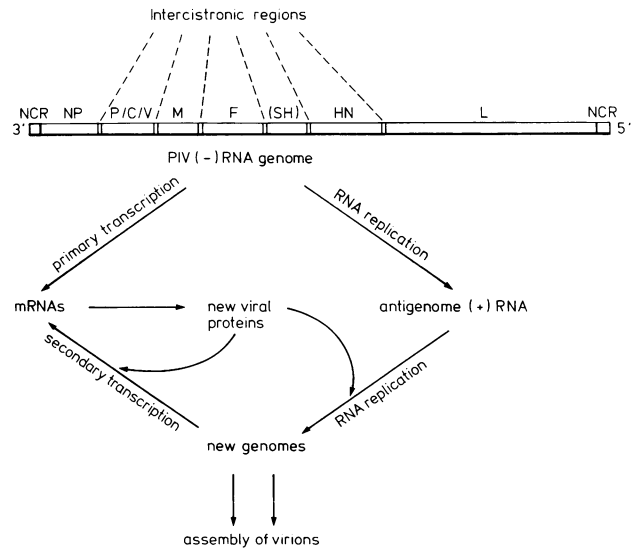

FIG. 2. Schematic representation of the PIV genome and the replicative cycle.

the end of every gene, and a short sequence between the genes. The control regions are conserved (42, 54, 63, 96, 116) and are transcribed to mRNAs, whereas the short intergenic sequences are not copied.

The first gene at the 3′ end of the genome is the NP gene, which is highly conserved among PIVs (32, 54, 55, 90, 92). The length of the monocistronic NP gene of PIV3 is in the range of 1,641 to 1,850 nt, and it encodes a 515-amino-acid protein (32, 55, 90).

The P gene is a multicistronic genome region with a unique coding strategy. The P genes have continuous, discontinuous, and overlapping cistrons (36), and, according to their coding strategy, PIVs can be divided in two groups. In the first group, PIV1 and PIV3 encode their 500- to 600-amino-acid P proteins from continuous P cistrons. The second start codon in the P gene region initiates a shorter overlapping open reading frame coding for a smaller, nonstructural C protein (33, 65, 66, 97). The proportional quantities of the P and C proteins produced are evidently controlled at the level of translation. The C protein (about 204 amino acids) is not found within the virion but is detected in infected cells.

The second group, which includes PIV2, PIV4A, and PIV4B, produces more than one mRNA species by editing the P mRNA with insertion of two additional, non-template-dependent G residues at a precise site in the mRNA molecule; the nonstructural V protein is encoded directly by the genomic sequence (61, 76, 78, 95, 108). A similar editing mechanism has been described for measles virus (9), which is also a member of the *Paramyxoviridae*, suggesting that this phenomenon has a more general biological significance. The mechanism responsible for addition of the nontemplate residues is unknown, nor is it known whether it occurs cotranscriptionally or posttranscriptionally, but virus-encoded proteins are probably needed for this process (36, 77, 108). The P and V proteins are amino coterminal and share 164 amino acids before the sequences diverge. The V proteins have a cysteine-rich region, which is similar to the metal-binding domains present in proteins known to bind to DNA or RNA. This cysteine-rich region has been found in all paramyxovirus P genes, suggesting that it is biologically important (78).

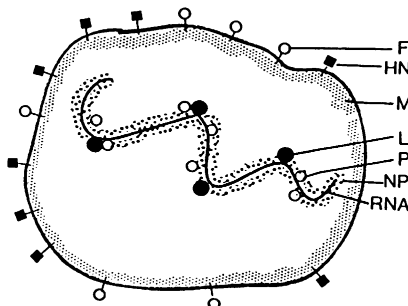

FIG. 1. Structural model of PIV.

The M gene is the most highly conserved gene among paramyxoviruses (34, 64, 99), and the homology is most extensive at the carboxy-terminal third of the protein sequences. Most conserved regions represent hydrophobic regions, which may be structurally involved in membrane interactions.

The PIV3 F gene is 1,845 to 1,851 nt long and encodes a 539-amino-acid protein (18, 98). The F gene sequences reported for a number of clinical PIV3 isolates show a very high degree of conservation at the nucleotide (>94%) and amino acid (>97%) levels (82). The F protein contains two hydrophobic regions and a signal sequence, which is later removed through a proteolytic cleavage. Comparison of the F genes of Sendai virus and NDV indicates a close evolutionary relationship between these human and animal pathogens (5, 10, 73, 93).

