

Rapid Changes in Peripheral Lymphocyte Concentrations During Interferon-Free Treatment of Chronic Hepatitis C Virus Infection

Eric G. Meissner^{1,2}, Anita Kohli,³ Jeanette Higgins,⁴ Yu-Jin Lee,⁵ Olga Prokunina,⁵ David Wu,⁵ Cody Orr,¹ Henry Masur,² and Shyam Kottlil^{5,6}

Treatment of chronic hepatitis C virus (HCV) infection with direct-acting antivirals results in a rapid decline in viral load and markers of hepatic inflammation, including serum chemokine (C-X-C motif) ligand 10 (CXCL10) concentration, which is followed in most cases by a sustained virologic response. Whether parallel changes of significance occur in the cellular composition of peripheral blood is relatively unknown. We hypothesized that longitudinal characterization of peripheral blood during treatment would provide insight into cellular migration and immune activation, which would have implications for understanding host immunity both before and after HCV treatment and may relate to HCV clearance. We analyzed longitudinal peripheral innate and adaptive immune cell populations by flow cytometry from 95 subjects enrolled in two direct-acting antiviral clinical trials and examined chemokine receptor expression on T lymphocytes in 43 patients. Within 1-2 weeks of initiating treatment, significant increases were observed in the concentration of peripheral cluster of differentiation 4-positive (CD4⁺) and CD8⁺ T lymphocytes but not monocyte or natural killer cells. In tandem with these changes, the percent of both CD4⁺ and CD8⁺ T lymphocytes with an activated phenotype (human leukocyte antigen [HLA] DR⁺ and CD38⁺) decreased, and T-lymphocyte surface expression of chemokine (C-X-C motif) receptor 3, the chemokine receptor for CXCL10, increased. *Conclusion:* Rapid changes in peripheral cellular populations occur during direct-acting antiviral treatment of HCV infection, which could potentially relate to hepatic efflux of tissue lymphocytes due to altered inflammation and chemokine receptor signaling, providing critical insight into the relationship between host immunity and viral clearance during HCV infection. (*Hepatology Communications* 2017;1:586-594)

Over 70 million people worldwide are chronically infected with hepatitis C virus (HCV), which can result in hepatic cirrhosis and hepatocellular carcinoma if untreated.⁽¹⁾ While eradication of chronic HCV infection can be achieved in >90% of patients with oral direct-acting antiviral (DAA) therapy, virologic relapse after treatment and reinfection are causes of viral persistence and sustained

Abbreviations: CD, cluster of differentiation; CXCR3, chemokine (C-X-C motif) receptor 3; CXCL10, chemokine (C-X-C motif) ligand 10; DAA, direct-acting antiviral; HCV, hepatitis C virus; HLA, human leukocyte antigen; IFN, interferon; NK, natural killer; SVR, sustained virologic response.

Received March 3, 2017; accepted June 30, 2017.

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1074/supinfo.

Supported by the National Institute of Allergy and Infectious Diseases (K08AI121348, to E.G.M.), the National Cancer Institute, National Institutes of Health (HHSN261200800001E), and the National Institute of Health Critical Care Medicine Department. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US government.

Copyright © 2017 The Authors. *Hepatology Communications* published by Wiley Periodicals, Inc., on behalf of the American Association for the Study of Liver Diseases. This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs License](https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep4.1074

Potential conflict of interest: Nothing to report.

hepatic inflammation, and patients with cirrhosis remain at risk for complications.⁽²⁻⁵⁾ Although relapse after cessation of therapy is the most common cause of treatment failure, the exact mechanism(s) for this is not fully understood. Prior work has indicated that host and viral factors impact the odds of relapse after treatment and influence the duration of treatment necessary to achieve a sustained virologic response (SVR).^(6,7) Changes correlated with restoration of innate and adaptive immune function have also been observed in parallel with HCV serum clearance and in some instances associate with favorable treatment outcome.⁽⁸⁻¹³⁾

Previous work has indicated that cellular components of the intrahepatic T-cell compartment, including regulatory CD4⁺ T cells and HCV-specific CD8⁺ T cells, do not normalize in liver tissue after SVR.^(14,15) Furthermore, while studies have demonstrated a decline in histological activity index inflammatory scores after SVR in most treated patients,⁽¹⁶⁾ the fate of tissue-infiltrating lymphocytes during treatment has not been fully explored. A greater understanding of changes in hepatic and peripheral immunity as a result of DAA treatment remains critically important to help inform the risk of subsequent reinfection, regression of fibrosis post-SVR, and prospects for HCV vaccination.

