

Immunodominance of HLA-A2-Restricted Hepatitis C Virus-Specific CD8⁺ T Cell Responses Is Linked to Naïve-Precursor Frequency

Julia Schmidt, Christoph Neumann-Haefelin, Tayibe Altay, Emma Gostick, David A. Price, Volker Lohmann, Hubert E. Blum and Robert Thimme
J. Virol. 2011, 85(10):5232. DOI: 10.1128/JVI.00093-11.
Published Ahead of Print 2 March 2011.

Updated information and services can be found at:
<http://jvi.asm.org/content/85/10/5232>

	<i>These include:</i>
REFERENCES	This article cites 25 articles, 12 of which can be accessed free at: http://jvi.asm.org/content/85/10/5232#ref-list-1
CONTENT ALERTS	Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»

Information about commercial reprint orders: <http://journals.asm.org/site/misc/reprints.xhtml>
To subscribe to to another ASM Journal go to: <http://journals.asm.org/site/subscriptions/>

Immunodominance of HLA-A2-Restricted Hepatitis C Virus-Specific CD8⁺ T Cell Responses Is Linked to Naïve-Precursor Frequency[∇]

Julia Schmidt,^{1,2} Christoph Neumann-Haefelin,¹ Tayibe Altay,¹ Emma Gostick,³ David A. Price,³
Volker Lohmann,⁴ Hubert E. Blum,¹ and Robert Thimme^{1*}

Department of Medicine II, University of Freiburg, Freiburg, Germany¹; Faculty of Biology, University of Freiburg, Freiburg, Germany²; Department of Infection, Immunity and Biochemistry, Cardiff University School of Medicine, United Kingdom³; and Department of Molecular Virology, University of Heidelberg, Germany⁴

Received 14 January 2011/Accepted 21 February 2011

The impact of naïve-precursor frequency on human virus-specific CD8⁺ T cell immunodominance is not well understood. Using a recently developed major histocompatibility complex (MHC) class I tetramer enrichment protocol, we found a conserved hierarchy and a >10-fold difference in naïve-precursor frequencies across three HLA-A2-restricted hepatitis C virus (HCV)-specific epitopes. Importantly, the NS3₁₄₀₆ epitope with the highest naïve-precursor frequency in healthy donors was also the most frequently targeted epitope in a large cohort of chronically HCV-infected patients, both *ex vivo* and after *in vitro* stimulation. These results indicate for the first time that immunodominance in a human viral infection is linked to naïve-precursor frequency.

CD8⁺ T cells play an important role in the outcome of human viral infections, including hepatitis C virus (HCV) infection (23). Although viral pathogens encode thousands of potentially immunogenic determinants, CD8⁺ T cells recognize and respond to only a very small fraction of these potential viral epitopes. This phenomenon is called immunodominance. Factors that contribute to CD8⁺ T cell immunodominance include antigen processing and presentation, abundance of peptide-major histocompatibility complex class I (pMHC) molecules on antigen-presenting cells, and the number of naïve T cells that express complementary T cell receptors (25). The study of naïve antigen-specific CD8⁺ T cell frequency as a determinant of immunodominance has only recently become possible through the use of improved pMHC tetramer enrichment protocols. Importantly, these studies, performed in different mouse models, have largely suggested that CD8⁺ T cell response magnitude is dictated by naïve-precursor frequency (8, 16, 18). One recent study, however, performed in a mouse model of influenza virus infection, suggested that T cell immunodominance is determined instead by recruitment from the available pool of epitope-specific precursors and the duration of their continued expansion over the course of viral infection (10). Thus far, the impact of naïve-precursor frequency on T cell immunodominance has not been analyzed in human viral infections. Recently, however, the group of Matthew Albert reported a new strategy based on the combination of tetramer staining, magnetic-bead enrichment, and multiparametric flow cytometry (1). This experimental approach allowed for the first time the detection and analysis of naïve human CD8⁺ T cells specific for different epitopes (1). The authors reported a >100-fold differences in naïve-precursor frequencies against epitopes derived from MART1 (Melan-A), HIV, HCV, NY-

ESO-1, and cytomegalovirus (CMV) that were conserved among individuals. Here, using the same novel approach, we set out to determine whether immunodominance hierarchies within HLA-A2-restricted HCV-specific CD8⁺ T cell responses are linked to naïve-precursor frequency.

