Regulatory T-Cell Responses to Low-Dose Interleukin-2 in HCV-Induced Vasculitis

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ORIGINAL ARTICLE

Regulatory T-Cell Responses to Low-Dose Interleukin-2 in HCV-Induced Vasculitis

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ABSTRACT

BACKGROUND

Patients with vasculitis induced by the hepatitis C virus (HCV) have reduced levels of regulatory T cells (Tregs). Resolution of HCV infection correlates with cure of vasculitis and the recovery of Treg levels. We reasoned that interleukin-2, a cytokine that promotes Treg survival and function, could be beneficial for patients with vasculitis that is resistant to HCV therapy.

METHODS

We investigated the safety and immunologic effects of the administration of low-dose interleukin-2 in a prospective open-label, phase 1–phase 2a study. Ten patients with HCV-induced vasculitis that was refractory to conventional antiviral therapy, rituximab therapy, or both and who were not receiving glucocorticoid or immunosuppressant therapy, received one course of interleukin-2 (1.5 million IU per day) for 5 days, followed by three 5-day courses of 3 million IU per day at weeks 3, 6, and 9. Both the safety of the treatment and its effectiveness were evaluated, the latter by monitoring the Treg response and the clinical signs of HCV vasculitis.

RESULTS

No adverse events reached a level higher than grade 1. The treatment did not induce effector T-cell activation, vasculitis flare, or increased HCV viremia. We observed a reduction in cryoglobulinemia in 9 of 10 patients and improvement of vasculitis in 8 of 10. Administration of low-dose interleukin-2 was followed by an increase in the percentage of CD4+, $CD25^{high}$, forkhead box P3 (FOXP3+) Tregs [E_{max} (maximum value) ÷ baseline value × 100 = 420%] with potent suppressive activity in all subjects and by a concomitantly decreased proportion of marginal-zone B cells. Transcriptome studies of peripheral-blood mononuclear cells revealed that interleukin-2 induced a global attenuation of the signatures for inflammation and oxidative stress mediators.

CONCLUSIONS

The trial showed that low-dose interleukin-2 was not associated with adverse effects and led to Treg recovery and concomitant clinical improvement in patients with HCV-induced vasculitis, an autoimmune condition. (Funded by the French Agency for Research on AIDS and Viral Hepatitis [ANRS] and others; ClinicalTrials.gov number, NCT00574652.)

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Interleukin-2 has been identified for its capacity to stimulate T cells in vitro[1] and has been used to boost effector immune responses in patients with cancers and infectious diseases.[2,3] It is a registered indication when used as an adjunct for the treatment of renal-cell carcinoma, but there is a response to treatment in less than 10% of those with the disease, a finding partly explained by the discovery that interleukin-2 mediates the survival and suppressive function of regulatory T cells (Tregs),[4] which are known to suppress antitumor effector responses.[5,6] A marked increase in levels of Tregs has been documented during interleukin-2 treatment in patients with cancer.[7,8]

The paradox that interleukin-2 has been approved by the Food and Drug Administration and similar government agencies worldwide for immunotherapy of cancer even though it activates immune cells that block anticancer immune responses relates to the fact that interleukin-2 receptor signaling is important to the responses of both Tregs and effector T cells.[9] However, mice deficient in interleukin-2 or interleukin-2 receptor lack Tregs but are able to mount effector immune responses,[9] indicating that interleukin-2 could play a more critical role in relation to Tregs than to effector T cells. Tregs have a low threshold of response to interleukin-2 receptor signaling, which supports their development and peripheral homeostasis.[9] Low-dose interleukin-2 might thus represent a novel class of immunoregulatory drug that is specific to the expansion and activation of Tregs and thus could be beneficial in diseases associated with Treg deficiency, such as vasculitis induced by infection with hepatitis C virus (HCV).[10]

Chronic HCV infection is uniquely associated with an array of extrahepatic complications whose pathogenic mechanisms appear to be largely driven by the immune system. Among these complications, cryoglobulinemia and its clinical sequelae have the strongest association with infection. Cryoglobulins are readily detectable in 40 to 60% of HCV-infected patients,[11] whereas overt cryoglobulinemic vasculitis (or mixed cryoglobulinemia) develops in only 5 to 10% of these cases.[11] The most common target organs are skin, joints, nerves, and kidneys. Disease expression is variable, ranging from mild clinical symptoms (e.g., purpura and arthralgia) to fulminant life-threatening complications (e.g., glomerulonephritis and widespread vasculitis). The observation of T cells in vascular infiltrates, the presence of autoantibodies, and the genetic association between some HLA alleles and susceptibility to mixed cryoglobulinemia in HCV-infected patients support the suggestion that there is an autoimmune component to this virus-linked condition.[10,12] Mixed cryoglobulinemia appears to result from the interaction between HCV and lymphocytes, which directly modulates the function of B cells and T cells and results in the activation and expansion of B cells that produce IgM with rheumatoid-factor activity.[11,13] We previously reported a quantitative defect in Tregs in persons with HCV-induced mixed cryoglobulinemia.[10,14,15] In patients with mixed cryoglobulinemia who could be successfully treated for HCV infection, clearance of the virus was associated with the cure of vasculitis and the recovery of Treg levels.[15,16]

