Specific recruitment of regulatory T cells into the CSF in lymphomatous and carcinomatous meningitis

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Whereas regulatory T (Treg) cells play an important role in the prevention of autoimmunity, increasing evidence suggests that their down-regulatory properties negatively affect immune responses directed against tumors. Treg cells selectively express chemokine receptors CCR4 and CCR8, and specific migration occurs following the release of various chemokines. Neoplastic meningitis (NM) resulting from leptomeningeal spread of systemic non-Hodgkin lymphoma (NHL) or carcinoma has a poor prognosis. We hypothesized that Treg-cell accumulation within the subarachnoid space as a result of interfering with tumor immunity may be relevant for survival of neoplastic cells. We collected cerebrospinal fluid (CSF) from 101 patients diagnosed with lymphomatous/carcinomatous NM and various inflammatory diseases (IDs) and noninflammatory neurologic disorders (NIDs). CSF Treg-cell counts were determined by flow cytometry, Treg-cell–specific chemokines by enzyme-linked immunosorbent assay (ELISA), and Treg-cell trafficking by chemotaxis assay. Both frequencies of Treg-cell and Treg cell–specific chemotactic activities were significantly elevated in CSF samples of patients with NM. Local Treg-cell accumulation occurred without concomitant rise of conventional T (Tconv) cells, coincided with elevated concentrations of Treg cell–attracting chemokines CCL17 and CCL22 and correlated with numbers of atypical CSF cells. We conclude that Treg cells are specifically recruited into the CSF of patients with NM, suggesting that the presence of Treg cells within the subarachnoid space generates a microenvironment that may favor survival and growth of malignant cells. (Blood. 2008;111:761-766)

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Introduction

Dissemination of malignant non-Hodgkin lymphoma (NHL) or systemic carcinoma to the leptomeninges carries a poor prognosis and is rapidly fatal in the most patients. The predictors of metastatic tumor spread to the meninges are elusive, and little is known as to what extent the microenvironment of the subarachnoid space affects local accumulation and expansion of neoplastic cells. One way in which tumors escape physiologic defense mechanisms is through counteracting antitumor immunity.1-5 Recent findings demonstrate that naturally suppressive T cells of CD4+CD25+FOXP3+ phenotype (regulatory T [Treg] cells) are critically involved in this process and, in addition to their functional significance in the prevention of murine and human autoimmunity, may promote poor antitumor immunity by down-regulating the functional activity of tumor-infiltrating CD4+ T cells. Thus, experimental depletion of Treg cells in tumor-bearing mice enhances both immune-mediated tumor clearance and responses to immune-based therapies.6-8 Likewise, tumor Treg cells are detectable in both solid human tumors and human B-cell NHL.9,15 and in patients with ovarian cancer, intratumoral Treg cell accumulation correlates with an unfavorable clinical outcome.16 In contrast with these observations, a recent study in Hodgkin lymphoma reported that increased frequencies of infiltrating cytotoxic T cells in parallel with low numbers of local Treg cells negatively predict disease-free survival.17

Human Treg cells selectively express chemokine receptors CCR4 and CCR8, and specific migration of Treg cells occurs in response to release of CCR4 and CCR8 ligands, including the chemokines CCL17 (thymus and activation-regulated chemokine [TARC]), CCL22 (macrophage-derived chemokine [MDC]), CCL1 (I-309), and vMIP-I (viral macrophage inflammatory protein I).18 Secretion of Treg cell–attracting chemokines is not restricted to immune cells, but may also occur in carcinoma cells and neoplastic B-lymphocytes.19-21 Curiel et al demonstrated that Treg cell migration to ovarian tumors is mediated by CCL22 released by cancer cells and tumor-associated macrophages.16 Secretion of CCR4 and CCR8 ligands was further demonstrated for activated and neoplastic B lymphocytes.22-25

In this study, we used cerebrospinal fluid (CSF) specimens from patients with lymphomatous or carcinomatous meningitis to assay Treg-cell migration into the CSF compartment. We comparatively tested the chemotactic activity toward Treg cells prompted by lymphoma/tumor infiltration of the leptomeninges versus Treg-cell trafficking to the CSF occurring in association with various inflammatory diseases (IDs) and noninflammatory neurologic
disorders (NIDs). We also determined CSF $T_{reg}$-cell prevalences and chemokine profiles in these conditions.

