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Mutations in Immunodominant T Cell Epitopes Derived from the Nonstructural 3 Protein of Hepatitis C Virus Have the Potential for Generating Escape Variants That May Have Important Consequences for T Cell Recognition

Huiru Wang and David D. Eckels

One of the most disturbing features of hepatitis C virus (HCV) is its long-term persistence in the host. One hypothesis to explain this phenomenon is that HCV escapes immune recognition through its intrinsic hypermutability. To determine whether immunodominant T cell epitopes derived from HCV nonstructural 3 (NS3) protein might be subject to sequence variations leading to escape mutants, we examined sequence variations of one IL-2-producing epitope, NS3358–375, and one IL-10-producing epitope, NS3505–521. By PCR amplification, cloning, and sequencing, we observed significant sequence variations in the two epitopes, although the selection intensity for each epitope was different. For NS3358–375, more variants were observed, and for NS3505–521, fewer mutations were observed. Moreover, functional studies revealed that three NS3358–375 and one NS3505–521 variants failed to stimulate T cell proliferation, and two other NS3358–375 and NS3505–521 variants weakly stimulated T cell responses. Our results are consistent with immune selection of viral variants at the epitope level, which may enable HCV to evade host defenses over time. *The Journal of Immunology*, 1999, 162: 4177–4183.

Hepatitis C virus (HCV) was identified in 1989 as the major etiologic agent of non-A, non-B hepatitis. HCV infects not only hepatocytes, but also lymphocytes and monocytes of humans and chimpanzees. One of the most disturbing features of HCV is its long-term persistence in the host, followed by development of chronic liver disease and the associated possibilities of hepatocellular carcinoma, cryoglobulinemia, and autoimmunity. Extensive structural and functional studies on the HCV nonstructural 3 protein (NS3) have revealed that NS3 is a protein of ~70 kDa in size and possesses three known catalytic activities consisting of a serine protease in the first 180 amino acids followed by a nucleotide triphosphatase-dependent RNA helicase in the C-terminal region. Therefore, NS3 is critical to HCV infection and replication.

Immune responses to HCV are poorly understood. Despite the fact that strong Ab responses are observed in most patients, up to 90% of Ab-positive patients are persistently viremic. Studies on cellular immunity to HCV demonstrate that specific CD4 (helper) and CD8 (cytotoxic) T cells can be found in peripheral blood or liver from infected individuals. Why such T cells are ineffective at eliminating HCV in vivo is unknown. One hypothesis is that escape mutants arise due to hypermutability in the HCV RNA genome. A similar mechanism has been proposed to explain persistence of human immunodeficiency virus and hepatitis B virus.

We have seen that certain epitopes of HCV NS3, identified using overlapping synthetic peptides, caused secretion of IL-2, but not IL-10, and others that stimulated IL-10 release but not IL-2. We have also observed extensive sequence variation in the HCV NS3 region throughout the infective course of a chronic HCV patient, and most nonsynonymous mutations were found to be clustered within specific regions of NS3. All such variable regions occurred in epitopes recognized by host T cells. Because it is thought that escape variants may arise in response to immune selection, we wanted to determine whether T cell epitopes that stimulated release of different cytokines might be subject to different selective pressures and, if so, what the functional implications might be. In a patient infected over the course of 2 years, we examined sequence variations in one IL-2-producing epitope and one IL-10-producing epitope. Significant variations were observed in both epitopes, and functional studies revealed that synthetic peptides corresponding to these epitope variants either failed to stimulate or elicited weak T cell proliferation, suggesting that mutations in the HCV genome have the potential for generating escape variants with significant functional consequences for CD4 T cells.

Materials and Methods

Samples

Peripheral blood samples were collected from a patient (P.B3019) with chronic HCV at about 12 (P.B3019.1), 22 (PB.3019.2), 28 (PB.3019.3), and 34 (P.B3019.4) mo after infection. The presence of HCV-specific Abs in the patient’s serum was determined by second and third generations of passenger performance. The Blood Research Institute, The Blood Center, Milwaukee, WI 53201

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1 This work was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health Program Project Grant HL44612 and The Blood Center Research Foundation.