We therefore reasoned that induction of Tregs could have beneficial effects for patients with HCV-induced vasculitis that is resistant to HCV therapy, and we carried out an open-label phase 1–phase 2a trial to assess the safety and immunologic and clinical effects of repeated administration of low-dose interleukin-2 in HCV-infected patients with associated autoimmunity.

METHODS

STUDY PATIENTS

To be included in the study, patients had to have chronic HCV infection, as defined by the presence of HCV RNA in serum; a history of mixed cryoglobulinemic vasculitis, as defined by serum cryoglobulin levels of 0.05 g per liter or higher in at least two determinations; the triad of purpura–arthralgia–asthenia or, in the absence of purpura, biopsy-proven vasculitis (in the kidneys, nerves, or skin); clinically active vasculitis, with resistance or intolerance to conventional antiviral therapy (e.g., peginterferon alfa and ribavirin) or rituximab; and no treatment with antiviral therapy for a minimum of 2 months and no treatment with rituximab for a minimum of 6 months.[16,17]

Criteria for exclusion from the study included coinfection with hepatitis B virus or the human immunodeficiency virus, evidence of liver cirrhosis as revealed by analysis of biopsy specimens and noninvasive tests, the presence of cancer or lymphoma, the use of any glucocorticoids or im-

munosuppressants during the study or in the previous 6 months, drug addiction, alcohol abuse, or pregnancy.

STUDY DESIGN

We conducted a single-center, open-label, prospective phase 1–phase 2a trial in which four courses of interleukin-2 (aldesleukin [Proleukin, Novartis]) were administered subcutaneously. Patients received a dose of 1.5 million IU per day for 5 days (starting on a Monday) during a week-long hospital stay; the purpose of hospitalization was to allow evaluation of their tolerance to treatment. The patients were not hospitalized when they received the three subsequent courses of 3 million IU per day. The second course was initiated after a 9-day washout (week 3), and the third and fourth courses were each initiated after a 16-day washout (weeks 6 and 9). The study started in April 2008 and ended in July 2010. It was approved by the hospital's institutional review board, and written informed consent was obtained from all patients. The study was performed in accordance with the protocol and the statistical analysis plan (both available with the full text of this article at NEJM.org). All authors vouch for the accuracy and completeness of the reported results and for the fidelity of this report to the protocol.

The primary end point — an absolute increase of 4 percentage points in the proportion of CD4+, $CD25^{high}$, forkhead box P3 (FOXP3+) Tregs detected at the end of treatment — was selected because we observed a mean increase of 4 percentage points in persons with mixed cryoglobulinemia who were cured of vasculitis after successful HCV treatment.[15,16] Secondary end points included safety, the extent of cellular and humoral immunity at week 9, a persistent increase of Treg levels at week 19, and the extent of the clinical response of the vasculitis.

We evaluated patients on day 1 and day 5 of each treatment course, before the first and last administration of interleukin-2 for that particular course. We also evaluated them between 48 and 90 days after the last administration of the study treatment. We analyzed the response to treatment by comparing clinical, immunologic, and virologic data at the initial evaluation, at the end of each course of treatment, and at the end of follow-up. The clinical response was defined by the regression or disappearance of the main signs of vasculitis — that is, skin involvement (no purpura or leg ulcers), peripheral neuropathy (clinical and electrophysiological improvement on two successive examinations), renal involvement (normalization of serum creatinine levels and the disappearance of proteinuria and hematuria), and arthralgia.

STATISTICAL ANALYSIS

We calculated that a sample of 10 patients would provide 94% power to detect a mean of paired differences of 4 percentage points with an estimated standard deviation of differences of 3 percentage points and a significance level (alpha) of 0.05 with the use of a two-sided Wilcoxon signed-rank test, assuming that the actual distribution of the percentage of CD4+, $CD25^{high}$, FOXP3+ Tregs is normal.

