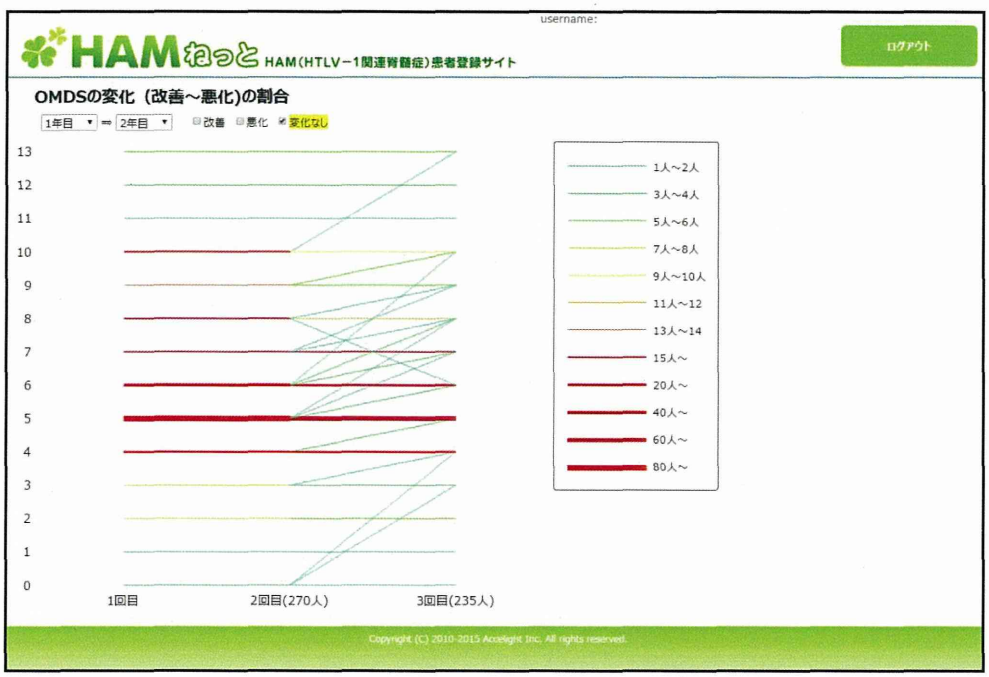
グラフ表示　OMDS変化　変化なしのみ表示（始点1回目、終点2回目）



【添付資料 3】

新　聞　記　事

2014年(平成26年)6月24日　火曜日
南日本新聞

難病HAM ウイルスで細胞変質

聖マリ医大研究班解明 治験中の新薬有望


山野嘉久准教授

鹿児島や宮崎に患者が多いとされ、国の難病にも指定されているHTLV－1関連脊髄症（HAM）で、原因ウイルスの感染細胞内で生み出されるタンパク質が、感染した細胞を、脊髄の炎症を増幅させる異常細胞へと変質させていることを、聖マリアンナ医科大学（神奈川県）の山野嘉久准教授＝鹿児島大学大学院出身＝の研究グループが解明した。ウイルスによって、細胞が変質しているのを突き止めたのは初めてという。論文は24日付の米科学誌ジャーナル・オブ・クリニカル・インベスティゲーションに掲載された。

HTLV－1ウイルスは、異物から体を守る免疫反応をつかさどるT細胞（リンパ球の一つ）に感染。特に、表面にCCR4というタンパク質を出し、免疫反応を抑える種類のT細胞に感染する。免疫反応を進めるT細胞もあり、通常はバランスが保たれている。

山野准教授らのグループは、HAM患者の髄液や脊髄病変部のCCR4陽性T細胞に、健康な人の細胞では一緒に現れるはずのない、炎症を引き起こすインターフェロン・ガンマを誘導するT－bet転写因子があることに着目。ウイルス感染細胞内で生み出されるTaxタンパク質が増えると、T－bet転写因子が増えることも突き止めた。

T－bet転写因子がHAM治療薬として、CCR4をターゲットにした分子標的薬「抗CCR4抗体KW－0761」（ポテリジオ）を治験中。この薬が、感染細胞を減らし、インターフェロン・ガンマの量を減らすことも確認した。

山野准教授は「HAM病態の主軸を解明し、新薬の有望性を裏付ける根拠を証明できた」と話している。（西元貴子）

2014年6月25日　水曜日
西日本新聞

指定難病「HAM」

細胞変質の仕組み解明

成人T細胞白血病ウイルス HTLV1 制圧へ

聖マリアンナ医大

九州に感染者が多いウイルスHTLV1が健康な細胞を病原性細胞に変質させ、厚生労働省の指定難病「脊髄症（HAM）」を引き起こす仕組みを、聖マリアンナ医科大の山野嘉久准教授（神経内科）のグループが突き止めた。24日付の米科学誌ジャーナル・オブ・クリニカル・インベスティゲーションに論文を発表した。

山野准教授によると、HTLV1は人体の免疫反応を制御する普通T細胞（リンパ球）に主に感染し、細胞内にTaxというタンパク質を作り出す。このTaxがT－betという遺伝子に作用することで①リンパ球が悪玉T細胞（病原性細胞）に変わり、HAMを発症の脊髄の炎症を増幅させるインターフェロンγというタンパク質が急増－する。

HAM患者の髄液を解析すると、T細胞の7割以上が病原性細胞だった。これまでに病原性細胞が発生した後に症状が進行する仕組みは解明していたが、研究が進んだことで、より根本的な治療が期待できる。

一方、HAMと同じくHTLV1が原因で起きる難治性血液がん・成人T細胞白血病（ATL）用の新薬「抗CCR4抗体KW－0761」が病原性細胞をほぼ死滅させ、インターフェロンγを減らすことも確認できた。山野准教授らはこの新薬をHAM患者に投与する臨床試験中で、今のところ重篤な副作用はないという。

山野准教授は「HAMのメカニズムを分子レベルで解明できたことで、新薬の有望性を立証できた」と話している。（坂本信博）

HTLV1関連脊髄症（HAM）　主に母乳を介して母子感染し、国内感染者が推計100万人を超えるウイルスHTLV1が、血液中のリンパ球に感染して脊髄に慢性の炎症を起こす神経難病。つえや車椅子が必要になる下半身まひと排尿・排便障害が主症状で、進行すると寝たきりになる。疫学調査によると、発症した国内患者数は推定約3千人で半数が九州に在住。かつては九州の風土病と誤解されていた。

【添付資料４】

講　演　資　料

HAMの新薬とロボットスーツによる治療法についての講演会

日本からHTLVウイルスをなくす会
スマイルリボン

アトムの会会員以外の方もご参加ください。

参加費無料　　定員100名

日時）平成26年4月20日（日）10：30～12：30/13：30～15：30

場所）鹿児島県民交流センター大研修室第3　099-221-6600（代表）

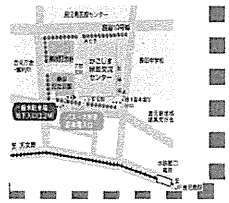
午前の部　10：30～12：30　医療講演

1. 講演
HAMに対する日本発の革新的治療の実用化に向けて～医師主導治験の概要～
聖マリアンナ医科大学難病治療研究センター　山野嘉久先生

2. 講演
HAM歩行障害に対する新しい治療、ロボットスーツHAL-HN01による治験準備について　独立行政法人国立病院機構新潟病院　中島　孝先生

午後の部　13:30 ～15：30　患者と家族・医師との交流・相談会

主催：■ＮＰＯ法人スマイルリボン（全国ＨＡＭ患者友の会「アトムの会」鹿児島支部）
■平成26年度厚生労働科学研究費補助金（難治性疾患等実用化研究事業）「HAMの革新的な治療法となる抗CCR4抗体療法の実用化に向けた開発」■希少性難治性疾患－神経・筋難病疾患の進行抑制治療効果を得るための新たな医療機器、生体電位等で随意コントロールされた下肢装着型補助ロボット（HAL-HN01）に関する医師主導治験の実施研究
共催：■　かごしま難病支援ネットワーク



※当日受付OKですが予約された方を優先いたします。
★問い合わせ：スマイルリボン事務局
099－800－3112
メール nakusukai@po.minc.ne.jp

HAMの臨床研究に関する打ち合わせ並びにセミナーのお知らせ

【日時】2014年9月22日（火）17:00～

【会場】福岡大学医学部3階B会議室

- 17:00～
- 演者：聖マリアンナ医科大学　難病治療研究センター　山野嘉久先生
- 「HTLV-1関連脊髄症（HAM）の有効性評価指標に関する前向き多施設共同臨床研究」打ち合わせ

17:30～
- 演者：聖マリアンナ医科大学　難病治療研究センター　山野嘉久先生
- セミナー「HAMの総論とupdate」

- 18:00～
- 演者：独立行政法人国立病院機構新潟病院　中島孝　先生
- 「希少性難治性疾患－神経・筋難病疾患の進行抑制治療効果を得るための新たな医療機器、生体電位等で随意コントロールされた下肢装着型補助ロボット（HAL-HN01）に関する医師主導治験の実施研究班　NCY-2001試験」打ち合わせ

- 18:45-19:30（4階東病棟リハビリテーション室へ移動）
- 演者：独立行政法人国立病院機構新潟病院　中島孝　先生
- CRC、PT、サイバーダイン、治験責任医師などとの打ち合わせ