Precursor frequency of naïve HLA-A2-restricted hepatitis C virus-specific CD8⁺ T cells. In a first set of experiments, we analyzed the naïve-precursor frequencies of CD8⁺ T cells specific for three well-described HLA-A2-restricted HCV epitopes (4, 13, 20) (Table 1) in seven healthy donors. The analysis was limited to three epitopes only, because a major limitation of the novel tetramer enrichment protocol for the analysis of naïve CD8⁺ T cells is the requirement for high volumes of blood. We did not include the frequently recognized HLA-A2-restricted NS3₁₀₇₃ epitope in our analysis, since CD8⁺ T cell responses to this epitope may be influenced by past exposure to influenza virus (22, 24). However, the cross-reactivity between these viral epitopes is weak and mostly unidirectional, e.g., CD8⁺ T cells primed with the HCV epitope can be activated by the influenza epitope but not *vice versa*, questioning the relevance of heterologous immunity against this epitope (7). Importantly, by using the previously described combination of tetramer staining, magnetic-bead enrichment, and multiparametric flow cytometry (1), we were able to detect naïve HCV-specific CD8⁺ T cells in all seven healthy donors (Fig. 1). Although the cells were not detectable before the enrichment step, they could be clearly identified after enrichment, as shown for one representative subject in Fig. 1A. The cells displayed a naïve phenotype characterized by high expression of CD45RA, CD27, and CCR7 and low expression of CD11a (Fig. 1B). Of note, the median calculated precursor frequency of NS3₁₄₀₆ epitope-specific CD8⁺ T cells was 1.47×10^{-5} , and thus >10-fold higher than the calculated precursor frequencies of CD8⁺ T cells specific for NS5B₂₅₉₄ (5.12×10^{-7}) and the Core₁₃₂ epitope (5.46×10^{-7}) (Fig. 1C). Interestingly, the frequently recognized HLA-A2-restricted NS3₁₀₇₃ epitope displayed similar precursor frequencies (5.06×10^{-6}) compared to NS3₁₄₀₆ (data not shown). Since CD8⁺ T cell

* Corresponding author. Mailing address: University Hospital Freiburg, Department of Medicine II, Hugstetter Str. 55, 79106 Freiburg, Germany. Phone: 49-761-270-3280. Fax: 49-761-270-3725. E-mail: robert.thimme@uniklinik-freiburg.de.

[∇] Published ahead of print on 2 March 2011.

TABLE 1. HLA-A2-restricted epitopes

Epitope ^a	Peptide length	Sequence	IC ₅₀ (nM) ^b
Core ₁₃₂	9	DLMGYIPLV	14.5
NS3 ₁₄₀₆	10	KLVALGINAV	52.8
NS5B ₂₅₉₄	9	ALYDVVTKL	11.8

^a The subscript numerals indicate the amino acid positions.

^b IC₅₀, 50% inhibitory concentration, determined using the Immune Epitope Database (IEDB [http://www.iedb.org]).

responses to this epitope were suggested to be influenced by past exposure to influenza virus by some studies, as discussed above, we did not analyze this epitope in our further experiments. Although precursor frequencies varied significantly between epitopes, frequencies for each of the specific epitopes were conserved across the healthy donors tested. These results suggest that a clear hierarchy of naïve HCV-specific CD8⁺ T cell precursor frequencies exists in healthy donors.

