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T Cell Recognition of Hypervariable Region-1 from Hepatitis C Virus Envelope Protein with Multiple Class II MHC Molecules in Mice and Humans: Preferential Help for Induction of Antibodies to the Hypervariable Region

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Hepatitis C virus (HCV) is responsible for a large portion of acute and chronic viral hepatitis, as well as cryp
tic cirrhosis and hepatocellular carcinoma (1–4). The envelope, especially the hypervariable domain (hypervariable region 1; HVR1) of approximately 28 amino acids in the E2 region, which is directly downstream from a putative signal peptide sequence in the junction between E1 and E2, is highly variable in sequence (5, 6). Several lines of evidence suggest that HVR1 is also an important determinant for B cells, as well as a neutralizing Ab epitope (7–10), as previously reported for the hypervariable V3 domain of HIV-1 gp160, although no practical widely available neutralizing Ab assay for HCV is available.

It has been demonstrated that a collaboration between helper T cells (Th) (CD4 class II-restricted help in vivo) and CTL or B cells results in activation of CTL or Ab production to mediate protection in vivo against certain virus infections (11–17). Even though helper epitopes anywhere on a protein could in principle provide help for Ab production to any epitope on the protein, there is evidence that helper epitopes proximal to Ab epitopes may have some special advantage, or disadvantage, depending on how the binding of the Ab affects processing of the Ag (18–25). This bias is believed to be due to the fact that the Ag-specific B cell takes up Ag via its surface Ig specific for that Ag and then internalizes the monoclonal immune complexes into endosomes, which are processed as complexes rather than free Ag as might occur in other APCs. Thus, the susceptibility of regions of the protein to proteolytic processing will be influenced by the steric hindrance of the bound Ab. In this way, B cells of different epitope specificity may present Ag to, and receive help from, helper T cells specific for different epitopes on the same protein molecule, in a process we called T-B reciprocity (18). Thus, it was of interest to determine whether helper T cells also recognized the HVR1 of HCV and whether that help had any preferential influence on the production of Abs to the putative neutralizing HVR1 site.

Four HVR1 peptides (HCV aa 385–416) were synthesized on the basis of the sequence from different isolates (a genotype 1b, a genotype 2a, and two genotype 1a isolates). However, it should be emphasized that the HVR1 sequence varies independently of geno
type, which is defined on the basis of more conserved sequences within the core and NS5 proteins (26, 27). To explore the range of class I and class II MHC molecules that could present these pep
tides, we attempted to generate helper T cells specific for HVR1 in two strains of B10 congenic mice, and BALB/c mice, representing three MHC types. Two distinct murine class II MHC molecules, as well as human class II molecules, presented this peptide
cross-reactively to CD4+ T cells, but class I presentation was not detected under the conditions used. The helper responses were more cross-reactive than expected for a hypervariable region, and these could be mapped to the more conserved ends of the region. Further, the presence of Abs to HVR1 was correlated with helper T cell responsiveness to this region in both mice and humans, whereas Abs to other parts of the envelope protein were not correlated. This result demonstrates the principle of T-B reciprocity in a human disease setting. Therefore, cross-reactive helper T cells against this region of HCV may be important in induction of potentially neutralizing Abs and will be important in the characterization of the natural immune response to HCV infection.

Materials and Methods

Peptide synthesis

Peptides were synthesized on an automated peptide synthesizer (Model 430A; Applied Biosystems, Foster City, CA) utilizing t-boc chemistry (28). The peptides were cleaved from the resin with HF and finally purified by size exclusion chromatography. Alternatively, peptides were synthesized using F-moc chemistry on a Rainin Symphony (Emeryville, CA) automated peptide synthesizer. Purification to single peaks was achieved by reverse-phase HPLC on μBondapack reverse-phase C18 columns (Waters Associates, Milford, MA).