We previously observed a rapid decline in hepatic markers of endogenous interferon (IFN) activation during DAA-HCV therapy. Serum CXCL10 declined to levels observed in healthy volunteers within the first week of therapy in parallel with HCV viral load decline and hepatic transaminase normalization.^(9,17) CXCL10 is among the chemokines that influence leukocyte migration to sites of tissue inflammation, acting primarily through chemokine (C-X-C motif) receptor 3

(CXCR3), which is expressed on activated naive CD4⁺ T cells, CD8⁺ T cells, natural killer (NK) cells, NK-T cells, and macrophages; and the CXCL10–CXCR3 chemokine–receptor axis has been implicated in hepatic T-lymphocyte migration during inflammation.⁽¹⁸⁻²⁰⁾

Here, we asked whether changes in hepatic immunity are accompanied by corresponding changes in peripheral leukocytes during DAA treatment of HCV, in order to gain insight into cellular migration and inform our understanding of the relationship of host immunity and viral clearance.

Patients and Methods

CLINICAL TRIALS AND SAMPLES

Subjects from trial 1 (SYNERGY group A-C, NCT01805882) had genotype 1 HCV infection and received either 12 weeks of ledipasvir/sofosbuvir (group A), 6 weeks of ledipasvir/sofosbuvir + GS-9669 (group B), or 6 weeks of ledipasvir/sofosbuvir + GS-9451 (group C).⁽⁶⁾ For this report, data from 52 of 60 enrolled subjects were available for analysis, and all 52 subjects achieved SVR. Seventeen patients from group A, 18 patients from group B, and 17 patients from group C were included; and 8 subjects had advanced liver disease (all in group A, Metavir stage F3-4). Data from the three groups were combined for analysis and are presented as “trial 1.”

Subjects from trial 2 (SYNERGY group F, NCT01805882) had genotype 1 HCV infection, had advanced liver disease (Metavir stage F3-4), and received ledipasvir/sofosbuvir + GS-9451 for 6 weeks.⁽⁷⁾ Twenty-five patients were treatment-naïve, and 25 had a history of receiving IFN-based therapy. In the overall study 38/50 (76%) patients achieved

ARTICLE INFORMATION:

From the ¹Division of Infectious Diseases, Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC; ²Critical Care Medicine Department, NIH Clinical Center, Bethesda, MD; ³St. Joseph's Hospital and Medical Center, Department of Hepatology, Creighton University School of Medicine, Phoenix, AZ; ⁴Applied and Developmental Research Directorate, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD; ⁵Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD; ⁶Division of Clinical Care and Research, Institute of Human Virology, University of Maryland, Baltimore, MD.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Eric G. Meissner
Division of Infectious Diseases, Medical University of South Carolina
135 Rutledge Ave, MSC752

Charleston, SC, 29425
E-mail: meissner@muscc.edu
Tel: +1-843-792-4541

TABLE 1. TRIAL SUMMARY OF SUBJECTS USED FOR ANALYSIS

	Treatment Regimen	Treatment Duration	No. of subjects	SVR	Relapse	Advanced Fibrosis
Trial 1	SOF/LDV alone or + GS-9451 or GS-9669	6-12 weeks	52	52	0	8/52 (15%)
Trial 2	SOF/LDV + GS-9541	6 weeks	43	34	9	43/43 (100%)

Abbreviations: LDV, ledipasvir; SOF, sofosbuvir.

SVR, 11/50 (22%) relapsed after treatment, and 1/50 (2%) was lost to follow-up. No differences in SVR were observed in patients who were treatment-naïve versus treatment-experienced. Data from 43 subjects, including 34 who achieved SVR and 9 who relapsed, were available for this analysis.

In both trials, patients were predominantly male (71% trial 1, 66% trial 2), black (88% trial 1, 60% trial 2), and overweight (mean body mass index 27 trial 1, 30.5 trial 2) and had genotype 1a infection (70% trial 1, 76% trial 2). Advanced fibrosis (F3-F4 disease) was present in 30% of patients enrolled in trial 1 and 98% of patients enrolled in trial 2. Most patients (57/60 trial 1, 43/50 trial 2) had an HCV viral load that was either undetectable or below the limit of quantitation by week 4 of treatment.

Both studies received approval from the institutional review board at the National Institute of Allergy and Infectious Diseases, National Institutes of Health, and were conducted in concordance with the 1975 Declaration of Helsinki; and all patients provided written informed consent, as reported with initial publication of the clinical trial results.^(6,7,16)

FLOW CYTOMETRY

Immunophenotyping of peripheral blood drawn into ethylenediaminetetraacetate-containing blood collection tubes was performed according to the manufacturer's instructions, using a modification of the Centers for Disease Control and Prevention guidelines in a Clinical Laboratory Improvement Act-certified laboratory, as described.^(21,22) Cells were stained with combinations of monoclonal antibodies and then lysed after staining with Optilyse C (Beckman Coulter, Brea, FL), washed twice, and resuspended in 500 μ L of phosphate-buffered saline (Cambrex, Walkersville, MD). Samples were analyzed immediately on a Becton Dickinson FACS Canto flow cytometer (BD Biosciences, San Jose, CA). Four-color antibody panels used for cellular identification and enumeration are shown in [Supporting Table S1](#).