会終了後に場所を移動して懇親会を予定しています。

福岡大学医学部　神経内科学教室

HAMの新薬とロボットスーツによる治療法についての講演会

日本からHTLVウイルスをなくす会
スマイルリボン

参加費無料　　定員100名

日　時：平成26年9月23日（祝）13：00～15：30

会　場：福岡市市民福祉プラザ『ふくふくプラザ』　5階502研修室

講演1
HAM歩行障害に対する新しい治療、ロボットスーツHAL-HNO1による
治験開始についてのお知らせ
独立行政法人国立病院機構新潟病院　中島　孝先生

講演2
HAMに対する日本発の革新的治療の実用化に向けて～医師主導治験の概要～
聖マリアンナ医科大学難病治療研究センター　山野嘉久先生

主催：NPO法人　スマイルリボン（http://smile-ribbon.org/）
共催：スマイルリボン福岡支部（http://www.ac.auone-net.jp/~srfuk.）アトムの会福岡支部
難治性疾患等実用化研究事業　「HAMの革新的な治療法となる抗CCR4抗体療法の実用化に向けた開発」研究班、「希少性難治性疾患－神経・筋難病疾患の進行抑制治療効果を得るための新たな医療機器、生体電位等で随意コントロールされた下肢装着型補助ロボット（HAL-HNO1）に関する医師主導治験の実施研究」研究班

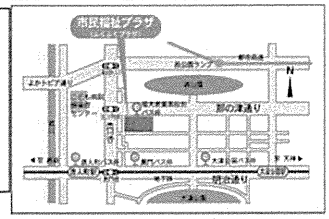
★問い合わせ

※予約された方を優先いたします。

スマイルリボン福岡　（森永）
080-4296-1918
（当日の連絡先は上記へお願いします）

事務局　099-800-3112
メール nakusukai@po.minc.ne.jp

会場　福岡市中央区荒戸3丁目3-39



V.　研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
山野嘉久.	ヒト細胞白血病ウイルスⅠ型関連脊髄症.	水澤英洋	最新医学別冊 新しい診断と治療のABC 神経関連感染症	最新医学社	大阪	2014	200-205
中川正法.	ヒトTリンパ球向性ウイルス脊髄症（HTLV-1関連脊髄症）.	永井良三他	神経内科研修ノート	診断と治療社	東京	2014	178-180
中村龍文.	痙性対麻痺（HAMを含む）.	福井次矢，高木誠，小室一成.	今日の治療指針	医学書院	東京	2015	897-899

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Araya N, Sato T, Ando H, Tomaru U, Yoshida M, Coler-Reilly A, Yagishita N, Yamauchi J, Hasegawa A, Kannagi M, Hasegawa Y, Takahashi K, Kunitomo Y, Tanaka Y, Nakajima T, Nishioka K, Utsunomiya A, Jacobson S, Yamano Y.	HLVL-1 induces a Th1-like state in CD4+CCR4+ T cells.	J Clin Invest	124(8)	3431-3442	2014
Yamauchi J, Coler-Reilly A, Sato T, Araya N, Yagishita N, Ando H, Kunitomo Y, Takahashi K, Tanaka Y, Shibagaki Y, Nishioka K, Nakajima T, Hasegawa Y, Utsunomiya A, Kimura K, Yamano Y.	Anti-CCR4 antibody mogamulizumab targets human T-lymphotropic virus type I-infected CD8+ as well as CD4+ T cells to treat associated myelopathy.	J Infect Dis	211(2)	238-248	2015
Ishihara M, Araya N, Sato T, Saichi N, Fujii R, Yamano Y, Sugano S, Ueda K.	A plasma diagnostic model of human T-cell leukemia virus-1 associated myelopathy.	Ann Clin Transl Neurol	2(3)	231-240	2015
Bangham C, Taylor G, Yamano Y, Araujo A.	HTLV-I-Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP).	Nature Reviews Disease Primers	in press		2015
Coler-Reilly A, Ando H, Yamano Y.	Positive feedback loop via astrocytes causes chronic inflammation in human T lymphotropic virus type 1-associated myelopathy/tropical spastic paraparesis.	Clin Exp Neuroimmunol	5	108-109	2014
Kawamata T, Ohno N, Sato K, Kobayashi M, Jo N, Yuji K, Tanosaki R, Yamano Y, Tojo A, Uchimaru K.	A case of post-transplant adult T-cell leukemia/lymphoma presenting myelopathy similar to but distinct from human T-cell leukemia virus type I (HTLV- I)-associated myelopathy.	SpringerPlus	3	581	2014
Tezuka K, Xun R, Tei M, Ueno T, Tanaka M, Takenouchi N, Fujisawa JI.	An animal model of adult T-cell leukemia-humanized mice with HTLV-1 specific immunity.	Blood	123(3)	346-355	2014

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Ando R, Nishikawa N , Tsujii T, Iwaki H, Yabe H, Nagai M, Nomoto M.	HTLV-I-associated Myelopathy with Bulbar Palsy-type Amyotrophic Lateral Sclerosis-like Symptoms: A Case Report.	Internal Med	54(9)	1105-1107	2015
Nakamura H, Takahashi Y, Yamamoto-Fukuda T, Horai Y, Nakashima Y, Arima K, Nakamura T, Koji T, Kawakami A.	Direct Infection of Primary Salivary Gland Epithelial Cells by Human T Lymphotropic Virus Type I in Patients With Sjögren's Syndrome.	Arthritis Rheumatol	67(4)	1096-1106	2015
Nakamura T, Satoh K, Nakamura H, Yamasaki H.	Intracellular cyclic adenosine monophosphate regulates the efficiency of intercellular transmission of human T-lymphotropic virus type I.	Clin Exp Neuroimmunol	5	209-215	2014
Nakamura T, Satoh K, Fukuda T, Kinoshita I, Nishiura Y, Nagasato K, Yamauchi A, Kataoka Y, Nakamura T, Sasaki H, Kumagai K, Niwa M, Noguchi M, Nakamura H, Nishida N, Kawakami A.	Pentosan polysulfate treatment ameliorates motor function with increased serum soluble vascular cell adhesion molecule-1 in HTLV-1 associated neurologic disease.	J Neurovirol	20	269-277	2014
Nozuma S, Matsuura E, Matsuzaki T, Watanabe O, Kubota R, Izumo S, Takashima H.	Familial clusters of HTLV-1-associated myelopathy/tropical spastic paraparesis.	PLOS ONE	9(5)	e86144	2014
Matsuura E, Kubota R, Tanaka Y, Takashima H and Izumo S.	Visualization of HTLV-1 Specific Cytotoxic T Lymphocytes in the Spinal Cords of Patient With HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis.	J Neuropathol Exp Neurol	74(1)	2-14	2015
Aach M, Cruciger O, Sczesny-Kaiser M, Hoffken O, Meindl RCh, Tegenthoff M, Schwenkreis, Sankai Y, Schildhauer TA.	Voluntary driven exoskeleton as a new tool for rehabilitation in chronic spinal cord injury: a pilot study.	The Spine Journal	14(12)	2847-2853	2014
Saito M.	Neuroimmunological aspects of human T cell leukemia virus type 1-associated myelopathy/tropical spastic paraparesis.	J Neurovirol	20(2)	164-174	2014
Tanaka Y, Takahashi Y, Tanaka R, Kodama A, Fujii H, Hasegawa A, Kannagi M, Ansari AA, Saito M.	Elimination of human T cell leukemia virus type-1-infected cells by neutralizing and antibody-dependent cellular cytotoxicity-inducing antibodies against human T cell leukemia virus type-1 envelope gp46.	AIDS Res Hum Retroviruses	30(6)	542-552	2014
Saito M, Tanaka R, Fujii H, Kodama A, Takahashi Y, Matsuzaki T, Takashima H, Tanaka Y.	The neutralizing function of the anti-HTLV-1 antibody is essential in preventing in vivo transmission of HTLV-1 to human T cells in NOD-SCID/γcnull (NOG) mice.	Retrovirology	11(1)	74	2014
Ma G, Yasunaga J-I, Akari H, Matsuoka M.	TCF1 and LEF1 act as T-cell intrinsic HTLV-1 antagonists by targeting Tax.	Proc. Natl. Acad. Sci. USA	112(7)	2216-2221	2015

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Takachi T, Takahashi M, Takahashi-Yoshita M, Higuchi M, Obata M, Mishima Y, Okuda S, Tanaka Y, Matsuoka M, Saitoh A,	Human T-cell leukemia virus type 1 Tax oncoprotein represses the expression of the BCL11B tumor suppressor in T-cells.	Cancer Sci	106(4)	461-465	2015
Kinpara S, Ito S, Takahata T, Saitoh Y, Hasegawa A, Kijiyama M, Utsunomiya A, Masuda M, Miyazaki Y, Matsuoka M, Nakamura M, Yamaoka S, Masuda T, Kannagi M.	Involvement of double-stranded RNA-dependent protein kinase and anti-sense viral RNA in the constitutive NFκB activation in adult T-cell leukemia/lymphoma cells.	Leukemia	in press		2015
Suehiro Y, Hasegawa A, Iino T, Sasada A, Watanabe N, Matsuoka M, Takamori A, Tanosaki R, Utsunomiya A, Choi I, Fukuda T, Miura O, Takaishi S, Teshima T, Akashi K, Kannagi M, Uike N, Okamura J.	Clinical outcomes of a novel therapeutic vaccine with Tax peptide-pulsed dendritic cells for adult T cell leukaemia/lymphoma in a pilot study.	Br J Haematol	169(3)	356-367	2015
Niederer HA, Laydon DJ, Melamed A, Elemans M, Asquith B, Matsuoka M, Bangham CR.	HTLV-1 proviral integration sites differ between asymptomatic carriers and patients with HAM/TSP.	Virol J	11	172	2014
Lavorgna A, Matsuoka M, Harhaj EW.	A critical role for IL-17RB signaling in HTLV-1 Tax-induced NF-κB activation and T-cell transformation.	PLoS Pathogens	10(10)	e1004418	2014
Cook LB, Melamed A, Niederer H, Valganon M, Laydon D, Foroni L, Taylor GP, Matsuoka M, Bangham CR.	The role of HTLV-1 clonality, proviral structure and genomic integration site in adult T cell leukemia/lymphoma.	Blood	123	3925-3931	2014
Furuta RA, Ma G, Matsuoka M, Otani S, Matsukura H, Hirayama F.	Re-evaluation of screening of plasma positive for human T-cell leukemia virus type 1 using a luciferase immunoprecipitation system in blood donors.	Transfusion	in press		2015
Zhao, T, Satou Y and Matsuoka M.	Development of T cell lymphoma in HTLV-1 bZIP factor and Tax double transgenic mice.	Arch Virol	159	1849-1856	2014
Tanaka-Nakanishi A, Yasunaga J-I, Takai K and Matsuoka M.	HTLV-1 bZIP factor suppresses apoptosis by attenuating the function of FoxO3a and altering its localization.	Cancer Res	74	188-200	2014
Kawamata T, Ohno N, Sato K, Kobayashi M, Jo N, Yuji K, Tanosaki R, Yamano Y, Tojo A, Uchimaru K.	A case of post-transplant adult T-cell leukemia/lymphoma presenting myelopathy similar to but distinct from human T-cell leukemia virus type I (HTLV- I)-associated myelopathy.	Springerplus	4:3	581	2014
Ishigaki T, Zaike Y, Nojima M, Kobayashi S, Ohno N, Uchimaru K, Tojo A, Nakauchi H, Watanabe N.	Quantification of adult T-cell leukemia/lymphoma cells using simple four-color flow cytometry.	Clin Chem Lab Med	53(1)	85-93	2015

