Host Immune Status and Incidence of Hepatocellular Carcinoma among Subjects Infected with Hepatitis C Virus: A Nested Case-Control Study in Japan

Robert Y. Suruki,[1] Nancy Mueller,[1] Katsuhiro Hayashi,[5] Donald Harn,[2] Victor DeGruttola,[3] Christina A. Raker,[1] Hirohito Tsubouchi,[5,6] and Sherri O. Stuver[1,4]

Departments of [1]Epidemiology, [2]Immunology and Infectious Diseases, and [3]Biostatistics, Harvard School of Public Health; [4]Department of Epidemiology, Boston University School of Public Health, Boston, Massachusetts; [5]Department of Internal Medicine II, University of Miyazaki Faculty of Medicine, Miyazaki, Japan; and [6]Digestive Disease and Life-style related Disease Health Research Human and Environmental Science, Kagoshima University Graduate School of Medical and Dental Science, Kagoshima, Japan

Abstract

A nested case-control study was conducted to examine the association between host immune status, as characterized by serum immune marker levels, and the development of hepatocellular carcinoma (HCC) up to 8 years later in persons with chronic hepatitis C virus (HCV) infection. Cases (n = 39) and matched controls (n = 117) were selected from participants of the Town C HCV Study in Japan between 1996 and 2004 and matched on age at first available sample ($\pm$1 year), gender, and length of follow-up. Separate analyses were done for each of three serum immune markers: soluble tumor necrosis factor-receptor II (sTNF-R2) and soluble intercellular adhesion molecule-1 (sICAM-1), as indicators of type 1, cell-mediated immune response, and soluble CD30 (sCD30), as an indicator of type 2, humoral immune response. The median concentrations of sTNF-R2, sICAM-1, and sCD30 among controls were 3,170 pg/mL, 305 ng/mL, and 3.0 units/mL, respectively, and were higher among cases (3,870 pg/mL, 372 ng/mL, and 3.3 units/mL, respectively). The risk of developing HCC among subjects with immune marker concentrations above the median levels of the controls was >2-fold greater than among subjects with lower concentrations for all three markers [sTNF-R2: odds ratio (OR), 6.9; 95% confidence interval (95% CI), 2.4-20.5; sICAM-1: OR, 2.0; 95% CI, 0.9-4.1; and sCD30: OR, 2.1; 95% CI, 1.0-4.7]. Simultaneous adjustment for all three markers revealed only sTNF-R2 to be associated with HCC risk (OR, 6.4; 95% CI, 2.0-20.6). Adjustment for alcohol consumption and HCV serotype did not materially alter these associations. Results from this prospective, community-based study suggest that a dysregulation in both type 1–related and type 2–related host immunity contributes to the development of HCV-associated HCC. (Cancer Epidemiol Biomarkers Prev 2006;15(12):2521–5)

Introduction

Hepatocellular carcinoma (HCC) is the predominant histologic subtype of primary liver cancer (1). Although relatively infrequent in developed countries, the incidence of HCC has increased in the United States and Japan over the past 20 to 30 years (1, 2). Such increases have been partially attributed to the emergence of the hepatitis C virus (HCV), an established risk factor for HCC (3, 4). Despite worldwide endemicity, the prevalence of HCV infection varies significantly by geographic region. For example, higher HCV infection prevalence rates have been reported in African and Asian countries, whereas prevalence rates among industrialized nations in North America, northern and western Europe, and Australia have generally been lower (5).

The great majority of HCV-infected individuals fail to eliminate the virus and progress onto chronic HCV infection (6-9). Explanations for this phenomenon include the presence of HCV quasispecies, the development of mutations in key areas of the viral genome, and the direct interference by the virus of the host immune response (10-12). A strong cell-mediated immune response is thought to lead to clearance of HCV, whereas an elevated humoral response or an only moderately increased cell-mediated response pattern has been reported in patients with chronic infection (7, 8, 13-16). The tension between the continued replication of the virus and a persistent attempt by a less than optimal immune response to eliminate HCV-infected cells within chronically infected persons is implicated in hepatocyte damage and, in some instances, progression to HCC. This continuous inflammation and hepatocyte regeneration in the setting of chronic hepatitis and progression to cirrhosis is thought to lead to an accumulation of chromosomal damage and possibly to initiate hepatic carcinogenesis (17).

The immune response to virus infection consists of two major components: the innate and adaptive response. The innate response is the first to respond to invading pathogens and involves natural killer cells, complement, cytokines, and apoptosis (18). Natural killer cells rely on antigen-independent mechanisms to inhibit viral replication (19, 20). In contrast, the adaptive response requires recognition of a specific viral epitope and is divided into two effector types: cell-mediated type 1 response and humoral type 2 response (18). The function of these two effector responses is tightly regulated within immunocompetent persons; however, dysregulation of type 1 and/or type 2 response can occur in cases of infectious, neoplastic, and inflammatory diseases (21).