The HN genes of PIV3, Sendai virus, and simian virus 5 encode a 565- to 572-amino-acid protein containing one major hydrophobic region and no signal sequence (4, 26, 51). The highest homology, 62% at the amino acid level, occurs between PIV3 and Sendai virus (73).

The L gene is located at the 5′ end of the genome and is the largest PIV gene (6,799 nt). It represents about 40% of the coding capacity of the entire genome (72, 93, 116). Because of

TABLE 2. Structural proteins of PIVs

Protein	Designation	Chemical nature	Location	Function
Hemagglutinin-neuraminidase	HN	Glycosylated	Envelope	Attachment to host cell receptors, hemagglutinating and neuraminidase activities
Fusion protein	F_1F_2	Glycosylated	Envelope	Fusion, hemolysis, penetration
Nucleoprotein	NP	Phosphorylated	Nucleocapsid	Tightly complexed with genomic RNA, structural component in nucleocapsid
Phosphoprotein	P	Phosphorylated	Nucleocapsid	Component of polymerase complex
Large protein	L	Phosphorylated	Nucleocapsid	Component of polymerase complex
Matrix protein	M	Phosphorylated	Inside the envelope	Assembly

the length of the gene and its location, the number of L mRNAs is the smallest among the transcripts.

Proteins and Their Antigenic Structure

The PIV genomes encode six structural proteins, which are found in virions, and two (or more) nonstructural proteins, which are detected only in infected cells. Table 2 summarizes the chemical nature, location in the virion, and functions of the structural proteins.

The HN glycopolypeptide is a receptor-binding protein that mediates virus-cell attachment. It is anchored to the envelope by the hydrophobic region near its amino terminus (4, 51, 70), whereas the F glycoprotein is anchored near its carboxy terminus. The HN protein has hemagglutinating and neuraminidase activities. Its putative three-dimensional structure is thought to be broadly similar to that of influenza virus neuraminidase, but the framework residues of the active site are less strictly conserved (17). By using recombinant vaccinia viruses expressing the HN and F glycoproteins of PIV3, it has been shown that the HN protein is also required for complete fusion activity (25).

The PIV3 HN protein contains six antigenic sites (A to F), most of which are conformational (15, 16, 106). Three of these sites (A, B, and C, together containing 11 epitopes) are involved in neutralization and hemagglutination activities; in PIV3, these regions are highly conserved among clinical isolates (14, 88, 104). However, in PIV1, HN protein genetic variation and evolution occur (49, 50). On the other hand, the HN proteins of Sendai virus and NDV contain only four antigenic sites, but antibodies against all of them are needed for complete neutralization of the virus (52, 53, 75, 79). In addition, glycosylation of the protein is important for antigenicity. Monoclonal antibodies to site A epitopes also inhibit neuraminidase activity, suggesting that site A is located on the PIV3 HN molecule in a region topographically close to the site of neuraminidase activity (14, 15).

The F glycoprotein is involved in hemolysis, cell fusion, and virus penetration into the cell. It is a disulfide-linked protein composed of a distal polypeptide, F_2, and a larger, proximal polypeptide, F_1, anchored to the envelope near its C-terminal end (10). It induces neutralizing antibodies, and the PIV3 F protein contains eight antigenic sites. The existence of identical changes in F epitopes in naturally occurring PIV3 strains raises the possibility that mutations arise easily (104, 105).

NP is the most abundant component in the nucleocapsid structure. It is tightly bound to genomic RNA, having an essential role in the stability of the nucleocapsid. NP also interacts with the P and L proteins in the nucleocapsid structure, but the precise role of NP in transcription and replication is not completely understood. It has been proposed that this polypeptide might function as a switching factor in the change from transcription to replication. The NP of PIV3 exhibits extensive homology (70%) with that of Sendai virus in the first 424 amino-terminal amino acids, but no homology is observed between the 95 carboxy-terminal amino acids (90). Moreover, NP contains two other identical, highly conserved regions, suggesting their structural or functional importance. Like other internal components of the virions, M, P, and L proteins, NP would not be expected to induce a good humoral responses. On the other hand, these proteins may act in the induction of T-cell immunity, as has been shown with Sendai virus and measles virus NPs (22, 89).