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Takahashi R, Yamagishi M, Nakano K, Yamochi T, Yamochi T, Fujikawa D, Nakashima M, Tanaka Y, Uchimaru K, Utsunomiya A, Watanabe T.	Epigenetic deregulation of Ellis Van Creveld confers robust Hedgehog signaling in adult T-cell leukemia.	Cancer Sci	105(9)	1160-1169	2014
Kobayashi S, Nakano K, Watanabe E, Ishigaki T, Ohno N, Yuji K, Oyaizu N, Asanuma S, Yamagishi M, Yamochi T, Watanabe N, Tojo A, Watanabe T, Uchimaru K.	CADM1 expression and stepwise downregulation of CD7 are closely associated with clonal expansion of HTLV-I-infected cells in adult t-cell leukemia/lymphoma.	Clin Cancer Res	20(11)	2851-2861	2014
Kobayashi S, Watanabe E, Ishigaki T, Ohno N, Yuji K, Nakano K, Yamochi T, Watanabe N, Tojo A, Watanabe T, Uchimaru K.	Advanced HTLV-1 carriers and early-stage indolent ATLs are indistinguishable based on CADM1 positivity in flow cytometry.	Cancer Sci	in press		2015
石塚賢治、山野嘉久、宇都宮與、内丸薫.	HTLV-1キャリア外来の実態調査.	臨床血液	in press		2015
山野嘉久.	HTLV-1関連脊髄症（HAM）.	別冊日本臨牀	30	153-156	2014
山野嘉久.	HTLV-1の神経障害.	内科	113(6)	1431	2014
山野嘉久.	HTLV-1関連脊髄症（HAM）の分子病態に基づく治療戦略.	細胞	46(6)	258-261	2014
新谷奈津美，山野嘉久.	HTLV-1関連脊髄症（HAM）に対する分子標的治療薬開発の現状と将来.	血液内科	68(1)	30-35	2014
山野嘉久.	希少な慢性進行性の神経難病HAMにおける治療有効性評価モデルの探索.	臨床評価　別冊	41(3)	504-508	2014
中川正法.	レトロウイルス感染症（HTLV-1とHIV）.	化学療法の領域	30(8)	1584-1594	2014
松浦英治，髙嶋博.	HTLV-1関連脊髄症.	別冊BIO Clinica 慢性炎症と疾患	3(1)	29-35	2014
松浦英治，　出雲周二.	HTLV-1 associated myelopathy(HTLV-1関連脊髄症;HAM)－日常臨床におけるHTLV-1の理解とHAM診断のピットフォール－.	脊椎脊髄ジャーナル	27(8)	747- 753	2014
中島孝.	難病ケアにおけるコペルニクス的転回 臨床評価を患者・家族の主観的評価に変える.	総合診療	25(3)	206-209	2015
内丸薫.	わが国におけるHTLV-1キャリアとATL患者に対する相談機能と知識の普及.	血液内科	68(1)	58-64	2014
内丸薫.	成人T細胞白血病（ATL）.	検査と技術	42	1370-1375	2014
内丸薫.	成人T細胞白血病.	Medicina	52(4)	in press	2015

VI.　研究成果の刊行物・別刷

HTLV-1 induces a Th1-like state in CD4$^+$CCR4$^+$ T cells

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Human T-lymphotropic virus type 1 (HTLV-1) is linked to multiple diseases, including the neuroinflammatory disease HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T cell leukemia/lymphoma. Evidence suggests that HTLV-1, via the viral protein Tax, exploits CD4$^+$ T cell plasticity and induces transcriptional changes in infected T cells that cause suppressive CD4$^+$CD25$^+$CCR4$^+$ Tregs to lose expression of the transcription factor FOXP3 and produce IFN-γ, thus promoting inflammation. We hypothesized that transformation of HTLV-1–infected CCR4$^+$ T cells into Th1-like cells plays a key role in the pathogenesis of HAM/TSP. Here, using patient cells and cell lines, we demonstrated that Tax, in cooperation with specificity protein 1 (Sp1), boosts expression of the Th1 master regulator T box transcription factor (T-bet) and consequently promotes production of IFN-γ. Evaluation of CSF and spinal cord lesions of HAM/TSP patients revealed the presence of abundant CD4$^+$CCR4$^+$ T cells that coexpressed the Th1 marker CXCR3 and produced T-bet and IFN-γ. Finally, treatment of isolated PBMCs and CNS cells from HAM/TSP patients with an antibody that targets CCR4$^+$ T cells and induces cytotoxicity in these cells reduced both viral load and IFN-γ production, which suggests that targeting CCR4$^+$ T cells may be a viable treatment option for HAM/TSP.

Introduction

The flexibility of the CD4$^+$ T cell differentiation program that underlies the success of the adaptive immune response has recently been implicated in the pathogeneses of numerous inflammatory diseases (1–3). The majority of CD4$^+$ T lymphocytes belong to a class of cells known as Th cells, so called because they provide help on the metaphorical immune battlefield by stimulating the other soldiers — namely, B cells and cytotoxic T lymphocytes — via secretion of various cytokines. Interestingly, there is also a minority group of CD4$^+$ T cells with quite the opposite function: Tregs actively block immune responses by suppressing the activities of CD4$^+$ Th cells as well as many other leukocytes (4). Tregs are credited with maintaining immune tolerance and preventing inflammatory diseases that could otherwise occur as a result of uninhibited immune reactions (5). Thus, the up- or downregulation of certain CD4$^+$ T cell lineages could disrupt the carefully balanced immune system, threatening bodily homeostasis.

The plasticity of CD4$^+$ T cells, particularly Tregs, makes CD4$^+$ T cell lineages less clean-cut than they may originally appear. CD4$^+$ T cells are subdivided according to various lineage-specific chemokine receptors and transcription factors they express, as well as the cytokines they produce (6). Th1 cells, for example, can be identified by expression of CXC motif receptor 3 (CXCR3) and T box transcription factor (T-bet; encoded by *TBX21*) and are known to secrete the proinflammatory cytokine IFN-γ (6). While both have been known to express CC chemokine receptor 4 (CCR4) and CD25, Th2 cells and Tregs can usually be distinguished from each other by their expression of GATA-binding protein 3 (GATA3) and forkhead box p3 (FOXP3), respectively (6, 7). CCR4 is coexpressed in the majority of CD4$^+$FOXP3$^+$ cells and in virtually all CD4$^+$CD25$^+$FOXP3$^+$ cells, making it a useful — albeit not fully specific — marker for Tregs (8, 9). FOXP3 is a particularly noteworthy marker because its expression is said to be required for Treg identity and function (10). In fact, *Foxp3* point mutations are reported to cause fatal multiorgan autoimmune diseases (11). Even partial loss of FOXP3 expression can disrupt the suppressive nature of Tregs, representing one of several pathways by which even fully differentiated Tregs can reprogram into inflammatory cells (12). There have been several reports of Tregs reprogramming in response to proinflammatory cytokines such as IL-1, IL-6, IL-12, and IFN-γ (12, 13); it is thought that this reprogramming may have evolved as an adaptive mechanism for dampening immune suppression when protective inflammation is necessary (12). However, this same plasticity can lead to pathologically chronic inflammation, and several autoimmune diseases have been associated with reduced FOXP3 expression and/or Treg function, including multiple sclerosis, myasthenia gravis, and type 1 diabetes (14, 15).

Of the roughly 10–20 million people worldwide infected with human T-lymphotropic virus type 1 (HTLV-1), up to 2%–3% are affected by the neurodegenerative chronic inflammatory dis-

Conflict of interest: The authors have declared that no conflict of interest exists.
Submitted: January 17, 2014; **Accepted:** May 8, 2014.
Reference information: *J Clin Invest.* 2014;124(8):3431–3442. doi:10.1172/JCI75250.