The type 1/type 2 cytokine balance in sera, liver tissue, and culture supernatant of lymphocytes has been studied extensively in HCV-infected patients, but inconsistent results have failed to provide definitive information about the role of cytokines in HCV disease pathology (13-15, 22-29). Nevertheless, data from several studies suggest that a dysregulation of

Received 6/12/06; revised 9/13/06; accepted 10/9/06.

Grant support: NIH grant CA-87982. R.Y. Suruki was supported by NIH training grant T32 CA09001-28 and a Career Development Award from the Hepatitis Foundation International.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Robert Y. Suruki, GlaxoSmithKline, P. O. Box 13398, Five Moore Drive, Research Triangle Park, NC 27709. Phone: 919-483-7620; Fax: 919-315-8747. E-mail: suruki@post.harvard.edu

doi:10.1158/1055-9965.EPI-06-0485

the host immune status may be important in the progression of HCV-related liver disease (14, 22-25). A shift to a type 1 cytokine profile in patients with chronic hepatitis C is correlated with liver disease activity and progression (14). Similarly, an elevation of soluble CD30 (sCD30), a marker of type 2 response, has also been reported to be correlated with liver disease progression and severity in HCV-infected patients (25).

Tumor necrosis factor (TNF)-α is a mediator of innate inflammation and cellular immune response produced primarily by activated monocytes and Kupffer cells and plays a role in initiating fibrogenesis through binding to specific cellular receptors [TNF-receptors (TNF-R); ref. 26]. After cellular stimulation, extracellular domains of these receptors can be proteolytically cleaved, resulting in two soluble forms: soluble TNF-R1 (sTNF-R1) and sTNF-R2. High concentration of sTNF-R2 has been observed for prolonged periods in the circulation of patients with various inflammatory diseases, including HCV infection, making sTNF-R2 an ideal serum biomarker to characterize the type 1 immune response (27-30). Activation of the immune response in chronic hepatitis has also been shown by means of using circulating levels of intercellular adhesion molecule (ICAM)-1 (31, 32). Soluble ICAM-1 (sICAM-1) is an important adhesion molecule that is thought to be involved in liver inflammation. sCD30 is a member of the TNF/nerve growth receptor family and is preferentially expressed and secreted by human CD4 T cells producing type 2 cytokines (33, 34). Elevated levels of sCD30 have been detected in patients with conditions attributed to type 2 cytokine immunity, such as systemic lupus erythematosus and Omenn's syndrome, as well as in patients with HCV-associated liver disease (25, 35, 36).

We undertook the present study to elucidate the role of host immune status in the incidence of HCV-associated HCC in a prospective community-based cohort of HCV-infected persons in Japan. Given their extremely short half-life and the potential effects of freeze/thaw cycles (37), direct measurement of cytokines is not feasible in a community-based study using archived frozen serum samples. Serum proteins that are less labile and documented to be correlated with type 1 and type 2 response, particularly with respect to HCV infection, represent a more feasible alternative (28-32, 35, 36). For these reasons, we selected sTNF-R2 and sICAM-1 as markers of a type 1 cytokine milieu and sCD30 as a surrogate marker of a type 2 cytokine environment. Using prediagnostic serum levels of these serologic immune markers, we hypothesized that host immune dysregulation suggesting an up-regulated type 1 (cell mediated) and/or type 2 (humoral) response against HCV in a community-based setting increases HCC incidence. The propensity of HCV to cause clinically inapparent disease underlines the importance of assessing the informativeness of these biomarkers in identifying chronically infected subjects who may be predisposed to develop HCC.

Materials and Methods

Study Population. Data collected as part of the Town C HCV Study were used for the present analysis (38). Briefly, this community-based cohort study is being conducted within the adult population of a community (Town C) in southwestern Miyazaki Prefecture, Japan. Beginning in 1994, anti-HCV-positive residents identified at annual government-sponsored general health examinations in Town C were invited to participate in a liver disease screening program to monitor the development of HCC. In 2001, as a collaborative effort between Harvard School of Public Health (Boston, MA) and University of Miyazaki Faculty of Medicine (Miyazaki, Japan), a research component was incorporated into the ongoing liver disease screenings, which augmented these annual ultrasonography-based liver examinations with a self-administered questionnaire and the collection of a blood sample. Frozen aliquots of the blood samples are retained and stored at −80°C at the University of Miyazaki Faculty of Medicine, with duplicate aliquots shipped to Harvard School of Public Health where they are also stored at −80°C. In addition, between 1995 and 2000, serum samples collected from identified anti-HCV-positive Town C residents, in conjunction with the government-sponsored general health examinations, also have been frozen and stored at −20°C at the University of Miyazaki Faculty of Medicine. This population-based study was approved by the Human Subjects Committees of Harvard School of Public Health and University of Miyazaki Faculty of Medicine.