2 The nucleotide sequence data reported in this paper can be found in the DDBJ, EMBL, and GenBank nucleotide sequence databases with accession numbers AF035122 through AF035150.

3 Address correspondence and reprint requests to Dr. David D. Eckels, The Blood Research Institute, The Blood Center, P.O. Box 2178, Milwaukee, WI 53201-2178. E-mail address: ddeckels@bcsew.edu

4 Abbreviations used in this paper: HCV, hepatitis C virus; NS, nonstructural.

5 H. Wang, T. H. Bian, and D. D. Eckels. Sequence heterogeneity in the nonstructural 3(NS3) gene of the hepatitis C virus genome: evidence for positive and negative selection. Submitted for publication.
were pulsed overnight with \(^{3}H\)TdR.

Cultures were incubated at 37°C in 5% humidified CO\(_2\) for 6 days, pulsed tissue culture medium. Medium without Ag was used as a negative control.

To 96-well round-bottom plates, 100 \(\mu\)l aliquots of PBMC were resuspended at a concentration of 1 \(\times\) 10\(^6\)/ml in RPMI 1640 tissue culture medium containing 25 mM HEPES, 2.0 mM L-glutamine, 1.0 mM sodium-pyruvate, 10 U/ml sodium-heparin, 100 U/ml penicillin, 100 \(\mu\)M gentamicin sulfate, and 10% pooled human plasma. To Ags at varying dilutions also in 100 \(\mu\)l aliquots of PBMC from samples P.B3019.1, P.B3019.2, P.B3019.3, and P.B3019.4 at 1 \(\times\) 10\(^6\)/well were stimulated with synthetic peptides NS3\(_{358–375}\) and NS3\(_{505–521}\). After 6 days, cultures were pulsed overnight with \(^{3}H\)TdR. Radioactive label incorporation was measured (counts/4 min) and the results are represented as the mean ± SEM of triplicate cultures. Controls consisted of a normal individual P1115 and culture medium without Ag.

ELISA testing using diagnostic kits provided by Abbott Laboratories (Irving, TX) and used by The Blood Center in its routine screening of prospective blood donors. Viremia was also evaluated using a previously published set of nested primers that amplify a conserved segment of the 5′ noncoding region of the HCV RNA genome (22). Other than Ab and viremia, this patient has no other symptoms. Blood was collected in acid citrate dextrose anticoagulant, centrifuged at 400 \(\times\) g for 15 min, and divided into plasma and buffy coat fractions. After isolation of PBMC over Lymphocyte Separation Medium (Organon Teknika, Durham, NC), plasma and PBMC were stored at −70°C or in liquid nitrogen, respectively.

Cytokine analysis

PBMC from samples P.B3019.1, P.B3019.2, P.B3019.3, and P.B3019.4 were resuspended at a concentration of 1 \(\times\) 10\(^6\)/ml in RPMI 1640 tissue culture medium containing 25 mM HEPES, 2.0 mM L-glutamine, 1.0 mM sodium-pyruvate, 10 U/ml sodium-heparin, 100 U/ml penicillin, 100 \(\mu\)g/ml streptomycin, 5.0 \(\mu\)g/ml gentamicin sulfate, and 10% pooled human plasma. To 96-well round-bottom plates, 100 \(\mu\)l aliquots of PBMC were added to Ags at varying dilutions also in 100 \(\mu\)l 10% pooled human plasma tissue culture medium. Medium with Ag was used as a negative control. Cultures were incubated at 37°C in 5% humidified CO\(_2\) for 6 days, pulsed overnight with 1.0 \(\mu\)Ci/well \(^{3}H\)TdR, and harvested onto glass fiber filters. Radioactive label incorporation was measured by gas scintillation spectrometry by counting for 4 min; results are represented as the mean ± SEM of triplicate cultures.

Cytokine analysis

To measure secreted cytokines, PBMC from P.B3019.1 and P.B3019.3 were cultured in the presence of synthetic peptides as in the T cell proliferation assays described above. Results from P.B3019.2 have been published elsewhere (21). Supernatants were characterized in duplicate for secreted IFN-γ, IL-2, and IL-10 using a commercial Ag-capture human T cell proliferation assay (Caltag Laboratories, San Francisco, CA) and human IFN-γ and IL-10 ELISA sets from PharMingen (San Diego, CA). Experimental values were compared with a standard curve derived using recombinant cytokines. Negative controls consisted of background levels derived from cultures of PBMC in the presence of tissue culture medium alone.