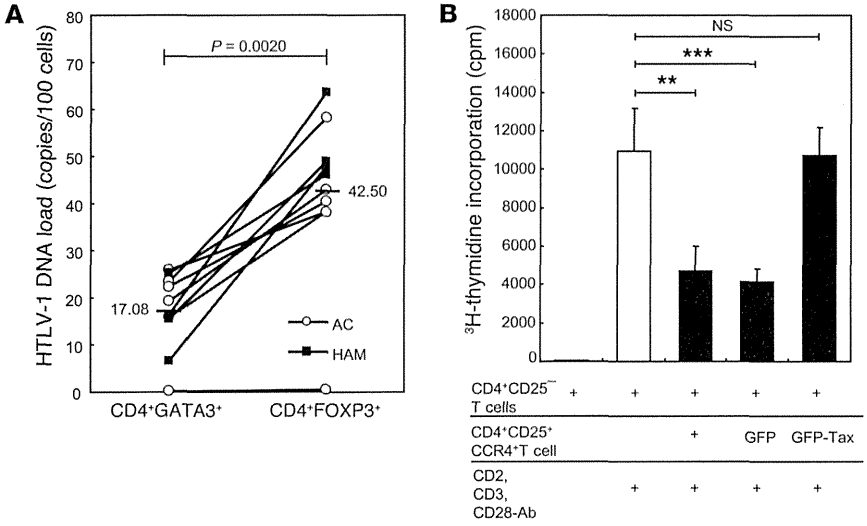

Figure 1. HTLV-1 mainly infects Tregs and inhibits their regulatory function. (**A**) Higher HTLV-1 proviral DNA load in $CD4^+FOXP3^+$ cells (Tregs) compared with $CD4^+GATA3^+$ cells ($P = 0.0020$, Wilcoxon test) from asymptomatic carriers (AC; $n = 6$) and HAM/TSP patients ($n = 4$). PBMCs were FACS sorted, and proviral load was measured using quantitative PCR. Horizontal bars represent the mean value for each set. (**B**) Loss of regulatory function in Tax-expressing $CD4^+CD25^+CCR4^+$ cells (Tregs). $CD4^+CD25^-$ T cells from an HD were stimulated with CD2, CD3, and CD28 antibodies and cultured alone or in the presence of equal numbers of $CD4^+CD25^+CCR4^+$ T cells, GFP lentivirus–infected HD $CD4^+CD25^+CCR4^+$ T cells, or GFP-Tax lentivirus–infected HD $CD4^+CD25^+CCR4^+$ T cells. As a control, $CD4^+CD25^-$ T cells alone were cultured without any stimulus. Proliferation of T cells was determined using ^{3}H-thymidine incorporation by adding ^{3}H-thymidine for 16 hours after 4 days of culture. All tests were performed in triplicate. Data are mean ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$, ANOVA followed by Tukey test for multiple comparisons.

ease HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP). The main other condition associated with the retrovirus is adult T cell leukemia/lymphoma (ATLL), a rare and aggressive cancer of the T cells. HAM/TSP represents a useful starting point from which to investigate the origins of chronic inflammation, because the primary cause of the disease — viral infection — is so unusually well defined. HAM/TSP patients share many immunological characteristics with FOXP3 mutant mice, including multiorgan lymphocytic infiltrates, overproduction of inflammatory cytokines, and spontaneous lymphoproliferation of cultured $CD4^+$ T cells (16–18). We and others have proposed that HTLV-1 preferentially infects $CD4^+CD25^+CCR4^+$ T cells, a group that includes Tregs (7, 19). Samples of $CD4^+CD25^+CCR4^+$ T cells isolated from HAM/TSP patients exhibited low FOXP3 expression as well as reduced production of suppressive cytokines and low overall suppressive ability — in fact, these $CD4^+CD25^+CCR4^+FOXP3^-$ T cells were shown to produce IFN-γ and express Ki67, a marker of cell proliferation (19). The frequency of these IFN-γ–producing $CD4^+CD25^+CCR4^+$ T cells in HAM/TSP patients was correlated with disease severity (19). Finally, evidence suggests that the HTLV-1 protein product Tax may play a role in this alleged transformation of Tregs into proinflammatory cells in HAM/TSP patients: transfecting Tax into $CD4^+CD25^+$ cells from healthy donors (HDs) reduced FOXP3 mRNA expression, and Tax expression in $CD4^+CD25^+CCR4^+$ cells was higher in HAM/TSP versus ATLL patients despite similar proviral loads (19, 20). Therefore, we hypothesized that HTLV-1 causes chronic inflammation by infecting $CD4^+CD25^+CCR4^+$ T cells and inducing their transformation into Th1-like, IFN-γ–producing proinflammatory cells via intracellular Tax expression and subsequent transcriptional alterations including but not limited to loss of endogenous FOXP3 expression.

In this study, we first sought to discover the detailed mechanism by which Tax influences the function of $CD4^+CD25^+CCR4^+$ T cells. We used DNA microarray analysis of $CD4^+CD25^+CCR4^+$ T cells from HAM/TSP patients to identify *TBX21*, known as a master transcription factor for Th1 differentiation, as a key intermediary between Tax expression and IFN-γ production. We demonstrated that Tax, in concert with specificity protein 1 (Sp1), amplified *TBX21* transcription and subsequently IFN-γ production. Next, we established the presence of Th1-like $CD4^+CCR4^+$ T cells in the CSF and spinal cord lesions of HAM/TSP patients. The majority of these $CD4^+CCR4^+$ T cells coexpressed CXCR3 as well as T-bet and IFN-γ. Finally, we investigated the therapeutic potential of an anti-CCR4 monoclonal antibody with antibody-dependent cellular cytotoxicity (ADCC) (21). Applying this antibody in vitro diminished the proliferative capacity of cultured PBMCs and reduced both proviral DNA load and IFN-γ production in cultured CSF cells as well as PBMCs. In conclusion, we were able to elucidate a more detailed mechanism for the pathogenesis of HAM/TSP and use our findings to suggest a possible therapeutic strategy.

Results

HTLV-1 preferentially infects Tregs and alters their behavior via Tax. Experiments were conducted to determine which among $CD4^+CD25^+CCR4^+$ T cells were infected by HTLV-1, and how the infection influenced their functionality. Analysis of fluorescence-activated cell sorting (FACS)-sorted PBMCs obtained from asymptomatic carriers ($n = 6$) as well as HAM/TSP patients ($n = 4$) revealed that Tregs ($CD4^+FOXP3^+$) carried much higher proviral loads than Th2 cells ($CD4^+GATA3^+$) ($P = 0.0020$; Figure 1A). As it is well established that each infected cell contains only 1 copy of the HTLV-1 provirus (22, 23), these results indicate that a larger proportion of $FOXP3^+$ than $GATA3^+$ $CD4^+$ T cells are infected. As expected, proliferation of $CD4^+CD25^-$ cells after stimulation, as measured by ^{3}H-thymidine incorporation, was suppressed upon coculture with $CD4^+CD25^+CCR4^+$ cells, including Tregs ($n = 3$, $P < 0.01$; Figure 1B). However, after being transduced with lentiviral vector expressing GFP-Tax, the $CD4^+CD25^+CCR4^+$ cells no longer suppressed cell proliferation; conversely, cells transduced with the control vector expressing only GFP retained full suppressive function ($P < 0.001$; Figure 1B).

The HTLV-1 protein product Tax induces IFN-γ production via T-bet. Experiments were conducted to determine if and how Tax affects IFN-γ production in infected T cells. First, the existence of a functional link between *Tax* and *IFNG* was established by using the