A total of 70 incident HCC cases has been identified among the study population of HCV-seropositive Town C residents (n = 1,311; mean age, 62 years) between 1994 and 2004. Of these, 39 cases occurred between the years 1996 and 2004 and had a prediagnostic serum sample obtained at least 1 year before the HCC diagnosis, for measuring the selected immune markers, and had evidence of chronic HCV infection, defined as having at least one HCV RNA or HCV core antigen-positive result between 1995 and 2004. For 32 of the 39 cases, the diagnosis was determined based on information collected via biopsy and/or imaging analysis using magnetic resonance imaging, computed tomography scan, angiography, or ultrasonographic tomography. An additional seven HCC cases were identified by means of death certificate information; for these cases, the year of death was used as the year of diagnosis.

We used incidence density sampling to select controls from the set of subjects at risk at the time of diagnosis of each HCC case (39). Subjects with evidence of chronic HCV infection, at least 1 year of follow-up, and an available sample were eligible for inclusion in the risk set (n = 676; mean age, 64 years). Three controls were randomly selected from the risk set for comparison with the index HCC case. A risk set was defined by the gender, age (±1 year) at first available sample, and length of follow-up (equal or greater than case) of the case. A total of 117 controls was matched to the 39 cases. Due to the matching criteria, the number of potential subjects within a risk set was relatively small; thus, the controls were made up of 99 unique individuals and included 15 controls that were selected more than once.

Laboratory Methods. Specimens were tested for HCV RNA using a reverse transcription-PCR assay (Amplicore HCV, Nippon Roche, Tokyo, Japan). Between 1995 and 2001, HCV core antigen level was measured by a fluorescent enzyme immunoassay (Immunocheck F-HCV Core Antigen, Kokusai Shiyaku, Kobe, Japan); starting in 2002, an immunoradiometric assay replaced the fluorescent enzyme immunoassay to measure HCV core antigen (Ortho HCV Ag IRMA Test, Ortho-Clinical Diagnostic, K.K., Tokyo, Japan). HCV serotype was determined by an enzyme immunoassay (Immunocheck F-HCV Grouping, International Reagents Co., Kobe, Japan). When the serologic group could not be clearly classified by this assay, HCV genotypes were determined by the reverse transcription-PCR method (40). Genotypes 1a and 1b were defined as serologic HCV group 1 and genotypes 2a and 2b as group 2. The above serum testing was completed by a commercial laboratory in Japan.

Serum immune marker testing of archived baseline specimens was completed by the General Clinical Research Center Core Laboratory at Massachusetts Institute of Technology (Boston, MA). The samples were sent in randomly ordered batches, and laboratory personnel were blinded to the case-control status of the specimens. The levels of sTNF-R2 and sICAM-1 were measured by means of ELISA (Quantikine and Paramter, respectively, R&D Systems, Minneapolis, MN); these assays have an interassay variability that ranges from 6% to

Table 1. Baseline characteristics of cases and matched control subjects, including median and interquartile range of serum immune marker levels, in the Town C HCV Study, Japan

Characteristics	Cases (n = 39)	Controls (n = 117)
Gender, n (%)		
Men	25 (64.1)	75 (64.1)
Women	14 (35.9)	42 (35.9)
Age (y), mean (SD)	65.3 (7.5)	65.2 (7.5)
Length of follow-up (y), mean (SD)	4.4 (2.1)	4.4 (2.0)
Alcohol consumption, n (%)*		
None	21 (55.3)	53 (51.5)
Occasional	4 (10.5)	18 (17.5)
Daily, low (≤60 g alcohol per day)	10 (26.3)	26 (25.2)
Daily, high (>60 g alcohol per day)	3 (7.9)	6 (5.8)
HCV serotype, n (%)†		
1	29 (76.3)	78 (66.7)
2	9 (23.7)	39 (33.3)
sTNF-R2 (pg/mL)		
Median	3,870.0	3,170.0
Interquartile range	3,350-4,820	2,670-3,970
Minimum, maximum	2,330, 6,740	1,740, 7,150
sICAM-1 (ng/mL)		
Median	372	305
Interquartile range	287-449	228-367
Minimum, maximum	55, 891	58, 699
sCD30 (units/mL)		
Median	3.3	3.0
Interquartile range	2.67-4.89	2.17-3.87
Minimum, maximum	0.5, 10.0	0.5, 18.8

*One case and 14 controls missing alcohol information.
† One case missing serotype information.

