the most important, being a major cause of severe gastroenteritis in infants and young children worldwide [16, 26]. Adenovirus, astrovirus, norovirus and sapovirus, however, are also considered to be significant global enteropathogens [1–4, 10]. Transmission routes of these viruses are classified into foodborne, water-borne, person-to-person spread and might be some other unknown modes [10, 12, 21, 22, 27].

Norovirus (NV, formed known as "Norwalk-like viruses") and *Sapovirus* (SV, formerly known as "Sapporo-like viruses") are the two distinct genera within the family *Caliciviridae*. The prototype strain of sapovirus is the Sapporo virus (Hu/SV/Sapporo virus/1977/JP), which was originally discovered from an outbreak in a home for infants in Sapporo, Japan in 1977 [3]. Morphologically similar viruses were detected in a subsequent series of diarrheal outbreaks in the same institution between 1977 and 1982. They have a typical "Star of David" configuration by electron microscopy (EM) and are antigenically identical to each other by immune EM [4]. Sapovirus contains a positive sense single-strand RNA genome surrounded by an icosahedral capsid. Based on the sequence analysis of the capsid gene, sapovirus is divided into five genogroups, among which only genogroups I, II, IV and V are known to infect humans [16, 23, 29]. Recently, Phan et al. [24] described the diversity of sapoviruses in which genogroup I and II sapoviruses could be classified into 7 and 4 genotypes, respectively [24]. Based on phylogenetic analysis and molecular distance method, the sapovirus isolate which had a low identity with other reference sapovirus strains previously registered in the DDBJ DNA database and did not belong to any the genetic clusters might be tentatively called the new genotype [6, 23, 24].

Immunological and seroepidemiologic studies have indicated a worldwide distribution of sapovirus [7, 10, 18, 28]. The age-related prevalence of antibody against this virus also has shown that infections commonly occur in children less than 5 years old. Furthermore, it was found that serum antibody level to sapovirus was lowest in the first year of life, rising after two years of age [7, 13, 19, 28].

The objectives of this study were: to determine the incidence of diarrheal virus infections in infants and children with acute gastroenteritis in three different places of Japan during 2002 and 2003; to characterize the detected sapovirus according to genogroup and genotype and to describe the genetic diversity among them. Additionally, the age-related distribution and seasonal pattern of sapovirus infection were also determined.

A total of 371 fecal specimens were collected from infants and children with acute gastroenteritis in three different places (Tokyo, Osaka and Maizuru), Japan during the period of July 2002 to June 2003. The fecal specimens were diluted with distilled water to 10% suspensions, and clarified by centrifugation at 10,000 × g for 10 min. The supernatants were collected and stored at −30 °C until use for the detection of diarrheal viruses. The viral genomes were extracted from 140 μl of 10% fecal suspensions using a spin column technique according to the manufacturer's instructions (QIAGEN®, Germany).

Human astrovirus, norovirus, sapovirus, rotavirus and adenovirus were detected by a reverse passive hemagglutination (RPHA), Rotalex (a commercial latex

agglutination), reverse transcription-polymerase chain reaction (RT-PCR), reverse passive hemagglutination (PRHA), RNA-polyacrylamide gels (RNA-PAGE) methods. For Rotalex test (Daiich Kagaku Co., Ltd, Japan) and RPHA (Denka Seiken Co., Ltd, Japan), fecal specimens were processed according to the manufacturer's instructions. Electropherotyping of viral RNA was carried out in 10% polyacrylamide gel electrophoresis (PAGE), and silver staining was performed as described by Theil et al., 1981 [31]. Multiplex RT-PCR with specific primers as previously reported was used to perform the identifications of these viral groups [33, 34]. The PCR was performed at 94 °C for 3 min followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 60 s, and a final extension at 72 °C for 7 min, and then held at 4 °C. PCR products were electrophoresed in a 1.5% agarose gel, followed by staining with ethidium bromide (EtBr, 5 mg/ml) for 20 min then visualized under ultraviolet (UV) light, and the results were recorded by photography.

The nucleotide sequences of PCR products (DNA) positive for sapovirus were determined with the Big-Dye terminator cycle sequencing kit and an ABI Prism 310 Genetic Analyzer (Applied Biosystems Inc.). Sequence analysis was performed using CLUSTAL W (Version 1.6). The nucleotide sequence data for the capsid region from strains 4588/Tokyo/JP, 4508/Maizuru/JP, 4408/Maizuru/JP, 4715/Osaka/JP, 4761/Osaka/JP and 4724/Osaka/JP had been submitted to the DDBJ DNA database and had been assigned accession numbers AB180207, AB180208, AB180209, AB180210, AB180211 and AB180212, respectively. Reference strains and accession numbers used in this study were as follows: Southampton (L07418), PEC (AF182760), Bristol/89 (AJ249939), Lyon/598/97/F (AJ271056), London/92 (U95645), Mex340/90 (AF435812), Cruise ship/00 (AY289804), Hou7-1181/90 (AF435814), Arg39 (AY289803), Mex14917/00 (AF435813), Houston/90 (U95644), Parkville/94 (U73124), Houston/86 (U95643), Sapporo/82 (U65427), Manchester/93 (X86560), Southampton (L07418), Karachi/877/1990 (AB181133), Karachi/730/1992 (AB126249), Karachi/879/1993 (AB181132) and Karachi/874/1992 (AB126249).