The lymphocyte population was identified by side scatter and CD45 staining, with confirmation that <5% of cells within the lymphocyte gate expressed CD14.⁽²²⁾ Absolute concentrations of specific lymphocyte populations (CD3⁺, CD4⁺, CD8⁺, CD4⁺/8⁺, CD19, and CD16⁺/56⁺ lymphocytes) were calculated using the measured percentage of each cell type within the lymphocyte gate and the absolute lymphocyte concentration. The percent and absolute concentration of CD3⁺CD4⁺ and CD3⁺CD8⁺ T cells with an activated phenotype were determined by co-surface expression of human leukocyte antigen (HLA)-DR and CD38, as described.⁽²¹⁾ CD27 and CD45RO were stained to identify memory cell subsets within the CD4⁺ and CD8⁺ lymphocyte populations.

Staining for CXCR3 and CXCR4 expression for trial 2 was performed using CXCR3-FITC (R&D Systems), CD3-PerCp (BD Biosciences), and CXCR4-BV421 (Biolegend) along with isotype controls.

STATISTICAL ANALYSIS

Data were analyzed using GraphPad Prism 6 statistical software with either nonparametric or parametric assumptions with a multiple-test correction, as indicated in each individual figure legend.

Results

To assess the possibility that reduced inflammation and HCV replication during DAA treatment would impact peripheral blood composition, we examined peripheral lymphocyte concentrations using longitudinal blood specimens collected during two DAA clinical trials (initially reported in Kohli et al.⁽⁶⁾, Kattakuzhy et al.⁽⁷⁾, and Osinusi et al.⁽¹⁶⁾ and summarized in Table 1). In both trials, we observed a significant early increase in the peripheral lymphocyte concentration 1-2 weeks posttreatment (Fig. 1), a change not consistently sustained over the course of treatment. While NK-cell, CD4⁺ T-cell, and CD8⁺

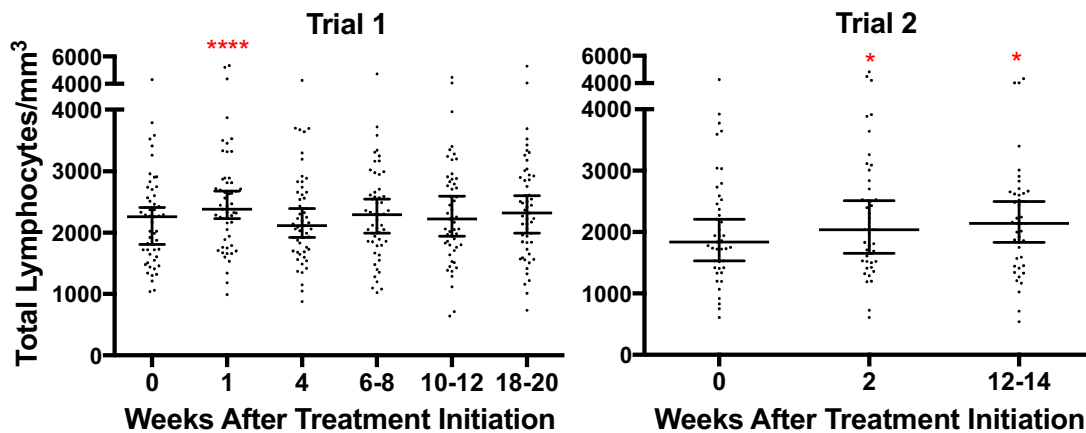


FIG. 1. Peripheral lymphocyte concentrations increase significantly 1-2 weeks after treatment in both trials. Statistical analysis was performed for each time point relative to week 0 using Friedman's test with Dunn's multiple test correction. **** $P < 0.0001$, * $P < 0.05$.

T-cell functional capacity has been assessed early after DAA therapy,^(8,10,11) bulk changes in peripheral concentration of specific lymphocyte populations have not been characterized. To address this, we performed flow cytometry with combinations of four-color antibody panels to enumerate cellular populations. A representative plot delineating gating strategies for CD4⁺ and CD8⁺ lymphocytes is shown in Fig. 2A. We observed early and significant increases in peripheral concentrations of CD4⁺ and CD8⁺ T cells (Fig. 2B,C), less consistent changes in CD19⁺ B cells (Supporting Fig. S1), and no significant changes in CD16/56⁺ NK-cell or CD14⁺ monocyte concentrations (Supporting Fig. S1). Relative frequencies of CD4⁺ and CD8⁺ T lymphocytes with a specific memory phenotype, assessed by CD45RO/CD27 staining, did not change over the course of treatment; and no differences were observed in trial 2 when results were analyzed by treatment outcome (data not shown).