Mice

Mice were purchased from Japan Charles River Laboratories (Tokyo, Japan) and Japan SLC (Shizuoka, Japan), or from The Jackson Laboratory, Bar Harbor, ME. Mice used were 8 wk old.

Immunizations with an adjuvant

Mice were immunized twice 3 wk apart in the footpads and i.p. with 30 nmol of peptide emulsified 1:1 in CFA (Difco Laboratories, Detroit, MI).

T cell proliferation assay in mice

For the assay of Ag-induced T cell proliferation, 11 days after the second immunization with peptide in adjuvant, immune spleen (or lymph node) cells were resuspended at 2 × 10^6 cells/well in 96-well flat-bottom culture plates containing each peptide at various concentrations in triplicate in complete T cell medium (1:1 mixture of RPMI 1640 and EHA A medium containing 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5 × 10^{-3} M 2-ME). After 78 h of incubation at 37°C in 5% CO2, [3H]thymidine (1 μCi) was added to each well. Eighteen hours later, the cells were harvested on an automated harvesting device (Skatron, Sterling, VA), and thymidine incorporation into DNA was determined by scintillation counting. The immune cells were stimulated in vitro with peptide at 10 μM after treatment with either anti-CD8 mAb (3.155; rat IgM) (29) plus complement or anti-CD4 mAb (RL-174; rat IgM) (30) plus complement, or complement alone, or stimulated in vitro with peptide at 10 μM in the presence of anti-I-E (anti-I-E44, 14-4-4 for BALB/c (31), or anti-I-E, AMS-16 (PharMingen, San Diego, CA) for B10 mice) or anti-I-A (anti-I-A4, MK-D6 (32) for BALB/c, or anti-I-Ae, AP6-120-1 (PharMingen) for B10 mice) at 25 μg/ml to block the proliferative activity, as described previously (33, 34).

Helper T cell lineage generation in BALB/c mice

For the induction of Ag-specific helper T cell lines, 11 days after the second immunization with peptide (SS3) in adjuvant, immune spleen cells (5 × 10^6/m/l) were stimulated in vitro in 24-well culture plates in complete T cell medium with irradiated (3000 rad) autologous naive spleen cells (2.5 × 10^6/m/l) pulsed with peptide (SS3) at 1 μM for 10 days. Then cells were washed twice with PBS and resuspended at 5 × 10^6/m/l in 24-well culture plates and reincubated in the presence of irradiated (3000 rad) autologous naive spleen cells (2.5 × 10^6/m/l) without peptide (resting) for 10 days. The line was used for mapping the epitope after at least seven rounds of stimulation/rest cycles.

HCV-seropositive patients

We tested 32 individuals, followed in Kagawa Medical School Medical Center (Kagawa, Japan), for HCV-specific serum Abs, detected by second-generation enzyme immunoassay tests (Abbott Laboratories, North Chicago, IL) specific for the putative core, NS3, and NS4 proteins (C22, C33, and C100-3 Abs) and for serum HCV RNA, detected by the double PCR method with two pairs of external and internal (nested) primers deduced from the 5’-noncoring region (35). Individuals coinfected with hepatotropic viruses other than HCV detected by serological testing were excluded from the study. The patients with hepatitis C who had elevated serum levels of alanine aminotransferase (ALT) for >1 yr were tested.

HCV genotypes were determined by using a PCR of the core genome region. Serum-derived HCV RNA was amplified with each type-specific primer in the second stage of PCR, as described previously (36). Genotypes I, II, III, and IV were comparable to genotypes 1a, 1b, 2a, and 2b, respectively, which were determined on the basis of NS5 sequence (27).

HVR1 sequencing

HCV HVR1 sequences were determined by PCR amplification of the E2 genome region. Serum-derived HCV RNA was amplified with specific primers and sequenced, as described previously (37).