10% according to the manufacturer. Levels of sCD30 were also determined by means of an ELISA (ZyQuick sCD30 ELISA kit, Zymed Laboratories, Inc., San Francisco, CA), with an inter-assay variability ranging from 9.4% to 17.5%.

Statistical Analysis. Cases and controls were compared by medians for continuous variables and by contingency tables for qualitative data. The association between biomarker levels and the risk of HCC was analyzed using conditional logistic regression, which accounts for the matching within the risk sets. With risk set sampling, the odds ratio (OR) derived from the conditional logistic regression analysis directly estimates the hazard ratio (39, 41). Because serum immune marker levels were skewed and no cutoff levels for an elevated value have previously been determined, the serologic biomarkers were modeled as dichotomous variables using the median value among the controls. We also evaluated alcohol consumption (none, occasional, or daily) at baseline and HCV serotype (serotype 1 versus serotype 2) as potential confounders in multivariable regression models. Alcohol consumption was determined based on responses to a questionnaire administered by the public health nurses at the first liver disease screening program examination attended by the resident. The ''daily'' drinkers were further categorized into high (>60 g alcohol per day) and low (≤60 g alcohol per day) groups. In instances where data from the public health nurses' questionnaire were not available, ''never'' drinkers could be identified using the study-related questionnaire obtained beginning in 2001 and were thus included in the ''none'' category (n = 7). To evaluate the potential effect of reverse causation (i.e., preclinical HCC causing the elevation of serum immune markers), the analyses also were restricted to HCC cases diagnosed >2 years after their first available prediagnostic sample. All P values are two tailed, and P values of <0.05 were considered to indicate statistical significance. All analyses were done with the use of Statistical Analysis System software version 8.2 (SAS Institute, Cary, NC).

Results

The baseline (i.e., at first available serum sample) characteristics for the 39 cases and 117 matched controls are shown in Table 1. The mean age of the study participants was 65.2 years (SD, ±7.4) and 64.1% were men. Cases and controls were comparable with respect to age, gender, and length of follow-up by the matched design. There also was no difference in alcohol consumption between cases and controls; however, cases were more likely to be infected with HCV serotype 1 than were controls, although the difference was not statistically significant.

With respect to the type 1 immune markers, the median concentration of sTNF-R2 and sICAM-1 was statistically significantly higher among HCC cases than among controls (Table 1). The risk of developing HCC among subjects with type 1 immune marker levels above the median value of the controls was approximately 6- and 2-fold greater than among subjects with lower levels for sTNF-R2 and sICAM-1, respectively (Table 2). These observations suggest that HCC cases experience an elevated type 1 immune response before the development of HCC. Levels of sTNF-R2 and sICAM-1 were positively correlated with one another; the age- and gender-adjusted partial correlation coefficient among controls was 0.44 (P < 0.0001). Multivariable regression analysis revealed that adjusting for alcohol consumption and HCV serotype did not materially change these associations (data not shown).

About the type 2 serum immune marker among HCC cases, the median level of sCD30 was marginally significantly greater compared with controls (Table 1). An increased sCD30 level was also positively associated with a 2-fold greater HCC risk compared with subjects with levels below the median value of the controls in the conditional logistic regression analysis (Table 2). sCD30 levels were significantly correlated with the type 1 immune markers; the age- and gender-adjusted partial correlation coefficients among controls for sCD30 with sTNF-R2 and sICAM-1 were 0.64 (P < 0.0001) and 0.40 (P < 0.0001), respectively. Again, adjustment for alcohol consumption and HCV serotype did not notably alter the observed association for sCD30 (data not shown).

There were seven HCC cases that were diagnosed within 2 years of their first available prediagnostic sample. To evaluate the potential effect of reverse causation, we removed these seven cases from the analysis and found that the observed associations remained unchanged: $OR_{sTNF\text{-}R2}$, 6.0 [95% confidence interval (95% CI), 2.0-17.9]; $OR_{sICAM\text{-}1}$, 2.2 (95% CI, 1.0-5.0); and OR_{sCD30}, 2.3 (95% CI, 1.0-5.5).

