Table 1
The age-specific prevalence of confirmed anti-HCV positivity in 1993–1995 in Town C, Japan

Age (years)[a]	Male	Female	All
–29	2/119 (1.7%)	6/202 (3.2%)	8/321 (2.5%)
30–39	22/193 (11.4%)	29/442 (6.6%)	51/635 (8.0%)
40–49	52/403 (12.9%)	46/503 (9.1%)	98/906 (10.8%)
50–59	81/407 (19.9%)	130/607 (21.4%)	211/1014 (20.8%)
60–69	185/693 (26.7%)	277/919 (30.1%)	462/1612 (28.7%)
70–	122/385 (31.7%)	199/704 (28.3%)	321/1089 (29.5%)
Total	464/2200 (21.1%)	687/3377 (20.3%)	1151/5577 (20.6%)

[a] In 1995.

by RT-PCR in 1995. These 591 anti-HCV antibody-positive individuals who also tested positive for either HCVcAg or HCV RNA 6 months or more after the initial anti-HCV antibody testing were judged as having a persistent HCV infection.

3.2. Serum ALT and HCV core antigen in anti-HCV positive residents

Those individuals harboring persistent HCV infections were similar in both gender and age to previously infected individuals (Table 2). Although the mean titers of anti-HCV antibodies were greater in those individuals positive for HCVcAg in comparison to those negative for HCVcAg, but HCV RNA-positive, AST, ALT, and γ-GTP levels were similar between these two groups (Table 2). The frequency of HCV serotype I was significantly higher in the HCVcAg-positive individuals in comparison to the HCVcAg-negative, HCV RNA-positive individuals. The increased sensitivity of the HCVcAg FEIA in carriers infected with HCV serotype I may explain this observation [13]. The serum levels of both AST and ALT were significantly higher in individuals with persistent HCV infections than in those lacking any evidence of HCVcAg or HCV RNA. The serum levels of γ-GTP were significantly higher in HCVcAg-positive individuals than in those lacking detectable HCVcAg or HCV RNA. In chronically infected individuals, however, no correlation between HCVcAg concentrations and ALT levels could be observed (data not shown).

Of the 591 individuals diagnosed with persistent HCV infection in 1995, 511 had at least four available annual ALT measurements between 1993 and 2000. Sixty-three of these individuals reported having received interferon (IFN) treatment before 2002.

Of the 448 individuals who had not been treated with IFN, 162 (36.2%) had normal ALT levels in all tests (<35 IU/L) (Group N), while 286 (63.8%) had at least one abnormal ALT level (≥35 IU/L) during the examination period (Group A). There were no differences between these two groups in the HCVcAg levels determined in 1995 (data not shown).

3.3. Spontaneous elimination of HCV RNA

In 591 individuals who were judged as having a persistent HCV infection in 2000, serum samples in 2001 or 2002 were obtained from 302 individuals who had at least four available annual ALT measurements between 1993 and 2000 and had not been treated by IFN until 2002. These 302 serum samples were tested of HCV RNA using RT-PCR and a positive HCV RNA was detected in 282 of these 302 serum samples. Of the 20 individuals who were HCV RNA-negative, four individuals (case A, C, F and G in Fig. 1 and Table 3) were only positive for HCV RNA in 1995, 3 were confirmed positive for HCV RNA in any of the available stored samples until 2000 and 13 did not have detectable HCV RNA in any of the available stored samples spanning from 1995 to 2000. As a result, seven individuals (2.4%, 7/289) were determined to have spontaneously eliminated the HCV infection, based on RT-PCR (Fig. 1). For cases A, C, D, F, and G, there were insufficient serum quantities to re-test the 1995 samples. The titers of anti-HCV in the samples of cases F and G were very low in 1993 and became negative after 2001. The titers of anti-HCV in cases A–C also appeared to decrease over time (Fig. 1). In the seven individuals with apparent

Table 2
Characteristics of anti-HCV antibody-positive Town C residents, separated by HCV core antigen and HCV RNA status in 1995[a]