We compared measures taken at baseline with those taken at week 9 and with those taken after the last dose of interleukin-2 was administered with the use of the Wilcoxon signed-rank test. The F approximation of the Friedman test was used to make comparisons across all repeated measurements.[18] We performed approximations of the critical region of the Friedman statistic and multiple comparisons where appropriate.[19] We determined time-to-peak values (T_{max}) directly from the experimental data as the time of the maximum Treg percentage (E_{max}).

RESULTS

PATIENTS

At inclusion, the median (interquartile range) age of the 10 study patients was 58.5 years (interquartile range, 49.5 to 66.2), with a 1:1 ratio of men to women. Baseline characteristics and outcomes for each of the 10 patients are provided in Table 1. The clinical manifestations of mixed cryoglobulinemic vasculitis in these patients included peripheral neuropathy (8 patients), purpura (8), asthenia (6), arthralgia (3), and kidney involvement (1, with daily proteinuria [1.5 g of protein per 24 hours], microscopic hematuria, and creatinemia [74 μmol per liter]). The median cryoglobulin level was 0.53 g per liter (interquartile range, 0.26 to 2.77), characterized as type II cryoglobulinemia with monoclonal IgM kappa in all cases. The median level of complement protein C4 was 0.07 mg per liter (interquartile range, 0.02 to

Table 1. Characteristics and Outcomes for the 10 Study Subjects.

Characteristic or Outcome	Patient 1	Patient 2	Patient 3	Patient 4
Age at diagnosis (yr)	48	74	63	50
Sex	Female	Female	Male	Male
Symptoms				
At baseline	Arthralgia, fatigue	Purpura, neuropathy, fatigue	Purpura, neuropathy, fatigue	Neuropathy, fatigue
After administration of interleukin-2	—	—	Neuropathy	Neuropathy
Previous therapy	Peginterferon alfa, ribavirin	Peginterferon alfa, ribavirin	Peginterferon alfa, ribavirin	Peginterferon alfa, ribavirin
Serum cryoglobulin (g/liter)				
At baseline	0.56	1.61	0.17	0.16
After administration of interleukin-2	0	1.00	0.91	0
C4 complement (mg/liter)				
At baseline	0.20	0.06	0.08	0.20
After administration of interleukin-2	0.21	0.05	0.12	0.27
HCV genotype	1	5	1	4
HCV viral load (log copies/ml)				
At baseline	5.3	6.2	7.2	6.3
After administration of interleukin-2	5.2	5.8	5.6	6.2
Treatment side effects*				
Course 1	—	—	—	—
Course 2	—	Fatigue, myalgia	Fatigue	Fatigue
Course 3	—	Fatigue	Fatigue	Fatigue
Course 4	—	—	—	—

* The treatment provided in course 1 consisted of 1.5 million IU of interleukin-2 per day for 5 consecutive days and that provided in courses 2, 3, and 4 consisted of 3.0 million IU of interleukin-2 per day for 5 consecutive days.

0.16), rheumatoid factor activity was present in 9 patients, and a test for antinuclear antibodies was positive in 1 patient (with a titer of 1:640). The mean (±SE) estimated duration of HCV infection was 30±2 years. The median HCV viral load was 6.3 log copies per millimeter (range, 5.5 to 6.8). HCV subtypes included genotype 1 (in 7 patients), genotype 4 (in 2), and genotype 5 (in 1). No patients had cirrhosis.

Safety

All patients completed all four courses of interleukin-2 (Table 1). We observed no statistically significant changes in circulating levels of granulocytes (including eosinophils), red cells, or liver enzymes throughout the study. We did observe asthenia in 4 patients, transient local reactions at injection sites in 4 patients, flu-like syndrome in 4 patients, myalgia in 1 patient, and hypertension in 1 patient, all at the higher dose of interleukin-2; all of these adverse events resolved (Table 1). We observed no biologic or clinical signs (such as vasculitis flare) indicating activation of pathogenic T cells, and we observed no increase in HCV viral load (Table 1 and Fig. 1).