A total of 371 fecal specimens were examined for the presence of diarrheal viruses. Diarrheal viruses were detected in 225 out of 371 (60.6%) specimens tested. Among diarrheal viruses detected, group A rotavirus was the most prevalent (42.2%) followed by norovirus (28.9%). Group C rotavirus was the next with 8.4%. Sapovirus and adenovirus were closely behind with 6.7% and 5.3%, respectively. Astrovirus was the last and accounted for 0.9%. No group B rotavirus was found in these subjects. Of interest, the high rate (7.6%) of viral mixed infections was shown in this study (Table 1).

Table 2 shows that 17 fecal specimens were found positive for sapovirus. Of these, 2 cases (4508 and 4464) had mixed infection with group A rotavirus. The highest incidence of sapovirus was in the 12–23 month old group (35.3%), and the lowest fell into the infants aged less than 6 months (5.9%). It was also found that infants and children aged less than 3 years had a rather high rate of sapovirus infection (70.6%).

A total of 17 sapovirus sequences were analyzed by phylogenetics and grouped using the recent sapovirus capsid region classification scheme of Phan et al.,

Table 1. Distribution of viral infection in infants and children with acute gastroenteritis in Maizuru, Tokyo and Osaka, Japan

No of specimen tested	No of viral positive (%)	Monoinfection (%)								Mixed infection (%)						
		RV			NV		SV	Ad	Ast	Ad NVGII	RC NVGII	RA NVGII	RA RC	SV RA	NVGI RA	Ad, NVGII and Ast
		A	B	C	I	II										
371	225 (60.6)	95 (42.2)	0 (0)	19 (8.4)	1 (0.4)	64 (28.5)	15 (6.7)	12 (5.3)	2 (0.9)	1 (0.4)	2 (0.9)	4 (1.8)	5 (2.3)	2 (0.9)	2 (0.9)	1 (0.4)

No, Number; RV, Rotavirus; NV, Norovirus; SV, Sapovirus; Ad, Adenovirus; Ast, Astrovirus; NVGI, Norovirus genogroup I; NVGII, Norovirus genogroup II; RC, Group C rotavirus; RA, Group A rotavirus

Table 2. Characteristics of seventeen sapovirus infections among infants and children with acute gastroenteritis in three places of Japan during 2002 and 2003

No	Patient	Sex	Age	Month	Year	Isolate	Sapovirus genogroup	Sapovirus genotype	Other viruses				
									PCR	Rotalex[#]	PRHA[&]	PAGE[$]	Serotype[#]
1	4333	F	6 m	August	2002	4333/Maizuru/JP	GI	1	−	−	−	ND	ND
2	4408	F	11 m	November	2003	4408/Maizuru/JP	GII	5*	−	−	−	ND	ND
3	4457	M	1 y	April	2003	4457/Maizuru/JP	GI	1	−	−	−	ND	ND
4	4464	M	1 y	April	2003	4464/Maizuru/JP	GI	1	RA	+	−	+	G3, G9
5	4508	F	4 y	April	2003	4508/Maizuru/JP	GI	5	RA	+	−	+	G3, G9
6	4542	M	1 y	June	2003	4542/Maizuru/JP	GI	1	−	−	−	ND	ND
7	4557	F	3 y	October	2002	4557/Tokyo/JP	GII	1	−	−	−	ND	ND
8	4568	F	10 m	November	2002	4568/Tokyo/JP	GI	1	−	−	−	ND	ND
9	4570	M	4 m	December	2002	4570/Tokyo/JP	GI	1	−	−	−	ND	ND
10	4585	M	1 y	October	2002	4585/Tokyo/JP	GI	1	−	−	−	ND	ND
11	4586	M	1 y	November	2002	4586/Tokyo/JP	GI	1	−	−	−	ND	ND
12	4588	M	5 y	January	2003	4588/Tokyo/JP	GI	4	−	−	−	ND	ND
13	4699	M	1 y	August	2002	4699/Osaka/JP	GII	1	−	−	−	ND	ND
14	4715	M	8 y	November	2002	4715/Osaka/JP	GII	5*	−	−	−	ND	ND
15	4716	F	2 y	November	2002	4716/Osaka/JP	GI	1	−	−	−	ND	ND
16	4724	M	2 y	November	2002	4724/Osaka/JP	GI	6	−	−	−	ND	ND
17	4761	F	7 y	March	2003	4761/Osaka/JP	GI	6	−	−	−	ND	ND

No, Number; M, Male; F, Female; m, Month; y, Year; *New genotype; [#]Only used to detect and serotype group A rotavirus; [$]Only used to characterize rotavirus; [&]Only used to detect group C rotavirus; RA, Group A rotavirus; +, Positive; −, Negative; ND, Not done