**Methods**

**Human samples**

We collected CSF and serum samples from 101 patients who underwent diagnostic lumbar puncture. Patients were diagnosed with lymphomatous meningitis (LM; $n = 24$) resulting from B-NHL; carcinomatous meningitis (CM; $n = 18$) due to breast ($n = 9$), lung ($n = 5$), gastric ($n = 1$), testicular ($n = 1$), or renal ($n = 1$) cancer, and cancer of unknown primary origin ($n = 1$); IDs of the nervous system (infections, $n = 26$); multiple sclerosis (MS; $n = 16$); and various NIDs ($n = 17$).

Mean CSF cell counts in samples obtained from patients with LM and CM were 62.9/μL (range, 1-375/μL) and 42.3 μL (range, 1-1462/μL), respectively. The presence of NM was established by unequivocal cytologic evidence of atypical cells in routine CSF cytospins. Mean percentages of NM were 30.2% (LM; range, 1%-100%) and 48.1% (CM; range, 1%-100%), respectively. The blood-CSF barrier (BCB) function was determined by nephelometric assessment of serum and CSF albumin concentrations and calculation of the CSF-to-serum albumin ratio ($Q_{alb} = \text{albumin CSF [mg/L]} / \text{albumin serum [mg/L]} \times 10^{-3}$; normal = 2.0-8.0). Mean $Q_{alb}$ was 19.5 (range, 4.8-45.1) in LM and 22.6 (range, 4.7-60.0) in CM samples and revealed BCB dysfunction in 6 of 12 patients with LM and 13 of 16 patients with CM. Lack of serum samples (range, 4.7-60.0) in CM samples and revealed BCB dysfunction in 6 of 12 and 13 of 16 patients, respectively; mean Qalb was 4.9 (range, 3.1-9.2) for patients with MS and 6.9 (range, 2.9-15.3) for patients with NID. None of the patients with NM underwent intrathecal chemotherapy or radiation of the neuraxis prior to lumbar puncture. Likewise, treatment with anti-infectious agents or corticosteroids did not precede CSF analysis in patients with infections and MS.

The protocol was approved by the University of Heidelberg ethics committee, and written informed consent was obtained from all individuals.

**Flow cytometry**

For flow cytometric analysis, freshly isolated peripheral blood mononuclear cells (PBMCs) or CSF cells were stained with monoclonal antibodies (mAbs) specific for human CD4 and CD25 (purchased from BD Pharmingen, Heidelberg, Germany) and FOXP3. FOXP3 staining was performed with a FOXP3 staining kit from eBioscience (San Diego, CA) according to the manufacturer’s protocol. A minimum of 5000 CSF cells per staining was required, with at least 1000 events measured. Fluorescence-activated cell sorter (FACS) acquisition was performed immediately with a FACS Canto II instrument (BD Biosciences, Palo Alto, CA). CSF cells were stained with monoclonal antibodies (anti-CD25, anti-FOXP3, and anti-CD4) and analyzed with FACS Diva software (BD Biosciences, Palo Alto, CA). CSF cells were first gated according to forward and side light-scattering properties and CD4 expression and then analyzed for CD25 and intracellular FOXP3. For quantification of $T_{reg}$ cells, stained CSF cells were first gated for CD4 and then assessed by CD25 expression, and then coexpression of FOXP3 and CD25 was assessed. After staining for CD4, CD25, CD127, and FOXP3, cells were gated for CD4+ lymphocytes. Figure 1 shows $CD4^+CD25^{hi}CD127^{lo}$ cells. In vitro proliferation assay with $10^4$ control-derived $T_{reg}$ cells and increasing numbers of CD4+ $CD25^{hi}CD127^{lo}$ $T_{reg}$ cells isolated from CSF samples obtained from patients with viral infections. CSF-derived $T_{reg}$ cells suppress proliferation of activated $T_{eff}$ cells in a dose-dependent manner.