To further explore the observed changes in CD4⁺ and CD8⁺ T-lymphocyte concentrations, we determined the frequencies of cells which coexpressed the activation markers HLA-DR and CD38. We found that the percent of CD4⁺ and CD8⁺ T lymphocytes with an activated (HLA-DR⁺ and CD38⁺) phenotype decreased rapidly with treatment in both CD4⁺ and CD8⁺ T-lymphocyte populations (Fig. 3A,B). While the activated CD4⁺ T-lymphocyte concentration also decreased during treatment, no significant change in CD8⁺ T-lymphocyte concentration was observed in either trial (Supporting Fig. S2). In trial 2,

no differences were observed between patients achieving SVR versus relapsers at baseline or at week 2 of treatment (data not shown), while a significant increase was observed in relapsers after patients had already experienced virologic rebound posttreatment at week 12-14 (Fig. 3C).

To explore whether changes in peripheral lymphocyte numbers may relate to the early decline in CXCL10 serum concentration during DAA treatment,^(9,17) we measured peripheral T-lymphocyte surface expression of CXCR3, the chemokine receptor for CXCL10, as well as CXCR4, the receptor for CXCL12, whose gene expression did not change in liver as a result of DAA treatment.^(9,23) Samples from subjects enrolled in trial 2 were available for use in this analysis.⁽⁷⁾ While the percent of CD3⁺ lymphocytes expressing CXCR3 had increased by week 2 of treatment, no differences were seen in the percent of cells expressing CXCR4 (Fig. 4A,B). No difference in CXCR3 staining was observed based on treatment outcome at any time point.

Discussion

Our data demonstrate rapid changes in peripheral T-lymphocyte concentrations during DAA treatment for chronic HCV infection that correlate with surface expression of CXCR3 and previously observed changes in CXCL10 concentration. The increase in peripheral cellular concentrations observed 1-2 weeks after