Proliferation assay of human lymphocytes

For the assay of Ag-induced T cell proliferation, the PBL were separated on lymphocyte-separating medium (LeucoPREP, Becton Dickinson, Mountain View, CA), washed twice, counted, and resuspended in complete T cell medium. Cells (2 × 10^6) in complete T cell medium (200 μl) were added to wells of 96-well flat-bottom culture plates containing each peptide in triplicate. After 48 h of incubation at 37°C in 5% CO2, [3H]thymidine (1 μCi) was added to all the wells. Eighteen hours later, the cells were harvested on an automated harvesting device (Skatron), and thymidine incorporation into DNA was determined by scintillation counting. The stimulation index (SI) is the ratio of cpm incorporated in the presence of Ag to cpm incorporated with medium alone.

IL-2 production by human PBL

For the assay of Ag-induced IL-2 production by human PBL, PBL were resuspended at 4 × 10^6/ml in complete T cell medium. In triplicate wells of a 96-well flat-bottom plate (Costar, Cambridge, MA), 0.15 ml of PBL was added as well as peptide in culture and without stimulation or with each peptide at a final concentration of 1 μM for 24 h. The supernatant IL-2 activity was assessed as previously described (38). Immunofluorescence staining of PBL with FITC-labeled mouse mAbs to human CD4 or CD8 (anti-Leu-3a, anti-Leu-2a, Becton Dickinson) and CD4+ (or CD8+) cell fractions were sorted using an EPICS ELITE flow cytometer (Coulter Electronics, Hialeah, FL) by standard techniques. The isolated viable cells were washed and used for the assay described above.

ELISA of the human sera for Abs against the synthetic peptides of patient’s isolates within position 390–410 of HVR1 and Abs against recombinant E1E2 protein of HCV H strain

A hyperimmune rabbit serum against a synthetic peptide corresponding to HVR1 of HCV isolate H77 prevented infection with H77 virus, and the reactivity of the serum by ELISA was mapped to positions 390–410 (8). Therefore, we synthesized peptides corresponding to 390–410 of each patient’s HCV isolate to measure binding by ELISA. Wells of the ELISA plate (Falcon 3911, MicroTest III, Oxnard, CA) were coated with the synthetic peptides corresponding to positions 390–410 of the HVR1 of patient’s isolates (Fig. 1) (5 μg/well) or recombinant E1E2 protein of HCV H strain (59) (1 μg/well) in sodium carbonate buffer (15 mM NaHCO3, 35 mM Na2CO3, pH 9.6) at 4°C overnight, and unoccupied binding sites were saturated with PBS including 0.05% Tween 20, 5% low fat milk, 4% goat serum for 3 h at room temperature. After three washes with PBS containing 0.05% Tween 20, each well received 100 μl of test serum (200-fold dilution) diluted with PBS including 0.05% Tween 20 and 5% low fat milk, and the plate was incubated at 37°C for 1 h. The plate was washed three times with PBS including 0.05% Tween 20, and each well received 100 μl PBS containing 0.05% Tween 20 and 5% low fat milk supplemented with goat anti-human IgG labeled with horseradish peroxidase (1:1000). The plate was incubated at 37°C for 1 h and was washed three times, and then 100 μl 2.2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) was introduced to each well and left at room temperature for 30 min in the dark. Then 100 μl of 1% SDS was added to stop the reaction, and the absorbance at 405 nm was measured in an automated system.

ELISA of mouse serum for Abs against the synthetic peptides of HVR1 (position 385–410) of genotype II(1b) of HCV