The P and L proteins are crucial components in RNA-dependent RNA polymerase activity. P protein is the most extensively phosphorylated protein in the virion structure, although NP and L protein are also phosphorylated to some extent. L protein is the largest virus-specific protein and is believed to be responsible, together with P protein, for transcriptase activity as well as for capping, methylation, and polyadenylation of virus-specific mRNAs.

The hydrophobic M protein is found in close association with the envelope. M protein plays a critical role in the structure of the virion by mediating interaction between the nucleocapsid core and the surface glycoprotein spikes. The attachment of the nucleocapsid to glycoproteins through the M protein is thought to start the budding process of the virus particle through the plasma membrane; this is the final step in virion maturation. Tight association of M protein with the nucleocapsid structure in infected cells suggests that it may also have a regulatory role in transcription and/or replication. The fact that the M protein is highly conserved among PIV proteins also indicates that it plays an important role in virus replication (34, 99). Furthermore, it has been speculated that decreased synthesis or stability of M protein plays a role in the establishment and maintenance of persistent infections.

Replication

Replication of RNA viruses takes place in the cytoplasm of the host cell, and the mature virus particles are released by budding through the plasma membrane. The first step in the infection cycle is the attachment of the virus particle to its cellular receptor, a process mediated by the HN glycoprotein in PIVs. The F protein catalyzes subsequent fusion of the virus envelope and cell membrane. After the uncoating event, the nucleocapsid structure containing the genome is released into the cytoplasm.

The genome of negative-sense RNA viruses cannot function directly as mRNA but must first be transcribed to virus-specific mRNA species by RNA-dependent RNA polymerase. Because eukaryotic cells do not contain this enzyme, the virus nucleocapsid structure has to transport the activity into cells. The viral P and L proteins, and possibly also NP, are needed for this activity. During primary transcription, the genomic RNA is sequentially transcribed starting from the 3′ end of the genome

to produce individual mRNA species. According to the Kingsbury-Kolakofsky model of replication, the polymerase has a single site of attachment to the genomic RNA, and it copies the viral genes one by one (60). At the gene boundaries the transcriptase moves to the next gene without copying the short intergenic sequences, and at each of the gene junctions the transcription efficiency decreases. Viral mRNA molecules are modified to contain a 5'-end methylated cap structure and a polyadenylated 3' end resembling eukaryotic messengers. The mRNAs are translated subsequently to full-size proteins on host cell ribosomes, except for the F protein, which is synthesized as a precursor (F_0) and later cleaved to its active form (F_1F_2) by host cell proteolytic enzymes. Proteolytic cleavage of the inactive precursor protein F_0 is important for virus infectivity, because cells lacking this activity are not able to support virus replication. Thus proteolytic activation is a determinant for PIV tissue tropism and pathogenesis. The glycoproteins are processed (e.g., glycosylation, attachment of fatty acids) during their complex transportation process through the Golgi complex to the plasma membrane.

Genome replication takes place in two steps. First the negative-strand RNA is copied to a complementary positive-sense RNA, and then this molecule functions as a template for the synthesis of genomic RNA. In genome replication, the polymerase must ignore the transcription stop signals at the gene boundaries in order to make full-length genomic RNA. On the basis of studies with Sendai virus, it has been argued that the concentration of NP regulates the activities of polymerase, with an excess favoring production of full-length genome copies by a read-through mechanism of the replicase. On the other hand, NP scarcity favors production of monocistronic mRNAs (109). Figure 2 represents a schematic model of the PIV genome and its replicative cycle. The relative gene sizes have been drawn to scale.

DIAGNOSIS

Although some clinical signs and symptoms (e.g., croup [see below]) are more frequent in illnesses caused by PIVs than in other respiratory infections, specific etiological diagnosis always requires detection of infectious virus or virus components in clinical samples or a serological response. PIVs grow and produce a syncytial cytopathic effect in certain cell lines, enabling isolation of viruses from specimens taken from the respiratory tract of patients. Primary rhesus monkey kidney (MK) cells were once the only choice for growing PIVs. The LLC-MK2 cell line is now also widely used for PIV isolation (28), and a continuous line of mucoepidermoid human lung carcinoma cells, NCI-H292 (8), has recently been shown to be equivalent to MK cells. In culture, the presence of the virus may be detected by hemadsorption with guinea pig erythrocytes (11) or by using specific immunological reagents. The isolation procedure can be accelerated by using the immunoperoxidase method to stain cultures with monoclonal antibodies 2 days after inoculation of the sample, when the cytopathic effect is not always visible. This method has been used successfully at the Department of Virology, University of Turku, for other respiratory viruses (113) and recently also for PIV1, PIV2, and PIV3 grown in NCI-H292 cells (112).