Treg Levels

Interleukin-2 induced an increase in circulating levels of Tregs CD4+, CD25^{high}, and FOXP3+ (Fig. 1A, and Fig. S1 in the Supplementary Appendix, available at NEJM.org). The mean baseline percentage of Tregs in this group of patients was 3.6±0.2% of CD4+ T cells, significantly lower than normal values (4.6±0.6) and consistent with

Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
59	67	51	66	58	43
Male	Male	Female	Male	Female	Female
Purpura, neuropathy, fatigue	Purpura, neuropathy	Purpura, neuropathy	Neuropathy	Arthralgia, purpura	Arthralgia, purpura, neuropathy, kidney involvement, fatigue
—	—	—	Neuropathy	—	Neuropathy
Peginterferon alfa, ribavirin	Peginterferon alfa, ribavirin	Peginterferon alfa, ribavirin	Peginterferon alfa, ribavirin	Peginterferon alfa, ribavirin	Peginterferon alfa, ribavirin/rituximab
0.30	0.3	6.99	2.77	2.78	0.51
0.34	0	3.87	1.99	2.94	0.19
0.06	0.15	0.07	0.02	0.03	0.02
0.09	0.19	0.10	0.06	0.04	0.03
1	1	1	4	1	1
5.6	6.9	5.8	6.3	6.8	5.4
5.6	5.8	6.0	5.1	4.6	5.5
—	—	—	—	—	—
Fatigue	Flulike syndrome, local reaction	Flulike syndrome, local reaction	Flulike syndrome, local reaction	Arterial hypertension	Flulike syndrome, local reaction
Fatigue	Local reaction	Flulike syndrome	Flulike syndrome, local reaction	Arterial hypertension	—
—	Local reaction	—	Local reaction	—	—

previous findings.[10,15] At week 9, we observed that Tregs made up 11.8±2.0% of CD4+ cells ($P=0.004$), reaching the criterion for efficacy of the primary end point. Notably, Treg proportions had increased by approximately a factor of 2 after the first 5-day course of 1.5 million IU of interleukin-2 per day (Fig. 1A), continued to increase during the washout period between courses, and were further boosted after the administration of subsequent courses (Fig. 1A). As compared with baseline values, these increases in the proportions of Tregs were statistically significant throughout treatment ($P=0.02$ at week 1 and $P<0.001$ at weeks 3, 6, and 9). Thus, the primary end point of our study was met. An increase in CD4+ Tregs leads to an increase in the ratio of Tregs to effector T cells (Fig. 1B). We observed no modification of the ratio of CD4+ to CD8+ cells (Table S1 in the Supplementary Appendix).

The percentage of Tregs peaked at the end of the third course of interleukin-2 in all patients. The median peak value ($E_{max}=14\%$) corresponded to a 420% increase in the proportion of Tregs [(E_{max} value ÷ baseline value) × 100]. The proportion of Tregs remained significantly elevated at 129 to 150 days after initial treatment, at twice the baseline value (6.1±0.5%, $P=0.008$), and within the normal range of values for healthy blood donors. Finally, we tested the functionality of the Tregs induced by interleukin-2 and observed them to be highly suppressive (Fig. S2 in the Supplementary Appendix). A population of rare CD8+, CD25+, FOXP3+ T cells with suppressor function can be detected in healthy persons[20] (Fig. S3 in the Supplementary Appendix). We monitored the levels of these cells and observed their marked increase during the trial, which was concomitant with the increase in levels of CD4+ Tregs. The