2005 [24]. In the present study, all of the sapovirus sequences were classified into two distinct genogroups I and II. Majority of the sapovirus sequences (76.5%, 13 of 17) belonged to sapovirus GI. Our results indicated that sapovirus group I was a dominant genogroup. The sapovirus GI sequences clustered into 4 genotypes (GI/1, GI/4, GI/5, GI/6) and these presented 69.2%, 7.7%, 7.7% and 15.4%, respectively. Two of the sapovirus GII sequences were classified into GII/1 (known as the Lyon/598/97 virus cluster). Interestingly, other two isolates 4408/Maizuru/JP and 4715/Osaka/JP in the present study did not belong to any genetic clusters and showed a novel sapovirus GII genotype tentatively called GII/5. Using CLUSTAL W, it was noticed that these sapoviruses had a low identity on the amino acid with other reference sapovirus strains previously registered in the DDBJ DNA database in the same genogroup ranged from 66% to 80%.

Viral gastroenteritis is still the health burden in developed and developing countries [9, 16, 30]. In this study, diarrheal viruses were detected in 60.6% fecal specimens tested. Other etiologic agents caused about 39.4% of diarrheal cases. Among diarrheal viruses detected, group A rotavirus was the most prevalent and became a leading cause of viral gastroenteritis in infants and children in Japan followed by norovirus, group C rotavirus, sapovirus, adenovirus and astrovirus. These viruses are associated with sporadic cases and outbreaks of gastroenteritis in such settings as kindergartens, schools, hospitals, cruise ships, restaurants, nursing homes for the elderly and among military recruits [4, 8, 14, 15, 20, 32].

Sapovirus is one of the global causes of viral gastroenteritis and also is associated with sporadic cases and outbreaks of gastroenteritis worldwide [1, 3, 6, 18]. Sapovirus infection causes acute gastroenteritis in all age group, through it occurs predominantly in infants and young children [4]. Out of 371 fecal specimens in this study, 17 were determined to be positive for sapovirus and the majority of infants and children with sapovirus infection (70.6%) was aged less than 36 months. This result was consistent with previously published reports on sapovirus epidemiology worldwide in which its prevalence was shown to be ranging from 0.3 to 9.3% and usually much lower than norovirus [1, 11, 20, 22]. Our findings also confirmed sapovirus as one of the enteropathogens responsible for viral gastroenteritis among infants and children in Japan. According to some reports, sapovirus was prevalent in cold season, and several studies did not find a seasonal correlation [4, 20, 22, 24]. In this study, however, sapovirus was identified throughout the year except in February, July and September and was highest in November as autumn.

To date, the mixed infections with different enteric viruses have been reported by several groups of investigators [1, 22, 23]. Interestingly, the high incidence (7.6%) of mixed infections with different enteric viruses was detected. Of these, mixed infections with sapovirus and group A rotavirus in a relatively high percentage of 11.8% (2 of 17) were identified in patients 4464 and 4508 with 1 and 4-years old, respectively. In addition, the presence of two group A rotaviruses associated with mixed infections with sapovirus was also determined by other methods with different principles, the Rotalex test and RNA-PAGE. These two cases were further