Immunodominance of HLA-A2-restricted hepatitis C virus-specific CD8⁺ T cell responses during chronic infection. Next, we set out to determine whether the clear hierarchy of naïve-precursor frequencies for these three epitopes might be linked to their relative immunodominance in chronically HCV-infected patients. For this purpose, we performed *ex vivo* tetramer analyses in a prospectively and randomly recruited cohort of patients with chronic HCV genotype 1 infection (*n* = 26). As shown in Fig. 2A and B, we observed clear patterns of immunodominance, with most responses targeting the NS3₁₄₀₆ epitope (8/26 patients), followed by the NS5B₂₅₉₄ epitope (2/26 patients) and finally the Core₁₃₂ epitope (0/18 patients). Importantly, the same immunodominance profile was also detectable after 2 weeks of antigen-specific stimulation. Accordingly, as shown in Fig. 2C and D, most expanded responses targeted the NS3₁₄₀₆ epitope (8/20 patients), while substantially fewer responses targeted the NS5B₂₅₉₄ (4/20 patients) and Core₁₃₂ (1/19 patients) epitopes. Although NS3₁₄₀₆ epitope-specific CD8⁺ T cell responses were not detectable in all patients, these results indicate a clear immunodominance hierarchy with respect to these three epitopes. Notably, these results are in agreement with previous studies of HLA-A2⁺ patients with chronic HCV infection, in which NS3₁₄₀₆ has been shown to be recognized more frequently than NS5B₂₅₉₄. For example, Spangenberg et al. (21), Lechner et al. (13), and Racanelli et al. (19) found NS3₁₄₀₆ epitope-specific responses in 8/27, 5/19, and 5/20 chronically HCV-infected HLA-A2⁺ patients, respectively; in contrast, NS5B₂₅₉₄ epitope-specific responses were observed in 7/27, 0/10, and 3/20 patients, respectively. It is also notable that the Core₁₃₂ epitope was rarely targeted in most studies (5, 11–13, 21).

Expansion capability and antiviral efficacy of naïve HCV-specific CD8⁺ T cells is linked to precursor frequency. Since the immunodominance of the NS3₁₄₀₆ epitope in chronically HCV-infected patients was present not only *ex vivo* but also after antigen-specific expansion *in vitro*, we next asked whether naïve CD8⁺ T cells would also be able to proliferate after antigen-specific stimulation and whether expansion capability could be linked to naïve-precursor frequency. For these experiments, we cocultured enriched CD8⁺ T cells derived from healthy donors for 12 days with mature autologous dendritic

cells (DCs) pulsed separately with each of the three HLA-A2-restricted HCV peptides. As shown in Fig. 3A (data are for one representative patient), the number of NS3₁₄₀₆ epitope-specific CD8⁺ T cells expanded *in vitro*. Peptide-specific stimulation and expansion led to downregulation of CD45RA and CCR7 and to upregulation of CD11a, indicating that these