To examine whether the helper responders to HVR1 synthetic peptides can help to produce the Abs against this region, we performed an ELISA to
detect the Abs directed against this region. Wells of ELISA plate (Falcon 3911, MicroTest III) were coated with SS3 (Fig. 1) (0.5 mg/well) in carbonate buffer (15 mM NaHCO₃, 35 mM Na₂CO₃, pH 9.6) at 4°C overnight, and unoccupied binding sites were saturated with PBS containing 0.05% Tween 20, 5% low fat milk, and 4% goat serum for 3 h at room temperature. After three washes with PBS containing 0.05% Tween 20, each well received 100 μl of test serum (50-, 500-, or 5000-fold dilution) diluted in PBS containing 0.05% Tween 20 and 5% low fat milk, and the plate was incubated at 37°C for 1 h. The plate was washed three times with PBS containing 0.05% Tween 20, and each well received 100 μl PBS including 0.05% Tween 20 and 5% low fat milk supplemented with goat anti-mouse IgG labeled with alkaline phosphatase (1:2000). The plate was incubated at 37°C for 1 h and was washed three times, and 50 μl of a Tris buffer and p-nitrophenyl phosphate (pNPP) (1:1) mixture was introduced to each well and left at room temperature for 30 min in the dark, after which 100 μl of 1% SDS was added to stop the reaction, and the absorbance at 405 nm was measured in an automated system.

Results

Four peptides, SS1, -2, -3, and -4 (HCV aa 385–416) covering HVR1, were synthesized on the basis of the sequence from isolates of three different genotypes (SS1 and -2, genotype 1a; SS3, genotype 1b; SS4, genotype 2a) (Fig. 1). To explore the range of MHC molecules that could present this peptide of HVR1, we attempted to generate helper T cells specific for HVR1 in two strains of B10 congenic mice, and BALB/c and C3H/HeJ mice, together representing 3 distinct MHC types, H-2d, H-2b, and H-2k, as well as in humans.

Mice were immunized with SS3 emulsified in CFA twice. The immune lymph node and splenic cells were stimulated in vitro with SS peptides for Th cell proliferation. When their immune spleen cells were restimulated in vitro with peptides, two of the three strains with distinct MHC, H-2d and H-2b, showed peptide specific proliferation (Fig. 2). We determined the phenotype and mapped the restriction element of T cells specific for SS3 in the context of class II MHC molecules. Treatment of the immune cells specific for SS3 with anti-CD4 mAb, but not anti-CD8 Ab, reduced or abrogated proliferative activity in the presence of the specific peptide, SS3 (not shown). Therefore, the specific T cells were conventional CD4⁺CD8⁻ Th cells. Also, since the proliferative activity was inhibited by anti-I-A d or anti-I-A b, not anti-I-E, the Th cell must be restricted by the I-A molecule (data not shown).

Titration of the peptide concentration used for proliferation of immune spleen cells demonstrated that proliferation specific for HVR1 was induced at concentrations of peptide greater than 0.1 μg/ml.
amino acid residues, respectively. The three variant peptides could be used to define the effect of naturally occurring virus variation on peptide presentation by, or affinity for, the H-2d and H-2b class II molecules and on recognition by the TCR. In the titration study, SS3 and -4 (genotype 1b and 2a, both Japanese isolates) could each stimulate T cells for comparable levels of proliferation between 0.1 and 10 μM in B10 mice (H-2b) (Fig. 3b) whereas SS4 was 10-fold less potent on a molar basis than SS3 for cross-reactive stimulation of peptide-specific proliferation in BALB/c mice (H-2b) (Fig. 3a). Thus, the sequence variations between SS3 and SS4 in the HVR1 did not seem to abrogate peptide interaction with MHC class II or recognition by the TCR in these two distinct mice. SS1 (United States isolate, genotype 1a) showed much less (10- to 100-fold) activity than SS3 to stimulate T cells in H-2d and some cross-reactivity, but less than SS4, in H-2b.

We could map the minimal epitopes in H-2b mice using long-term T cell lines raised against SS3 (Fig. 3c). In BALB/c mice, the dose-response curve for SS3–3 was comparable to that of whole SS3, whereas SS3–2 was about 10-fold less active than SS3 on a molar basis. Therefore, the minimal epitope is likely to be the segment shared by SS3–2 and SS3–3.