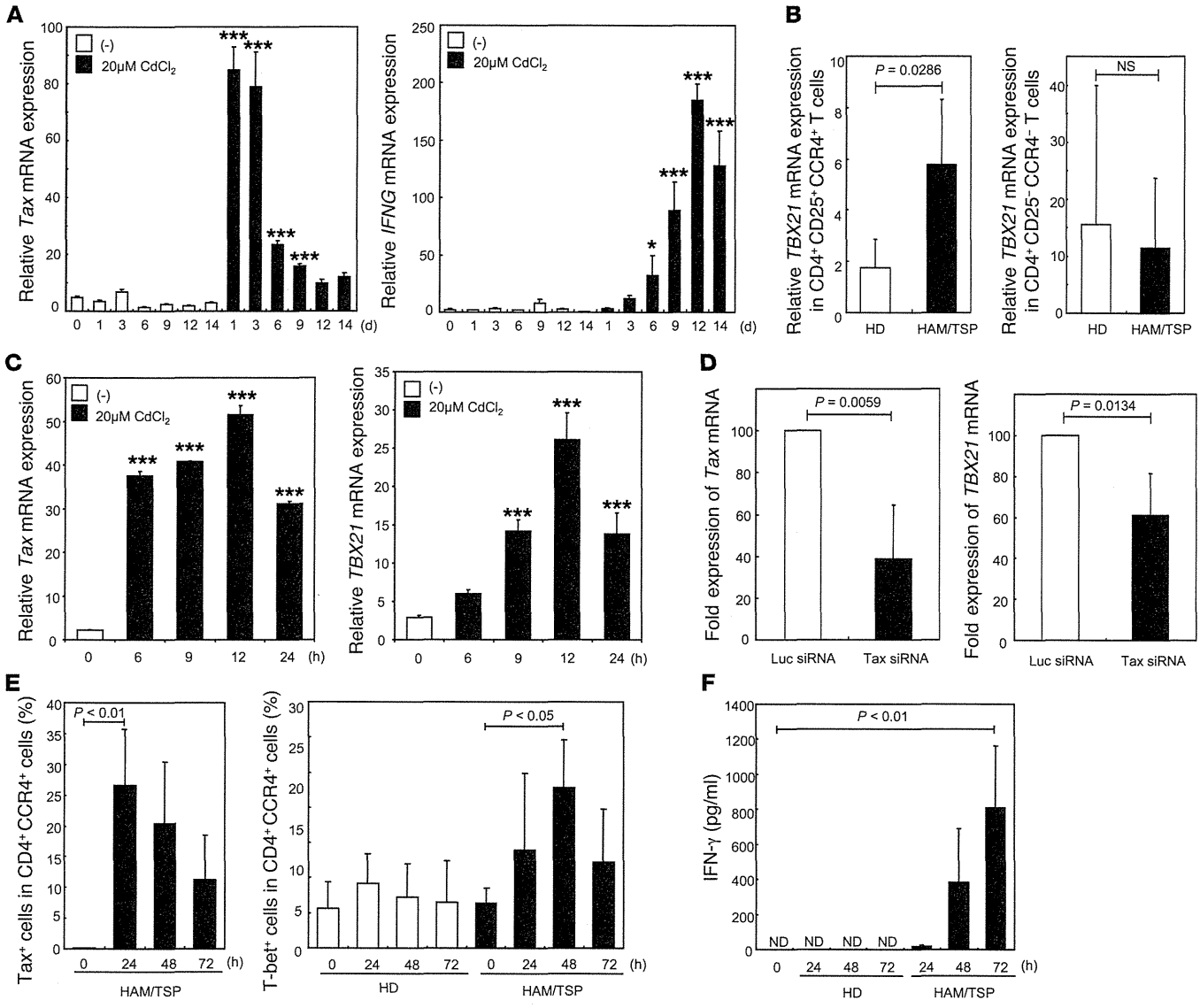

Figure 2. Tax induces IFN-γ production via T-bet. (**A**) Tax-dependent *IFNG* mRNA expression in JPX-9 cells. Experiments were performed in triplicate. (**B**) Elevated *TBX21* mRNA expression in CD4$^+$CD25$^+$CCR4$^+$ T cells from HAM/TSP patients relative to HDs (n = 4 per group). (**C**) Tax-dependent *TBX21* mRNA expression in JPX-9 cells. Experiments were performed in triplicate. (**D**) Reduced *TBX21* mRNA expression after silencing Tax in CD4$^+$CD25$^+$CCR4$^+$ T cells from HAM/TSP patients. PBMCs from HAM/TSP patients (n = 5) were FACS sorted, transfected with either Luc or Tax siRNA, and incubated for 24 hours. (**E** and **F**) Tax expression correlated with T-bet expression and IFN-γ production in CD4$^+$CCR4$^+$ T cells from HAM/TSP patients. CD4$^+$CCR4$^+$ T cells isolated from HDs and HAM/TSP patients (n = 4 per group) were cultured before being stained for Tax and T-bet protein and analyzed using FACS. IFN-γ production in the culture medium was measured using a CBA assay. ND, not detectable. All data are mean ± SD. P values were calculated using (**A** and **C**) 1-way ANOVA followed by Dunnett test for multiple comparisons, (**B**) Mann-Whitney U test, (**D**) paired t test, or (**E** and **F**) Friedman test followed by Dunn test for multiple comparisons. *$P < 0.05$, ***$P < 0.001$ vs. time point 0.

JPX-9 cell line possessing a stably integrated $CdCl_2$-inducible *Tax* construct and measuring *IFNG* mRNA expression. Inducing *Tax* expression with $CdCl_2$ periodically over 2 weeks yielded a steady rise in *IFNG* expression (Figure 2A). Although there was clearly a correlation between Tax and IFN-γ expression, the *IFNG* expression level was not proportional to that of *Tax*, and the steepest rise in the former was delayed several days after the steepest rise in the latter. Thus, we suspected that expression of 1 or more additional genes may represent an important middle step on the pathway linking Tax and IFN-γ production. DNA microarray results revealed that expression of *TBX21*, which is known to be associated with IFN-γ production, was elevated in CD4$^+$CD25$^+$CCR4$^+$ cells from the HAM/TSP patient, but not the ATLL patient, compared with the HD (Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI75250DS1). *TBX21* mRNA expression, measured via real-time RT-PCR, was elevated in CD4$^+$CD25$^+$CCR4$^+$ cells, but not CD4$^+$CD25$^-$CCR4$^-$ cells, from HAM/TSP patients compared with HDs (Figure 2B). A direct correlation between *Tax* and *TBX21* mRNA expression was then established using the JPX-9 cell line, as described above (Figure 2C). Silencing the *Tax* gene with siRNA in CD4$^+$CD25$^+$CCR4$^+$ cells from HAM/TSP patients reduced *TBX21* as well as *Tax* expression (Figure 2D). Similarly,

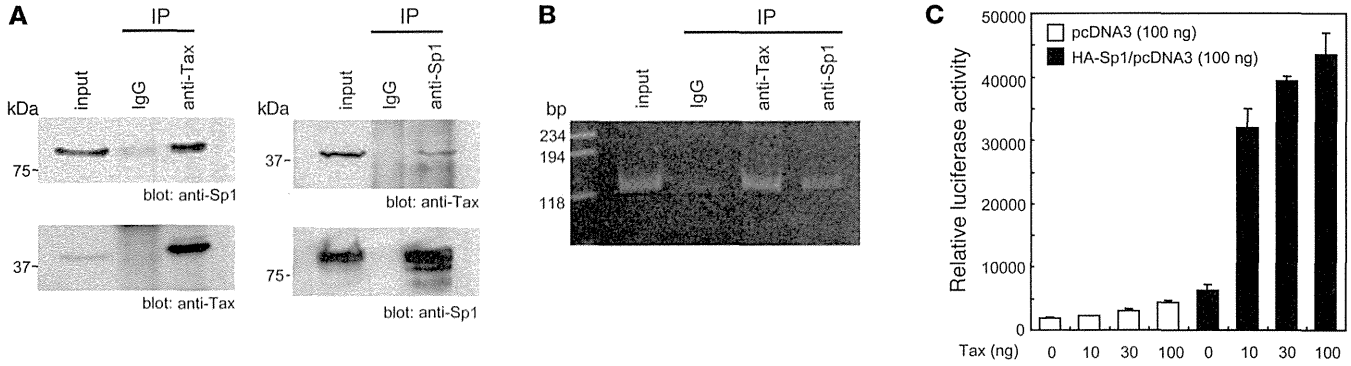

Figure 3. Tax and Sp1 cooperatively enhance *TBX21* promoter activity. (**A**) Co-IP of endogenous Tax and Sp1. Nuclear extracts from MT-2 cells were immunoprecipitated with anti-Tax or anti–Sp1 antibodies or with normal IgG as a control, then immunoblotted with anti-Tax or anti-Sp1 antibodies as indicated. (**B**) Tax bound to the *TBX21* promoter in vivo. ChIP assay using anti-Tax antibody followed by primers encompassing the *TBX21* promoter region (–179 to –59) was performed on genomic DNA isolated from MT-2 cells. DNA (input) and IP with anti-Sp1 served as positive controls, and normal IgG served as a negative control. (**C**) Coactivation of *TBX21* promoter by Sp1 and Tax. HEK293 cells were transfected with 100 ng of *TBX21*-Luc reporter plasmid or Sp1 expression plasmid, as well as 0–100 ng of Tax expression plasmid as indicated. Values were normalized to β-galactosidase activity as an internal control. Data are mean ± SD.

elevation of *Tax* expression via transduction of a GFP-Tax construct into $CD4^+CD25^+CCR4^+$ cells from a HD increased expression of *TBX21* as well as *Tax* (Supplemental Figure 2). Thus, a functional relationship between *Tax* and *TBX21* was confirmed. Finally, among $CD4^+CCR4^+$ cells from HAM/TSP patients, the appearance of Tax^+ cells was associated with a rise in the percentage of $T\text{-}bet^+$ cells, which was associated with a delayed but roughly proportional rise in the amount of IFN-γ protein (Figure 2, E and F). The production of Tax versus T-bet in these $CD4^+CCR4^+$ cells from HAM/TSP patients was compared at 0 versus 48 hours of culturing. At 0 hours, the overwhelming majority of the $CD4^+CCR4^+$ cells were both Tax^- and $T\text{-}bet^-$; by 48 hours, a substantial presence of $Tax^+T\text{-}bet^+$ cells had emerged, and there were very few $T\text{-}bet^+$ cells that were not also Tax^+ (Supplemental Figure 3).

Tax in concert with Sp1 induces TBX21 transcription. Experiments were conducted to investigate the mechanism by which Tax may be involved in *TBX21* transcription in HTLV-1–infected T cells. First, co-IP reactions were performed using nuclear extracts from the HTLV-1–infected MT-2 T cell line to confirm a suspected interaction between endogenous Tax and Sp1, which is known to both form a complex with Tax and to activate *TBX21* transcription (24, 25). Precipitation with anti-Tax or anti-Sp1 antibodies yielded bands corresponding to both Tax and Sp1, whereas precipitation with the nonspecific IgG antibody as the negative control yielded neither band (Figure 3A), thus demonstrating the existence of a Tax-Sp1 complex in HTLV-1–infected T cells. Second, a ChIP assay using primers encompassing the *TBX21* promoter region (–179 to –59) was performed on the MT-2 cells to confirm the suspected interaction between this Tax-Sp1 complex and the *TBX21* promoter. Precipitation with anti-Tax or anti-Sp1, but not IgG, yielded a PCR product corresponding to the *TBX21* promoter (Figure 3B), which suggests that a Tax-Sp1 complex does bind to the *TBX21* promoter site. Finally, a reporter assay was performed using cells transfected with *TBX21*-Luc, a luciferase reporter plasmid containing the *TBX21* promoter region, to confirm a functional relationship among Tax, Sp1, and *TBX21* transcription. Cotransfection with Sp1 resulted in elevated luciferase activity compared with transfection with the reporter alone, and addition of Tax heightened this effect in a concentration-dependent manner (Figure 3C). These findings suggested that Tax, in concert with Sp1, induces *TBX21* transcription.