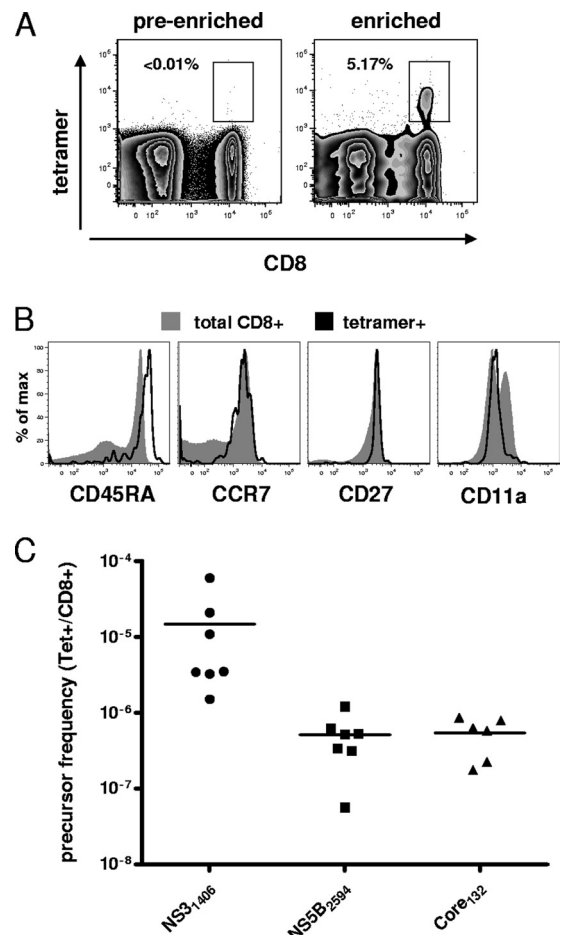


FIG. 1. Enrichment and enumeration of naïve HCV-specific CD8⁺ T cells from healthy donors (*n* = 7). (A) Representative flow cytometry plots of peripheral blood mononuclear cells (PBMCs) from a healthy donor showing staining with the NS3₁₄₀₆ HLA-A*0201 tetramer before and after magnetic-bead enrichment. Procedural details are similar to those described by Alanio et al. (1). In brief, PBMCs were incubated with FcR blocking reagent and then stained with pMHC tetramer labeled with allophycocyanin (APC). Subsequently, labeled cells were incubated with anti-APC microbeads and passed over a magnetically activated column. The percentage of tetramer-positive CD8⁺ T cells is indicated before and after enrichment. (B) Representative phenotypic analysis of enriched tetramer-positive CD8⁺ T cells. Enriched tetramer-positive CD8⁺ T cells (shown in black) were stained for CD3, CD45RA, CCR7, CD27, and CD11a; a naïve phenotype (CD45RA^{hi} CCR7^{hi} CD27^{hi} CD11a^{low}) was apparent in all cases. For reference, bulk CD8⁺ T cells are shown in gray. (C) Precursor frequencies of CD8⁺ T cells specific for NS3₁₄₀₆, NS5B₂₅₉₄ and Core₁₃₂ detected in healthy donors after tetramer staining, magnetic-bead enrichment, and multiparametric flow cytometry analysis (*n* = 7; one donor was not tested for Core₁₃₂). The total number of phenotypically naïve tetramer-positive CD8⁺ T cells (Tet+) relative to the total number of CD8⁺ T cells is indicated. A calculation similar to that of Alanio et al. (1) was used.