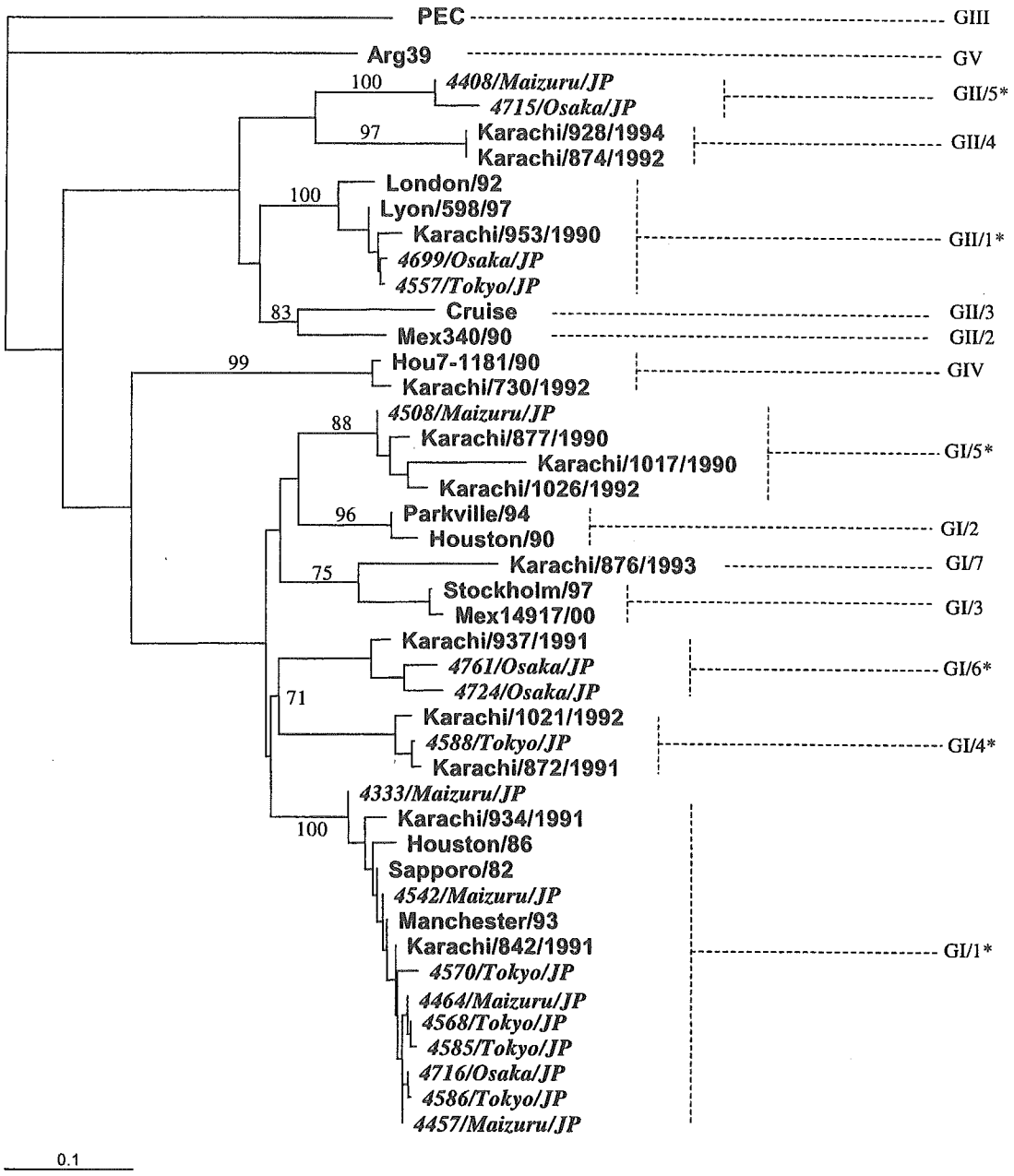

Fig. 1. Phylogentic tree of amino acid sequences of 17 isolates of sapovirus based on the recent sapovirus capsid region classification scheme of Phan et al. [24]. The tree was constructed from partial amino acid sequences of the capsid region (144aa) of the seventeen sapoviruses detected in three places of Japan during 2002 through 2003. Sapoviruses in this study are highlighted in italic. The numbers in the branches indicate the bootstrap values. The bootstrap values show how well supported parts of the tree are, considering the method used for generating the tree and values >70% are considered to be supportive. The scale indicates the genetic distance between the isolates. Reference strains of sapovirus were selected from DDBJ/GenBank under the accession number indicated in the text. Note: *The genotype has sapoviruses detected in this study

characterized for serotype specificity using previously published serotype specific primers presented by Das et al., 1994 [5]. Remarkably, these group A rotaviruses were identified with mixed serotypes G3 and G9. Apparently, these specimens represented the triple mixed infections. These results were in good agreement with other previous published reports in which mixed infection among sapovirus and other enteropathogens is not rare [23].

The results in this study showed all Japanese sapovirus sequences belonged to two distinct sapovirus genogroups I and II. Of these, the sapovirus positives were further classified into 4 sapovirus GI genotypes (GI/1, GI/4, GI/5 and GI/6) and 1 sapovirus GII genotype (GII/1) according to the recent sapovirus capsid region classification scheme of Phan et al., 2005 [24]. Moreover, other two sapovirus isolates 4408/Maizuru/JP and 4715/Osaka/JP did not cluster with any sapovirus references indicated in the text and presented one novel sapovirus genotype tentatively called GII/5. These sapoviruses had a low identity on the nucleotide as well as the amino acid with other reference strains in the same genogroup previously registered in the DDBJ DNA database. It was noteworthy to point out that sapovirus in genogroup II could be classified into 5 genotypes (Fig. 1).

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Herpes Simplex Virus 2–Associated Hemophagocytic Lymphohistiocytosis in a Pregnant Patient

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BACKGROUND: Uncontrolled phagocytosis of normal hemopoietic cells by activated histiocytes in bone marrow is collectively referred to as hemophagocytic lymphohistiocytosis.

CASE: We present a case of hemophagocytic lymphohistiocytosis associated with herpes simplex virus-2 infection in the second trimester. Cytopenia, elevated C-reactive protein, ferritin, soluble interleukin-2 receptor, and interleukin-6 with high-grade fever were observed following genital herpes infection, and the existence of hemophagocytes in bone marrow confirmed the diagnosis of hemophagocytic lymphohistiocytosis. Corticosteroid therapy failed to arrest the hemophagocytic process, whereas cyclosporin A was effective. The patient delivered a healthy infant after remission and has not experienced exacerbation.

CONCLUSION: It is often important to take into consideration hemophagocytic lymphohistiocytosis when encountering cytopenia with high-grade fever. Cyclosporin A was a safe and available strategy for this corticosteroid-resistant case. (Obstet Gynecol 2005;105:1241–4. © 2005 by The American College of Obstetricians and Gynecologists.)