**Cell separation**

CD4+ $CD25^{hi}$ cells were isolated from CSF samples with high cell counts obtained from 2 patients with viral infections of the central nervous system (CNS; 2.6 × 10⁵ and 3.8 × 10⁵ white CSF cells) as described previously. In short, CSF cells were sedimented for 10 minutes at 4° and

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Figure 1. $T_{reg}$ cells accumulate in the CSF of patients with NM. (A) Typical FACS staining of CSF cells (sample obtained from 1 patient with LM) for surface CD25 and intracellular FOXP3. For quantification of $T_{reg}$ cells, stained CSF cells were first gated according to forward and side light-scattering properties and CD4 expression, and then coexpression of FOXP3 and CD25 was assessed. (B) After staining for CD4, CD25, CD127, and FOXP3, cells were gated for CD4+ lymphocytes. (B) R1 shows $CD4^+CD25^{hi}CD127^{lo}$ cells. (C) R2 shows $CD4^+FOXP3^+CD127^{lo}$ cells. (D) In vitro proliferation assay with $10^4$ control-derived $T_{reg}$ cells and increasing numbers of CD4+ $CD25^{hi}CD127^{lo}$ $T_{reg}$ cells isolated from CSF samples obtained from 2 patients with viral infections. CSF-derived $T_{reg}$ cells suppress proliferation of activated $T_{eff}$ cells in a dose-dependent manner. (E) Percentages of CD25+FOXP3+ $T_{reg}$ cells among CD4+ CSF cells in patients with NM and control diseases. Symbols indicate percentages of individual patients. Medians are indicated.
400g, and CD25<sup>hi</sup> cells were then immunomagnetically separated using a T<sub>reg</sub> cell isolation kit (Dynal, Dylam, Germany). Immune magnetic separation yielded 2.8 × 10<sup>4</sup> and 5.3 × 10<sup>4</sup> highly pure T<sub>reg</sub> cells (87.5% and 90.2% CD4<sup>+</sup>CD25<sup>hi</sup> cells). For isolation of effector T (T<sub>eff</sub>) cells, CD4<sup>+</sup> cells were enriched from the peripheral blood of 1 healthy donor using a negative CD4<sup>+</sup> T-cell isolation kit (Dynal). CD4<sup>+</sup>CD25<sup>med</sup>/CD4<sup>+</sup>CD25<sup>hi</sup> T<sub>reg</sub> cells were obtained by depleting CD25<sup>hi</sup> cells with the T<sub>reg</sub> cell isolation kit.

**Proliferation assay**

For functional characterization, CD4<sup>+</sup>CD25<sup>hi</sup> cells isolated from CSF were tested in subsequent in vitro proliferation assays as described previously, with the following modifications. A total of 10<sup>4</sup> freshly isolated T<sub>reg</sub> cells were cultured alone or together with T<sub>eff</sub> cells (T<sub>eff</sub>/T<sub>reg</sub> ratios: 1:2, 1:4, and 1:8) and stimulated with soluble anti-CD3 (1 μg/mL) and anti-CD28 mAbs (1 μg/mL). After 4 days at 37°C in 5% CO2, 0.0185 MBq (0.5 μCi) [H<sub>3</sub>] thymidine per well was added for additional 16 hours, and proliferation was measured using a scintillation counter.

**Migration assay**

T<sub>reg</sub>-cell trafficking was assessed by using a transwell assay to measure the specific chemotactic activity in CSF. A total of 10<sup>4</sup> freshly isolated PBMCs obtained from 1 healthy standard donor were suspended in 200 μL RPMI and transferred into the upper chambers of 6.5-mm diameter, 5.0-μm pore-size polycarbonate membrane filter transwell plates (Costar Corning, Cambridge, MA). RPMI (600 μL), CCL22 (100 ng/mL; R&D Systems, Minneapolis, MN), or CSF supernatants with or without blocking mAbs were added to the lower chamber. After 4 hours at 37°C, migrated cells were collected in the lower chamber, stained with anti-CD4 and anti-CD25 mAbs, and analyzed by flow cytometry analysis. The chemotactic index for T<sub>reg</sub> cells (CI-T<sub>reg</sub>) was defined as the ratio of percentages of T<sub>reg</sub> cells in CD4<sup>+</sup> T cells of migrated cells and percentages of T<sub>reg</sub> cells in CD4<sup>+</sup> T cells of original PBMCs. Specific mAbs for inhibition of CCL17 (500 ng/mL) and CCL22 (500 ng/mL) were purchased from R&D Systems.