In the HCV-seropositive patients with chronic hepatitis, 7 of 32 patients mounted proliferative responses of PBL to either SS1, SS2, SS3, or SS4 peptide but did not recognize other types of peptide cross-reactively (Table I). To determine the phenotypes of human T cells specific for SS peptide, we used mAbs for inhibition. As shown in Figure 4a, the proliferative activity was inhibited by anti-class II HLA-DR Ab. Thus, the T cells must be restricted by a class II HLA-DR molecule (Fig. 4a), possibly DR4, which is shared in at least four patients (Table I), based on known sequence motifs (Fig. 1c).

IL-2 production was analyzed using PBL stimulated with SS peptides in HCV patients responsive to the SS peptides in the terms of proliferation. In patients 2, 3, 4, and 6, there was significant IL-2 production in response to peptide SS3 (patients 2, 3, and 6) or SS1 (patient 4) (Fig. 4b). Also, in patients 1 and 5, marginal IL-2 production in response to peptide SS2 and -4, respectively, was observed. To determine the cell types involved in expression of IL-2, we analyzed IL-2 production of CD4+ or CD8+ cell fractions, separated using mAbs to CD4 or CD8. Secretion of IL-2 was predominantly restricted to the CD8+ cell fraction containing CD8+ T cell subset in those patients (data not shown).

To see whether immune selective pressure by the T cell response is involved in the genetic variability of HVR1 and to understand the cross-reactivity, we further investigated the sequence variability of the HCV genome encoding HVR1 on a sample of responder and nonresponder patients and mapped the epitopes in two patients. Sequences 2, 4, 5, and 6 (Fig. 1b) were derived from SS peptide responder patients, respectively patients 2, 4, 5, and 6 in Table I. Sequences 8 (DR9, 15), 9 (DR13, 15), and 10 (DR4+) were derived from nonresponders. Frequent changes of amino acid residues occurred from positions 386 to 405 in patients 2, 5, and 6 (DR4+) and in patient 4 (DR1+), who generated anti-HVR1 T cell response, but the T cells were found to see a short peptide (SS3–7) covering the more conserved C-terminal sequence and DR4-binding motif (position 403–411) in patients 2 and 6, the only two with remaining cells to test (Fig. 5), using the overlapping panel of peptide fragments in Fig. 1a. Also, HLA-binding peptide motif analysis predicted that a sequence adjacent to the C terminus contains a DR1-binding motif that is conserved between SS1 and HVR of patient 4 (position 407–415; SS1 AKQNIQLIN vs patient 4 ASQNIIQPIN) (Fig. 1) and could be the T cell determinant. Thus, anti-HCV helper T cell response was found likely to be against relatively conserved C-terminal epitopes and probably
restricted by DR4 and DR1. The cross-reactivity between variants may be accounted for by the focusing of the response on the relatively more conserved C-terminal portion of the hypervariable region. Moreover, the greater sequence variations in the responder patients suggest the possibility that the anti-HVR1-specific T cell response may exert immunological pressure against HCV to drive the selection of specific adaptive amino acid substitutions.