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CASE

A primigravida in midgestation was transferred to our hospital, complaining of headache, fatigue, and fever up to 39°C for 2 weeks (Fig. 1). The first physician diagnosed a herpes simplex viral infection at the external genitals, preceded by high-grade fever, and the skin lesions of viral infection were improved on admission. The vital signs were as follows: body temperature 39.5°C, blood pressure 112/60 mm Hg, and heart rate 120 beats per minutes. No significant swelling of superficial lymph nodes, hepatomegaly, or splenomegaly were observed on physical examination. Initial laboratory studies showed cytopenia with hemoglobin of 8.0 g/dL, a white blood cell (WBC) count of 2,620/μL (69.5% neutrophils, 26.0% lymphocytes, 0% atypical lymphocytes). A platelet count of 123,000/μL, slight liver dysfunction with aspartate aminotransferase (AST) 66 U/L (normal range 10–31 U/L), and alanine aminotransferase (ALT) 47 U/L (normal range 6–26 U/L) were recorded. Elevation of triglyceride 180 mg/dL (normal range 36–112 mg/dL), lactate dehydrogenase (LDH) 460 U/L (normal range 115–245 U/L), serum ferritin 865.8 ng/mL (normal range 8–74 ng/mL), and soluble interleukin-2 receptor (sIL-2R) 2,710 U/mL (normal range 220–530 U/mL) were noted. Serum cytokine levels for interferon-γ (INF-γ), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-10 were all within normal ranges, whereas IL-6 was elevated (15.8 pg/mL, normal range $<$ 4 pg/mL). Slightly increased levels of fibrinogen degradation products (29.4 μg/mL, normal range $<$ 4 μg/mL) were observed. However, results from other coagulation studies, such as prothrombin times, partial thromboplastin times, and fibrinogen were at appropriate levels for

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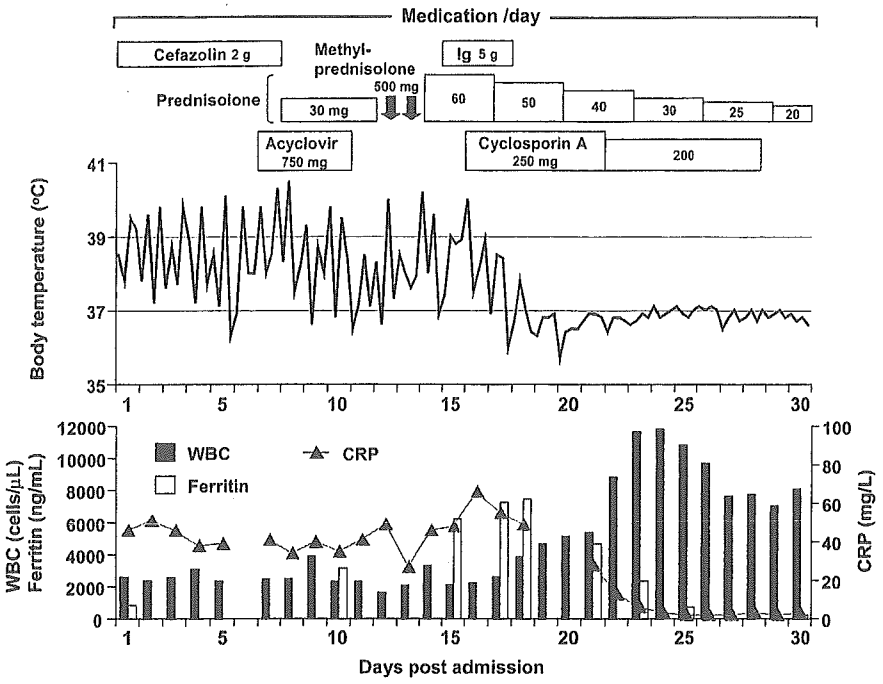

Fig. 1. Clinical course. Changes of body temperature, number of white blood cells (WBC),. and serum levels of ferritin and C-reactive protein (CRP), as well as medication dosages are shown.

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midgestation. Antinuclear antibody and double-stranded DNA antibody were negative, and hypergammaglobulinemia and increase of complement 1q immune complex were not observed. Rather, serum complement titer was increased (52.3 U/mL, normal range 27–42 U/mL). The percentage of CD4 positive cells, CD4/CD8 ratio, and percentage of activated NK cells in peripheral blood were normal. Herpes simplex virus (HSV) antibody titer of immunoglobulin (Ig)M and IgG were 0.46 and 79.7, respectively, (normal range < 0.8 and 2), and the neutralizing antibodies titer for herpes simplex virus-1 (HSV-1) and HSV-2 were 1:16 and 1:8 (normal range < 1:4). Antibody titer for Epstein-Barr viral capsid antigen for IgM and IgG were 0 and 12.8 (normal range < 1), and that for nuclear antigen IgG was 4.0 (normal range < 1). Viral serology for human immunodeficiency virus (HIV), human T-cell leukemia virus, cytomegalovirus, and hepatitis B and C virus were all negative.

