To the editor:

IFN-γ and STAT1 are required for efficient induction of CXC chemokine receptor 3 (CXCR3) on CD4$^+$ but not CD8$^+$ T cells

The CXC chemokine receptor 3 (CXCR3) regulates migration and function of T cells during inflammation. Resting T cells express low levels of CXCR3 but they up-regulate CXCR3 upon activation. Several studies suggest that T helper 1 (Th1)/Th2-associated cytokines regulate levels of CXCR3 on T cells.[1-3] Interferon-γ (IFN-γ), which signals via STAT1, enhances CXCR3 expression on T cells,[2] but its role in regulating CXCR3 on CD4$^+$ versus CD8$^+$ T cells is not clear. We therefore examined the role of IFN-γ and STAT1 in regulating CXCR3 levels on CD4$^+$ and CD8$^+$ T cells.

Naive CD4$^+$ and CD8$^+$ T cells from C57BL/6 mice were stimulated as described previously[2] with anti-CD3e (3 μg/mL; Biolegend, San Diego, CA) and anti-CD28 (4 μg/mL; Biolegend) in the presence of anti–IFN-γ neutralizing or control antibody, and CXCR3 expression was analyzed by flow cytometry. CD4$^+$ T cells activated in the presence of anti–IFN-γ Ab (clone no. XMG1.2; Pharmingen, San Diego, CA) expressed less CXCR3 than controls (Figure 1A). However, IFN-γ blockade had no effect on CXCR3 levels in CD8$^+$ T cells (Figure 1B). Control CD4$^+$ and CD8$^+$ T cells up-regulated CXCR3 (Figure 1A,B) and produced comparable IFN-γ (data not shown).

Next, we compared CXCR3 surface expression and mRNA levels in stimulated CD4$^+$ and CD8$^+$ T cells from wild-type (WT) and STAT1$^{-/-}$ C57BL/6 mice. Activated STAT1$^{-/-}$ CD4$^+$ T cells failed to up-regulate CXCR3 as efficiently as WT CD4$^+$ T cells (Figure 1C). In contrast, STAT1$^{-/-}$ CD8$^+$ T cells showed a significant induction of CXCR3 similar to WT CD8$^+$ T cells (Figure 1D). Low CXCR3 expression on STAT1$^{-/-}$ CD4$^+$ T cells also correlated with low CXCR3 mRNA levels (Figure 1E,F). Activated WT and STAT1$^{-/-}$ CD4$^+$ as well as CD8$^+$ T cells produced significant IFN-γ, but levels were lower in STAT1$^{-/-}$ T cells (WT CD4, 19908.17 [± 2582.868] pg/mL; WT CD8, 27361.05 [± 3484.116] pg/mL; STAT1$^{-/-}$ CD4, 8012.928 [± 1627.75] pg/mL; STAT1$^{-/-}$ CD8, 17152.14 [± 3680.819] pg/mL). Blockade of IFN-γ did not inhibit CXCR3 expression on STAT1$^{-/-}$ CD8$^+$ T cells, suggesting that IFN-γ was not inducing CXCR3 via a STAT1-independent pathway (data not shown).

We also determined whether the transcription factors T-bet and eomesodermin (Eomes) are involved in CXCR3 induction on STAT1$^{-/-}$ CD8$^+$ T cells by measuring mRNA levels by real-time reverse transcription–polymerase chain reaction (RT-PCR). Both these factors control CD4$^+$ and CD8$^+$ T-cell activity by regulating expression of many genes, including *Cxcr3*,[4] and their expression in T cells can be induced via STAT1-depdendent and -independent mechanims.[5,6] STAT1$^{-/-}$ CD4$^+$ T cells showed less induction of T-bet mRNA than WT CD4$^+$ T cells (Figure 1G). In contrast, STAT1$^{-/-}$ CD8$^+$ T cells contained more T-bet mRNA than WT CD8$^+$ T cells, but the difference was not significant (Figure 1H). STAT1$^{-/-}$ CD4$^+$ and CD8$^+$ T cells also contained less Eomes mRNA than WT T cells, but these differences were not significant (Figure 1I,J).

These findings show that IFN-γ and STAT1 are critical for efficient induction of CXCR3 on CD4$^+$, but not CD8$^+$ T cells upon activation. To best of our knowledge, this is the first study to demonstrate differential requirement for IFN-γ and STAT1 in up-regulation of CXCR3 on CD4$^+$ versus CD8$^+$ T cells. These findings are important in designing therapeutic strategies to control