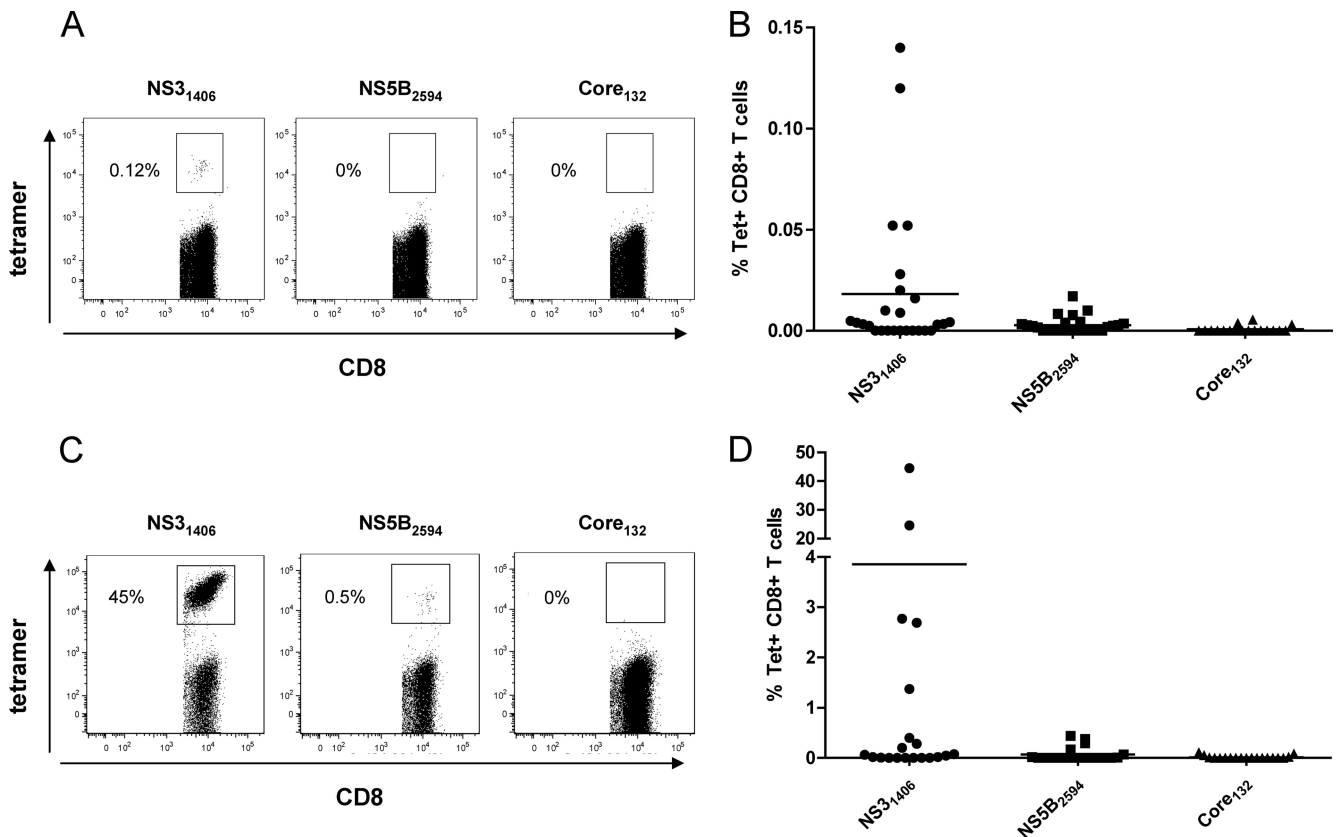


FIG. 2. Immunodominance patterns of HLA-A2-restricted HCV-specific CD8⁺ T cell responses in patients with chronic HCV genotype 1 infection ($n = 26$). (A) Representative *ex vivo* staining of PBMCs from a chronically HCV-infected patient with NS3₁₄₀₆, NS5B₂₅₉₄, and Core₁₃₂ HLA-A*0201 tetramers. (B) *Ex vivo* frequencies of CD8⁺ T cells specific for the NS3₁₄₀₆, NS5B₂₅₉₄, and Core₁₃₂ epitopes defined by staining with the corresponding HLA-A*0201 tetramers; responses were identified in 8/26, 2/26, and 0/18 patients, respectively (cutoff, 0.01% tetramer-positive CD8⁺ cells). (C) Representative staining with NS3₁₄₀₆, NS5B₂₅₉₄, and Core₁₃₂ HLA-A*0201 tetramers of PBMCs from the patient shown in panel A after antigen-specific stimulation for 2 weeks as described previously (17). (D) Frequencies of CD8⁺ T cells specific for the NS3₁₄₀₆, NS5B₂₅₉₄, and Core₁₃₂ epitopes defined by staining with the corresponding HLA-A*0201 tetramers after 2 weeks of antigen stimulation; responses were identified in 8/20, 4/20, and 1/19 patients, respectively (cutoff, 0.1% tetramer-positive CD8⁺ cells).

cells lost their naïve phenotype and acquired an effector-memory phenotype (Fig. 3B). Figure 3C summarizes the results derived from seven healthy donors. Of note, we were able to expand the pool of naïve NS3₁₄₀₆ epitope-specific CD8⁺ T cells in 5/7 cases. In contrast, expansion of NS5B₂₅₉₄ and Core₁₃₂ epitope-specific CD8⁺ T cells was barely detectable. Nevertheless, using higher numbers of enriched CD8⁺ T cells in the assay, we were able to expand the population of NS5B₂₅₉₄-specific CD8⁺ T cells. This indicates that NS5B₂₅₉₄-specific CD8⁺ T cells exist in naïve individuals and confirms the biological relevance of low precursor frequencies detected *ex vivo* (data not shown). To further confirm the functionality and specificity of these expanded HCV-specific CD8⁺ T cell populations, we also analyzed their antiviral efficacy. For these experiments, HuH7 cells that were transduced with a JFH1-based selectable subgenomic luciferase replicon and with the HLA-A*0201 gene (HuH7_{A2}HCV cells) were pulsed separately with each of the three HCV epitopes. Subsequently, these cells were cocultured for 48 h with HCV-specific CD8⁺ T cells previously expanded from the blood of healthy donors. Importantly, as shown in Fig. 3D, only the expanded NS3₁₄₀₆ epitope-specific CD8⁺ T cells were able to significantly inhibit

HCV replication, as measured by luciferase activity. This effect was lost when the NS3₁₄₀₆ epitope-specific CD8⁺ T cells were depleted from the expanded cell pool, clearly indicating that these cells mediated the observed antiviral efficacy.

In summary, the results of this study show for the first time that CD8⁺ T cell immunodominance hierarchies observed in a chronic human viral infection such as HCV infection can be linked to naïve-precursor frequency. Indeed, the NS3₁₄₀₆ epitope, for which the highest naïve CD8⁺ T cell precursor frequencies were consistently identified, was also the most frequently targeted of the three epitopes in chronically HCV-infected patients. Although a causal link cannot be proven in the human setting, it is interesting that these results are in agreement with a series of elegant mouse studies that support the important role of naïve T cell frequency as a determinant of T cell immunodominance (8, 16, 18). However, other factors, such as antigen abundance, processing efficiency, and HLA binding affinity, can also influence immunodominance. In this context, it is noteworthy that the three epitopes analyzed here display comparable HLA-A2 binding affinities (Table 1). Another factor that might influence immunodominance in viral infections is the degree of epitope sequence conservation.