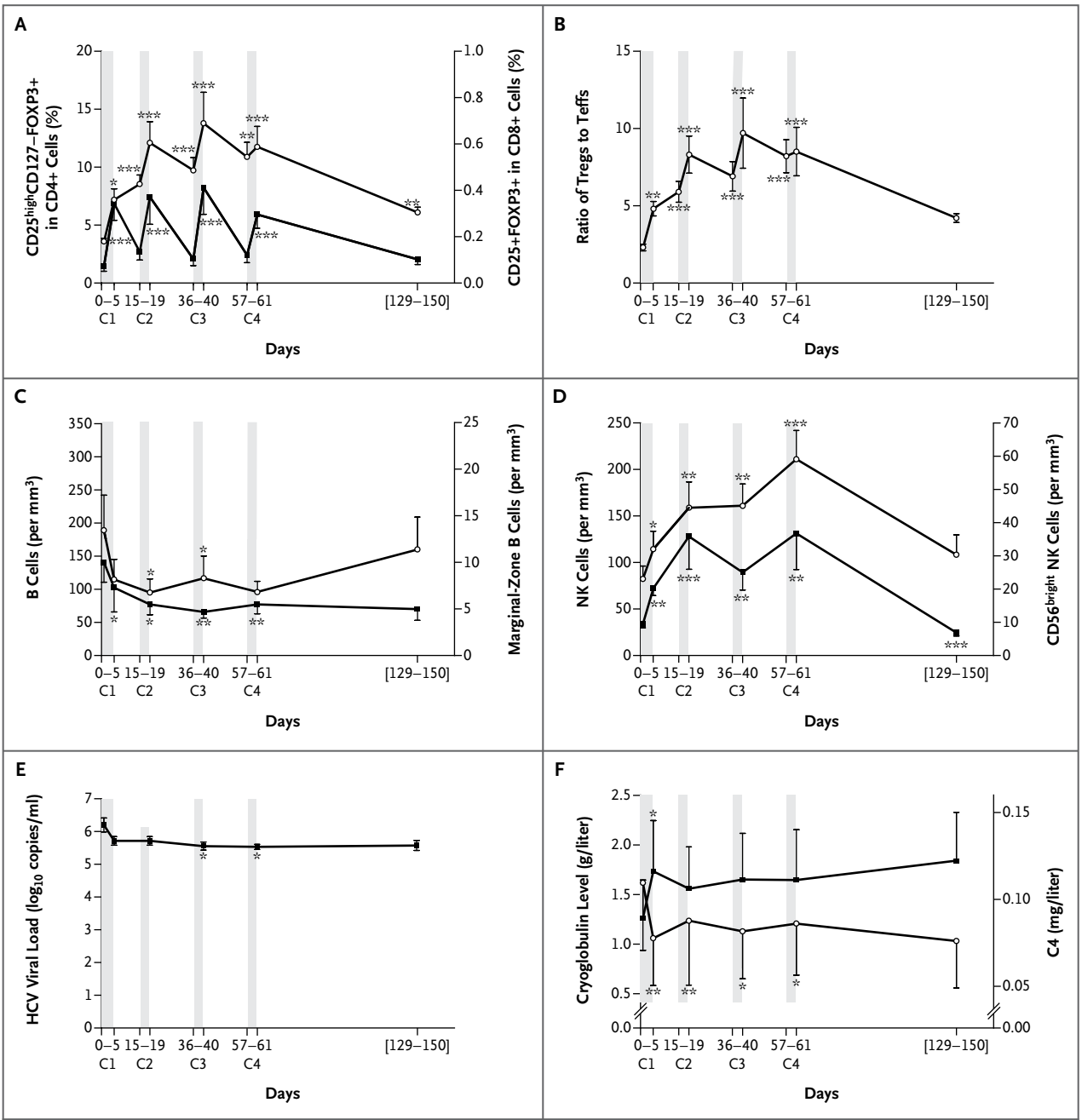

Figure 1. Effects of Low-Dose Interleukin-2 on Levels of Biologic Markers and on Lymphocyte Subpopulations in Patients with Vasculitis Induced by Infection with Hepatitis C Virus (HCV), According to Treatment Course.

Changes in biologic markers and lymphocyte subpopulations are shown for each of the four courses of treatment, with course 1 (C1) consisting of 1.5 million IU of interleukin-2 per day for 5 days and courses 2, 3, and 4 (C2, C3, and C4) consisting of 3.0 million IU of interleukin-2 per day for 5 days. Changes in percentages of $CD25^{high}CD127$–Forkhead box P3+ (FOXP3) within CD4+ cells (circles) and of CD25+FOXP3+ within CD8+ (squares) are shown in Panel A, the ratio of regulatory T cells (Tregs) to the sum of the effector T cells (Teffs) CD4+ and CD8+ in Panel B, absolute numbers of CD19+ total B cells (circles) and marginal-zone B cells (squares) in Panel C, absolute numbers of natural killer (NK) cells (circles) and $CD56^{bright}$ NK cells (squares) in Panel D, the HCV viral load in Panel E, and serum levels of cryoglobulin (circles) and C4 complement (squares) in Panel F. Data are expressed as means ±SE for 10 patients. One asterisk (*) indicates P<0.05, two indicate P<0.01, and three indicate P<0.001.

proportion of CD8+, CD25+, FOXP3+ T cells increased by approximately 500% during the first course of treatment and remained elevated throughout all four courses of treatment (Fig. 1A, and Table S1 in the Supplementary Appendix).

The number of B cells decreased immediately after the first course of treatment with interleukin-2 and then recovered after therapy (mean number of weeks until recovery, 12; median, 12). This decrease particularly affected the IgD+ and CD27+ marginal-zone B cells that are implicated in the pathophysiology of vasculitis[21] (Fig. 1C, and Fig. S4 in the Supplementary Appendix). In contrast, there was a significant and continuous increase in the numbers of natural killer cells over time; their numbers returned to baseline levels after discontinuation of interleukin-2 (Fig. 1D, and Table S1 in the Supplementary Appendix). We observed an increase in the numbers of the CD56 bright natural killer cells that produce high levels of immunoregulatory cytokines and are poorly cytotoxic[22] (Fig. 1D, and Table S1 in the Supplementary Appendix).