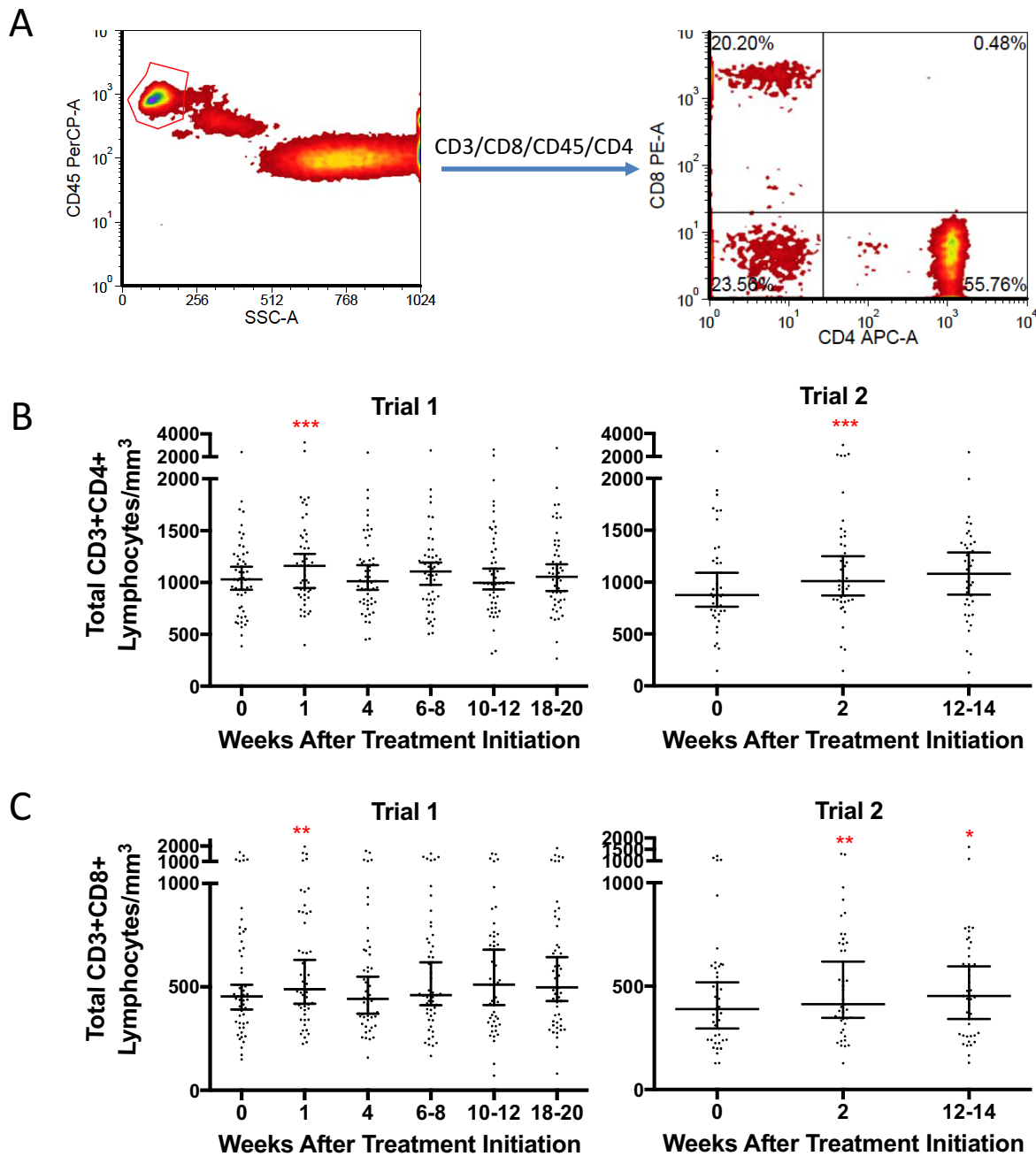


FIG. 2. Early increase in total peripheral CD4⁺ and CD8⁺ T-lymphocyte concentrations. (A) Gating strategy used to delineate cell subsets with four-color flow panel detecting CD3, CD45, CD4, and CD8. CD4⁺ (B) and CD8⁺ (C) T-lymphocyte concentrations are shown over time for both trials. Statistical analysis was performed for each time point relative to week 0 using Friedman’s test with Dunn’s multiple test correction. ****P* < 0.001, ***P* < 0.01, **P* < 0.05. Abbreviations: APC, allophycocyanin; PE, phycoerythrin; SSC, side scatter.

treatment initiation correlated with declines in hepatic transaminase concentrations as well as the temporally slower HCV decline observed by Talal et al. in liver relative to peripheral blood.⁽²⁴⁾ This pattern was not

observed for all cellular populations as CD16/56⁺ NK cells, CD14⁺ monocytes, and CD19⁺ B cells had less striking or no changes in concentration during treatment. Taken together, these data suggest that the