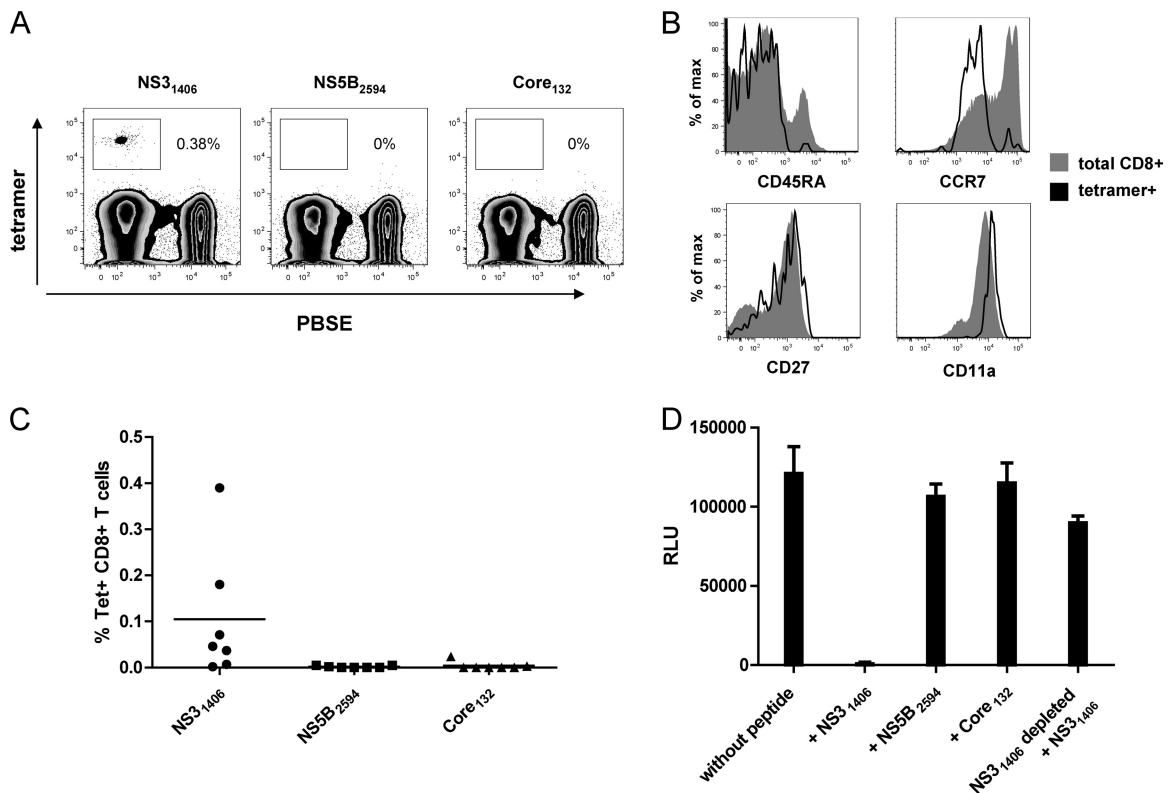


FIG. 3. Proliferation and maturation of HCV-specific CD8⁺ T cells from healthy donors after stimulation with peptide-pulsed DCs (*n* = 7). (A) Representative tetramer staining of CD8⁺ T cells cocultured for 12 days with NS3₁₄₀₆, NS5B₂₅₉₄, and Core₁₃₂ peptide-pulsed DCs. For the maturation of monocyte-derived-DCs (MD-DCs), CD14-enriched PBMCs were cultured for 6 days with rhGM-CSF (recombinant human granulocyte-macrophage colony-stimulating factor) and rhIL-4 (recombinant human interleukin 4) and for 1 day with rhTNF- α (recombinant human tumor necrosis factor alpha) in addition. Mature MD-DCs were pulsed with individual peptides and then cocultured with CD8-enriched PBMCs in the presence of anti-CD28. rhIL2 was added on day 4 and day 8. Proliferation of CD8⁺ T cells upon peptide stimulation was measured by Pacific Blue succinimidyl ester (PBSE) dilution. The percentage of tetramer-positive PBSE^{low} CD8⁺ cells is indicated. (B) Representative phenotypic analysis of tetramer-positive CD8⁺ T cells stimulated in culture. Tetramer-positive CD8⁺ T cells (shown in black) were stained for CD45RA, CCR7, CD27, and CD11a; an effector-memory phenotype (CD45RA^{low} CCR7^{low} CD27^{hi} CD11a^{hi}) was apparent in all cases. For reference, bulk CD8⁺ T cells are shown in gray. (C) Frequencies of tetramer-positive CD8⁺ T cells after 12 days of expansion with NS3₁₄₀₆, NS5B₂₅₉₄, and Core₁₃₂ peptide-pulsed DCs. The percentage of tetramer-positive CD8⁺ cells is indicated. (D) Antiviral efficacy of expanded HCV-specific CD8⁺ T cells. HuH7_{A2}HCV cells were pulsed with NS3₁₄₀₆, NS5B₂₅₉₄, or Core₁₃₂ peptides and cocultured for 48 h with CD8⁺ T cells that had previously been expanded for 12 days with NS3₁₄₀₆, NS5B₂₅₉₄, or Core₁₃₂ peptide-pulsed DCs. In an additional experiment, NS3₁₄₀₆ epitope-specific CD8⁺ T cells were depleted from the expanded culture by tetramer staining and magnetic-bead separation. Inhibition of viral replication was measured by luciferase activity.