**ELISA**

Enzyme-linked immunosorbent assays (ELISAs) of CSF supernatants and serum samples were performed using Quantikine Immunoassay kits specific for human CCL17 (TARC) and CCL22 (MDC), according to the manufacturer’s instructions (all from R&D Systems).

**Statistical analysis**

To determine whether differences and correlations in CSF cell counts, migration indices, and chemokine levels were statistically significant, data were analyzed with the Sigma STAT software (SPSS, Chicago, IL). Mann-Whitney U tests and Pearson correlation analyses were used for statistical assessment. P values less than .05 were considered significant.

**Results**

**Enhanced T<sub>reg</sub>-cell frequencies within the CSF of patients with NM**

T<sub>reg</sub> cell frequencies in CSF samples were flow-cytometrically defined as proportions of CD25<sup>hi</sup>FOXP3<sup>+</sup> cells among CD4<sup>+</sup> T cells (Figure 1A). CSF-derived T<sub>reg</sub> cells were CD127<sup>low</sup> (Figure 1B,C) and functionally suppressive (Figure 1D), and thus represent T<sub>reg</sub> cells.

The CSF of patients with NID contained a median of 2.8% T<sub>reg</sub> cells of all CD4<sup>+</sup> cells (range, 0.1%-5.1% of CD4<sup>+</sup> T cells; Figure 1E). T<sub>reg</sub>-cell levels were significantly elevated in CSF specimens from patients with LM (median, 5.1%; range, 2.6%-7.4%; P = .018) and were also enhanced in CM (median, 4.5%; range, 2.5%-8.2%; P = .040). In contrast, T<sub>reg</sub>-cell frequencies obtained from both individuals with infections and MS did not differ compared with the NID cohort (infections: median, 3.0%; range, 0.7%-8.3%; P = .467; MS: median, 2.8%; range, 0.3%-6.3%; P = .939).

There was a strong correlation between the amount of atypical cells and the prevalence of T<sub>reg</sub> cells in CSF of patients with LM (r<sup>2</sup> = .96) and also with CM (r<sup>2</sup> = .67).

Proportions of CD4<sup>+</sup> cells were equivalent between NID (median, 61.2%; range, 33.6%-77.2%), CM (median, 60.3%, range, 34.6%-85.1%; P = .571), and LM (median, 53.3%; range, 28.6%-76.5%; P = .324), but were increased in CSF of patients with infections (median 70.9%, range 13.4%-100.0%, P = .046; not depicted).

**Increased T<sub>reg</sub>-cell–specific chemotactic activity in CSF supernatants from patients with NM**

T<sub>reg</sub>-cell–specific chemotactic activities in CSF supernatants were tested by in vitro cell migration assays. Compared with individuals with NID (CI-T<sub>reg</sub>, 1.2 ± 0.4), the mean CI-T<sub>reg</sub> was moderately elevated in patients with infections (1.8 ± 0.4; P = .020; Figure 2), whereas differences were not statistically significant in patients with MS (CI-T<sub>reg</sub>, 1.3 ± 0.5; P = .743). In contrast, a pronounced increase of CI-T<sub>reg</sub> was detectable in both individuals with LM (CI-T<sub>reg</sub>, 3.0 ± 1.2; CI-T<sub>reg</sub>, 0.008) and CM (CI-T<sub>reg</sub>, 2.5 ± 1.1; P = .015) versus NID.