BIOLOGIC AND CLINICAL EFFICACY

The HCV viral load modestly decreased throughout the treatment period and was significantly lower at week 9 as compared with baseline ($P=0.02$), in the absence of any antiviral treatment (Fig. 1E). Serum levels of cryoglobulin decreased ($P=0.003$) and C4 increased ($P=0.03$) after the administration of the four courses of interleukin-2 (Fig. 1F). Cryoglobulin levels continued to decrease through week 9 ($P=0.01$). No antinuclear antibodies developed in any patients, and antinuclear antibodies could no longer be detected in one patient.

Consistent with the improved biologic measures of HCV-induced mixed cryoglobulinemic vasculitis (Fig. 1), 8 of 10 patients showed clinical improvement after interleukin-2 therapy: purpura was not noted in all 8 patients who had it at baseline and arthralgia was not reported in all 3 patients who reported having it at baseline. Measures of kidney function normalized (daily proteinuria <0.3 g of protein per 24 hours, and no detection of hematuria) in 1 patient. The only patients who showed no clinical response to treatment were 2 patients who had presented with neuropathy as the sole symptom of vasculitis at the time of inclusion in the study. Clinical improvement started after the first course of treatment in 2 of the 10 patients and after the second course in 6 of the remaining 8 patients, and these improvements coincided with the maximum rate of increase in levels of Tregs (Fig. 2).

INFLAMMATION

We analyzed the transcriptome of peripheral-blood mononuclear cells before and after the administration of interleukin-2 (Table 2, and Fig. S5 in the Supplementary Appendix) (MIAMExpress database, accession number E-MTAB-563). We first performed a supervised analysis, comparing the two sets of data directly to identify genes that were up-regulated or down-regulated. The results were consistent with our phenotypic observations, showing increased expression of genes related to Treg and natural killer cell function, together with a decreased expression of genes related to B-cell function (not shown). Hierarchical clustering revealed a decrease in the expression of genes associated with mediators of inflammation or oxidative stress (Fig. S5 in the Supplementary Appendix), implicating the nuclear factor-κB pathway in this regulation. We confirmed these results by using an unsupervised analysis (i.e., not hypothesis-driven) in which we mixed the transcriptome data from samples taken before and after the administration of interleukin-2 and sought signatures maximizing the segregation of the data in independent groups by means of independent component analysis.[23] We examined Gene Ontology terms and pathways (as designated by the Gene Ontology Consortium) in the signatures that differentiated the groups according to pretreatment and post-treatment (Table 2). The ratio of up-regulated to down-regulated terms and pathways was 0:251 for inflammation ($P=1.30\times10^{-40}$), 16:684 for immune responses ($P=3.40\times10^{-94}$), and 77:555 for lymphocyte activation ($P=7.00\times10^{-49}$) (Table 2). Conversely, we obtained 1701 up-regulated signatures and 208 down-regulated signatures enriched with the Gene Ontology terms and pathways related to cell cycle ($P=1.50\times10^{-138}$). A similar analysis conducted with randomly chosen control terms showed no enrichment. We then carried out a similar analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways terms related to autoimmune and transplantation-related pathologic conditions and to inflammatory infectious diseases (Table 2). These signatures were