Characteristics	HCV core antigen ≥8 pg/mL (n = 528)	HCV core antigen (–) and HCV-RNA (+) (n = 63)	HCV core antigen (–) and HCV RNA (–) (n = 245)	p value*
Gender, male female	209/319	26/37	90/155	0.69
Age	65.2 ± 10.4	66.5 ± 10.3	64.0 ± 10.9	0.19
Anti-HCV titer	9.0 ± 1.3[b,c]	6.6 ± 2.0[d]	5.0 ± 2.5	<0.001
HCV Serotype (I/II)[e]	303/115	30/22	NT[f]	0.03
AST	48.4 ± 41.2 (493)[c]	52.1 ± 35.1 (60)[d]	27.2 ± 149(231)	<0.001
ALT	42.9 ± 40.4 (493)[c]	44.8 ± 36.8 (60)[d]	25.0 ± 16.5 (231)	<0.001
γ-GTP	38.6 ± 51.5(493)[c]	41.4 ± 25.4 (60)	25.4 ± 293 (231)	<0.001

[a] Data shown as the means ± S.D. (number of individuals examined).
[b] $p < 0.001$ vs. HCV core antigen (–) and HCV RNA (+) group, by Scheffe's test.
[c] $p < 0.001$ vs. HCV core antigen (–) and HCV RNA (–) group, by Scheffe's test.
[d] $p < 0.01$ vs. HCV core antigen (–) and HCV RNA (–) group, by Scheffe's test..
[e] Excluding individuals whose HCV serotype was undetermined.
[f] Not tested.
* Based on one-factor ANOVA, χ^2 test, or Fisher's exact test, as appropriate for the comparison across the groups.