Evaluation of the independent effect of each serum immune marker after adjusting for the other two markers showed that only sTNF-R2 was significantly associated with HCC

Table 2. OR estimates for the association of greater than median level of serum immune markers with HCC among HCV-infected subjects in the Town C HCV Study

	Cases/controls	OR* (95% CI)
sTNF-R2		
≤3,170 pg/mL†	6/60	1.0
>3,170 pg/mL	33/57	6.9 (2.4-20.5)
sICAM-1		
≤305 ng/mL†	13/59	1.0
>305 ng/mL	26/58	2.0 (0.9-4.1)
sCD30		
≤3.0 units/mL†	13/59	1.0
>3.0 units/mL	26/58	2.1 (1.0-4.7)

*Conditional logistic regression, matched on age, gender, and minimum follow-up.
† Median value of controls.

incidence. Subjects with an elevated sTNF-R2 level experienced a HCC risk that was ~6.5 times greater than that of subjects with a lower sTNF-R2 level following adjustment for all markers (OR, 6.4; 95% CI, 2.0-20.6), whereas an association with increased HCC risk was no longer observed for sICAM-1 and sCD30 (OR, 1.3; 95% CI, 0.6-3.1 and OR, 1.0; 95% CI, 0.4-2.6, respectively). These associations were not substantially different after multivariable adjustment for alcohol consumption and HCV serotype or when restricted to those HCC cases diagnosed >2 years after their first available prediagnostic sample (data not shown).

Discussion

In this prospective, community-based study, we found that an apparent up-regulated immune response, as characterized by the studied serum immune markers, was positively associated with an increased HCV-related HCC risk. Markers of both type 1 and type 2 immune responses were shown to be elevated in prediagnostic serum samples of HCC cases compared with HCV-infected controls. The increased levels of these serum immune markers may reflect an activated immune response that could predispose individuals to more severe liver disease, ultimately resulting in the development of HCC.

In its function as a receptor for the proinflammatory cytokine TNF-α, sTNF-R2 has been shown in many studies to be directly associated with HCV-related chronic hepatitis and histologic fibrosis (27, 28, 42). Itoh et al. (43) reported high correlations between sTNF-R2 and several liver disease markers (e.g., alanine aminotransferase, aspartate aminotransferase, and γ-glutamyl transpeptidase) as well as with Knodell's histologic activity index score in HCV chronically infected subjects. It has also been suggested that, at low concentrations, as observed during chronic HCV infection (13), TNF-α preferentially binds TNF-R2 over TNF-R1 (42). Furthermore, studies in murine models have shown that the binding of TNF-R2 initiates signals for the proliferation of thymocytes and cytotoxic T cells (44). Thus, the binding of TNF-R2 by TNF-α could contribute to a persistent low-level immune response that exacerbates ongoing liver injury.

ICAMs, which are readily expressed on the surface of hepatocytes, also seem to play a major role in HCV-associated chronic inflammation and persistent liver damage. Although sICAM-1 is secreted by various cell types, circulating levels have been suggested to parallel the level of liver inflammation (31, 45-47). For example, circulating levels of sICAM-1 have been reported to increase in patients who are progressing from chronic hepatitis to cirrhosis and HCC and are strongly correlated with indices of hepatic injury, including alanine aminotransferase (48, 49). In addition, Hamazaki et al. (50) observed a strong correlation between sICAM-1 serum level and tumor size in HCC patients. In the present prospective evaluation to examine the effects of prediagnostic levels of sICAM-1 on HCV-associated HCC risk, we found that subjects with elevated levels of prediagnostic sICAM-1 experienced a greater risk of HCC compared with individuals with lower levels. This finding is consistent with our hypothesis that an activated type 1 or cell-mediated immune response during chronic HCV infection increases the risk for developing HCC.

High levels of circulating sCD30 levels were also positively associated with increased HCC risk. Patients who fail to eliminate the virus and progress to chronic HCV infection have been found to have peripheral evidence of a strong type 2 immune response (25). The present findings agree with that of Gramenzi et al. (51) who recently reported that a predominant type 2 profile was associated with more severe liver disease. However, Gramenzi et al. also reported that a shift to a type 1 cytokine profile of peripheral blood mononuclear cells was associated with a more favorable clinical outcome, which is not consistent with the present findings. In fact, the current findings suggest that, in addition to an elevated sCD30 level, elevated type 1 immune markers may also contribute to a general dysregulation of the host immune status before HCC diagnosis, which ultimately predisposes the subject to increased immunopathogenesis of the liver.