Specific migration of all CD4<sup>+</sup> cells was similar between NID (CI-T<sub>reg</sub>, 1.1 ± 0.4), LM (CI-T<sub>reg</sub>, 1.2 ± 0.4; P = .882), and CM (CI-T<sub>reg</sub>, 1.3 ± 0.3; P = .384), and significantly enhanced in CSF samples of patients with infections (1.8 ± 0.3; P = .001; not depicted).

Spontaneous migration of PBMCs toward RPMI medium always resulted in T<sub>reg</sub>-cell– and CD4<sup>+</sup>-specific indices between 0.9 and 1.1 (data not shown).

**T<sub>reg</sub>-cell–specific trafficking toward CSF supernatants from patients with NM depends on CCL17 and CCL22**

As several studies implicate an important role of CCL17 and CCL22 in migration of human T<sub>reg</sub> cells, we tested the effect of anti-CCL17 and anti-CCL22 mAbs on T<sub>reg</sub>-cell–specific trafficking toward CSF supernatants from 9 patients with NM. In all patients studied, T<sub>reg</sub>-cell–specific trafficking was significantly reduced when both blocking Abs were added to CSF supernatants prior to
chemotaxis assays. The mean CI-T\textsubscript{reg} dropped from 3.7 ± 0.9 to 1.5 ± 1.0 (P = .006) in patients with LM (n = 6) and from 6.0 ± 1.9 to 1.6 ± 1.2 (P = .029; Figure 3) in patients with CM (n = 3). The use of isotypic control antibodies had no effect (data not shown).

**Elevated concentrations of T\textsubscript{reg} cell–specific chemokines in CSF of patients with NM**

Concentrations of T\textsubscript{reg} cell–specific chemokines CCL17 and CCL22 in CSF samples were determined by ELISA. Compared with the NID cohort (CCL17: 234.6 ± 12.1 pg/mL; CCL22: 100.3 ± 3.8 pg/mL), CCL17 and CCL22 levels were increased in the CSF of patients with LM (CCL17: 305.5 ± 105.4 pg/mL; P = .044; CCL22: 281.5 ± 124.9 pg/mL; P = .001) and CM (CCL17: 285.1 ± 44.7 pg/mL; P = .035; CCL22: 158.7 ± 57.5 pg/mL; P = .012; Figure 4). Elevations were also detectable in infections (CCL17: 287.0 ± 79.4 pg/mL; P = .080; CCL22: 132.8 ± 26.4 pg/mL; P = .002), but not in MS (CCL17: 225.4 ± 4.9 pg/mL; P = .470; CCL22: 110.5 ± 18.8 pg/mL, P = .100).

High concentrations of T\textsubscript{reg} cell–specific chemokines CCL17 and CCL22 correlated well with both CSF T\textsubscript{reg} cell–frequency (CCL17: LM r\textsuperscript{2} = 0.64, CM r\textsuperscript{2} = 0.50; CCL22: LM r\textsuperscript{2} = 0.51, CM r\textsuperscript{2} = 0.55) and numbers of atypical cells (CCL17: LM r\textsuperscript{2} = 0.55, CM r\textsuperscript{2} = 0.40; CCL22: LM r\textsuperscript{2} = 0.53, CM r\textsuperscript{2} = 0.48). Moreover, there was a clear correlation between chemokine concentrations and T\textsubscript{reg} cell–specific trafficking toward NM CSF samples (LM: CCL17 r\textsuperscript{2} = 0.42, CCL22 r\textsuperscript{2} = 0.65; CM: CCL17 r\textsuperscript{2} = 0.59, CCL22 r\textsuperscript{2} = 0.31).

Chemokine concentrations were also measured in 38 serum samples. Levels of CCL17 and CCL22 did not differ between patients with NM and the NID cohort (CCL17: NID, 196.3 ± 62.7 pg/mL; CM, 281.4 ± 150.3 pg/mL; P = .290; LM, 223.6 ± 88.8 pg/mL; P = .624; CCL22: NID, 601.7 ± 77.2 pg/mL; CM, 585.7 ± 304.5 pg/mL; P = .919; LM, 509.7 ± 77.4 pg/mL; P = .206; not depicted). Moreover, there was no correlation between chemokine levels in CSF and Q\textsubscript{abs}, indicating that the elevated T\textsubscript{reg} cell–specific chemokine concentrations observed in CSF specimens from patients with NM originate predominantly from synthesis within the CNS. Concentrations of CCL17 and CCL22 were also not elevated in serum samples from patients with MS (CCL17: 271.5 ± 149.3 pg/mL; CCL22: 591.0 ± 91.0 pg/mL) and from patients with infections of the CNS (CCL17: 249.1 ± 97.5 pg/mL; CCL22: 580.1 ± 82.9 pg/mL).