RNA extraction and synthesis of cDNA

HCV genomic RNA was isolated from 1 ml each of P.B3019.1 and P.B3019.3 sera using RNAzol B (Tel-Test, Friendswood, TX). cDNA was synthesized using reverse transcriptase from Moloney’s murine leukemia virus (Life Technologies, Gaithersburg, MD) and random hexadeoxynucleotide primers (Pharmacia Biotech, Piscataway, NJ).

Amplification of HCV cDNA by PCR

Specific primers were designed to amplify the HCV NS3 region based on previously reported HCV sequence information. Primers NS3-3A (5′-CG GACCTTTACCTGGTCACG-3′) and NS3-2M (5′-CGCCCCCTCAAA AATCAAGATGG-3′) were used for the first PCR amplification in which HCV cDNA was subjected to 35 cycles of amplification in a buffer with 10 mM Tris, pH 8.3, 50 mM KCl, 2.5 mM MgCl\(_2\), 0.1 mg/ml gelatin, 0.02% NP40, and 5% DMSO. Each cycle consisted of 30 s at 94°C, 60 s at 55°C, and 60 s at 72°C with an initial denaturation (94°C, 5 min) and a final extension step (72°C, 7 min). Then, 2 μl of the products from the first PCR was used for the second PCR amplification with the same conditions using primers NS3-3C (5′-CAAGTTCCTTGCCGACGCCGG-3′) and NS3-2M. The amplified fragments were 790 bp in length and encompassed nucleotides 813-1602 of the NS3 region.

Cloning and sequencing

PCR products were cloned using a T-vector cloning kit (Invitrogen, San Diego, CA). Ligation and transformation were performed essentially according to the manufacturer’s instructions. Recombinant clones were screened and selected by specific PCR amplification of the inserted fragment. Plasmid DNA was prepared from the selected clones with a Wizard Plus miniprep DNA purification kit (Promega, Madison, WI) according to the standard protocol. Fifteen independent clones for each sample of P.B3019.1 and P.B3019.3 were sequenced in both directions by dye-termination cycle sequencing using an automated DNA sequencer (373A, Applied Biosystems, Foster City, CA). The sequencing results were analyzed using the ABI Gene Works software (IntelliGenetics, Mountain View, CA). Plasmid DNA from the Hutchinsion strain (1a) of HCV (23) was diluted to 10\(^{-14}\) g/ml then amplified, cloned, and sequenced as a control for polymerase errors. This concentration of plasmid DNA produced PCR amplification bands with intensities comparable to those obtained from viral cDNA under essentially identical conditions. The Taq error rate under such conditions was calculated as [no. of sporadic changes ÷ (no. of clones × sequence length × PCR cycles)] according to Smith et al. (24).
Peptide synthesis

Peptides with variant sequences of NS3358–375 and NS3505–521 were synthesized locally using Fmoc chemistry and purified by HPLC. Peptide powder was dissolved in a drop of DMSO and adjusted to ~1 mg/ml with RPMI 1640 tissue culture medium. Peptide was used at indicated concentration to stimulate PBMC in T cell proliferation or cytokine assays.

Results

T cell proliferative responses to HCV NS3 synthetic peptides

Our previous studies have reported that two synthetic peptides, NS3358–375 and NS3505–521, respectively corresponding to residues 358–375 and 505–521 of HCV strain 1a NS3, could be preferentially recognized by peripheral blood T cells from donor P.B3019 (21). To know if there were any differences of T cell recognition between early and later stage of infection, we analyzed T cell proliferative responses to NS3358–375 and NS3505–521 at about 12 (P.B3019.1), 22 (PB.3019.2), 28 (P.B3019.3), and 34 (P.B3019.4) mo after infection with HCV. Proliferative results are shown in Fig. 1. Peptides NS3358–375 stimulated strong dose-dependent proliferation of T cells from P.B3019.1 to P.B3019.4, while the response to peptide NS3505–521 was definite but comparably weak over the period from which these results were obtained. In contrast, these peptides did not stimulate proliferation of cells from the normal individual P1115.