Interestingly, the epitope with the highest naïve CD8⁺ T cell precursor frequency also displays the highest degree of sequence diversity in HCV genotype 1-infected patients (<http://hcv.lanl.gov> [9]), providing one possible explanation for why this epitope is not targeted in all chronically HCV-infected patients. On the other hand, viral escape mutations within the epitope could also contribute to the maintenance of the antiviral functions of HCV-specific CD8⁺ T cells, which otherwise can undergo exhaustion and lose their antiviral functionality (3, 6). Of note, naïve-precursor frequency also seems to be associated with the expansion capability of virus-specific CD8⁺ T cells. Indeed, NS3₁₄₀₆ epitope-specific CD8⁺ T cell responses were dominant in cultures from healthy donors and chronically HCV-infected patients after *in vitro* stimulation. Thus, HLA-A2-restricted HCV-specific CD8⁺ T cell immunodominance appears to be a predetermined feature of the naïve CD8⁺ T cell repertoire that is relatively independent of virological factors. These findings may have implications for

vaccine development and adoptive T cell therapy, both of which require the generation of functionally active virus-specific CD8⁺ T cells from the naïve pool of antigen-specific precursors (2, 14–15).

We sincerely thank all the study subjects. Recombinant human IL-2 was kindly supplied by the NIH AIDS Research and Reference Reagent Program, Germantown, MD.

This work was supported by the Deutsche Forschungsgemeinschaft (R.T., Heisenberg-Professorship TH-719/3-1; R.T./C.N.-H./V.L., FOR1202 “Mechanisms of persistence of hepatotropic viruses”). D.A.P. is a Medical Research Council (United Kingdom) Senior Clinical Fellow.

The authors declare no financial or commercial conflict of interest.

REFERENCES

1. Alanio, C., F. Lemaitre, H. K. Law, M. Hasan, and M. L. Albert. 2010. Enumeration of human antigen-specific naïve CD8⁺ T cells reveals conserved precursor frequencies. *Blood* **115**:3718–3725.
2. Barnes, E., et al. 2008. Monocyte derived dendritic cells retain their func-

