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Hepatitis C Virus in Pelvic Lymph Nodes and Female Reproductive Organs

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Based on epidemiological evidence, hepatitis C virus (HCV) is thought to be involved in the etiology of not only hepatocellular carcinoma, but also lymphoproliferative diseases. In addition, our previous studies using recently developed cell culture systems that support HCV replication have indicated that HCV possesses both hepatotropism and lymphotropism. To determine whether HCV is present in extrahepatic organs, we conducted semi-quantitative reverse transcription-polymerase chain reaction for the 5′ non-coding region of the HCV genome in surgical specimens (lymph nodes, ovary, uterus, peripheral blood mononuclear cells [PBMCs] and serum) from three patients with gynecological cancer. We found relatively high HCV genome titers in the lymph nodes, not in the sera, irrespective of various titers in PBMCs. These results suggest that lymph nodes may play an important role in the carrier state and the persistence of HCV infection. Moreover, contrary to expectation, high titers of the HCV genome were observed in the ovaries and the uteri, suggesting the feasibility of mother-to-infant and spouse-to-spouse transmissions of HCV.

Key words: Hepatitis C virus (HCV) — Lymph node — Ovary — Uterus

Hepatitis C virus (HCV) is a positive-stranded RNA virus and a member of the *Flaviviridae*.[1] Its genome is 9.5 kb long and includes a large open-reading frame that encodes a polyprotein precursor of about 3000 amino acids.[2,3] In general, HCV is believed to be a frequent cause of chronic liver diseases, i.e., chronic hepatitis and liver cirrhosis, and subsequently hepatocellular carcinoma.[4,5] Recently, accumulating epidemiological evidence has indicated an association between HCV and certain lymphoproliferative diseases, such as cryoglobulinemia and non-Hodgkin's lymphoma.[6–10] In accordance with such epidemiological investigations indicating the association of HCV with both lymphocytic and hepatic disorders, we demonstrated *in vitro* replication of HCV in cultured lymphocytes and hepatocytes; MT-2 cells (human T cell leukemia virus type I infected T cell line)[11,12] and PH5CH cells (non-neoplastic human hepatocytes immortalized by SV40 large T antigen),[13] respectively. In this study, we examined the status of HCV infection in lymph nodes which do not drain the liver. Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) using normal pelvic lymph nodes from HCV-infected patients diagnosed with gynecological cancer was performed, and we also investigated whether female reproductive organs, such as ovary and uterus, are extrahepatic targets of HCV infection.

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Three patients diagnosed with gynecological cancer, who were anti-HCV antibody-positive, underwent radical hysterectomy (Table I). Patient 1 was diagnosed with ovarian cancer, while patients 2 and 3 were diagnosed with uterine cancer (no human papilloma virus was detected). None of them was infected with hepatitis B virus. Patients 1 and 3 were asymptomatic carriers of HCV, without marked elevation of serum alanine aminotransferase (ALT) or asparate aminotransferase (AST), and they had never been treated for HCV infection. Patient 2, on the other hand, had been diagnosed with chronic hepatitis previously and had been treated with interferon α-2a for six months. Informed consent was obtained from each patient before the study.

Intraoperatively, fresh tissue samples (non-cancerous portions of the ovary and the uterus, two uninvolved pelvic lymph nodes) were collected from the resected specimens, immediately frozen, and stored overnight at −70°C. Serum was also separated from 5 ml of non-heparinized blood taken from each patient intraoperatively. Peripheral blood mononuclear cells (PBMCs) were separated from 5 ml of heparinized blood on a Ficoll-Conray density gradient (Immuno-Biological Laboratories, Fujioka), and were washed four times in phosphate-buffered saline.[14] RNAs were then extracted from the frozen tissue specimens, the PBMCs and the sera, by using Isogen or Isogen-LS.[11] Semi-quantitative RT-nested PCR for the 5′ non-coding region of HCV

Table I. Clinical Profiles of Patients with Gynecological Cancer

Patient	Age (years)	Primary cancer	History of blood transfusion	Interferon pretreatment	ALT[a] (U/liter)	AST[b] (U/liter)	HBs-Ag[c]	Clinical diagnosis as to HCV
1	43	Ovary	(−)	(−)	9	20	(−)	Asymptomatic carrier
2	58	Uterus	(+)	(+)	63	69	(−)	Chronic hepatitis
3	56	Uterus	(−)	(−)	9	13	(−)	Asymptomatic carrier

a) Data on admission. Normal range: 5–35 U/liter.
b) Data on admission. Normal range: 10–30 U/liter.
c) HBs-Ag: hepatitis B surface antigen.