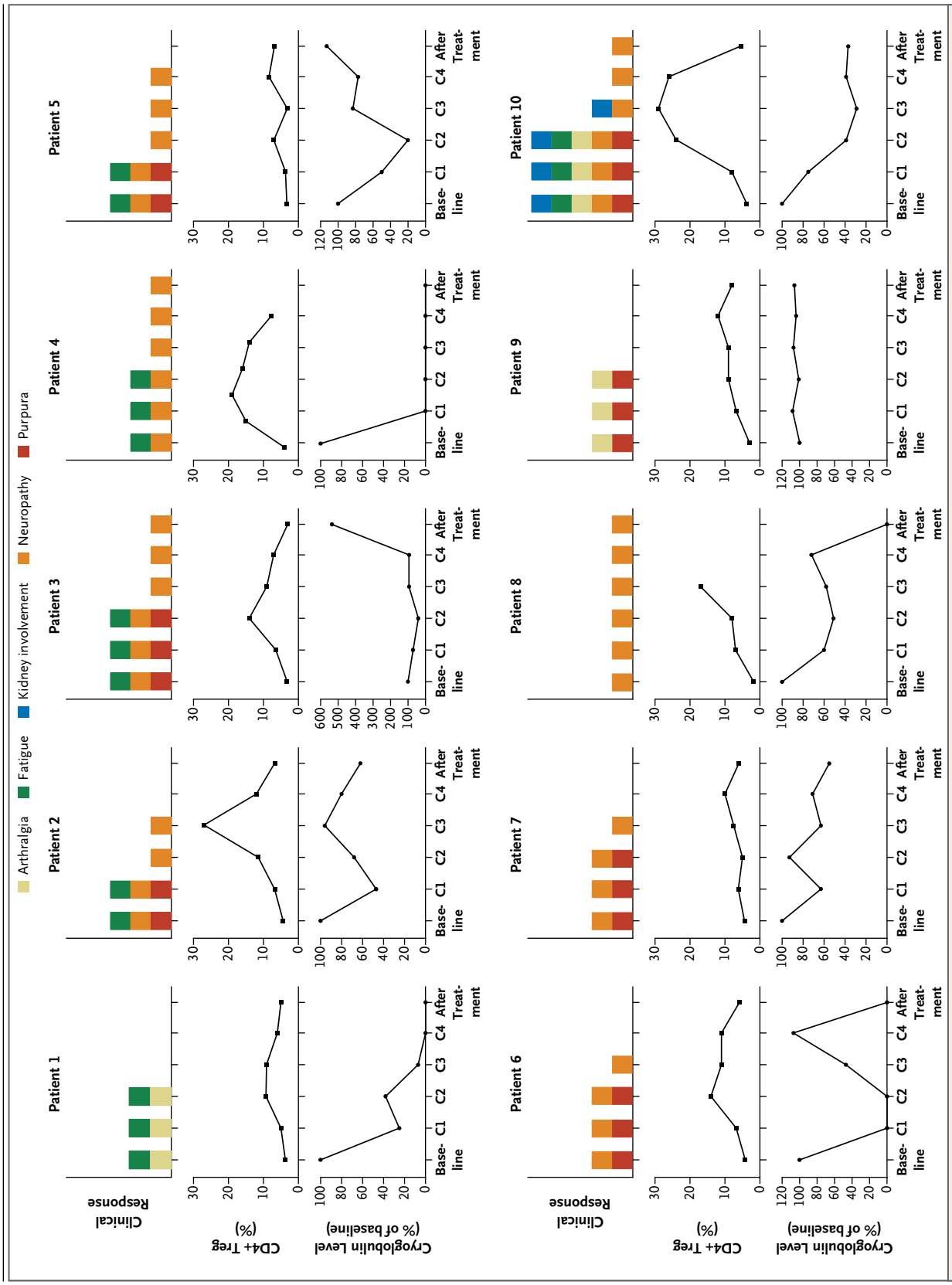

Figure 2. Temporal Effects of Low-Dose Interleukin-2 on Clinical Features, Levels of Regulatory T Cells, and Cryoglobulin for Each Study Patient.
For each patient and at each time point during follow-up, the upper rows of panels indicate the absence or presence of clinical signs, the middle rows of panels the percentages of CD4+ regulatory T cells (Tregs), and the lower rows of panels the levels of cryoglobulin after each administration of interleukin-2 as a percentage of levels at baseline.

Table 2. Antiinflammatory Effects of Low-Dose Interleukin-2 Revealed through Unsupervised Transcriptome Analyses of Peripheral-Blood Mononuclear Cells.*

Terms and Pathways	Up-Regulated Signature	Down-Regulated Signature	P Value
	number of signatures		
Gene Ontology			
Inflammation	0	251	1.30×10^{-40}
Immune response	16	684	3.40×10^{-94}
Lymphocytes	77	555	7.00×10^{-49}
Cell cycle	1701	208	1.50×10^{-138}
Control	226	343	2.50×10^{-1}
KEGG			
Autoimmune diseases or complications of transplantation	0	46	7.60×10^{-9}
Inflammatory infectious diseases	6	242	7.60×10^{-36}
Other diseases	190	211	4.15×10^{-2}

* The table shows the number of up-regulated or down-regulated signatures in peripheral-blood mononuclear cells that have significant enrichment for Gene Ontology terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways related to inflammation, immune response, autoimmune disease (type 1 diabetes mellitus, systemic lupus erythematosus, autoimmune thyroid disease), transplantation (graft-versus-host disease and allograft rejection), or inflammatory infection-related pathologic conditions (Chagas' disease, leishmaniasis, *Helicobacter pylori* infection, malaria, amebiasis, or shigellosis, all characterized by a high degree of inflammation). For controls, the same number of randomly selected Gene Ontology terms was tested, together with cell-cycle–related Gene Ontology terms and control pathologic conditions. For each term coined by the Gene Ontology Consortium and each pathway named in KEGG, the P value, calculated with the use of a chi-square test, indicates a possible enrichment bias for up-regulated or down-regulated signatures as compared with the overall up-regulated (2527) or down-regulated (3429) signatures.

preferentially down-regulated after treatment with interleukin-2 ($P=7.6\times10^{-9}$ and $P=7.6\times10^{-36}$, respectively), whereas the signatures for other diseases were not.