Given the precedent in murine systems that MHC-linked Ir genes can influence Ab specificity (18, 40, 41) and that B cells expressing Abs specific for different antigenic determinants on the same Ag molecule may present that Ag differently to helper T cells (18–25), we asked whether this helper T cell response to the HVR1 region might influence the production of potentially neutralizing Abs to this region of the HCV envelope E2 protein, despite the probability that other helper epitopes existed elsewhere in the molecule. To address this question, we synthesized peptides with unique sequences of each patient’s own HCV isolate (Fig. 1b) covering the region of residues 390–410 of HVR1, the region that had been found to show binding of Abs by ELISA that correlated with neutralizing activity (8). These were used to coat plates in ELISA assays to test sera from the same patients and from five normal uninfected controls (Fig. 6a). At a 1:200 dilution, only the sera of patients 2, 4, 5, and 6, who showed T helper responses, had detectable Abs binding to their own isolate’s sequence 390–410 peptide significantly above the mean of the five individually tested normal control sera. In contrast, none of the sera from T cell nonresponder patients 8, 9, and 10 showed binding of their respective peptides above the level of control sera. Similar results were obtained at a 1:50 dilution of the sera, except that patient 9’s serum showed modest reactivity, but still substantially less than that of the responder patients (data not shown). Thus, the patients whose T cells did not react with HVR1 also lacked Abs to HVR1 (patients 8 and 10) or had titers much lower (patient 9) than those of the T cell responder patients. As a specificity control, we tested binding of the same patient sera to a recombinant E1E2 envelope protein of HCV H strain (Fig. 6b). All patients’ sera tested had reactivity with the recombinant envelope protein greater than the mean of the individual control sera of normal subjects (n = 5) shown at the right of Fig. 6b, including all three T cell nonresponder subjects. Indeed, there was no significant difference in levels of reactivity to E1E2 in sera of T cell nonresponder and responder subjects, despite the presence of more reactivity to their own isolate HVR1.
We conclude that helper T cell reactivity to HVR1 correlates with production of Abs to this neutralizing epitope, despite the presence of sufficient T cell help to make Abs to other parts of the same envelope protein in all the patients. This correlation cannot prove cause and effect but is highly suggestive.

To explore the possibility of a similar relationship in the mice, we conducted ELISA assays of murine sera for binding to SS3 peptide (Fig. 7). The level of Ab correlated with the level of T cell proliferative response in the three strains, in that H-2d mice (BALB/c) had the highest level of both T cell response (Fig. 2) and Ab response (Fig. 7), whereas H-2b mice (B10 or B6) had intermediate levels of both responses and H-2k (B10.BR or C3H/He) mice did not make either type of response (Fig. 2 vs Figure 7).

However, this correlation may be more expected in the mice than in the human subjects, since the mice were immunized with SS3 peptide, rather than by infection with whole HCV as in the case of the humans, so that no helper sites outside SS3 were available to make Abs to other parts of the same envelope protein in all the patients. This correlation cannot prove cause and effect but is highly suggestive.

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**Discussion**

Recently it has been found that variations in the dominant HVR 1 sequence of the putative envelope glycoprotein (gp70) of HCV occur sequentially in the chronic phase of hepatitis at intervals of several months (42). It also has been reported that kinetic analysis of Ab levels and specificity in chronic hepatitis patients, in whom successive alterations of HVR1 amino acids have been observed, showed a lag of several months between the appearance of a new dominant HCV sequence isolate and the achievement of maximal titers of anti-HVR Abs against that isolate. This finding suggests that mutations in HVR1 to escape from anti-HVR Abs are involved in the mechanism of persistent HCV infection, and minor antigenic variation in HVR1 is involved in escape from the immunosurveillance system or immunoselection. Comprehensive sequence analysis of HCV genomes revealed the existence of at least...
six different genotypes, each with several subtypes (43, 44). The genotypes show differences of at least 28% and the subtypes between 14% and 25% in the nucleotide sequences of their virus genomes. Different isolates belonging to the same genotypes showed a few percent difference in the nucleotide sequences and the amino acid sequences (26). In particular, the HVR shows remarkable sequence diversity (6, 45, 46).

HVR1 was found in the N-terminal region of gp70 of the HCV genotype 1b, the most prevalent isolate in Japan (more than 80%) (6), whereas a similar HVR was also found to be present in the same site of the HCV genotype 1a, the major genotype in the United States (45, 46). Hypervariability seems to be due to immune selective pressure, as seen in the third hypervariable region (V3 loop) of the envelope protein gp160 of HIV-1 containing a neutralizing epitope as well as CTL and helper T cell determinants. The structural similarities between HCV and *Pestivirus* also suggest the presence of a neutralizing site in HVR1 (47, 48). Very recently, HVR1 was found to contain two Ab epitopes, and an HCV with mutation in HVR1 could escape recognition by preexisting anti-HVR Abs (42). Moreover, Abs binding by ELISA to the region 390–410 of the HVR1 were found to correlate with neutralizing activity (8). To complement all the evidence that the HVR1 is a major neutralizing Ab epitope, we now present evidence that the HVR is a helper T cell recognition site and that helper T cells specific for this region may play a preferential role in eliciting Abs to the HVR1.