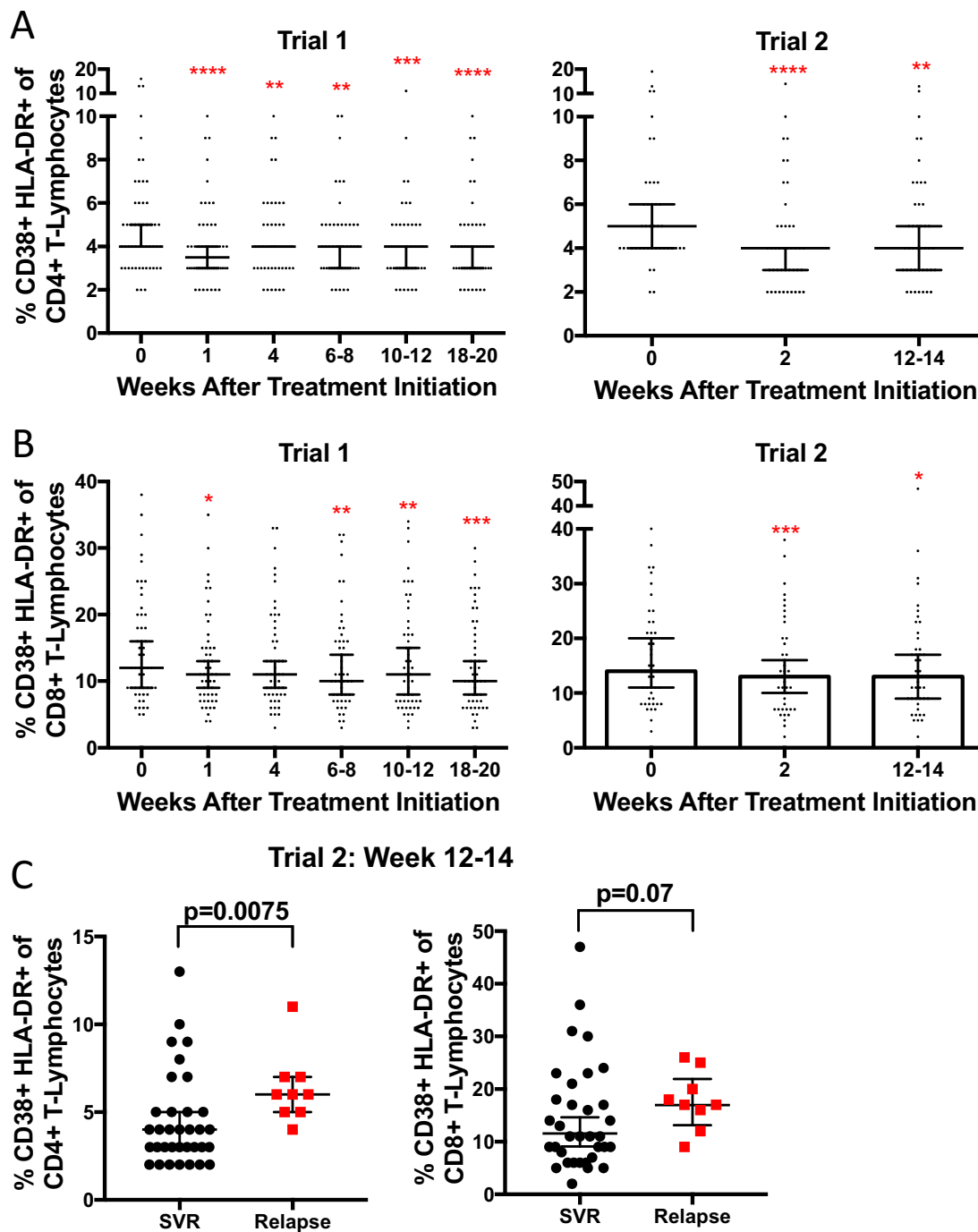


FIG. 3. The percent of CD4⁺ (A) and CD8⁺ (B) T lymphocytes with an activated (HLA-DR⁺ and CD38⁺) phenotype decreases rapidly with treatment initiation. Differences between patients who achieved SVR and relapse were noted at week 12-14 of treatment after patients had experienced virologic relapse (C). Statistical analysis for (A) and (B) was performed for each time point relative to week 0 using Friedman's test with Dunn's multiple test correction. Data in (C) were analyzed using the Mann-Whitney test. *****P* < 0.0001, ****P* < 0.001, ***P* < 0.01, **P* < 0.05.

T-lymphocyte increase observed in peripheral blood could reflect a combination of reduced hepatic migration of peripheral cells due to altered chemotaxis and potentially hepatic egress of tissue-resident

lymphocytes. Further analysis of these cells for specificity and functionality will be critical in enhancing our understanding of HCV pathogenesis and the natural history of liver disease post-SVR.

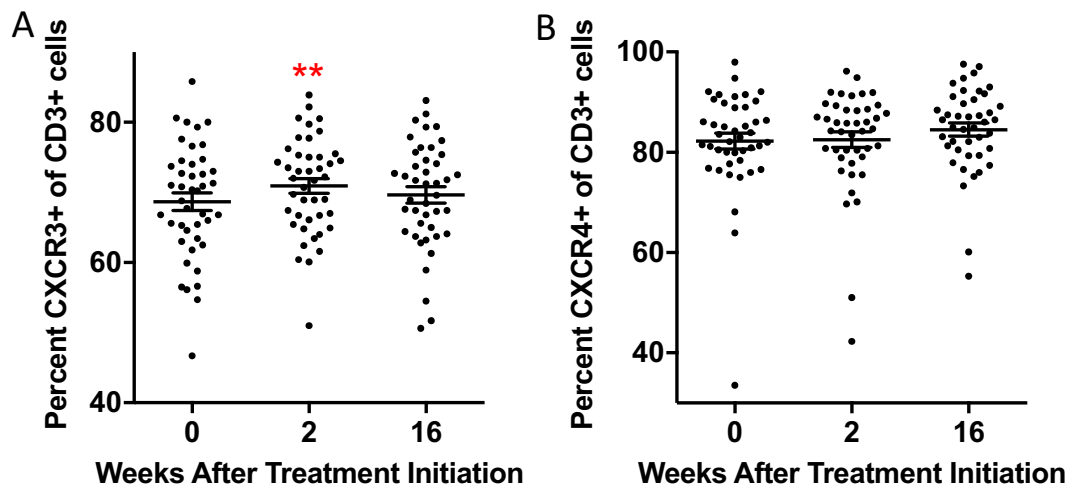


FIG. 4. Percent of CD3⁺ cells expressing CXCR3 increased by week 2 of treatment (A), whereas no change in CXCR4 frequency was observed (B). Data are from patients who participated in trial 2. Statistical analysis was performed for each time point relative to week 0 using one-way analysis of variance with Dunnett's multiple test correction. ** $P < 0.01$.

Interestingly, we previously observed an increase in gene expression for type 1 and type 2 IFN receptors in total peripheral blood mononuclear cells 1-2 weeks post DAA treatment with sofosbuvir and ribavirin.⁽⁹⁾ Considered with the observation here that peripheral T lymphocytes at this time point have an increase in CXCR3 expression, this suggests that peripheral T cells early after treatment may reflect recent hepatic emigrants that had exposure to hepatic inflammation, including endogenous IFNs and chemokines. In addition, the overall increase in peripheral CD4⁺ and CD8⁺ T cells observed early in treatment (Fig. 2), the decline in the percent of these cells with an activated phenotype (Fig. 3), and the less significant change in absolute number of activated CD8⁺ T cells early after treatment (Supporting Fig. S3) suggest that recent hepatic emigrants could have lost or could be in the process of losing activation status. If these cells do in fact represent recent hepatic emigrants, further analysis of cytokine secretion, functionality, and antigen specificity may provide novel insights into understanding hepatic fibrogenesis and potentially treatment outcome. We did not analyze liver lymphocytes as part of this study; thus, the possibility that changes observed in the periphery have a connection to intrahepatic cellular populations is an inference.