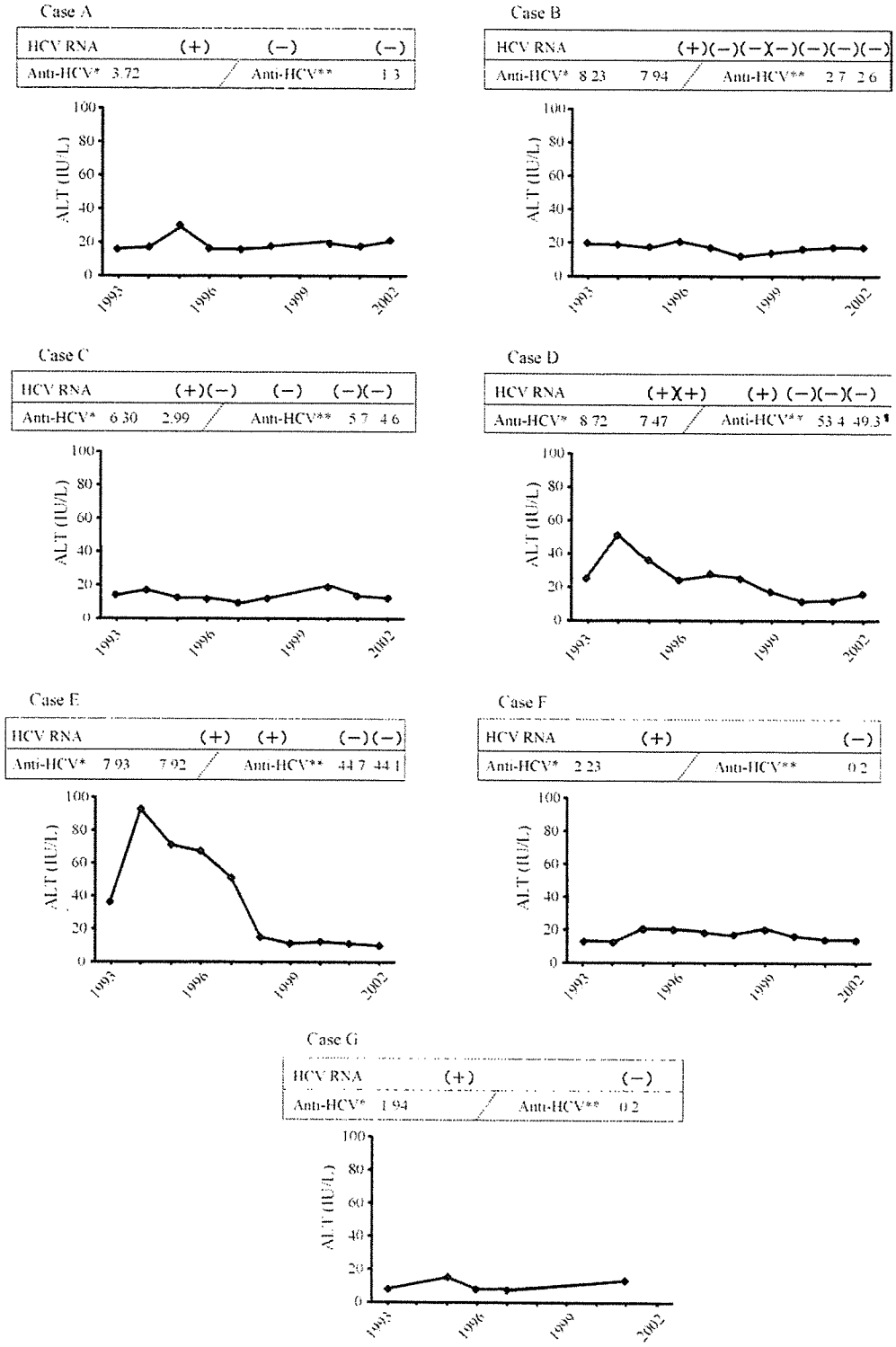

Fig. 1. Clinical course of individuals with spontaneous elimination of serum HCV RNA. Panels (A)–(G) represent the seven cases that were followed for 8 or 9 years. *Antibody against HCV (anti-HCV) were tested by a second generation enzyme immunoassay kit; titers higher than 1.0 were considered to be positive for anti-HCV antibody. **Since 2001, anti-HCV was evaluated by a third generation chemiluminescent enzyme immunoassay; samples with a signal/cut-off ratio of ≥1.0 were considered to be positive. ¶Data was from 2003.

Table 3
Characteristics of anti-HCV positive residents diagnosed with persistent HCV infection in 1995 whose serum HCV RNA were spontaneously eliminated between 1996 and 2002

Case	Age[a]/sex	HCV core antigen (pg/mL)	HCV serotype	ALT abnormality[b] (Group)	Platelets[c] ($\times 10^4/\mu L$)	Type IV collagen 7S[c] (ng/mL)
A	55/F	<8	ND[d]	N	25.5	4.7
B	60/F	33.4	I	N	25.0	3.9
C	64/F	<8	II	N	23.1	3.6
D	66/M	<8	II	A	25.3	3.9
E	68/M	28.2	II	A	17.1	3.3
F	73/M	<8	ND	N	20.6	4.5
G	76/M	<8	ND	N	20.7	3.8

[a] In 1995.
[b] Data were obtained between 1993 and 2000. N; persistently normal ALT levels, A; fluctuating or persistently abnormal ALT levels.
[c] Data were obtained in 2001, with the exception of case F for which data were obtained in 2002.
[d] Individuals whose HCV serotype was not determined.

Table 4
Comparison of demographic and virologic data of seven individuals whose serum HCV RNA were spontaneously eliminated to those of individuals remaining HCV RNA-positive

Characteristics	Elimination of HCV RNA ($n = 7$)	Remained positive for HCV RNA ($n = 282$)	p value*
Gender, male/female[a]	4/3	92/190	0.23
Age[a,b]	66.0 ± 7.2	65.3 ± 8.3	0.95
HCV core antigen (<20/>=20)[a]	5/2	33/249	<0.001
HCV Serotype (I/II)[a,c]	1/3	169/79	0.10
ALT[a,b]	28.9 ± 20.9	40.9 ± 38.7	0.16
ALT group (N/A)[d]	5/2	97/185	0.10