Two weeks later, only the neutralizing antibodies titer for HSV-2 was elevated to 1:16, and other antibodies tested were unaltered. The patient had been suffering from herpes simplex virus infection of her lips. The elevated neutralizing antibodies titer for HSV-2 suggested, in the light of the new genital herpes simplex infection on clinical observation, that the clinical manifestation was related to HSV-2. On admittance, we suspected hemophagocytic lymphohistiocytosis (HLH) by the initial results from peripheral blood tests and collected bone marrow for analysis. Although the first bone marrow studies revealed hypocellularity with a nucleated cell count of 45 × 10^9/L, an increased number of mature histiocytes with hemophagocytosis was not observed. We decided that intensive treatment was not necessary and expected natural remission with symptomatic treatment, because of the bone marrow observation and the fact that the patient was pregnant. The clinical manifestation had not improved after 1 week without medication, and so we administrated prednisolone as a 30-mg daily dose. Acyclovir (750 mg/d) was prophylactically used to reduce viral activity before initiating prednisolone treatment. Transient reduction of fever was observed. However, there was no effect on

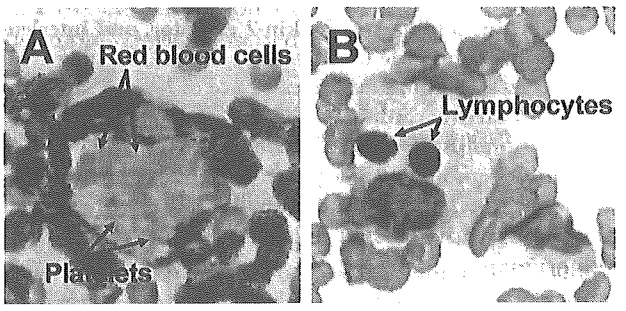

Fig. 2. Bone marrow features. Mature histiocytes with hemophagocytosis were revealed in bone marrow (Wright-Giemsa staining, × 1,000, original magnification). The phagocytes engulfed red blood cells, platelets **(A)**, and lymphocytes **(B)**.

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cytopenia and inflammation indicators such as C-reactive protein (CRP) and ferritin. Furthermore, serum levels of AST, ALT, triglyceride, and LDH elevated to 170, 110, 258, and 663, respectively. To evaluate the current status and to determine other therapeutic measures on day 10 postadmission, we performed magnetic resonance imaging (MRI) examination and repeated the bone marrow studies. Whole-body MRI revealed no swelling of deep lymph nodes, abscesses, or malignant lesions and absence of slight hepatomegaly. Progressive decrease in the number of nucleated cell counts (23×10^9/L) and greater than 3% of mature histiocytes (4%) engulfing red blood cells, platelets, and lymphocytes were shown in the bone marrow (Fig. 2). At the same time, viral DNA from Epstein-Barr virus and HSV in the bone marrow were not detected by polymerase chain reaction amplification. We performed intravenous pulse methylprednisolone and subsequent full-dose prednisolone treatments. However, the high-dose corticosteroid therapy also failed to arrest the hemophagocytic process. The patient's general status was worsening with elevated levels of CRP and ferritin, and reduction of amniotic fluid was noted. Ultimately, cyclosporin A was administered for this corticosteroid-resistant HLH case, and thereafter, fever gradually decreased to basal levels within a few days of treatment. With symptomatic improvement, laboratory data and the amniotic fluid came to within normal range. The patient conclusively achieved medication withdrawal by delivery without exacerbation and delivered, at 37 weeks and 0 days of gestation (by cesarean, owing to breech presentation), a 2,592-g male with Apgar scores of 9 and 10 at 1 and 5 minutes, respectively.

COMMENT

Hemophagocytic lymphohistiocytosis is characterized by exaggerated histiocytic proliferation and activation. High-grade fever and hepatosplenomegaly on physical findings, with cytopenia, elevated liver enzymes, hyperlipidemia, and coagulopathy on conventional peripheral blood tests, are observed. The unexplained progressive cytopenia is the fundamental finding on the blood tests, and supporting characteristic findings are elevated LDH, ferritin, and sIL-2R as a result of hemophagocytosis with various cytokines elevated (IL-1, IL-2, IL-6, IL-10, IFN-γ, TNF, macrophage colony-stimulating factor) as a sign of activated lymphocytes or macrophages/histiocytes. Causative factors of HLH fall into 3 main groups, inherited, reactive, and histiocytic medullary reticulosis.[1] The reactive group in HLH includes infection, malignancy, and autoimmune disease, and the prognosis of HLH varies from complete remission without medication to rapid deterioration and death. The criteria for the diagnosis of HLH in adults have been established as high fever of unknown etiology for more than a week, unexplained progressive cytopenia affecting at least 2 cell lineages, and the existence of greater than 3% or 2,500 cells/mL of mature histiocytes with prominent hemophagocytosis in bone marrow and/or hemophagocytosis in liver, spleen, or lymph nodes.[2] Accordingly, the diagnosis of HLH usually must be made on the basis of bone marrow studies. In clinical treatment during pregnancy, it is important to determine the differential diagnosis of hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome because of the similarity of clinical features with HLH.[3] In our case it was not difficult to suspect the diagnosis of HLH, given our knowledge of the preceding genital herpes infection. Nevertheless, conclusive diagnosis was made by the second bone marrow study, supporting the significance of bone marrow aspiration without hesitation.