Recently it was shown that the percent of lymphocytes expressing CD4 increased by week 4 of treatment in a ribavirin-free, IFN-free DAA cohort of 19 patients, while no such changes were observed for

CD8⁺ T cells, a study in which absolute cell concentrations were not reported.⁽¹⁰⁾ The percent of CD4 and CD8 cells with an effector memory phenotype was noted to increase, while surface expression of the coinhibitory molecule TIGIT decreased in both cell populations. While these data are not inconsistent with the observations reported here, differences in the time of sample collection (week 1-2 versus week 4), gating strategies, and sample sizes may have impacted the respective results.

Recent work has also correlated intrahepatic activated HCV-specific and polyclonal epitope-specific (not HCV-specific) CD8⁺ T cells with chronic inflammation during infection, measured by aspartate aminotransferase/alanine aminotransferase elevation and IFN-stimulated gene transcript expression, with the suggestion that this contributes to fibrosis progression.⁽²⁵⁾ We were unable to perform staining with Eomes/T-bet to assess the balance of functional and dysfunctional cells in our study due to sample availability. Because most CD8⁺ T cells in the liver are not HCV-specific, we suspect that the increase in peripheral CD8⁺ T-cell concentrations observed during therapy likely primarily reflects HCV-nonspecific CD8⁺ T cells drawn to the liver as a result of chronic inflammation and chemokine production,⁽²⁶⁾ a hypothesis that can be assessed in future work.

While the strength of this study is the large number of patients and the longitudinal nature of the samples analyzed, several weaknesses merit discussion. First,

because we analyzed whole blood at the time of collection and had not anticipated the results observed at week 1-2, our staining panel was not designed for an in-depth characterization of the functional phenotype of cells contributing to the increase in peripheral concentrations and, thus, could not clearly characterize these cells as recent hepatic emigrants. Second, while we sought preliminary associations of our findings with treatment outcome in trial 2, the relatively low number of relapsers ($n = 9$) limited our ability to draw firm conclusions regarding a functional correlation between these hepatic resident lymphocytes and HCV clearance. While patient age has been associated with relapse in trial 2, no association with gender, *IFNL4* genotype, body mass index, medication adherence, fibrosis stage (F3 versus F4), HCV genotype (1a versus 1b), viral load (over or under 6 million IU/mL), or pre-existing resistance-associated variants was identified,⁽⁷⁾ highlighting the need for further in-depth functional study of the observed immune changes here with treatment outcome. Finally, a number of other chemokines and chemokine receptors have been implicated in hepatic inflammation and immune cell migration in chronic HCV infection, including chemokine (C-C motif) receptor 5 and ligands, chemokine (C-C motif) receptor 6 and ligands, and chemokine (C-C motif) receptor 4 and ligands, none of which were examined in this study due to sample availability.⁽¹⁸⁾ Our focus on CXCR3 was based on its binding to CXCL9, CXCL10, and CXCL11, implicated in recruiting Th1 and Th17 cells in this liver as all three of these genes were significantly down-regulated in HCV liver over the course of treatment as demonstrated in our previous studies.^(9,23)

In conclusion, rapid changes in peripheral T-lymphocyte concentrations were observed during DAA treatment of chronic HCV infection, which could relate to early decreases in hepatic inflammation and egress of adaptive immune cells. Further kinetic and in-depth functional analyses of lymphocytes early in treatment are likely to provide additional meaningful insight into intrahepatic immunity during HCV infection and hepatic remodeling after eradication.