Cytokine responses to HCV NS3 synthetic peptides

To determine whether any differences in cytokine secretion existed in the early or later stages of infection, we analyzed IFN-γ, IL-2, and IL-10 secreted in response to the same peptides above, with B3019.1 and B3019.3 by cytokine-specific ELISA. The results are shown in Fig. 2. Consistent with our previous results (21), NS3358–375 could stimulate strong IL-2 and IFN-γ, but not significant IL-10. In contrast, NS3505–521 could stimulate neither significant IL-2 nor IFN-γ, while it could stimulate IL-10 secretion by PBMC from P.B3019.3.

Mutation rate in two HCV NS3 epitopes

To determine whether mutations were accumulating in HCV sequences in response to immune selection and to understand their impact on immune recognition, cloning and sequencing analysis of PCR products from specific epitope regions of NS3 were performed using cDNA templates synthesized from RNA isolates of P.B3019.1 and P.B3019.3.

Of 30 sequenced clones, 11 (37%) had nucleotide mutations in NS3358–375 producing 9 amino acid changes, and 5 (17%) had mutations in NS3505–521 causing 4 residue substitutions (Table I).

It was interesting that the general mutation rate for epitope NS3358–375, a strong IL-2-stimulating epitope, was twice as high as that of epitope NS3505–521, an IL-10-stimulating epitope, suggesting the possibility that different NS3 epitopes might be under different levels of immune selective pressure.

The amino acid changes in the regions encoding NS3358–375 and NS3505–521 as well as their flanking regions are shown in Fig. 3. More mutations were observed in epitope NS3358–375 throughout the whole infective course. In the early bleed P.B3019.1, there were both synonymous and nonsynonymous mutations and the ratio of synonymous/nonsynonymous was 4/6, whereas in the later bleed P.B3019.3, all mutations were nonsynonymous (5/5). In contrast, for the IL-10-inducing epitope NS3505–521, fewer mutations were observed and the majority occurred in the earlier isolate (Fig. 3 and Table I). Thus, for the IL-2-producing epitope NS3358–375, immune selection would seem to be stronger and persists longer than that for the IL-10-producing epitope NS3505–521, for which selective pressure may weaken with time.

Furthermore, six of eight nonsynonymous mutations were non-conservative changes, establishing the possibility that immune recognition of these epitopes could be affected.

To distinguish actual viral mutations from possible sporadic nucleotide substitutions caused by Taq misincorporation errors, we calculated the error rate using a known plasmid template (0.01 pg) from which amplified bands were comparable to those obtained from viral cDNA. Even with 70 cycles of amplification, we calculated a polymerase error rate of 2.16 × 10⁻⁵, which was well below that of epitope NS3505–521, an IL-10-stimulating epitope, for which the error rate was 3.53 × 10⁻⁴.

Table I. Mutation rate of two epitopes among 30 clones

<table>
<thead>
<tr>
<th></th>
<th>NS3358–375</th>
<th>NS3505–521</th>
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</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>Variant</td>
<td>Wild-type</td>
</tr>
<tr>
<td>B3019.1</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>B3019.3</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>MR (%)</td>
<td>37</td>
<td>17</td>
</tr>
</tbody>
</table>

MR, mutation rate.
within the usual \textit{Taq} error range of $0.2 - 2 \times 10^{-4}$ reported by others \cite{25, 26}. Thus, we discount the possibility of underestimating the number of artifacts due to saturation reached after only a few PCR cycles. The expected numbers of sporadic amino acid substitutions were calculated and compared with the actually observed nonsynonymous mutations in both peptide regions and flanking regions as shown in Table II and Fig. 3. In both cases, those regions encoding epitopes NS3\textsubscript{3358–375} and NS3\textsubscript{505–521} contained significantly increased numbers of nonsynonymous mutations.