As for the causative virus, the first serological examination was performed on admission (14 days after the initial infection), and the HSV IgG antibody titer (recognizing both HSV-1 and HSV-2) was significantly elevated. The neutralizing antibody titer for HSV-2 was 1:8 at the first examination, and it was elevated to 1:16 two weeks later, whereas the titer for HSV-1 was unaltered. The elevated neutralizing antibody titer was determined to be significant based on the assumption that the titer was negative when the patient had the initial infection 14 days before admission. Although we could not detect the viral gene in the second bone marrow examination, the virus is usually nonexistent in blood at initial diagnosis. Consequently, we considered that the causative virus was HSV-2 because of the serological findings with the clinical course. Nevertheless, it was not clarified whether it was a new or a recurrent infection by the elevated level of neutralizing antibodies titer.

We occasionally encounter viral infections and subsequent mild HLH-like symptoms in the first or second trimester of pregnancy, even though the incidence of HLH is very low in adults. Immune environments related to the function of T helper (Th) lymphocytes in pregnancy fundamentally shift to Th2-dominant to tolerate the fetus growing in the body, resulting in relatively decreased cell-mediated immunity by Th1 cells with increased susceptibility to viral infection.[4] Our hypothesis for the facility of HLH due to viral infection during pregnancy is that the decreased cell-mediated immunity may allow overactivation of hemophagocytes as the first immune response to infection instead of the relatively decreased Th1 immune response. The candidates for medical treatment of HLH, such as corticosteroid, high-

dose intravenous Ig, antithrombin, cyclosporin A, etoposide, and methotrexate, have been reported as successful treatments, and the combination therapy with dexamethasone,[2,5] cyclosporin A, and etoposide is recommended by the HLH-94 protocol.[6] We preferred prednisolone first, and then used it in combination with cyclosporin A in this case, because etoposide is classified as a category D risk factor during pregnancy by the U.S. Food and Drug Administration (FDA), whereas both prednisolone and cyclosporin A are classified as category C, and prednisolone can be inactivated in the placenta.[7] Although corticosteroid therapy did not have significant inhibitory effects on the hemophagocytosis, cyclosporin A was greatly effective without fetal distress and restriction of fetal growth in this case. Cyclosporine A has inhibitory effects on uncontrolled activation of the cellular immune system, and the mechanism is believed to involve binding to the intracellular receptor, cyclophilin, resulting in inhibition of calcineurin responsible for activation of NF-AT (nuclear factor of activated T cell) and subsequent inhibition of cytokines production. Although the specific inhibition mechanisms of HLH remain unclear, the potential effect of cyclosporine A therapy on HLH is likely inhibition of T-cell activation and normalizing of the Th1/Th2 balance.[8] It may have direct inhibitory effects on macrophages/histiocytes because of the presence of calcineurin in the cells. It is of course essential that targeted antimicrobial therapy be performed before chemotherapy or immunotherapy. We believe, considering the logical mechanism, that cyclosporin A has therapeutic utility for HLH in pregnancy without a sensitive period.

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Short communication

Studies on the prevalence of human papillomavirus in pregnant women in Japan

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Abstract

Aims: In order to evaluate the prevalence of human papillomavirus (HPV) in the pregnant population in Japan.
Methods: We examined cervical swabs of 1,183 pregnant women for HPV DNA using a PCR-RFLP method during October, 2000 and February, 2001. The overall prevalence of HPV in 1,183 pregnant women was analyzed and stratified by age.
Results: The overall prevalence of HPV in pregnant women was 12.5% (148 of 1,183 cases). The prevalence in pregnant women younger than 25 years (22.6%, 28 of 124 cases) was significantly higher compared with that in pregnant women ≥25 years (11.3%, 120 of 1057 cases, $P<0.0005$).
Conclusions: The data indicate a significantly higher prevalence of HPV infection in younger pregnant Japanese women.

Keywords: Human papillomavirus; infection; pregnancy; prevalence.

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Introduction

In recent years, estimates of the prevalence of HPV infection at the uterine cervix in the general population have varied widely, making comparisons difficult. The most important factor explaining the variation is the small sample size in the study population. In this context, we examined a significantly large sample of pregnant women in the Japanese population in order to clarify the prevalence of HPV in the pregnant population.

Materials and methods

During October, 2000 and February, 2001, 1,183 pregnant women were enrolled in this study. Informed consent was obtained at the obstetric outpatient clinics of the hospitals, to which the Cooperative Study Group members belong. The inclusion criteria were any pregnant women, without age or gestational week restriction, who consecutively visited the hospitals during the study period. The hospitals were major general hospitals in each district of Japan, and the pregnant women who visited these hospitals did not belong to a high risk group of sexually transmitted diseases. A sample for HPV DNA analysis was taken using Cytopick (cell sampling device from the uterine cervix, Lion Co. Ltd., Tokyo, Japan) from the uterine cervix by an obstetrician. The specimens were assayed using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) by the method of Yoshikawa et al. [15]. Briefly, the genomic DNA extracted from the cells was amplified by consensus primers for the L1 region of HPV. After amplification, the DNAs were digested by restriction enzymes. The subtypes of HPV were determined by the patterns of restriction fragment polymorphism. The prevalence of HPV was stratified by age, and the distribution of each HPV subtype was analyzed.