**Discussion**

Recent studies emphasize the unfavorable effect of T\textsubscript{reg} cells on tumor immunity, as local T\textsubscript{reg} cell accumulation driven by the presence of T\textsubscript{reg} cell–specific chemokines in the tumor environment promotes immune tolerance toward tumor antigens. In line with these observations, we show here that CD25\textsuperscript{low}FOXP3\textsuperscript{+} T cells are overrepresented in CD4\textsuperscript{+} T-cell populations within CSF samples from patients with both lymphomatous and carcinomatous NM versus control specimens. These cells are CD127\textsuperscript{low} and, when isolated from the CSF, are functionally T\textsubscript{reg} cells, exhibiting fully suppressive properties toward activated T\textsubscript{conv} cells in vitro. These findings reinforce previous reports implicating enhanced T\textsubscript{reg}-cell numbers in ovarian, breast, lung, gastric, esophageal, and pancreatic cancers as well as in Hodgkin lymphoma and B-NHL.\textsuperscript{10,12-17,32,33}

Irrespective of the underlying malignancy, the presence of neoplastic cells in the leptomeninges and CSF triggers trafficking of T\textsubscript{reg} cells from the systemic circulation to the subarachnoid...
space, as CSF supernatants from patients with LM and CM universally exhibit a marked chemotactic activity toward T_{reg} cells that is significantly more pronounced compared with specimens from patients with NID and ID. Consequently, prevalences of T_{reg} cells increase without a concomitant rise in CD4^{+} T-cell numbers. This is in sharp contrast with findings in CSF specimens from patients with infections, where CD4^{+} T cells increase, but T_{reg}-cell proportions do not compared with the NID cohort. We also did not detect redistribution of T_{reg} cells into the CSF in patients with MS, confirming our earlier observations. Both NM and infections coincided with a pathological Q_{alb} of variable extent in most patients, whereas enhanced T_{reg}-cell frequencies were detectable in NM only. Collectively, there was no correlation between Q_{alb} and prevalences of T_{reg} cells within CSF, indicating that the disturbed permeability of the BCB cannot fully explain the increase of T_{reg} cells in the CSF of patients with NM. Instead, T_{reg}-cell frequencies correlate with the amount of malignant cells in the CSF, suggesting that T_{reg} cells are selectively recruited in the CSF in response to leptomeningeal carcinomatosis or lymphomatosis.

In concordance with this assumption, T_{reg} cell–specific chemokines CCL17 and CCL22 are enriched in CSF specimens from patients with NM, and both their concentrations and the CI-T_{reg} are determined by the magnitude of malignant cell populations detectable in individual CSF samples. To exclude passive diffusion of chemokines across the BCB, we tested CCL17 and CCL22 in paired serum and CSF samples. There was no correlation between chemokine levels in CSF and Q_{alb}, and systemic chemokine concentrations were not elevated in the NM group, arguing for an intrathecal production of CCL17 and CCL22 in patients with LM and CM. Although we did not assess subsets of CSF cells independently for their respective chemokine profiles, the correlation between numbers of atypical cells and chemokine levels suggests that chemokine secretion from tumor cells contributes to the local synthesis of CCL17 and CCL22.

This conclusion is in concordance with a recent study demonstrating chemotaxis and migration of T_{reg} cells in B-NHL tissues resulting from secretion of CCL22 by lymphoma cells. Production mediating intratumoral T_{reg}-cell infiltration has also been detected in ovarian cancer cells. Moreover, CCL22 and/or CCL17 were found to be involved in trafficking of CCR4^{+} T_{reg} cells in diffuse large B-cell lymphoma, Hodgkin lymphoma, and B-cell chronic lymphocytic leukemia (B-CLL).