HTLV-1–infected Th1-like CCR4⁺ cells are in the CNS of HAM/TSP patients. We next sought to confirm that HTLV-1–infected $CCR4^+$ T cells infiltrate the spinal cords of HAM/TSP patients and exhibit Th1-like traits, such as T-bet and IFN-γ production. Fluorescent immunohistochemical staining of tissue sections from HAM/TSP patient spinal cord lesions revealed the presence of abundant $CCR4^+$ cells infiltrating around the small blood vessels and coexpressing T-bet and IFN-γ (Figure 4A and Supplemental Figure 4). Further investigation revealed that these $CCR4^+$ cells also expressed CXCR3, the marker for Th1 cells (6). It should be noted that both IFN-γ and CXCR3 expression are reported to be induced by T-bet expression (6). Immunofluorescent staining was also used to demonstrate the existence of HTLV-1–infected $CCR4^+$ cells in the CSF of HAM/TSP patients (Figure 4B). $CCR4^+CXCR3^+$ cells were numerous among cells isolated from the CSF of HAM/TSP patients, representing 73.90% of $CD4^+$ cells isolated from a representative patient (Figure 4C) and 63.63% ± 6.73% of $CD4^+$ cells isolated from all patients ($n = 8$; Figure 4D). However, nearly all of these $CD4^+CCR4^+CXCR3^+$ cells were negative for Ki67, a marker of cell proliferation, in the CSF of the HAM/TSP patients (93.94% ± 2.07%, $n = 3$; Figure 4E). The majority of these $CD4^+CCR4^+CXCR3^+$ cells were also $CD25^+$ (70.16% ± 14.08%, $n = 3$, Supplemental Figure 5), confirming the existence of a substantial $CD4^+CD25^+CCR4^+CXCR3^+$ cell population in the CSF of HAM/TSP patients. Importantly, $CD4^+CCR4^+CXCR3^+$ cells did not make up the majority of PBMCs in HAM/TSP patients nor in HDs; in fact, such cells were very few (HAM/TSP, 3.65% ± 1.96%, $n = 8$; HD, 6.88% ± 3.09%, $n = 4$; Figure 4D). PBMCs were also isolated from ATLL patients for comparison, and $CD4^+CCR4^+CXCR3^-$ cells made up the overwhelming majority (83.03% ± 18.61%, $n = 5$; Supplemental Figure 6).

CCR4 shows potential as a molecular target for HAM/TSP immunotherapy. Analysis of HTLV-1 proviral DNA load in subpopulations of $CD4^+$ PBMCs from HAM/TSP patients confirmed that $CCR4^+$ cells were heavily infected, compared with less than

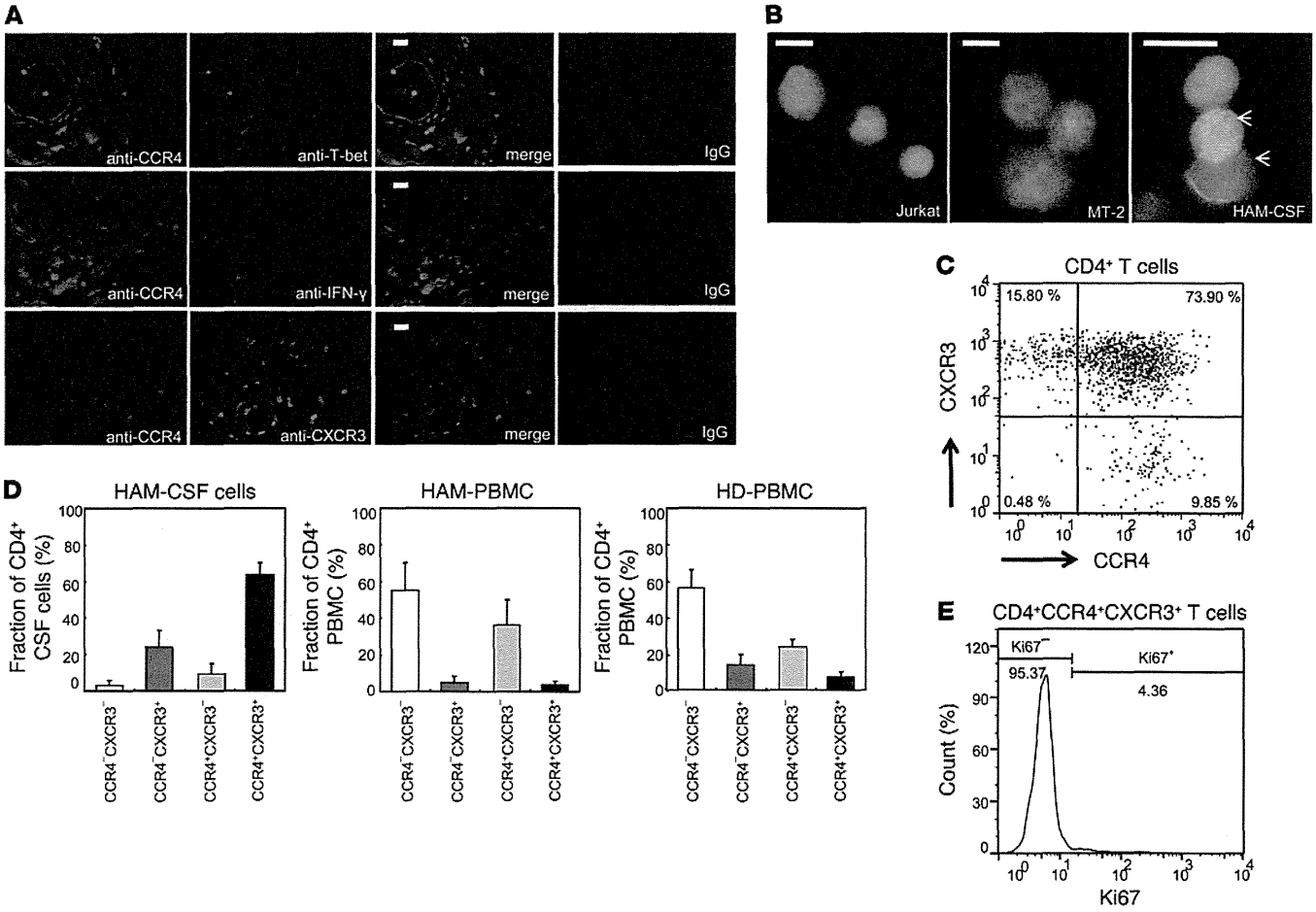

Figure 4. HTLV-1–infected Th1-like CCR4⁺ cells invade the CNS of HAM/TSP patients. (**A**) Detection of CCR4⁺ cells expressing T-bet, IFN-γ, and CXCR3 infiltrating the spinal cord of a HAM/TSP patient. Representative images show immunofluorescent codetection of CCR4 with T-bet, IFN-γ, and CXCR3, as well as the merged images, in thoracic spinal cord sections. Rabbit and goat IgG antibody served as a negative control. Scale bars: 20 μm. (**B**) Presence of HTLV-1–infected CCR4⁺ cells in HAM/TSP patient CSF. Representative images show immunofluorescence-FISH codetection of CCR4 (green) and HTLV-1 provirus (red) in Jurkat cells (uninfected control), MT-2 cells (infected control), and CSF cells from the patients. Arrows denote red provirus signal in the CSF sample. Scale bars: 20 μm. (**C**) CD4⁺ T cells in HAM/TSP patient CSF were mostly CCR4⁺CXCR3⁺. A dot plot of CCR4 and CXCR3 expression in CD4⁺ gated cells isolated from the CSF of a representative HAM/TSP patient is shown. (**D**) CD4⁺CCR4⁺CXCR3⁺ cells were numerous in CSF, but not elevated in peripheral blood, of HAM/TSP patients. Graphs show the percentages of CCR4⁻CXCR3⁻, CCR4⁻CXCR3⁺, CCR4⁺CXCR3⁻ and CCR4⁺CXCR3⁺ T cells among CD4⁺ PBMCs and CSF cells from HAM/TSP patients (n = 8) and PBMCs from HDs (n = 4). Analysis was performed using FACS. Data are mean ± SD. (**E**) Proliferation was not observed in CD4⁺CCR4⁺CXCR3⁺ cells from HAM/TSP patient CSF. The rate of Ki67 expression, a marker for cell proliferation, is shown for CD4⁺CCR4⁺CXCR3⁺ gated cells from the CSF of a representative HAM/TSP patient.

1% of CCR4⁻ cells (n = 7; Figure 5A). To predict the efficacy of a CCR4⁺ cell–targeting cytotoxic antibody as a treatment for HAM/TSP, PBMCs were isolated from patients (n = 9) and analyzed after being cultured with and without the defucosylated chimeric anti-CCR4 monoclonal antibody KM2760 (21) or, for comparison, the steroid therapy prednisolone (PSL). Addition of 1 μg/ml KM2760 significantly reduced the percentage of CCR4⁺ cells, as measured after 7 days (P = 0.0039; Figure 5B). As little as 0.1 μg/ml KM2760 was necessary to reduce the HTLV-1 DNA load (P < 0.05), whereas PSL had no significant impact (Figure 5C). Use of 1 μg/ml of either KM2760 or PSL was sufficient to suppress spontaneous proliferation of the PBMCs, as measured by ^{3}H-thymidine incorporation (P < 0.05 and P < 0.01, respectively; Figure 5D) as well as IFN-γ production (P < 0.05 and P < 0.001, respectively; Figure 5E). Similar results were observed in experiments using cells isolated from the CSF of HAM/TSP patients (n = 8): cultures to which 1 μg/ml of KM2760 had been added exhibited reduced HTLV-1 DNA load (P = 0.0078; Figure 5F) and IFN-γ production (P = 0.0391; Figure 5G). Certain samples shown in Figure 5G did not exhibit this reduction in IFN-γ production; those samples had particularly low cell counts (0.33–2.00 cells/μl), yielding less reliable data. Despite the presence of those lower-quality samples, statistical significance was still established for the sample group as a whole.