![Flanking region of NS3\textsubscript{3358–375}](image)

![Flanking region of NS3\textsubscript{505–521}](image)

**FIGURE 3.** Observed changes in regions encoding NS3\textsubscript{3358–375} (a) and NS3\textsubscript{505–521} (b) and their flanking sequences in comparison to wild type (Hutchinson strain, 1a) sequence. cDNA templates synthesized from RNA isolates of P.B3019.1 and P.B3019.3 were amplified by PCR. The amplified fragments encompassed epitopes NS3\textsubscript{3358–375} and NS3\textsubscript{505–521}. The PCR products were cloned, and 15 independent clones (30 in total) from each sample of P.B3019.1 and P.B3019.3 were sequenced in both directions.
Effect of variant peptides on T cell proliferation

According to the variant sequences shown in Fig. 3, peptides were synthesized and used to stimulate T cells in proliferation analysis. Of the seven NS3358–375 variants tested, three, H369R, S370P, and K371E, showed dramatically decreased T cell proliferation, and the K373R variant caused a threefold shift in the dose-response curve, but the level of response was comparable to wild-type, NS3358–375, at high peptide concentrations (Fig. 4a). Of the three NS3505–521 variants tested, L513P stimulated no T cell proliferation, while the response to V511A was weak (Fig. 4b). Taken together, these results indicate that residues 369, 370, 371, and 513 play critical roles in either MHC binding or contact with the T cell antigenic receptor.

Discussion

The failure of the immune system to eradicate HCV-infected cells is an intriguing problem. One explanation is that HCV, like HIV and hepatitis B virus, seems to be hypermutable, and escape mutants probably emerge under relative intense immune selection (27). It has been suggested that both HIV (20) and hepatitis B virus (28) may mutate in the course of infection and immune selection to form antagonist peptide epitopes that, when recognized by CD8 T cells, cause anergy rather than cytotoxicity, which results in a failure to eliminate virus-infected target cells. If such naturally occurring antagonist and escape mutants can be observed in peptide epitopes recognized by CD8 T cells, then it also seems plausible that a rapidly mutating virus such as HCV might be able to present functionally similar epitopes to CD4 as well as CD8 T cells.

In our present study, we have examined mutations in a region of the NS3 Ag that contains two functionally distinct epitopes, NS3358–375 and NS3505–521. We have shown that NS3358–375 preferentially stimulates IL-2 and IFN-γ, whereas NS3505–521 stimulates IL-10 production. Coincident with this functional difference, the localized mutation rate in these two NS3 epitopes is very different. The region corresponding to NS3358–375 underwent a consistently higher frequency of changes in both the early and later isolates and most of the mutations produced amino acid changes. In contrast, nonsynonymous changes in the nucleotides encoding NS3505–521 were frequent in the early isolate, but fewer were seen in the clones from the later sample. This observation is consistent with the possibility that NS3358–375 and NS3505–521 are under different intensities of selection pressure as exerted by CD4 T cells. We cannot at this time rule out an effect by CD8 T cells. We raise the question as to whether it is merely coincidental that selection intensity, as measured by mutational frequencies, correlates with the functional nature of the epitope, which presumes that stimulation of IL-2 and IFN-γ is more conducive to viral elimination (29).

It is important to distinguish viral mutations arising from immune selection from those that may be artifactual (24, 30, 31). We observed in two regions, one encompassing the functionally important DExH box and Switch region and another covering the RNA unwinding region of the HCV helicase, that the observed nonsynonymous mutation frequency was lower than that expected from sporadic amino acid substitutions, supporting a negative selection model in these functionally critical

Table II. Nonsynonymous mutations in different regions of HCV NS3

| Region (amino acid) | Length of Sequences (bp) | PCR Cycles | Clone No. | Expected Sporadic Mutations | Observed Nonsynonymous Mutations | p Value
<table>
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<tbody>
<tr>
<td>NS3381–380</td>
<td>150</td>
<td>70</td>
<td>30</td>
<td>4.5</td>
<td>3.0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>NS3331–380</td>
<td>150</td>
<td>70</td>
<td>30</td>
<td>4.5</td>
<td>22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NS3346–485</td>
<td>90</td>
<td>70</td>
<td>30</td>
<td>2.7</td>
<td>2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>NS3486–515</td>
<td>90</td>
<td>70</td>
<td>30</td>
<td>2.7</td>
<td>12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NS3358–375</td>
<td>54</td>
<td>70</td>
<td>30</td>
<td>1.6</td>
<td>9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NS3505–521</td>
<td>50</td>
<td>70</td>
<td>30</td>
<td>1.5</td>
<td>4</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

* The expected number of sporadic nonsynonymous mutations is calculated as the error rate of Taq (2.16 × 10⁻⁵) × length of compared region × number of PCR cycles × number of clones sequenced × proportion of sporadics expected to produce amino acid substitutions (24). The agreement between hypothesis and observation was tested by χ² goodness-of-fit.