Table 1 Positive Rate of HPV at the Uterine Cervix in Pregnant Women in Japan in Each Age Range.

Age	No. of cases	No. of positive cases	Positive rate (%)
~19	9	4	44.4
20–24	115	24	20.9
25–29	429	53	12.4
30–34	432	50	11.6
35–39	173	13	7.5
40~	25	4	16.0
Total	1183	148	12.5

n.b.: There is a statistically significant diffrence of positive rate between pregnant women group under 25 y.o. and equal to or older than 25 y.o. ($P<0.0005$, by Chi-square analysis).

Table 2 Positive Rate of HPV at the Uterine Cervix in Pregnant Women in Japan According to Gestational Week.

Gestational weeks	No. of cases	No. of positive cases	Positve rate (%)
~11W	89	9	10.1
12–15W	138	17	12.3
16–19W	110	25	22.7
20–23W	110	11	10.0
24–27W	126	20	15.9
28–31W	176	20	11.4
32–35W	237	22	9.3
36W~	197	24	12.2
Total	1183	148	12.5

n.b.: No significant difference was observed according to gestational week.

Results

Of 1,183 pregnant women, HPV was detected in 148 cases, for a prevalence of 12.5%. The prevalence was 44.0, 20.9, 12.4, 11.6, 7.5, and 16% in pregnant women <20, 20–24, 25–29, 30–35, 35–39, >39 years old, respectively (Table 1). The prevalence in pregnant women younger than 25 years was 22.6% (28 of 124 cases), significantly higher compared with pregnant women ≥25 years (11.3%, 120 of 1059 cases). ($P<0.0005$, by Chi-square test).

Table 2 shows the prevalence of HPV for each gestational week. The prevalence ranged from 9.3% (at 32–35 weeks) to 22.7% (at 16–19 weeks), but no significant difference was observed among gestational weeks.

In Table 3, the distribution of HPV subtypes is shown. The distribution of high-risk viruses for cancer, namely HPV types 16, 18, 31, 35, 45, 51, 52, and 56 was 9.9%, 3.9%, 0.7%, 2.0%, 0%, 2.6%, 17.8%, and 2.6%, respectively.

Discussion

Epidemiologic observation suggested that cervical cancer is associated with infection by sexually transmissible agents. A variety of molecular epidemiologic studies suggested that human papillomaviruses (HPVs) may be an important sexually transmissible agent related to the development of neoplastic changes in the cervix [1]. An increasing incidence of cervical intraepithelial neoplasia (CIN) among young women has been noticed in recent years [9]. For this reason, pregnancy might be a unique opportunity to detect HPV infection for those women who do not take part in a screening program for cervical carcinoma. Although a number of investigators reported the prevalence of HPVs in pregnant women, this varies from study to study [2, 6–8, 14], probably due to the samll number of cases in the study populations. In our study, we examined 1,183 pregnant women, and the prevalence of HPV was 12.5%, which is a relatively reliable figure as to the prevalence of HPV in the pregnant population in Japan. De Roda Husman et al. reported that the prevalence of HPV was 9.6% in 709 pregnant women, which was similar to our results [3].

None of the HPV prevalence differed significantly among the first, second and third-trimester. This finding is inconsistent with other observations demonstrating a higher prevalence of HPV in more advanced pregnancy [5, 10]. The discrepancy between our findings and other reports remains unclear. The prevalence in pregnant women younger than 25 years (22.6%, 28 of 124 cases) was significantly higher compared with that in pregnant women ≥25 years. It is suggested that further investigation should be conducted concerning the prevalence of HPV infection in younger pregnant women.

One must always take into consideration the effect of pregnancy, per se, on the infection of HPV. As pregnant women are considered to be immunosuppressed, a state which facilitates survival of 'semi-allogeneic' fetus, a possibility exists that new HPV infection or reactivation

Table 3 Number and Rate of Each HPV Genotype.

HPV genotype	Number of each genotype	Rate (%)
HPV16	15	9.9
HPV18	6	3.9
HPV31	1	0.7
HPV33	2	1.3
HPV35	3	2.0
HPV39	4	2.6
HPV51	4	2.6
HPV52	27	17.8
HPV53	5	3.3
HPV54	2	1.3
HPV56	4	2.6
HPV58	10	6.6
HPV59	3	2.0
HPV61	3	2.0
HPV66	3	2.0
HPV68	4	2.6
Unknown	45	29.6
Total	152	100.0

n.b.: Two genotypes were observed in 4 cases.

of HPV might occur during pregnancy. Although such effect of pregnancy on HPV infection is controversial [4, 7, 12], the comparative study of the prevalence of HPV between the pregnant and non-pregnant state of same population is mandatory.

Another major concern in such study is the outcome of the neonates born to the mother with positive HPV, i.e., a possibility exists that respiratory papillomatosis may occur in the neonates. Although this complication is reported to be very rare [11, 13], a follow up study of neonates born to HPV positive mother is also crucial.

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