When more rapid demonstration of the virus is needed, detection of viral antigens by immunoassays is recommended. Radioimmunoassays and enzyme immunoassays have been developed for the detection of PIV1, PIV2, and PIV3, using either polyclonal sera (91) or, more recently, monoclonal antibodies in a highly sensitive one-step time-resolved fluoroimmunoassay (46). In the assay, the nasopharyngeal sample is incubated with a europium-labeled detector antibody for 1 h in microtiter strips previously coated with the capture antibody. After the strips are washed, the result is measured with a time-resolved fluorometer.

A more recent development for detection of viruses in, e.g., nasopharyngeal samples is the use of PCR. In this assay as applied to the detection of PIVs, DNA complementary to viral RNA is first generated by using reverse transcriptase and an oligonucleotide primer. This product is further amplified in repeated cycles of DNA synthesis, initiated with a specific primer pair and catalyzed by a heat-stable DNA polymerase. The PCR product can then be detected by agarose gel electrophoresis or nucleic acid hybridization methods or used for direct sequencing, thus allowing exact comparisons of isolated strains for epidemiological purposes.

In a PCR approach, Karron et al. (58) used primers that detected a sequence at the 5' noncoding region of the PIV3 F protein gene. Specimens obtained from 10 children during a hospital outbreak were studied by sequence analysis after the PCR. The results showed that four different strains were present among the 10 isolates. Six isolates which represented one strain were obtained from a cluster of nosocomial cases in pediatric intermediate care unit. The presence of the other three strains in the community indicated that multiple strains can be found during a single epidemic.

Serological assays based on hemagglutination inhibition have been widely used for the detection of antibody responses against PIVs (11). In these assays, removal of nonspecific inhibitors is needed for optimal results. More recently, enzyme immunoassays have been developed for PIV serological assays. These tests are based either on the demonstration of an increase in the levels of specific immunoglobulin G (IgG)-class antibodies in serum samples collected during the convalescent phase of the disease over those in samples collected during the acute phase or on the detection of specific IgM present during the acute phase of infection. The former approach is in routine use in our laboratory for PIV1, PIV2, and PIV3 (110). Serum specimens are tested at a single dilution, and the results are expressed by comparison with a standard serum. In paired sera from patients with virologically confirmed PIV infection, increases in IgG antibody levels were detected in 69 to 87% of the patients while specific IgM antibodies were present in 42%.

CLINICAL AND EPIDEMIOLOGICAL ASPECTS

The clinical diseases caused by PIVs include rhinorrhea, cough, croup (laryngotracheobronchitis), bronchiolitis, and pneumonia. When 99 Finnish children with virologically confirmed PIV infection were studied, laryngitis was the clinical diagnosis in 64, 86, and 21% of PIV1, PIV2, and PIV3 infections, respectively (83). Upper respiratory tract infection was clinically diagnosed in 58% of the children with PIV3 infection. Otitis was detected in 10 to 34% of children with PIV infection while pneumonia was found in 12% of the children with PIV2 and 11% with PIV3 infections but not in those infected with PIV2. Bronchiolitis was not found in association with PIV infections in this study. The maximum temperature of these patients was approximately 40°C. Slightly elevated or normal C-reactive protein, leukocyte count, and erythrocyte sedimentation rate values were found in children with PIV infections (85).

Murphy et al. (74) have summarized the virological findings in pediatric inpatients with respiratory infections. In this report, PIV3 and respiratory syncytial virus were identified as the two leading causes of serious lower respiratory tract disease. PIV1, PIV2, and PIV3 were primarily associated with

croup, and PIV3, together with respiratory syncytial virus, was a major cause of bronchiolitis and pneumonia.

Sites of PIV infection other than the respiratory tract have been described. In rare cases, parotitis may occur. Meningitis, also a rare complication, has been reported in individual cases, and the etiology has also been confirmed by isolation of these viruses from the cerebrospinal fluid (2).