Discussion

Previously, we hypothesized that HTLV-1 gives rise to HAM/TSP by altering the behavior of infected cells via Tax expression to yield a new population of Th1-like proinflammatory cells (26). Evidence indicated that a significant portion of this population might be Tregs, as suggested by the CD4⁺CD25⁺CCR4⁺ expres-

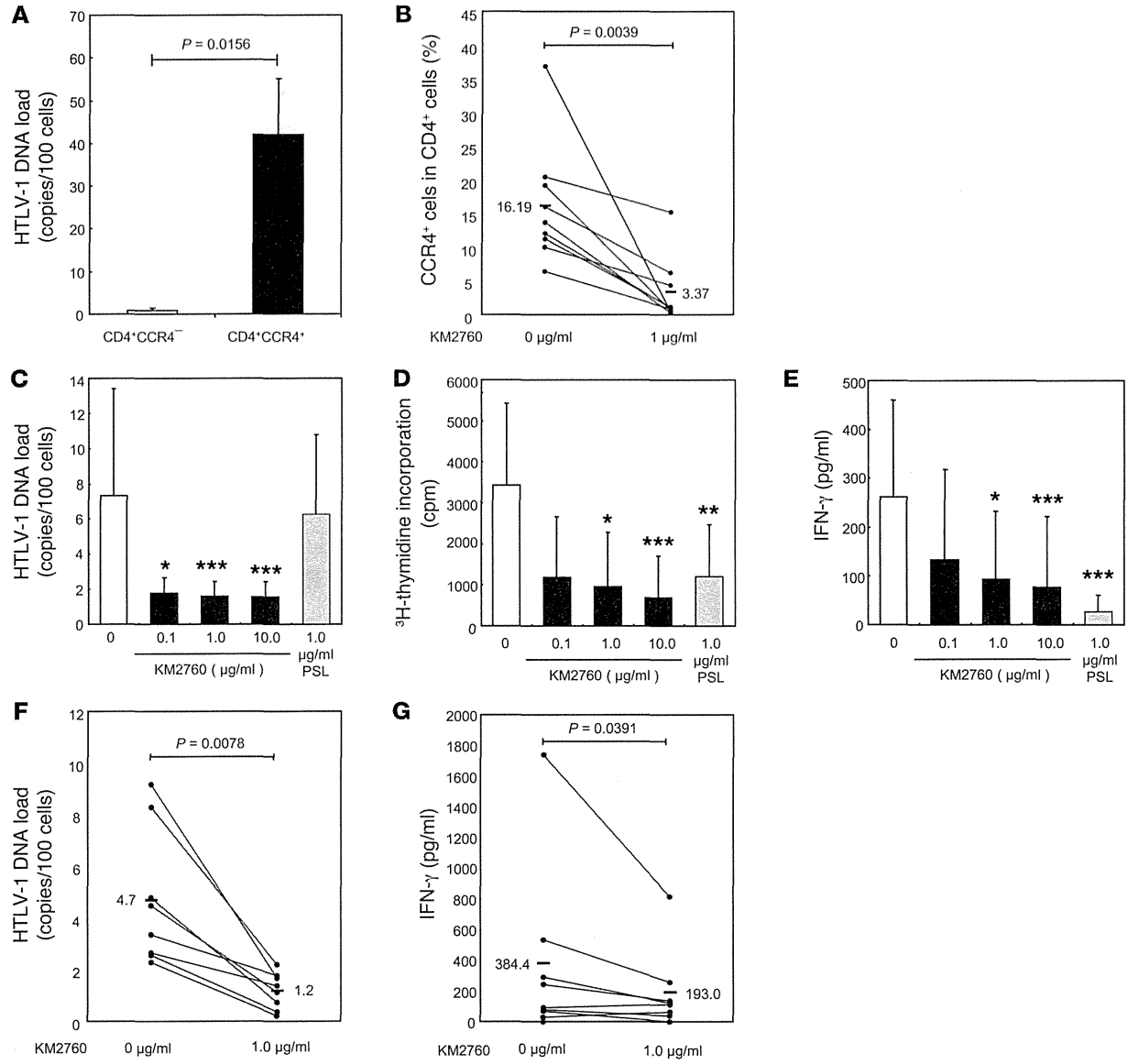

Figure 5. CCR4 shows potential as a molecular target for HAM/TSP immunotherapy. (**A**–**G**) Cells isolated from HAM/TSP patients were sorted via FACS (**A**; $n = 7$) or cultured for 7 days under the following conditions: PBMCs were cultured with various concentrations of KM2760 or 1 μg/ml PSL (**B**–**E**; $n = 9$), and CSF cells were cultured with 1 μg/ml KM2760 (**F** and **G**; $n = 8$). (**A**, **C**, and **F**) HTLV-1 proviral DNA loads were measured using quantitative PCR. (**D**) Degree of spontaneous proliferation was assessed by measuring ^{3}H-thymidine incorporation. (**E** and **G**) IFN-γ production in the culture media was evaluated using CBA assays. HTLV-1 resided in CD4$^+$CCR4$^+$ rather than CCR4$^-$ cells among PBMCs (**A**), and KM2760 treatment effectively targeted these cells (**B**). Consequently, KM2760 treatment successfully reduced HTLV-1 proviral DNA load (**C**), suppressed spontaneous proliferation (**D**), and decreased IFN-γ production (**E**) in PBMC cultures as well as reducing HTLV-1 DNA load (**F**) and IFN-γ production (**G**) in CSF cell cultures derived from HAM/TSP patients. (**A** and **C**–**E**) Data are mean ± SD. (**B**, **F**, and **G**) Thick horizontal bars represent mean value for all patients; line segments represent individual patients. Statistical analyses were performed using Friedman test followed by Dunn test for multiple comparisons (**C**–**E**) or Wilcoxon test (**A**, **B**, **F**, and **G**). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. untreated control.

sion profile (19). We suspected that these infected cells may infiltrate the CNS and trigger an inflammatory positive feedback loop, ultimately leading to chronic spinal cord inflammation (27). In the present study, we provided concrete evidence to support these theories on HAM/TSP pathogenesis, with a particular emphasis on the mechanism by which Tax can induce a proinflammatory phenotype intracellularly via transcriptional regulation.

There is strong evidence to support the conclusion that a substantial portion of the Treg population in HAM/TSP patients is infected with HTLV-1 (28, 29). In a previous study, we demonstrated that CD4$^+$CD25$^+$CCR4$^+$ cells were the main reservoir for HTLV-1 in HAM/TSP patients (19), but that expression profile is not exclusive to Tregs. Our present observation that CD4$^+$ T cells positive for FOXP3, a well-established marker for Tregs (10), were more thoroughly infected than the GATA3$^+$ subgroup (Figure 1A) strengthens the argument that Tregs may be the main viral reservoir. It remains debatable whether the virus preferentially infects these cells, promotes their survival (30), or even induces the expression of these

markers. One report postulates that HTLV-1 preferentially infects $CCR4^+$ cells by upregulating CCL22 to encourage cell-to-cell transfer via chemotactic attraction (31). More research is necessary to determine the true mechanism by which infected $CCR4^+$ and $FOXP3^+$ cells become so abundant in HAM/TSP patients.

We demonstrated that the suppressive ability of $CD4^+CD25^+$ $CCR4^+$ cells that characterizes Treg function was impaired by expression of the Tax protein, encoded in the pX region of the HTLV-1 genome (Figure 1B). Prior evidence indicates that Tax may exert these effects via downregulation of FOXP3 expression (20, 32). Transgenic mice expressing Tax exhibit reduced $CD4^+CD25^+FOXP3^+$ Tregs (33) and develop arthritis (34), and transgenic rats expressing HTLV-1 env-pX develop destructive arthropathies, Sjogren syndrome, vasculitis, and polymyositis (35). Collectively, these observations suggest that Tax expression can lead to inflammatory disease by weakening immune tolerance and disrupting homeostasis.

It has long been suspected that in addition to reducing FOXP3 expression, Tax may have the ability to induce IFN-γ production, thereby converting once-suppressive cells into proinflammatory cells. Indeed, intracellular Tax expression has been associated with the rapid upregulation of IFN-γ in infected cells, and researchers have theorized that this upregulation may contribute to the pathogenesis of HTLV-1–associated inflammatory disorders, including HAM/TSP (19, 36, 37). Here we showed at the mRNA level that *Tax* expression stimulated *IFNG* expression; moreover, the effect appeared delayed (Figure 2A), in a manner suggestive of 1 or more intermediate steps in the pathway, rather than direct transcriptional activation. Several candidate pathways have been proposed— such as via NF-κB, STAT1, or STAT5 — but none have been confirmed experimentally (38, 39).

We provided convincing evidence that Tax induces IFN-γ production in infected cells indirectly by amplifying the effects of Sp1 binding to — and increasing the activity of — the *TBX21* promoter: the resulting amplification of T-bet expression was responsible for the rise in IFN-γ production.

T-bet is said to be a Th1-specific T box transcription factor that controls the expression of the hallmark Th1 cytokine, IFN-γ (6). *TBX21*-deficient mice exhibit greater resistance to a variety of inflammatory and autoimmune diseases than their wild-type counterparts (40). Thus, it has been of interest that elevated *TBX21* levels have been found in the PBMCs of HAM/TSP patients (41). We showed that *TBX21* expression was elevated in the $CD4^+CD25^+CCR4^+$ cells of HAM/TSP patients, but not ATLL patients (Figure 2B and Supplemental Figure 1), which suggests that this trait is specific to HAM/TSP pathogenesis. Furthermore, we interpreted the lack of elevation in $CD4^+CD25^-CCR4^-$ cells to indicate that elevated *TBX21* is characteristic of infected cells. Importantly, we clearly demonstrated for the first time that Tax induced T-bet expression (Figure 2, C and E, and Supplemental Figures 2 and 3). Moreover, we showed that this pathway was active in $CD4^+CD25^+CCR4^+$ cells of HAM/TSP patients by silencing Tax expression and observed a corresponding reduction in *TBX21* expression; in the reverse scenario, inducing Tax expression in otherwise-normal $CD4^+CD25^+CCR4^+$ cells from HDs resulted in heightened *TBX21* expression (Figure 2D and Supplemental Figure 2). Finally, we confirmed that this correlation extended to protein production and clearly showed how Tax induces T-bet and subsequently IFN-γ production over time in culture (Figure 2E).

Tax has been reported to stably bind Sp1, a known positive transcriptional regulator of *TBX21* (25, 42). More specifically, interaction with Tax is thought to increase the DNA binding activity of Sp1 (42). Here we used co-IP with samples from the HTLV-1–infected MT-2 cell line to show that endogenous Tax interacted with Sp1 (Figure 3A). Subsequently, ChIP assays revealed that both Sp1 and Tax associated with the *TBX21* promoter region (Figure 3B), a novel finding that supports our theory that Tax and Sp1 together activate *TBX21* transcription. Finally, we showed that in the absence of Sp1, Tax had no significant effect on *TBX21* expression; however, in the presence of Sp1, Tax induced *TBX21* expression in a concentration-dependent manner (Figure 3C). This finding further substantiates our claim that Tax does not directly bind the promoter, but rather acts via Sp1. It should be noted that Tax may induce *TBX21* expression via multiple pathways: it has been reported that Tax enhances STAT1 gene expression in HTLV-1–transformed T cell lines and $CdCl_2$-stimulated JPX-9 cells (38), which suggests that Tax may also induce *TBX21* expression indirectly via STAT1.