- tional capacity in patients following infection with hepatitis C virus. *J. Viral Hepat.* **15**:219–228.
3. **Bensch, B., et al.** 2010. Coexpression of PD-1, 2B4, CD160 and KLRG1 on exhausted HCV-specific CD8+ T cells is linked to antigen recognition and T cell differentiation. *PLoS Pathog.* **6**:e1000947.
 4. **Cerny, A., et al.** 1995. Cytotoxic T lymphocyte response to hepatitis C virus-derived peptides containing the HLA A2.1 binding motif. *J. Clin. Invest.* **95**:521–530.
 5. **Chang, K. M., et al.** 2001. Differential CD4 and CD8 T-cell responsiveness in hepatitis C virus infection. *Hepatology* **33**:267–276.
 6. **Kasprowitz, V., Y. H. Kang, M. Lucas, J. Schulze zur Wiesch, et al.** 2010. Hepatitis C virus (HCV) sequence variation induces an HCV-specific T-cell phenotype analogous to spontaneous resolution. *J. Virol.* **84**:1656–1663.
 7. **Kasprowitz, V., et al.** 2008. Defining the directionality and quality of influenza virus-specific CD8+ T cell cross-reactivity in individuals infected with hepatitis C virus. *J. Clin. Invest.* **118**:1143–1153.
 8. **Kotturi, M. F., et al.** 2008. Naive precursor frequencies and MHC binding rather than the degree of epitope diversity shape CD8+ T cell immunodominance. *J. Immunol.* **181**:2124–2133.
 9. **Kuiken, C., K. Yusim, L. Boykin, and R. Richardson.** 2005. The Los Alamos hepatitis C sequence database. *Bioinformatics* **21**:379–384.
 10. **La Gruta, N. L., et al.** 2010. Primary CTL response magnitude in mice is determined by the extent of naive T cell recruitment and subsequent clonal expansion. *J. Clin. Invest.* **120**:1885–1894.
 11. **Lauer, G. M., et al.** 2004. High resolution analysis of cellular immune responses in resolved and persistent hepatitis C virus infection. *Gastroenterology* **127**:924–936.
 12. **Lauer, G. M., et al.** 2002. Comprehensive analysis of CD8(+)-T-cell responses against hepatitis C virus reveals multiple unpredicted specificities. *J. Virol.* **76**:6104–6113.
 13. **Lechner, F., et al.** 2000. Analysis of successful immune responses in persons infected with hepatitis C virus. *J. Exp. Med.* **191**:1499–1512.
 14. **Li, W., D. K. Krishnadas, R. Kumar, D. L. Tyrrell, and B. Agrawal.** 2008. Priming and stimulation of hepatitis C virus-specific CD4+ and CD8+ T cells against HCV antigens NS4, NS5a or NS5b from HCV-naive individuals: implications for prophylactic vaccine. *Int. Immunol.* **20**:89–104.
 15. **Li, W., D. K. Krishnadas, J. Li, D. L. Tyrrell, and B. Agrawal.** 2006. Induction of primary human T cell responses against hepatitis C virus-derived antigens NS3 or core by autologous dendritic cells expressing hepatitis C virus antigens: potential for vaccine and immunotherapy. *J. Immunol.* **176**:6065–6075.
 16. **Moon, J. J., et al.** 2007. Naive CD4(+) T cell frequency varies for different epitopes and predicts repertoire diversity and response magnitude. *Immunity* **27**:203–213.
 17. **Neumann-Haefelin, C., et al.** 2008. Virological and immunological determinants of intrahepatic virus-specific CD8+ T-cell failure in chronic hepatitis C virus infection. *Hepatology* **47**:1824–1836.
 18. **Obar, J. J., K. M. Khanna, and L. Lefrancois.** 2008. Endogenous naive CD8+ T cell precursor frequency regulates primary and memory responses to infection. *Immunity* **28**:859–869.
 19. **Racelli, V., et al.** 2007. Bone marrow of persistently hepatitis C virus-infected individuals accumulates memory CD8+ T cells specific for current and historical viral antigens: a study in patients with benign hematological disorders. *J. Immunol.* **179**:5387–5398.
 20. **Shirai, M., et al.** 1994. An epitope in hepatitis C virus core region recognized by cytotoxic T cells in mice and humans. *J. Virol.* **68**:3334–3342.
 21. **Spangenberg, H. C., et al.** 2005. Intrahepatic CD8(+) T-cell failure during chronic hepatitis C virus infection. *Hepatology* **42**:828–837.
 22. **Urbani, S., et al.** 2005. Heterologous T cell immunity in severe hepatitis C virus infection. *J. Exp. Med.* **201**:675–680.
 23. **Walker, C. M.** 2010. Adaptive immunity to the hepatitis C virus. *Adv. Virus Res.* **78**:43–86.
 24. **Wedemeyer, H., E. Mizukoshi, A. R. Davis, J. R. Bennink, and B. Rehermann.** 2001. Cross-reactivity between hepatitis C virus and Influenza A virus determinant-specific cytotoxic T cells. *J. Virol.* **75**:11392–11400.
 25. **Yewdell, J. W.** 2006. Confronting complexity: real-world immunodominance in antiviral CD8+ T cell responses. *Immunity* **25**:533–543.