The consequences of T_{reg}-cell accumulation within neoplastic tissues are not yet conclusively defined. However, available evidence demonstrates that intratumoral T_{reg} cells from patients with lymphoma as well as from patients with other malignancies are functionally capable of suppressing the proliferation and cytokine production of CD4^{+} T cells and to suppress tumor-specific T-cell immunity. Congruously, enhanced T_{reg}-cell frequencies were correlated with poor prognosis of patients with solid tumors and with B-CLL. Contradictory to these findings, high numbers of T_{reg} cells are associated with improved survival in Hodgkin lymphoma and follicular lymphoma, possibly indicating a different role for T_{reg} cells in the pathogenesis of these types of hemalogic malignancies.

Collectively, this study shows that leptomeningeal metastasis from B-NHL and from various carcinomas prompts selective recruitment of T_{reg} cells across the BCB to the subarachnoid space without concomitant accumulation of T_{conv} cells, and thereby might create a microenvironment which favors the survival and growth of malignant cells. Intriguingly, T_{reg} cells might even more efficiently counteract local antitumor immune responses than outside the CNS, where the movement of tumor-infiltrating conventional human T cells is less restricted by barrier structures. Further studies are required to prove that T_{reg} cells interfere with T-cell responses directed against the respective tumors. Proof of concept will provide a rationale for manipulating T_{reg} cells to enhance immunotherapy for patients with lymphomatous or carcinomatous NM.

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Authorship

Contribution: J.H. designed and performed research, analyzed data, and wrote the paper; L.S. performed research and analyzed data; B.S.-H. contributed clinical samples and assisted in writing the paper; B. Fritzsching performed flow cytometry and analyzed data; C.J. contributed expertise in ELISA; L.M. performed research and analyzed data; B. Fritz performed research; A.S. assisted in writing the paper; E.S.-P. assisted in writing the paper; M.H. contributed clinical samples and assisted in writing the paper; and B.W. designed research and wrote the paper.

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References

10. Ichihara F, Kono K, Takahashi A, Kawaida H, Sugai H, Fujii H. Increased populations of regulatory T cells in peripheral blood and tumor-infiltrating lymphocytes in patients with gastric and...

promised lymphocytes infiltrate hepatocellular 
carcinoma: the role of T-regulatory cells. Hepatol- 

nosuppressive regulatory T cells are abundant in 
the reactive lymphocytes of Hodgkin lymphoma. 

CD4+CD25+ T cells in tumors from patients with 
early-stage non-small cell lung cancer and late-

1030-1039.

15. Liyanage UK, Moore TT, Joo HG, et al. Preva-

16. Curiel TJ, Coukos G, Zou L, et al. Specific recruit-


18. Iellem A, Mariani M, Lang R, et al. Unique chem-


23. Lin L, Nonoyama S, Oshiba A, Kabasawa Y, 

24. Nakayama T, Hieshima K, Nagakubo D, et al. Se-

25. Peh SC, Kim LH, Poppema S. TARC, a CC che-

26. Reiber HPeter JB. Cerebrospinal fluid analysis: 

disease-related data patterns and evaluation pro-

27. Sakaguchi S. Naturally arising FOXP3-express-


30. Salustro F, Palermo B, Lenig D, et al. Distinct pat-

31. Haas J, Hug A, Viehöver A, et al. Reduced sup-

32. Viguier M, Lemaitre F, Verola D, et al. FOXP3-ex-

33. Yang ZZ, Novak AJ, Stenson MJ, Witzig TE, 

34. Ishida T, Inagaki H, Kusumoto S, et al. CC che-


36. van den Berg A, Visser L, Poppema S. High ex-

37. Beyer M, Kochanek M, Darabi K, et al. Reduced 

38. Maggio EM, van den Berg A, Visser L, et al. Com-

39. Sasada T, Kimura M, Yoshida Y, Kanai M, Tak-

40. Sasada T, Kimura M, Yoshida Y, Kanai M, Tak-


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