The presence of T cell infiltrates in the CNS, indicative of spinal cord inflammation, is a well-known feature of HAM/TSP. Researchers have worked to characterize these cells over the years; together, their findings suggest that the infiltrates are dominated by $CD4^+$ T cells with relatively high proviral loads and elevated Tax and IFN-γ expression (43–45). We hypothesized that a substantial portion of the infiltrate may be made up of infected $CD4^+CCR4^+$ T cells exhibiting Th1-like properties, including IFN-γ production. We used immunohistochemistry to investigate this theory and were able to establish the presence of $CD4^+CCR4^+CXCR3^+$T-$bet^+IFN\text{-}\gamma^+$ cells in spinal cord tissue and HTLV-1–infected $CCR4^+$ cells in the CSF of HAM/TSP patients (Figure 4, A and B). We used FACS analysis to confirm that $CD4^+CCR4^+CXCR3^+$ cells made up the majority of the $CD4^+$ T cells in the HAM/TSP patient CSF (Figure 4C). For the sake of continuity between this and our previous study (19), we also confirmed that the majority of these $CD4^+CCR4^+CXCR3^+$cellswerealso$CD25^+$(SupplementalFigure5), further suggestive of a Treg identity.

We interpret the observation that these $CD4^+CCR4^+CXCR3^+$ cells were virtually nonexistent among PBMCs in HAM/TSP patients (Figure 4D) to mean that the cells had migrated to the CNS, leaving few behind in the periphery. The surprising observation that the Ki67 marker for cell proliferation was negative in the overwhelming majority of $CD4^+CCR4^+CXCR3^+$ cells in the CSF (Figure 4E) signifies that the cells are indeed proliferating elsewhere and subsequently migrating to the CNS. It has in fact been said that HTLV-1–infected cells may be extraordinarily capable of crossing the blood-brain barrier (46). Due to the high proportion of CCR4 positivity among HTLV-1–infected cells (19), the high proviral load in the CSF of HAM/TSP patients (47), and the elevated levels of CCL22 in HAM/TSP patient peripheral blood (30), one might hypothesize that the infected cells migrate across the blood-brain barrier in response to chemokine ligands of CCR4, namely CCL22. However, we found that the CSF of HAM/TSP patients contained only negligible amounts of CCL22, instead favoring the CXCR3 ligand CXCL10 (48). We now postulate that

$CD4^+CCR4^+CXCR3^+$ T cells and other $CXCR3^+$ cells may migrate to the CNS via chemotaxis induced by CXCL10 secreted by astrocytes in the CNS. Previously, we showed that these astrocytes produce CXCL10 in response to IFN-γ, and these levels are further amplified by the invading $CXCR3^+$ cells (27). Together, these findings indicate that a positive feedback loop involving the recruitment of proinflammatory cells to the CNS is the source of chronic inflammation in HAM/TSP, and that the original trigger is the migration of IFN-γ–producing HTLV-1–infected cells to the CNS. Where these proinflammatory cells are primarily proliferating, and why they proliferate at different rates in different settings, are questions to be addressed in future studies.

Our findings in this and previous studies imply that targeting $CCR4^+$ cells could constitute an effective treatment for HAM/TSP. Indeed, this strategy is already in play for ATLL patients, the majority of whom suffer from $CCR4^+$ T cell–derived cancers (7). The humanized defucosylated anti-CCR4 monoclonal antibody KW-0761, which has been shown to induce CCR4-specific ADCC, has been approved as a treatment for ATLL (49, 50). The observation that the majority of infected $CD4^+$ PBMCs in HAM/TSP patients were $CCR4^+$ (Figure 5A) suggests that an anti-CCR4 antibody with ADCC properties might be used to effectively treat HAM/TSP patients as well. Steroids are currently the standard of care for HAM/TSP patients, but this approach is not considered optimal: as with many nonspecific treatments, the effectiveness is limited, and the side effects are numerous (51). Here we compared the effects of the defucosylated chimeric anti-CCR4 monoclonal antibody KM2760 (21) with those of the steroid PSL on ex vivo cultures of cells from HAM/TSP patients. Although PSL had more potent effects per microgram, both treatments successfully reduced cell proliferation and IFN-γ production (Figure 5, D, E, and G). In addition, even a small dose of the antibody effectively reduced proviral load, whereas PSL treatment had no significant effect (Figure 5, C and F). These findings support the main premise of this paper, namely, that $CCR4^+$ cells are major viral reservoirs and producers of IFN-γ. Our study is the first to test the effects of such an antibody-based treatment on cells from HAM/TSP patients; the results were promising, and a clinical trial investigating the in vivo effectiveness in HAM/TSP patients is now underway. Importantly, our research indicates that even if the antibody does not cross the blood-brain barrier, it could be therapeutically effective against spinal cord inflammation by eliminating the proinflammatory $CCR4^+$ cells in the peripheral blood that would have migrated to the CNS.

Until very recently, there had been no reports of T cell character changing from suppressive to inflammatory in response to internal transcriptional alterations induced intracellularly by viral products. There have been many reports of Tregs transforming in the presence of inflammation due to the influence of cytokines, including instances where FOXP3 expression is lost and even cases where IFN-γ production is gained (12, 13). The only report of a similar phenomenon occurring via an intracellular virus-induced pathway states that the HTLV-1 basic leucine zipper (HBZ) gene product can reduce the expression of FOXP3 in HBZ-transgenic mouse Tregs (52). Here we showed for the first time that the HTLV-1 virus can similarly affect gene expression in human cells, inducing IFN-γ production, as well as reduce suppressive function. Collectively, the research to date suggests that HTLV-1 may preferentially infect $CCR4^+$ cells, including Tregs, and induce transcriptional changes via Tax that not only reduce FOXP3 expression, but also induce T-bet expression and consequently IFN-γ production, yielding a proinflammatory immune imbalance. While there is considerable evidence to support this theory, further experiments are necessary to prove that this pathway is indeed the origin of HAM/TSP chronic inflammation. However, here we have directly shown that the HTLV-1 protein product Tax can induce the expression of the Th1 master transcription factor T-bet, which certainly implies that HTLV-1 is capable of activating inherent plasticity in T cells and shifting their gene expression profiles toward a Th1-like state.

Methods

Patient selection and sample preparation. The study included HTLV-1–noninfected HDs ($n = 8$, 4 male and 4 female; mean age, 36 yr), asymptomatic carriers ($n = 6$, 4 male and 2 female; mean age, 56 yr), ATLL patients ($n = 6$, 2 male and 4 female; mean age, 68 yr), and HAM/TSP patients ($n = 31$, 9 male and 22 female; mean age, 61 yr). Diagnosis of ATLL was based on the criteria established by Shimoyama (53). HTLV-1 seropositivity was determined by a particle agglutination assay (Serodia-HTLV-1) and confirmed by Western blot (SRL Inc.). HAM/TSP was diagnosed according to WHO guidelines (54).

Samples of PBMCs were prepared using density gradient centrifugation (Pancoll; PAN-Biotech) and viably cryopreserved in liquid nitrogen (Cell Banker 1; Mitsubishi Chemical Medience Corp.). CSF samples were taken from 17 HAM/TSP patients. CSF cells were isolated by centrifugation and cryopreserved in the aforementioned freezing medium until use. Thoracic spinal cord tissue samples from 1 HAM/TSP patient were obtained postmortem, fixed in 10% formalin, and embedded in paraffin.

Antibodies. For FACS studies, labeled anti-CD3 (UCHT1), anti-CD4 (OKT4), anti-GATA3 (TWAJ), and anti-FOXP3 (PCH101) were purchased from eBioscience, and labeled anti-CCR4 (1G1), anti-CD25 (BC96), anti-CXCR3 (1C6), anti-T-bet (4B10), and anti-Ki67 (B56) were purchased from BD Biosciences. For IP studies, anti-Sp1 (PEP2) and normal IgG were purchased from Santa Cruz Biotechnology Inc., and anti-Tax (Lt-4) was prepared as described previously (55). For immunofluorescence studies, anti-CCR4, anti-IFN-γ, and anti-CXCR3 were purchased from Abcam; anti–T-bet was purchased from Santa Cruz Biotechnology Inc.; and Alexa Fluor 488– and Alexa Fluor 594–conjugated secondary antibodies were purchased from Invitrogen. Kyowa Hakko Kirin Co. Ltd. provided KM2760, a chimeric anti-CCR4 IgG1 monoclonal antibody (21).

Plasmids. The *TBX21*-Luc reporter gene plasmid was constructed as described previously (25). The 100-bp promoter fragment (–101 to –1) in the 5′-flanking region of the human *TBX21* gene was obtained by PCR using human PBMC genomic DNA as the template. Primers used for PCR were 5′-CGCCTCGAGGGCGGGGTGGGGCGAGGCGG-3′ and 5′-CCCAAGCTTCTGTCACTAGAGTCGCAGCGCTTT-3′. The amplified PCR product was digested with XhoI/HindIII and cloned into pPicaGene-Basic vector II (Toyo-ink), which yielded *TBX21*-Luc. Creation of the human Sp1 construct with HA-tag added to the N terminus was accomplished via real-time RT-PCR amplification of human PBMC cDNA with the following primers: Sp1 forward, 5′-CGCGAATTCATGAGCGACCAAGATCACTCCATGGA-3′; Sp1 reverse, 5′-CGCCTCGAGTCAGAAGCCATTGCCACTGATATTAATGGAC-3′. The amplified fragment was digested with EcoRI/XhoI and