In industrialized countries, the epidemiology of PIV infections has been analyzed by several investigators. Surveillance of PIV3 infections in Houston, Tex., from 1975 to 1980 showed that most of the cases occurred during the late winter or spring (41). At least two-thirds of children were infected with PIV3 in each of the first 2 years of life. The risk of illness was approximately 30 per 100 children per year and decreased after 2 years of age. It is of interest that in the same region, PIV3 infections had previously followed an endemic rather than an epidemic pattern. PIV1 and PIV2 infections occurred in 2-year cycles. In another study, carried out in a small community in Tecumseh, Mich., PIV1 and PIV2 infections occurred together in alternate years from 1976 to 1981 (71). The peak of PIV1 infections was in October, and that of PIV2 infections was in December; the monthly distribution of PIV3 infections was more consistent. The viruses were isolated predominantly from children less than 2 years old. Analysis of virological data concerning PIV3 infections over a 12-year period (1978 through 1989) in Sydney, Australia, showed that the peak incidence was in the spring (21).

Easton and Eglin (24) have reported a different seasonal occurrence of PIV3 infections in England and Wales over a 10-year period from 1978 through 1987. They showed that PIV3 causes epidemics in the summer, which were detected yearly, whereas respiratory syncytial virus infections occur in the winter.

In Turku, Finland, 249 PIV1, 155 PIV2, and 812 PIV3 infections were diagnosed from 1981 to 1992 by using viral antigen detection (Fig. 3). An epidemic of PIV1 infection occurred in the winter of 1982; otherwise, only individual cases (a maximum of nine cases per month, usually in the early autumn) were observed. The findings concerning PIV2 infections were similar. PIV3 caused small epidemics every year in the spring. When compared with other respiratory viruses detected during the same period, the frequency of PIV infections is similar or lower (3,945 respiratory syncytial virus, 1,027 influenza A virus, 400 influenza B virus, and 1,805 adenovirus infections were diagnosed).

The epidemiology of PIV and other respiratory virus infections in the developing world has recently been investigated. Ruutu et al. (86) studied specimens from 312 Filipino children (less than 5 years old), living in periurban slums and middle-class housing, who fulfilled the criteria for acute lower respiratory tract infection. The etiological diagnosis was made on the basis of viral antigen detection, virus isolation, and serological assays. A total of 198 virus infections were confirmed in 162 patients, and PIVs were found in 8.8% of the patients. In the remaining patients, measles virus (21%), influenza A virus (16%), respiratory syncytial virus (7%), influenza B virus (6%), enteroviruses (5%), adenoviruses (4%), herpes simplex viruses (2%), and cytomegalovirus (1%) were found. De Arruda et al. (19) carried out an extensive study among an impoverished urban population in Brazil; in this study 175 children less than 5 years of age in 63 families were monitored for 29 months. Viruses were identified in 35% of the upper respiratory tract samples collected during symptomatic periods, and PIVs were found in 16% of the specimens. Other viruses detected were rhinoviruses (46%), enteroviruses (16%), adenoviruses (10%), herpes simplex viruses (7%), and influenza viruses (6%).

Patients with immunodeficiencies have a tendency to develop persistent and severe PIV and other respiratory virus infections (20, 56). Serious lower respiratory tract disease caused by PIVs has been reported in children and adults who undergo bone marrow transplantation (114). Of 1,253 transplant recipients, 27 had PIV infection as demonstrated by culture of the pathogen from nasopharyngeal or bronchoalveolar samples. Nineteen of the PIV-infected patients had lower respiratory tract involvement. Fatal respiratory failure developed in six of these patients.

It is known that infections caused by respiratory viruses are often complicated by bacterial infections, such as otitis and pneumonia. Korppi et al. (62) studied specimens collected from 37 children with serologically confirmed PIV infection for evidence of concomitant bacterial infection. They found no evidence of bacterial involvement in 24 children with croup, whereas in 3 of the 13 children with lower respiratory tract infection, serological findings supported *Streptococcus pneumoniae* infection, and in 1 case, the findings supported involvement of *Haemophilus influenzae.*

PATHOGENESIS AND IMMUNE RESPONSE

Primary inoculation of PIVs occurs through the nasal mucosal surface, and the first symptoms, including rhinorrhea, are observed after an incubation period of 2 to 4 days. The usual course involves slow recovery after upper respiratory tract involvement, but the illness is occasionally complicated by otitis. In more severe cases, the infection spreads to the lower respiratory tract, presumably through aspiration of secretions, and bronchiolitis or pneumonia may follow (69). PIV3 has also been isolated from the blood of children with acute respiratory infection (84).