It is of interest to note that simultaneous adjustment for all three immune markers revealed that only sTNF-R2 was significantly associated with increased incidence of HCC. That sTNF-R2 is independently associated with HCC after adjusting for sICAM-1 and sCD30 may reflect the significance of the immune response that is triggered by the binding of TNF-α to sTNF-R2 (44, 52). Because elevated levels of TNF-α are found in chronic HCV infection (26), it is possible that the cytotoxic T cells recruited by the TNF-α signaling system are more important in exacerbating ongoing liver injury. Given the correlation of sICAM-1 and sCD30 with sTNF-R2, as well as the stronger association of sTNF-R2 with HCC risk, it is not surprising that the association of sICAM-1 and sCD30 with HCC incidence became closer to the null with simultaneous adjustment for all three immune markers. Alternatively, the observed association between sTNF-R2 and HCC may be attributed to the bias resulting from imprecise measurement of correlated exposures (53). In the present study, sCD30 was measured with the greatest imprecision, whereas sTNF-R2 was measured with the smallest variability.

Unique to this study is the use of prediagnostic serum samples to measure the levels of sTNF-R2, sICAM-1, and sCD30. Therefore, the possibility that the tumor caused the elevation of circulating serum immune markers is unlikely. We also excluded HCC cases diagnosed within 2 years of the serum sample tested to minimize the possibility of reverse causation and found the associations with the immune markers to be unchanged. In addition, the community-based setting of the study provides a novel perspective in determining the natural history of HCV-induced HCC. The present findings show that prediagnostic serum levels of select immune biomarkers can be useful in predicting HCC incidence within a nonpatient population.

The present study has some limitations. Although information on alcohol consumption was obtained, the lack of quantitative data for all subjects may have resulted in residual confounding (i.e., 15 subjects were missing information on alcohol consumption). Nevertheless, because alcohol consumption is reportedly inversely associated with type 1 immune markers (54, 55), any residual confounding would be expected to result in an underestimation of the true relation between elevated type 1 markers and HCC incidence. In addition, although smoking was not adjusted for due to unavailable data, confounding by smoking was presumed to be minimal; Kuper et al. (56) found a significant dose-response, positive association between smoking and HCC risk only among HCV-negative subjects and concluded that smoking was less important as a risk factor for HCC among HCV-positive subjects. It is also important to consider that the immune marker data were obtained from the peripheral blood compartment, which may only partially reflect immune events occurring within the infected liver. However, Sobue et al. (14) reported a correlation in the helper T-cell type 1 and type 2 ratio between the peripheral blood and the liver and that the immune response of peripheral blood shifted toward a type 1 cytokine profile as liver damage progressed.

In summary, the observed association between elevated serum type 1 and type 2 immune markers and HCC risk supports the hypothesis that subjects with a dysregulated immune response experience greater hepatocyte damage, including hepatocarcinogenesis, as a result of HCV-induced immunopathogenesis. The association of sTNF-R2 and HCC risk after adjustment for sICAM-1 and sCD30 suggests a greater role for an activated type 1 response, although further

study is required. The present findings also show that prediagnostic serum levels of sTNF-R2, sICAM-1, and sCD30 can be useful in predicting HCC incidence within a community-based study population. This finding needs to be confirmed in other population studies.

Acknowledgments

We thank the public health nurses and staff of the Town C Health Department for their assistance in data collection and Yuriko Kuwabara, Bruce Molay, Keiko Toyama, and Jordan Mueller for expert assistance with data management.