DISCUSSION

We report an in vivo expansion of potent suppressive Tregs in response to low-dose interleukin-2 immunotherapy in patients with an autoimmune-related disease in the absence of concomitant use of glucocorticoids or immunosuppressive treatments. Interleukin-2 continues to be used largely for cancer immunotherapy and has recently been investigated for the treatment of chronic graft-versus-host diseases.[24] Its investigation as a potential therapeutic agent for human autoimmune diseases (particularly those in which there is an insufficiency of Tregs) has been limited, probably because of the perceived risks associated with such treatment. Indeed, because of its capacity to stimulate effector T cells, interleukin-2 carries the risk of activating the very cells that mediate autoimmunity. We provide biologic evidence showing that low doses of interleukin-2 can induce the activation of Tregs without activating other T cells and clinical evidence showing that there were no adverse events related to immune activation, such as vasculitis flare. Our data show that the use of low-dose interleukin-2 tips the Treg–effector T cell balance in favor of Tregs. The use of low-dose interleukin-2 in the treatment of chronic graft-versus-host disease is reported elsewhere in this issue of the *Journal* by Koreth et al.[24] In this study, in which the patients received glucocorticoids or immunosuppressants, there were no clinical flares and the Treg–effector T-cell balance was tipped in favor of Tregs, as was the case in our study, in which patients received neither glucocorticoids nor immunosuppressants.

We observed a biologic and clinical response of HCV-induced mixed cryoglobulinemia to therapy. Serum levels of cryoglobulin significantly decreased, whereas levels of complement C4 increased or normalized in the presence of low-dose interleukin-2 therapy. We observed a significant decrease of marginal-zone B cells (their clonal expansion has been documented during disease progression in persons with HCV-induced mixed cryoglobulinemia).[21] We also observed remission

of the main symptoms of vasculitis (i.e., arthralgia, purpura, and nephropathy) in 8 of the 10 study patients. We conclude that the administration of low-dose interleukin-2 was therapeutic for mixed cryoglobulinemic vasculitis associated with HCV infection. The moderate diminution of HCV viral load that we observed seems unlikely to have affected the course of mixed cryoglobulinemic vasculitis.[16,25] However, it has been reported that HCV-specific populations of T cells fail to produce interleukin-2 in chronic infection, and that the use of supplemental interleukin-2 in vitro can restore some of the anti-HCV reactivity of T cells.[26,27] It is thus possible that interleukin-2 therapy improved anti-HCV immune responses and thereby had a modest effect on viral load.

The cause of HCV-induced mixed cryoglobulinemic vasculitis remains unknown. The associated deficiency in Tregs is quantitative, not qualitative.[10] The exhaustion of type 1 helper T cells in late chronic HCV infection[26] could be a factor in the diminished levels of interleukin-2 and thereby the reduced numbers of Tregs. Alternatively, there could be an inherited risk of immunodeficiency that affects the subgroup of HCV-infected patients in whom this cryoglobulinemic syndrome develops.

When we designed the trial we had little sense of what constituted a dose of interleukin-2 that would safely induce the production of Tregs in persons with an immunopathologic condition. We defined a goal of 3 million IU per day and started with a 5-day course that included the administration of 1.5 million IU per day, with safety monitoring. This dose led to a significant increase of Treg percentages in all patients; adverse events were minor and transient. Although we monitored Treg levels only in peripheral-blood mononuclear cells, we believe the increase in these cells reflected a global increase in the proportion of Tregs, given previous observations in mice after exposure to low-dose interleukin-2. The initial course of treatment was followed by three 5-day courses involving the administration of 3 million IU per day. We observed minor adverse clinical events at this dose. During each treatment period we observed a further increase in proportions of Tregs. We do not know whether this increase was consequent to the increased dosage, to the repetition of treatment, or both. Further studies are needed to determine whether this intervention could be further modified and whether it would also be effective in the treatment of other inflammatory and autoimmune diseases, such as atherosclerosis[28] or type 1 diabetes.[29]

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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