Ciliated epithelial cells of the respiratory tract are infected (27), and a peribronchiolar infiltrate containing lymphocytes, plasma cells, and macrophages appears, together with edema and excess secretion of mucus. When pneumonia is predominant, a moderate hyperplasia of alveolar epithelia is observed and fluid containing some macrophages, erythrocytes, and leukocytes accumulates (117). The mechanisms of cell damage include both direct destruction by the virus and effects of the immune response (70). The latter may consist of formation of antigen-antibody complexes, allergic injury due to IgE, cytotoxic T cells, or delayed-type hypersensitivity. The release of soluble mediators may also play a role.

Several animal models closely mimic the course of PIV infection in humans. The most widely used model is Sendai virus infection in rodents, and a rat model is described here as an example. Garlinghouse et al. (37) and Giddens et al. (38) studied different parameters during infection of Sprague-Dawley rats. When 5- to 8-week-old animals were infected intranasally with the virus, severe rhinitis and pneumonia developed over 4 days. Signs of the respiratory tract involvement were still observed 3 weeks postinfection (p.i.), and bronchial lymphoid infiltration was still detected 42 days p.i. Viral antigens appeared in the respiratory tract cells during the first day after infection, and an increase in the level of virus-specific proteins was seen during the first 4 days; however, by 7 days p.i. the findings were negative. Infectious virus was recovered from the upper respiratory tract and lungs during the first 8 days, and virus antibodies appeared in serum 7 days p.i. In 5 of 12 rats, antibodies were present 9 months later. Cell-mediated immune responses were also observed 7 days p.i., and maximal values were measured 2 weeks later; no response was found 6 months p.i. Interferon appeared by 3 h