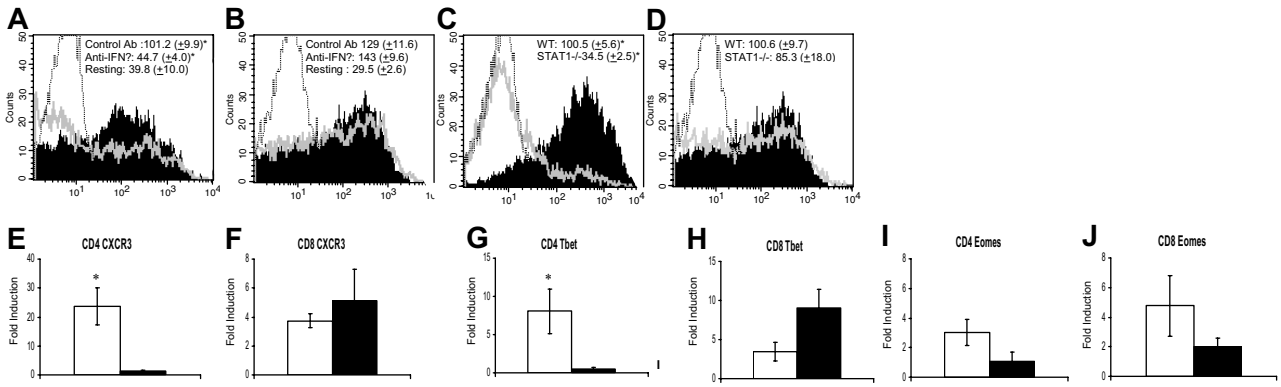

Figure 1. IFN-γ and STAT1 are required for efficient induction of CXCR3 on CD4$^+$ but not CD8$^+$ T cells and T-bet, but not Eomes, controls CXCR3 induction. After stimulation with anti-CD3 and anti-CD28 antibody, cells were rested 24 hours without stimulation. CXCR3 surface protein expression was measured by flow cytometric staining of wild-type CD4 (A) and CD8 (B) cells in the presence of 50 μg/mL IFN-γ–neutralizing antibody or an isotype control. Stained cells treated with anti–IFN-γ are represented by hollow gray peaks, cells stimulated in the presence of control antibody are depicted by solid peaks, and dotted lines represent a PE-labeled isotype control. Surface CXCR3 was also analyzed on STAT1$^{-/-}$ CD4 (C) and CD8 (D) T cells. STAT1$^{+/+}$ cells are represented by solid peaks, STAT1$^{-/-}$ cells are represented by hollow gray peaks, and isotype control staining is repressed by a dotted line. Levels of CXCR3 (E,F), T-bet (G,H), and *Eomes* (I,J) mRNA in activated CD4 and CD8 T cells from WT (□) as well as STAT1$^{-/-}$ mice (■) were measured by real-time RT-PCR. Data were normalized to the housekeeping gene *GAPDH*, and the results are presented as fold-induction of gene expression over nonactivated cells. Histograms in panels A and B are representative of 3 independent experiments, while panels C and D represent 1 of 5 independent experiments. Mean fluorescence intensity (MFI) data represent the mean (± SEM) for all experiments. Resting WT CD4$^+$ and CD8$^+$ T cells in these experiments yielded average MFIs of 39.8 (± 10.0) and 29.5 (± 2.6), respectively, while STAT1$^{-/-}$ CD4$^+$ and CD8$^+$ T cells resulted in 32.6 (± 10.7) and 34.1 (± 11.4), respectively, prior to activation. (E-J) Averaged results (± SEM) of 5 independent experiments. Statistical analysis was performed using the Mann-Whitney rank-sum test (SigmaStat; Systat Software, San Diego, CA). *$P < .05$ was considered significant.

CXCR3 expression in T cells for treatment of autoimmune and inflammatory diseases.

Joseph Barbi, Steve Oghumu, Claudio M. Lezama-Davila, and Abhay R. Satoskar

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Correspondence: Dr. Abhay R. Satoskar, Department of Microbiology, The Ohio State University, 484 West 12th Ave, Columbus, OH 43221; e-mail: satoskar.2@osu.edu.

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To the editor:

Epigallocathechin-3-gallate in AL amyloidosis: a new therapeutic option?

This unusual letter comes from Heidelberg, where the first description of amyloidosis was published in 1859.[1] The first autologous transplantation of blood-derived hematopoietic stem cells was conducted here in 1985 in my university hospital department and reported in *Blood* in 1986.[2]

At the present time, I am—as an emeritus professor of internal medicine and hematology—a patient, suffering from lambda light-chain amyloidosis since 2001, when I was 72, although it was not diagnosed until 2004. The illness has peculiarities that make it worth reporting. A short-term therapy with the "Boston scheme" (4 mg melphalan daily for 21 days) showed no response. Therapy according to the "Palladini scheme" (0.22 mg/kg melphalan and 40 mg dexamethasone for 4 days every 28 days) stabilized the disease, but at high cost because of the side effects. After 14 cycles (a total of 872 mg melphalan and 2400 mg dexamethasone), "drug holidays" were recommended by the German, Italian, and American physicians in charge of my treatment.

These "holidays" have been extended since September 2006. The drug treatment was not resumed because of the apparent effectiveness of an entirely different approach: since September 2006, I have ingested daily 1.5 to 2.0 L of green tea, effective presumably because of its contents of EGCG (epigallocatechin gallate) and other phenols.

Recommendation of this "therapy" came from former members of my staff on the basis of a lecture by Prof E. E. Wanker alerting them to in vitro experiments reporting the effects of EGCG on light chains and amyloid fibrils (Ehrnhoefer DE, Bieschke J, Boeddrich A, et al; submitted manuscript). These results impressed me, a hematologist rooted deeply in the natural sciences, so I decided to follow the advice to drink green tea on a daily basis.

The results are incredible. For 20 months, the interventricular septum had remained constant at 16.5 mm, which we considered the successful effect of chemotherapy. Since September 2006, it has decreased month by month to what is at present a constant thickness of 13.2 mm. The longitudinal myocardial deformation showed a clear improvement. The light chains do not exceed 50 mg/L; the kappa/lambda ratio remains steady between 0.74 and 0.50. The renal insufficiency remains unchanged with values of creatinine at 229.84 μmol/L (2.6 mg/dL) and urea at 24.99 μmol/L (70 mg/dL). And, most surprisingly, my quality of life has improved dramatically despite my now 78 years of age.

Since I am experiencing the objective and subjective improvements of my health, I am encouraged to present my case for your consideration. Because of the rarity of the disease, international clinical studies in amyloidosis should take note of this singular case, as with patients with lymphoma[3] after taking notice of "furtive green tea drinkers" among them. EGCG, a commercially available white powder, should be made a therapeutic option soon.

Werner Hunstein

Correspondence: University of Heidelberg, Schlosswolfsbrunnenweg 41, Heidelberg, D 69118, Germany; e-mail: werner.hunstein@urz.uni-heidelberg.de.

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