Table II. HCV Genome Titer in Various Organs

Patient	Lymph node 1[a]	Lymph node 2[a]	Ovary[a]	Uterus[a]	PBMCs[b]	Serum[b]
1	5.6	4.3	4.0	5.2	3.5	3.9
2	7.2	6.9	7.1	7.5	1.5	3.6
3	5.9	5.3	5.6	UD[c]	3.5	UD[c]

a) $\log_{10}$ HCV genomes/g tissue.
b) $\log_{10}$ HCV genomes/ml original blood.
c) UD: undetected.

genome[12, 13] was performed with 0.5 μg of total RNA from the tissue (lymph node, ovary and uterus) or RNA corresponding to 100 μl of original blood (PBMCs and serum). The detection limit of this analysis is 10 HCV genomes. The amounts of HCV are reported as the logarithms of equivalent HCV genomes per gram of original tissue (lymph node, ovary and uterus) or per milliliter of the original blood (PBMCs and serum). The HCV in every patient was found to be genotype 1b by RT-nested PCR using genotype-specific primers.

As shown in Table II, the HCV genome titer in the lymph nodes of all three patients was $10^{4.3}$–$10^{7.2}$ HCV genomes/g tissue, and was always higher than the titer in the serum (less than 10^4 HCV genomes/ml blood), and both of the lymph nodes examined from each patient contained similarly high levels of HCV. These results indicate that HCV can proliferate or at least preferentially exist in the lymph nodes, because the amount of HCV genomes in the lymph nodes was greater than could be explained merely by HCV contamination by serum. This suggests that the lymph nodes play an important role as reservoirs in the carrier state and in persistent HCV infection. If this is the case, they can be targeted for HCV treatment, in addition to the liver.

On the other hand, the HCV genome titers in the PBMCs of the three patients were relatively low (Table II). In patient 2, the HCV genome titer in the PBMCs was very much lower than in the serum, as observed in a previous study by another group, in which the HCV genome titer in PBMCs was shown to be generally much lower than in the serum.[15] In contrast, in patient 3, the HCV genome titer in PBMCs was $10^{3.5}$ HCV genomes/ml blood, in spite of HCV being undetectable in the serum. These results contrasted with the consistently high titers of HCV genomes in the lymph nodes, and suggested that lymph nodes are more susceptible to HCV than PBMCs.

Contrary to expectation, we found that HCV genome titers in the ovary and the uterus were higher than in the sera in all 3 patients, except for the uterus in patient 3 (Table II). It is unclear whether the observed HCV genome titers are attributable to replication of HCV in the parenchyma cells of the ovaries and the uteri. According to the results of previous studies supporting the lymphotropism of HCV[11, 12] and our present findings in the lymph nodes, it is more likely that the detected HCV genomes originated from lymphocytes that had infiltrated into the stroma of the ovary and the uterus. Actually, we observed lymphocytes infiltrating in the uterus and ovary to varying extents in each patient when we examined these organs microscopically (data not shown). It is also possible that HCV virions were trapped in connective tissues by a simple absorption mechanism.

Although HCV infection of extrahepatic organs other than lymphoid tissue has recently been reported,[16, 17] to the best of our knowledge this is the first study to detect HCV in female reproductive organs. Serum HCV contamination is always a problem and some pretreatments have been devised to avoid such contamination.[18] In the case of PBMCs, we washed the cells with phosphate-buffered saline four times, which is sufficient for HCV cell culture systems.[11, 13] However, it seems to be impossible to disperse cells from solid specimens completely and to eliminate HCV adsorbed on cell surfaces. Thus, further examinations will be needed to detect HCV *in situ*, for example, by using immunohistochemical methods or *in situ* hybridization techniques. Moreover, detection of negative-stranded HCV RNA, an intermediate form in HCV replication, is crucial to verify actual proliferation of HCV in extrahepatic organs.

In this study, we could not determine the HCV genome titers or histopathology of the liver, because liver speci-

mens were not available, and thus we were also unable to compare the amount of HCV in hepatic tissue and extrahepatic tissues. However, we were able to demonstrate that HCV in the lymph nodes, ovary, and uterus was more abundant than in circulating blood. More information regarding the amount of HCV in various organs needs to be accumulated to compensate for the lack of clinical specimens. In any event, the data obtained from the present study provide an important clue to the cell tropism of HCV, and tend to support the feasibility of mother-to-infant and spouse-to-spouse transmissions, which are still a matter of controversy.

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