![FIGURE 4.](https://www.jimmunol.org/)

PBMC from samples P.B3019.3 were used in proliferative assays with synthetic variant peptides of NS3358–375 (a) and NS3505–521 (b). Negative controls consisted of background levels derived from cultures of PBMC in the presence of tissue culture medium alone.
regions. In contrast, for the two variable regions containing NS3\(^{358-375}\) and NS3\(^{505-521}\) which are located neither near nor in the known functionally important portions of HCV helicase, the observed nonsynonymous mutation rate was significantly higher than expected, supporting a positive or over-dominant selection model for mutations in these epitopes (Table II and Fig. 3). Taken together, our observations support the hypothesis that nonsynonymous mutations in the regions encoding peptides NS3\(^{358-375}\) and NS3\(^{505-521}\) represent natural changes to the HCV RNA genome rather than artificial PCR errors.

We were able to examine whether nonsynonymous mutations in the NS3\(^{358-375}\) and NS3\(^{505-521}\) epitopes affect CD4 T cell recognition. Clearly, a large majority of the amino acids can be considered loss-of-recognition mutations as seen in our studies with the synthetic peptide variants. Loss of recognition could occur through decreased binding to MHC molecules or through disruption of critical TCR contact sites. A third possibility includes a shift in cytokine production such that the ability to stimulate IL-2 was lost while maintaining the ability to stimulate production of other cytokines, which is currently under investigation. Obviously, none of these is mutually exclusive. Furthermore, we would predict that after having elucidated which of the preceding mechanisms is operative, we should see emergence of viral isolates that would produce similar functional effects as we continue with longitudinal studies in this patient. While we did see an attenuation in the response to NS3\(^{505-521}\) over time, perhaps reflective of a developing anergic state, we observed a more intense response to wild-type NS3\(^{358-375}\) in PBMC derived later in the infection. The fact that we see such a vigorous recall response to NS3\(^{358-375}\) wild-type sequence probably reflects intense selection on this epitope, and thus we would expect to see that escape mutants should continue to emerge with time.

In addition to loss of recognition, mechanisms by which the T cell anti-viral response can be suppressed would include production of inhibitory cytokines. As an example, IL-10 is known to down-regulate essential costimulatory molecules such as CD80 on the surfaces of APCs with the result that transmembrane signaling via CD28 is impaired, which in turn leads to a failure to stabilize the IL-2 message induced by TCR ligation (32). Thus, viral mutation of the NS3\(^{505-521}\) epitope would not be driven as intensively due to stimulation of IL-10 by this and other epitopes and perhaps would abate with time. Inconsistent with this hypothesis is the fact that IL-2 production in response to NS3\(^{358-375}\) is virtually shut when the epitope is presented in the context of the intact NS3 Ag (33). Until we know whether the cytokine dysregulation that we observe in vitro differs from that which occurs in vivo, we cannot distinguish between these two alternatives.

The production of IL-10 is not a unique feature of HCV. EBV, for example, encodes an IL-10 homologue (34). Small RNA viruses like HCV lack the genomic capacity to carry their own immunomodulatory cytokines, but perhaps they have found ways to exploit this niche within the host environment by tolerating high mutational loads that allow IL-10-stimulating epitopes to evolve through extended host-viral interactions. In this regard, it is interesting to note that NS3\(^{358-375}\) and NS3\(^{505-521}\) are not localized within any known functional region of the NS3 helicase and thus may represent “expendable” regions of the Ag that can be allowed to mutate into forms that elicit cytokines conducive to virus survival. The fact that the NS3\(^{358-375}\) region is relatively conserved among diverse HCV and hepatitis G virus species, yet differs within a given patient, may imply that the virus has evolved a means by which to ensure this capacity.

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References