References

1. El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 1999;340:745–50.
2. Shibuya K, Yano E. Regression analysis of trends in mortality from hepatocellular carcinoma in Japan, 1972-2001. Int J Epidemiol 2005;34:397–402.
3. Bruix J, Barrera JM, Calvet X, et al. Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. Lancet 1989;2:1004–6.
4. Colombo M, Kuo G, Choo QL, et al. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. Lancet 1989;2:1006–8.
5. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005;5:558–67.
6. Sarih M, Bouchrit N, Benslimane A. Different cytokine profiles of peripheral blood mononuclear cells from patients with persistent and self-limited hepatitis C virus infection. Immunol Lett 2000;74:117–20.
7. Cucchiarini M, Kammer AR, Grabscheid B, et al. Vigorous peripheral blood cytotoxic T cell response during the acute phase of hepatitis C virus infection. Cell Immunol 2000;203:111–23.
8. Gruner NH, Gerlach TJ, Jung MC, et al. Association of hepatitis C virus-specific $CD8^+$ T cells with viral clearance in acute hepatitis C. J Infect Dis 2000;181:1528–36.
9. Tsai SL, Liaw YF, Chen MH, Huang CY, Kuo GC. Detection of type 2-like T-helper cells in hepatitis C virus infection: implications for hepatitis C virus chronicity. Hepatology 1997;25:449–58.
10. Pawlotsky JM. Hepatitis C virus infection: virus/host interactions. J Viral Hepat 1998;5 Suppl 1:3–8.
11. Rosen HR. Hepatitis C pathogenesis: mechanisms of viral clearance and liver injury. Liver Transpl 2003;9:S35–43.
12. Urbani S, Boni C, Missale G, et al. Virus-specific $CD8^+$ lymphocytes share the same effector-memory phenotype but exhibit functional differences in acute hepatitis B and C. J Virol 2002;76:12423–34.
13. Huang YS, Hwang SJ, Chan CY, et al. Serum levels of cytokines in hepatitis C-related liver disease: a longitudinal study. Zhonghua Yi Xue Za Zhi (Taipei) 1999;62:327–33.
14. Sobue S, Nomura T, Ishikawa T, et al. Th1/Th2 cytokine profiles and their relationship to clinical features in patients with chronic hepatitis C virus infection. J Gastroenterol 2001;36:544–51.
15. Spanakis NE, Garinis GA, Alexopoulos EC, et al. Cytokine serum levels in patients with chronic HCV infection. J Clin Lab Anal 2002;16:40–6.
16. Tangkijvanich P, Vimolket T, Theamboonlers A, Kullavanijaya P, Suwangool P, Poovorawan Y. Serum interleukin-6 and interferon-γ levels in patients with hepatitis B-associated chronic liver disease. Asian Pac J Allergy Immunol 2000;18:109–14.
17. Ahn J, Flamm SL. Hepatocellular carcinoma. Dis Mon 2004;50:556–73.
18. Romagnani S. T-cell subsets (Th1 versus Th2). Ann Allergy Asthma Immunol 2000;85:9–18; quiz 18, 21.
19. Trinchieri G. Biology of natural killer cells. Adv Immunol 1989;47:187–376.
20. Whiteside TL, Herberman RB. Role of human natural killer cells in health and disease. Clin Diagn Lab Immunol 1994;1:125–33.
21. Lucey DR, Clerici M, Shearer GM. Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. Clin Microbiol Rev 1996;9:532–62.
22. Quiroga JA, Martin J, Navas S, Carreno V. Induction of interleukin-12 production in chronic hepatitis C virus infection correlates with the hepatocellular damage. J Infect Dis 1998;178:247–51.
23. Napoli J, Bishop GA, McGuinness PH, Painter DM, McCaughan GW. Progressive liver injury in chronic hepatitis C infection correlates with increased intrahepatic expression of Th1-associated cytokines. Hepatology 1996;24:759–65.
24. Malaguarnera M, Di Fazio I, Romeo MA, Restuccia S, Laurino A, Trovato BA. Elevation of interleukin 6 levels in patients with chronic hepatitis due to hepatitis C virus. J Gastroenterol 1997;32:211–5.
25. Foschi FG, Gramenzi A, Castelli E, et al. Soluble CD30 serum level in HCV-positive chronic active hepatitis: a surrogate marker of disease activity? Cytokine 2000;12:815–8.
26. Nelson DR, Lim HL, Marousis CG, et al. Activation of tumor necrosis factor-α system in chronic hepatitis C virus infection. Dig Dis Sci 1997;42:2487–94.
27. Zylberberg H, Rimaniol AC, Pol S, et al. Soluble tumor necrosis factor receptors in chronic hepatitis C: a correlation with histological fibrosis and activity. J Hepatol 1999;30:185–91.
28. Kallinowski B, Haseroth K, Marinos G, et al. Induction of tumour necrosis factor (TNF) receptor type p55 and p75 in patients with chronic hepatitis C virus (HCV) infection. Clin Exp Immunol 1998;111:269–77.
29. Aderka D, Wysenbeek A, Engelmann H, et al. Correlation between serum levels of soluble tumor necrosis factor receptor and disease activity in systemic lupus erythematosus. Arthritis Rheum 1993;36:1111–20.
30. Kaplanski G, Marin V, Maisonobe T, et al. Increased soluble p55 and p75 tumour necrosis factor-α receptors in patients with hepatitis C-associated mixed cryoglobulinaemia. Clin Exp Immunol 2002;127:123–30.