[a] Data were obtained in 1995.
[b] Data shown as the means ± S.D.
[c] Excluding individuals whose HCV serotype was undetermined or was not examined.
[d] N: persistently normal ALT levels, A: fluctuating or persistently abnormal ALT levels.
* Based on Mann–Whitney U test or Fisher's exact test as appropriate.

clearance of HCV RNA, five had HCVcAg levels below the assay's limit of detection; only HCV RNA could be detected by RT-PCR at initial testing in 1995 (Table 3). The incidence of low HCVcAg levels (below 20 pg/mL) [14] determined in 1995 was significantly higher in the individuals with spontaneous elimination of serum HCV RNA (5/7, 71.4%) than in those who remained HCV RNA-positive (33/282, 11.7%) ($P < 0.001$) (Table 4). Five of the seven individuals (71.4%) also had persistently normal ALT levels (Group N), while two had at least one abnormal ALT level (Group A) in the measurements taken between 1993 and 2000 (Fig. 1, Table 3). In comparison to the individuals who remained HCV RNA-positive, those spontaneously eliminating HCV RNA were more likely to be male (57 versus 33%; $p = 0.23$), be infected with HCV serotype II (75 versus 32%; $p = 0.10$), and exhibit persistently normal ALT levels (71 versus 39%; $p = 0.10$), although none of these trends were statistically significant (Table 4). In addition, platelet counts and type IV collagen 7S levels, which reflect the degree of liver fibrosis [15,16], were normal in all available measurements taken from these seven individuals in 2001 or 2002 (Table 3). The serum ALT levels were also normal during this period (Fig. 1).

4. Discussion

This study was performed in an area of Japan hyperendemic for HCV infection, where the prevalence of anti-HCV antibody positivity is 4- to 12-fold higher than that seen in the surrounding areas. The frequency of anti-HCV antibody positivity in this community is similar to that reported for other endemic areas of Japan [5]. In this population, as most residents displayed asymptomatic infections, with less than 5% reporting a history of acute hepatitis or jaundice (data not shown), the exact date of infection were typically unknown. Residents who tested positive for HCVcAg and/or HCV RNA more than 6 months after demonstrating anti-HCV seropositivity were considered to have a persistent HCV infection. The 70.5% prevalence of persistent infection in this population was similar to that previously reported [5,17]. The HCV serotype I was the most common seen in the study population, as reported for other Japanese populations [18,19], suggesting that Town C is similar to other HCV endemic areas of Japan.

In the seven individuals who eliminated HCV RNA, the results of five individuals (cases A–C, F and G) were positive for HCV RNA only once, two of whom (cases F and

G) also were negative for HCV RNA only once. These data do not necessarily exclude the possibility of false positive or false negative HCV RNA results; however, the titers of anti-HCV in all five individuals were lower when the individuals were HCV RNA negative than when they were HCV RNA positive. Although the anti-HCV assays used were different before and after 2001, data from the individuals in the cohort who remained positive for HCV RNA did not show a similar decrease in titer values between the two assays (data not shown). Thus, the results of the HCV RNA and anti-HCV antibody testing, albeit not definitive, would seem to support the spontaneous elimination of HCV RNA in these individuals.

We demonstrated that serum HCV RNA was spontaneously eliminated in 7 of 20 individuals who were HCV RNA-negative in 2001/2002. The remaining 13 individuals who were HCVcAg-positive in 1995 were found to be HCV RNA-negative upon re-testing of all the available stored samples taken between 1995 and 2000. Although these 13 individuals may have undergone spontaneous HCV elimination, we could not confirm the elimination of HCV RNA using RT-PCR as the gold standard. Although the reason underlying this inconsistency is unclear, HCV RNA may have degraded in those blood samples that were stored at −30 °C until testing. As a result, we have conservatively estimated the frequency of HCV elimination in persistent infection at 2.4% (7/282).