Despite the variability of HVR1, Weiner et al. observed cross-reactivities of Abs with two different sequences of HVR1 of HCV genotype 1a (46). In the present study, we detected similar T cell cross-reactivity between SS3 and SS4 in mice (Fig. 3), although there is still high diversity between their sequences (genotype 1b and 2a; Japanese isolates). Furthermore, the same cross-reactive T cells respond partially to SS1, genotype 1a sequence (United States isolate), in spite of the marked variations.

To explore whether HVR is a T cell determinant and is cross-reactively presented by distinct multiple MHC molecules, we examined the T cell immune response to HVR1 in mice and humans. Proliferative responses to SS1, SS3, and SS4 were obtained from H-2d and H-2b but not from H-2k mice. We conclude that H-2d and H-2b are the most efficient at helping Ab production to this viral protein. The correlation of Ab and T cell responses to the HVR1, however, suggests that T cells specific for the HVR1 region itself are the most efficient at helping Ab production to this region and are responsible for most of the HVR1-specific Abs produced. The similar findings in the murine experiments (Fig. 7), while more expected because the mice were immunized with just the HVR1 peptide SS3, not the whole envelope protein, nevertheless indicate that the T cell proliferative response observed corresponds to T cells that can help for Ab production to HVR1. These findings are consistent with the studies from several labs including our own (18–25) that indicate a phenomenon we called “T-B reciprocity” (18), in which Abs on the surface of B cells bind specific Ag and are taken up with the Ag by receptor-mediated endocytosis into compartments where processing and loading of MHC class II molecules take place. These Abs then influence the susceptibility of different parts of the Ag protein to proteolytic processing and thus determine which peptides are presented on class II MHC molecules and thus which helper T cells can help that B cell. The net result is that Ag-specific B cells preferentially present Ag to helper T cells specific for certain epitopes, and helper T cells of different specificity preferentially help B cells specific for some epitopes more than others on the same protein. In the case of the HVR1 of HCV, T-B reciprocity could result in a significant MHC-linked genetic (Ir gene) difference among patients in the production of potentially neutralizing Abs to HCV, and thus in the susceptibility to or course of disease. Thus, while the phenomenon of T-B reciprocity has been observed in model Ag systems in mice, our current results suggest for the first time that it may occur in a human disease setting where it could actually influence the outcome of infection.
In summary, HVR1 from the HCV putative envelope protein was found to be presented to helper T cells by two different class II MHC molecules in mice as well as by class II HLA DR molecules in patients. Although there is no detectable clinical difference between responders and nonresponders in this sampling, in those patients whose T cells recognize these peptides, the T cells may play a role in the pressure that the immune system exerts on the virus and in the variability that results. This pressure may come from the T cells, themselves, or from neutralizing Abs whose production depends on T cells specific for this region. The finding of apparent T-B reciprocity in a human disease setting in which specificity of helper T cells influences the production of Abs to a non-B cell antigen is of greater interest. It is suggested that this HVR may be an important target on which to focus for understanding the evolution of protective immunity and virus variation during infection with HCV.

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References

CORRECTIONS


The authors have found that recent ELISA assays with sera obtained from the same patients 2–4 weeks later than the original blood was drawn did not show the same pattern as in Figure 6A, originally carried out by Dr. Tatsumi Arichi, a coauthor, and therefore the information in that figure may or may not be valid. In particular, the new sera from patients 4, 5, and 6 no longer appeared positive.


In footnote 1, the grant support of Preet M. Chaudhary was lacking. The corrected footnote is shown below.

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