REFERENCES

- World Health Organization. 2017. Hepatitis C: fact sheet number 164. WHO, Geneva, Switzerland. <http://www.who.int/mediacentre/factsheets/fs164/en/>.
- Kohli A, Shaffer A, Sherman A, Kottlilil S. Treatment of hepatitis C: a systematic review. *JAMA* 2014;312:631-640.
- Rehermann B. HCV in 2015: advances in hepatitis C research and treatment. *Nat Rev Gastroenterol Hepatol* 2016;13:70-72.
- Midgard H, Bjoro B, Maeland A, Konopski Z, Kileng H, Damas JK, et al. Hepatitis C reinfection after sustained virological response. *J Hepatol* 2016;64:1020-1026.
- Sacks-Davis R, Grebely J, Dore GJ, Osburn W, Cox AL, Rice TM, et al. Hepatitis C virus reinfection and spontaneous clearance of reinfection—the InC3 Study. *J Infect Dis* 2015;212:1407-1419.
- Kohli A, Osinusi A, Sims Z, Nelson A, Meissner EG, Barrett LL, et al. Virological response after 6 week triple-drug regimens for hepatitis C: a proof-of-concept phase 2A cohort study. *Lancet* 2015;385:1107-1113.
- Kattakuzhy S, Wilson E, Sidharthan S, Sims Z, McLaughlin M, Price A, et al. Moderate sustained virologic response rates with 6-week combination directly acting anti-hepatitis C virus therapy in patients with advanced liver disease. *Clin Infect Dis* 2016;62:440-447.
- Spaan M, van Oord G, Kreeft K, Hou J, Hansen BE, Janssen HL, et al. Immunological analysis during interferon-free therapy for chronic hepatitis C virus infection reveals modulation of the natural killer cell compartment. *J Infect Dis* 2016;213:216-223.
- Meissner EG, Wu D, Osinusi A, Bon D, Virtaneva K, Sturdevant D, et al. Endogenous intrahepatic IFNs and association with IFN-free HCV treatment outcome. *J Clin Invest* 2014; 124:3352-3363.
- Burchill MA, Golden-Mason L, Wind-Rotolo M, Rosen HR. Memory re-differentiation and reduced lymphocyte activation in chronic HCV-infected patients receiving direct-acting antivirals. *J Viral Hepat* 2015;22:983-991.
- Martin B, Hennecke N, Lohmann V, Kayser A, Neumann-Haefelin C, Kukolj G, et al. Restoration of HCV-specific CD8⁺ T cell function by interferon-free therapy. *J Hepatol* 2014;61: 538-543.
- Barrett L, Shivasabesan G, Wang C, Osinusi A, Kohli A, Meissner EG, et al. Altered HCV specific T cell immunity very early in interferon free HCV DAA therapy. *J Hepatol* 2013; 58(Suppl. 1):S1.
- Shrivastava S, Wilson E, Poonia B, Tang L, Osinusi A, Kohli A, et al. Augmentation of HCV specific immunity and sustained virological response (SVR). *J Viral Hepat* 2017; doi: 10.1111/jvh.12702.
- Spaan M, Claassen MA, Hou J, Janssen HL, de Knegt RJ, Boonstra A. The intrahepatic T cell compartment does not normalize years after therapy-induced hepatitis C virus eradication. *J Infect Dis* 2015;212:386-390.
- Callendret B, Eccleston HB, Hall S, Satterfield W, Capone S, Folgori A, et al. T-cell immunity and hepatitis C virus reinfection after cure of chronic hepatitis C with an interferon-free antiviral regimen in a chimpanzee. *Hepatology* 2014;60:1531-1540.
- Osinusi A, Meissner EG, Lee YJ, Bon D, Heytens L, Nelson A, et al. Sofosbuvir and ribavirin for hepatitis C genotype 1 in patients with unfavorable treatment characteristics: a randomized clinical trial. *JAMA* 2013;310:804-811.
- Meissner EG, Decalf J, Casrouge A, Masur H, Kottlilil S, Albert ML, et al. Dynamic changes of post-translationally modified forms of CXCL10 and soluble DPP4 in HCV subjects receiving interferon-free therapy. *PLoS One* 2015;10:e0133236.
- Marra F, Tacke F. Roles for chemokines in liver disease. *Gastroenterology* 2014;147:577-594.
- Van Raemdonck K, Van den Steen PE, Liekens S, Van Damme J, Struyf S. CXCR3 ligands in disease and therapy. *Cytokine Growth Factor Rev* 2015;26:311-327.

- 20) Hickman HD, Reynoso GV, Ngudiankama BF, Cush SS, Gibbs J, Bennink JR, et al. CXCR3 chemokine receptor enables local CD8⁺ T cell migration for the destruction of virus-infected cells. *Immunity* 2015;42:524-537.
- 21) Ganesan A, Crum-Cianflone N, Higgins J, Qin J, Rehm C, Metcalf J, et al. High dose atorvastatin decreases cellular markers of immune activation without affecting HIV-1 RNA levels: results of a double-blind randomized placebo controlled clinical trial. *J Infect Dis* 2011;203:756-764.
- 22) Swaminathan S, Hu Z, Rupert AW, Higgins JM, Dewar RL, Stevens R, et al. Plasma interleukin-27 (IL-27) levels are not modulated in patients with chronic HIV-1 infection. *PLoS One* 2014;9:e98989.
- 23) Meissner EG, Kohli A, Virtaneva K, Sturdevant D, Martens C, Porcella SF, et al. Achieving sustained virologic response after interferon-free hepatitis C virus treatment correlates with hepatic interferon gene expression changes independent of cirrhosis. *J Viral Hepat* 2016;23:496-505.
- 24) Talal AH, Dimova RB, Zhang EZ, Jiang M, Penney MS, Sullivan JC, et al. Telaprevir-based treatment effects on hepatitis C virus in liver and blood. *Hepatology* 2014;60:1826-1837.
- 25) Martini H, Citro A, Martire C, D'Ettorre G, Labbadia G, Accapezzato D, et al. Apoptotic epitope-specific CD8⁺ T cells and interferon signaling intersect in chronic hepatitis C virus infection. *J Infect Dis* 2016;213:674-683.
- 26) Knolle PA, Thimme R. Hepatic immune regulation and its involvement in viral hepatitis infection. *Gastroenterology* 2014;146:1193-1207.

Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1074/supinfo.