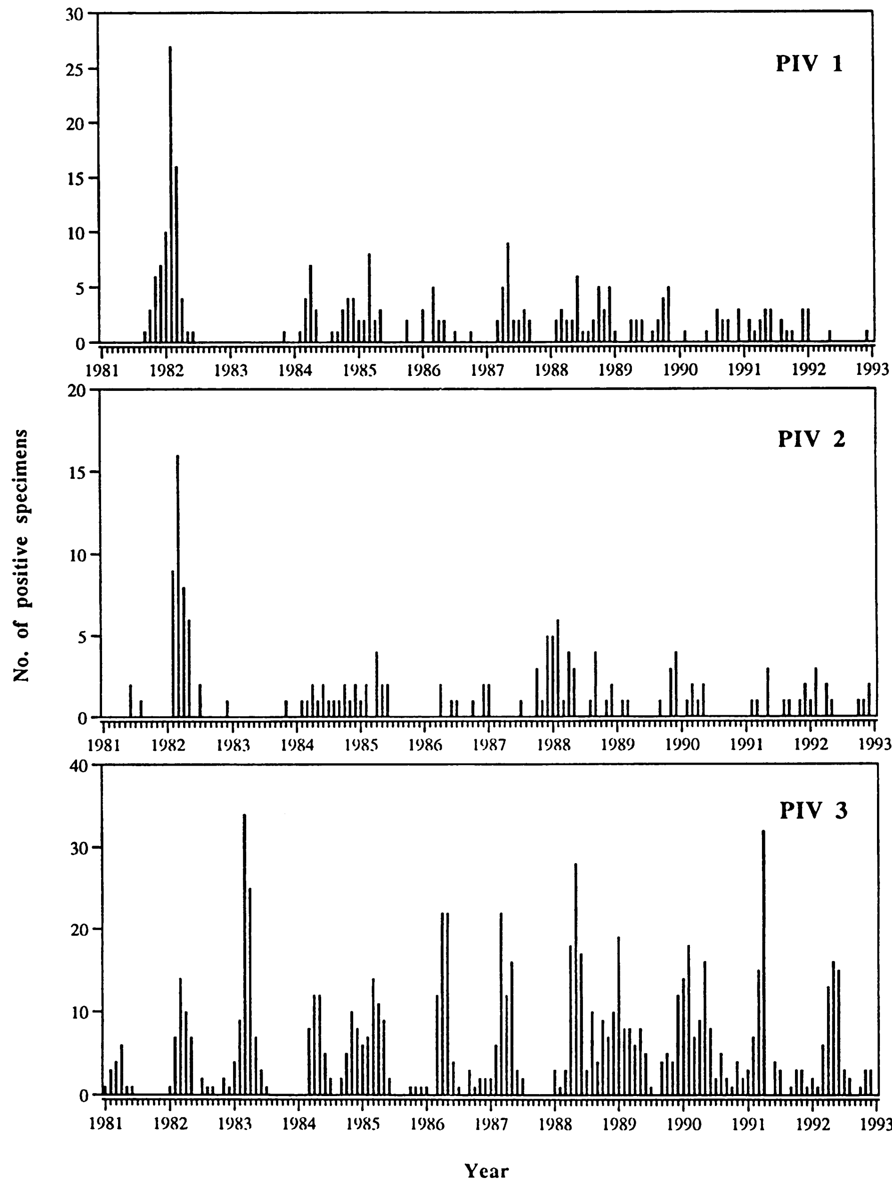

FIG. 3. Occurrence of PIV1, PIV2, and PIV3 infections from 1981 through 1992 as determined by virus antigen detection from nasopharyngeal specimens at the Department of Virology, University of Turku.

p.i. in lungs and serum, and the highest values were observed 6 h p.i.

Most reports on immunity to PIVs in humans concern local and circulating antibodies; the importance of cell-mediated immunity in clinical PIV infections is largely unknown. These viruses enter the body via the respiratory route, and so the first specific defense mechanism is the barrier of local antibodies. Smith et al. (94) studied the protective effect of antibodies in the serum and in nasal secretions in adult volunteers who were challenged intranasally with PIV1. They found that the presence of antibodies in nasal secretions is a better marker of host resistance to infection than the level of antibodies in serum. Yanagihara and McIntosh (115) have shown that in infants infected with PIV1 or PIV2, a significant increase in the level of virus-specific nasopharyngeal secretory IgA is detected. However, there was a discordance between the IgA level and neutralizing activity in individual specimens because many secretions containing IgA were not neutralizing and not all secretions with neutralizing activity contained IgA. It has been shown that in Sendai virus infection of mice, local administration of monoclonal IgA or IgG antibodies is effective in protecting the airways from viral infection (67). On the other hand, it has been proposed that passively acquired circulating maternal antibodies might participate in the pathogenesis of PIV infections. This mechanism was not supported in experiments involving PIV3 infection in hamsters, since partial passive immunization did not enhance the severity of the disease (40).

Reinfections with PIVs are common, as reported for infections caused by other respiratory viruses. To study the mechanism of this phenomenon and the specificity of the immune response in more detail, van Wyke Coelingh et al. (107)

analyzed the appearance of antibodies against individual antigenic sites of HN and F glycoproteins of PIV3 after primary and secondary infection in humans. They found that during primary infection, seronegative infants and children developed strong antibody responses with neutralizing activity whereas responses of young infants with maternal antibodies were weaker. Reinfected children exhibited antibody responses higher than those observed during primary infection. In adults, the antibody levels were lower, suggesting that they decay with time. In 90% of the children, the antibody response in primary infection was directed to four of the six antigenic sites studied in the HN glycoprotein, including three of the four neutralization sites. Response against the antigenic sites in the F glycoprotein was weaker than against HN sites and varied considerably from person to person. Reinfection broadened the site-specific responses, although the response against the F glycoprotein still remained restricted.

Rydbeck et al. (87) analyzed the protective effect of monoclonal antibodies against the glycoproteins of PIV3 in intracerebrally infected newborn hamsters. They observed that a significant degree of protection was obtained with antibodies against both HN and F glycoproteins but that none of the individual antibodies could completely protect the animals against disease.

Julkunen et al. (57) studied the nature of the antibody response in PIV1 infection in 10 patients. They observed diagnostic increases in the levels of IgG1 (nine patients), IgG2 (one patient), IgG3 (three patients), IgG4 (four patients), IgA1 (three patients), and IgM subclass antibodies (three patients) between acute- and convalescent-phase sera. The results were very similar to those obtained from patients with influenza A virus infection.

Local interferon production has been observed in 30% of PIV-infected children, whereas the corresponding percentages for respiratory syncytial and influenza virus infections were 4 and 55%, respectively (43).

PREVENTION AND TREATMENT

As outlined above, PIVs are a significant cause of morbidity, especially in infants and young children, and therefore several strategies have been used for the development of effective vaccines. These have included live attenuated viruses, noninfectious viral protein components, and vaccinia virus vectors expressing PIV glycoproteins.