31. Horiike N, Onji M, Kumamoto I, Masumoto T. Soluble intercellular adhesion molecule-1 in serum in chronic hepatitis B and C. J Gastroenterol 1994;29:455–9.
32. Kaplanski G, Farnarier C, Payan MJ, Bongrand P, Durand JM. Increased levels of soluble adhesion molecules in the serum of patients with hepatitis C. Correlation with cytokine concentrations and liver inflammation and fibrosis. Dig Dis Sci 1997;42:2277–84.
33. Horie R, Watanabe T. CD30: expression and function in health and disease. Semin Immunol 1998;10:457–70.
34. Manetti R, Annunziato F, Biagiotti R, et al. CD30 expression by $CD8^+$ T cells producing type 2 helper cytokines. Evidence for large numbers of $CD8^+CD30^+$ T cell clones in human immunodeficiency virus infection. J Exp Med 1994;180:2407–11.
35. Caligaris-Cappio F, Bertero MT, Converso M, et al. Circulating levels of soluble CD30, a marker of cells producing Th2-type cytokines, are increased in patients with systemic lupus erythematosus and correlate with disease activity. Clin Exp Rheumatol 1995;13:339–43.
36. Del Prete G, Maggi E, Pizzolo G, Romagnani S. CD30, Th2 cytokines, and HIV infection: a complex and fascinating link. Immunol Today 1995;16:76–80.
37. Bienvenu JA, Monneret G, Gutowski MC, Fabien N. Cytokine assays in human sera and tissues. Toxicology 1998;129:55–61.
38. Hayashi K, Hasuike S, Kusumoto K, et al. Usefulness of a new immuno-radiometric assay to detect hepatitis C core antigen in a community-based population. J Viral Hepat 2005;12:106–10.
39. Rothman KJ, Greenland S. Modern epidemiology. Philadelphia (PA): Lippincott-Raven; 1998.
40. Ohno O, Mizokami M, Wu RR, et al. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J Clin Microbiol 1997;35:201–7.
41. Prentice RL, Breslow NE. Retrospective studies and failure times models. Biometrika 1978;65:153–8.
42. Tartaglia LA, Goeddel DV. Two TNF receptors. Immunol Today 1992;13:151–3.
43. Itoh Y, Okanoue T, Ohnishi N, et al. Serum levels of soluble tumor necrosis factor receptors and effects of interferon therapy in patients with chronic hepatitis C virus infection. Am J Gastroenterol 1999;94:1332–40.
44. Tartaglia LA, Weber RF, Figari IS, Reynolds C, Palladino MA Jr, Goeddel DV. The two different receptors for tumor necrosis factor mediate distinct cellular responses. Proc Natl Acad Sci U S A 1991;88:9292–6.
45. Zohrens G, Armbrust T, Pirzer U, Meyer zum Buschenfelde KH, Ramadori G. Intercellular adhesion molecule-1 concentration in sera of patients with acute and chronic liver disease: relationship to disease activity and cirrhosis. Hepatology 1993;18:798–802.
46. Pata C, Yazar A, Altintas E, et al. Serum levels of intercellular adhesion molecule-1 and nitric oxide in patients with chronic hepatitis related to hepatitis C virus: connection fibrosis. Hepatogastroenterology 2003;50:794–7.
47. Hyodo I, Jinno K, Tanimizu M, et al. Detection of circulating intercellular adhesion molecule-1 in hepatocellular carcinoma. Int J Cancer 1993;55:775–9.
48. Huang YS, Wu JC, Chan CY, Chao Y, Chang FY, Lee SD. Circulating intercellular adhesion molecule-1 in chronic liver disease and hepatocellular carcinoma. Zhonghua Yi Xue Za Zhi (Taipei) 1999;62:487–95.
49. Piazzolla G, Tortorella C, Fiore G, et al. Immunoresponsiveness in chronic hepatitis C patients: correlation between tissue and serum findings. Immunopharmacol Immunotoxicol 1998;20:337–54.
50. Hamazaki K, Gochi A, Shimamura H, et al. Serum levels of circulating intercellular adhesion molecule 1 in hepatocellular carcinoma. Hepatogastroenterology 1996;43:229–34.
51. Gramenzi A, Andreone P, Loggi E, et al. Cytokine profile of peripheral blood mononuclear cells from patients with different outcomes of hepatitis C virus infection. J Viral Hepat 2005;12:525–30.
52. Bertoletti A, D'Elios MM, Boni C, et al. Different cytokine profiles of intraphepatic T cells in chronic hepatitis B and hepatitis C virus infections. Gastroenterology 1997;112:193–9.
53. Phillips AN, Smith GD. Bias in relative odds estimation owing to imprecise measurement of correlated exposures. Stat Med 1992;11:953–61.
54. Waltenbaugh C, Vasquez K, Peterson JD. Alcohol consumption alters antigen-specific Th1 responses: mechanisms of deficit and repair. Alcohol Clin Exp Res 1998;22:220–3S.
55. Dolganiuc A, Kodys K, Kopasz A, Marshall C, Mandrekar P, Szabo G. Additive inhibition of dendritic cell allostimulatory capacity by alcohol and hepatitis C is not restored by DC maturation and involves abnormal IL-10 and IL-2 induction. Alcohol Clin Exp Res 2003;27:1023–31.
56. Kuper H, Mantzoros C, Lagiou P, et al. Estrogens, testosterone, and sex hormone binding globulin in relation to liver cancer in men. Oncology 2001;60:355–60.