Although HCV RNA detection was not performed every year, the presence of HCV RNA was assessed more than 6 years after the initial testing for of HCVcAg or HCV RNA in 1995; thus, the elimination rate was estimated to be approximately 0.4%/year, which is similar to that reported by Watanabe et al. [5]. In contrast, some investigators have reported higher elimination rates than that seen in this study [6–9]. Hattori et al. [7] demonstrated that 14% of pregnant female patients with chronic HCV infections lost positivity for serum HCV RNA without treatment during the follow-up (duration of average follow-up; 5.8 years) of parturition. Fujisawa et al. [6] showed that 8.3% of children with chronic hepatitis C exhibit spontaneous clearance of serum HCV RNA during follow-up (duration of infection; 5.5 years). Fukuizumi et al. [8] estimated a natural disappearance rate of serum HCV RNA positivity at 2.8% per year. These differences may be related to differences in the immune systems of pregnant and non-pregnant women or children and adults. In addition, there may be differences in the initial serum HCV RNA levels, the rate of HCV mutation, the duration of infection, or the HLA allelic frequencies. Further investigations of the factors influencing spontaneous HCV clearance will be necessary to address these discrepancies, which are ongoing in this cohort study.

The spontaneous elimination of HCV RNA has been observed to occur more frequently in females and in individuals with persistently normal ALT levels [5,20]. These observations, however, included individuals who had recovered from acute HCV infection. Although these individuals were positive for anti-HCV antibodies, positive for HCV RNA were not shown before the infection was cleared. In this study, we did not observe a relationship between female gender and the spontaneous elimination of HCV in individuals with persistent infections, which correlated well with the results of Fukuizumi et al. [8]. These results indicate that there is no significant association between gender and spontaneous HCV elimination in individuals with persistent infections.

Yokosuka et al. reported that 6 (2%) of 310 patients with type C chronic liver disease became negative for HCV RNA detection over a 3-year period; all 6 patients exhibited liver cancer with chronic active hepatitis or liver cirrhosis [9]. Individuals became seronegative for HCV RNA in the terminal stages of liver cancer, prompting the hypothesis that a reduction in the amount of the virus occurs with the loss of a suitable environment in which the virus can replicate. In this study, none of the individuals displaying a spontaneous loss of HCV infection had any evidence of hepatocellular carcinoma or liver cirrhosis (data not shown). There were significant differences in the HCVcAg levels measured initially in 1995 between those who eliminated the virus and those supporting persisting infections. Thus, an initial low HCV viral load may be an important factor in the clearance of HCV infection [5–8].

Fukuizumi et al. reported that spontaneous elimination was associated with low ALT levels [8]. In our study, Town C residents exhibiting spontaneous elimination of HCV RNA also tended to have low ALT levels in 1995 and persistently normal ALT levels during 1995 and 2000. However, this tendency was not statistically significant and Fukuizumi et al. did not investigate the association between spontaneous elimination of HCV RNA and sequential ALT levels [8]. The exact mechanism that serum HCV RNA was spontaneously eliminated is unclear. However, HCV elimination in HCV carriers with normal or only mildly elevated ALT levels likely differs from that in individuals with acute infection, in whom cellular immune responses induce severe liver enzyme elevation and these acute responses are critical for the clearance of HCV. The role for HCV elimination in individual with persistent infection requires further investigation.

In summary, we have provided evidence for the spontaneous elimination of HCV RNA. This elimination occurred only rarely and was associated with low initial HCV viral loads, but was not associated with age, gender, or ALT levels. In addition, we could not observe an association between HCV viral load and ALT levels.

Acknowledgments

This work was supported in part by a grant (no. CA87982) from the United States National Institutes of Health and by a grant-in-aid (Research on Hepatitis and BSE) from the Ministry of Health, Labour, and Welfare, Japan. We thank Ms. Keiko Toyama and Ms. Yuriko Kuwabara for their technical assistance.

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