Live vaccines have the advantages that they induce more effective local mucosal immunity and that the duration of the protection is longer when compared with inactivated and subunit vaccines, although the inactivated and subunit vaccines may have fewer adverse effects. The first inactivated vaccines for PIV1, PIV2, and PIV3 were developed in the late 1960s (29). However, the antibody responses were variable and no protection against disease was observed (48).

Because bovine PIV3 (bPIV3) is antigenically related to human PIV3, attempts have been made to use bPIV3 as a vaccine against human disease (the Jennerian approach). It has been shown in a cotton rat model that bPIV3 is able to induce a high level of resistance to human PIV3 (106). Clinical evaluation of this vaccine candidate is under way (13).

Another approach is to use selected variants of human viruses with reduced virulence. Cold-adapted, temperature-sensitive mutants of PIV3 have been used for this purpose (74). Cold-adapted mutants were first produced by serial passages under suboptimal conditions in cell cultures. These viruses seem to have a stable phenotype, are attenuated when evaluated in animal models, and induce resistance to wild-type PIV3 challenge in experimental animals (45). Belshe et al. (3) evaluated passage level 18 of this cold-adapted vaccine candidate in a double-blind, randomized, placebo-controlled study in infants and young children. Of the seropositive children, none in the older age group became infected whereas some of the younger ones shed the virus. All four seronegative young children became infected with the vaccine strain, and two of them developed an illness characterized by rhinorrhea and wheezing. Furthermore, in one case, the virus spread to a sibling control (although it did not cause illness), indicating that this vaccine needs further development before it can be used safely.

In Sendai virus infection in mice, mutant viruses deficient in proteolytic processing of the F glycoprotein cause restricted infection but still are able to induce resistance to challenge by the wild-type virus (103). Therefore one possible approach for vaccine development could be selection of corresponding mutants of the human PIV strains. It is important to keep in mind, however, that for all vaccines containing live viruses, attenuation must be stable.

It has also become possible to construct recombinant viruses (by using, e.g., vaccinia virus as a vector) which express other viral polypeptides on the surface of infected cells. In monkeys immunized intradermally with a single dose of a vaccinia virus construct expressing the HN or F glycoprotein of PIV3, replication of challenge virus was significantly restricted (100, 101). Such new vaccines require further careful evaluation, and studies with other viruses (e.g., adenovirus) may show that they are more optimal vectors in this approach to immunization.

Subunit vaccines have also been developed for PIVs by using different strategies. Ambrose et al. (1) tested a PIV3 subunit vaccine consisting of detergent-solubilized, affinity-purified HN and F glycoproteins in cotton rats. Antibody levels observed after intramuscular immunization were similar to those observed in control animals infected intranasally with PIV3. After intranasal challenge, virus titers in the immunized animals were significantly reduced and inversely correlated with antibody levels in serum. In another study, cotton rats were immunized with the PIV3 F glycoprotein expressed in insect cells by using a baculovirus vector. Low levels of specific antibodies were detected, and the animals became moderately protected against subsequent PIV3 challenge (44). Brideau et al. (7) expressed a chimeric polypeptide containing the extracellular regions of the F and HN glycoproteins of PIV3 in insect cells by using a baculovirus vector. Immunization of cotton rats induced neutralizing antibodies, and the animals became protected against an intranasal challenge with PIV3. One risk of using inactivated virus or viral protein subunits as vaccines is the development of atypical and more severe forms of disease, as has been observed after immunization with formalin-inactivated respiratory syncytial virus preparations (59). This adverse phenomenon can now be evaluated by using the cotton rat model, thus simplifying the development of safe vaccines (80).

At present, the treatment of PIV infections is symptomatic. Some candidate antiviral drugs, including ribavirin, interferons, and protease inhibitors, have been tested, but more studies are needed to elucidate their efficacy.

Rapidly accumulating knowledge of PIV structure, replication, and immunopathogenesis offers a good opportunity to further develop new and more effective vaccines and antiviral drugs to prevent and treat PIV infections. Moreover, improved rapid diagnostic methods for PIVs permit specific diagnosis at an early stage of the illness and detection of single cases before the onset of an epidemic. This progress should facilitate

development of methods for prevention of respiratory infections caused by PIVs.

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