Journal of Neuroimmunology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

HTLV-1 induces a Th1-like state in CD4$^+$ CCR4$^+$ T cells that produces an inflammatory positive feedback loop via astrocytes in HAM/TSP

Yoshihisa Yamano *, Ariella Coler-Reilly

Department of Rare Diseases Research, Institute of Medical Science, St. Marianna University School of Medicine, Kanagawa, Japan

ARTICLE INFO

Article history:
Received 31 October 2015
Received in revised form 15 July 2016
Accepted 10 August 2016
Available online xxxx

Keywords:
HAM
HTLV-1
CCR4
CXCR3
CXCL10
Astrocyte

ABSTRACT

The main feature of Human T-lymphotropic virus type I (HTLV-1) -associated myelopathy/tropical spastic paraparesis (HAM/TSP) pathogenesis is a virus-induced hyperactive immune response that produces chronic inflammation in the central nervous system (CNS), but the mechanism by which HTLV-1 deregulates the immune response is unknown. We recently reported a high frequency of HTLV-1-infected CCR4$^+$ cells, including regulatory T cells. We showed that HTLV-1 induces a Th1-like state in these CCR4$^+$ cells via T-bet expression. We have also found that CXCL10 plays an important role in a positive feedback loop that maintains inflammation in the CNS. Astrocytes, which were found to be the main producers of CXCL10 in the CNS, are another key player in the loop. In short, we postulate that infected CCR4$^+$ Th1-like T cells produce interferon-γ, which stimulates astrocytes to produce CXCL10. We now have a much better understanding of the molecular mechanisms at play in HAM/TSP pathogenesis.

1. Introduction

Human T-lymphotropic virus type 1 (HTLV-1) is a human retrovirus endemic to Japan, the Caribbean, South America, and Africa. More than one million people in Japan alone and about 20 million people worldwide are infected with HTLV-1 (Gessain and Cassar, 2012). Although most infected individuals remain lifelong asymptomatic carriers (ACs), approximately 2–5% of those carriers develop an aggressive cancer known as adult T cell leukemia/lymphoma (ATLL) (Uchiyama et al., 1977), and 0.3–3.8% of HTLV-1 carriers develop the debilitating inflammation in the spinal cord that characterizes HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Gessain et al., 1985; Osame et al., 1986). Since the spinal cord is primarily affected in HAM/TSP patients, the main symptoms are lower limb paralysis, pain, dysuria, and constipation. As the disease progresses, patients may become wheelchair-bound or even bedridden (Bangham et al., 2015). Unfortunately, there is no cure for this disease. Furthermore, HAM/TSP patients are still at risk for developing ATLL. Thus, the prognosis for HAM/TSP patients is extremely poor, and there is a strong demand for a novel therapeutic strategy. To this end, it is important to elucidate the mechanism by which HTLV-1 causes HAM/TSP.

2. HAM/TSP is not a simple infection of the CNS

The primary neuropathological feature of HAM/TSP is chronic meningomyelitis of the white and gray matter, followed by axonal degeneration preferentially affecting the middle-to-lower thoracic cord. The lesions are associated with perivascular and mild parenchymal lymphocytic infiltration, with the presence of foamy macrophages, proliferation of astrocytes, and fibrillary gliosis (Izumo et al., 1992). Proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)-1β were detected in perivascular infiltrating cells (Umehara et al., 1994). There is direct evidence that HTLV-1-infected CD4$^+$ T cells are present in the spinal cord lesions (Matsuoka et al., 1998) and that CD8$^+$ T cells directed against HTLV-1 antigens accumulate in the CSF and spinal cord lesions (Nagai et al., 2001; Matsuura et al., 2015).

HAM/TSP is an unusual infectious disease in that the HTLV-1 virus disrupts the nervous system indirectly by infecting T cells and directly disrupting the immune system. The number of HTLV-1-infected T cells (i.e., proviral load) circulating in the peripheral blood is higher in patients with HAM/TSP than in ACs (Nagai et al., 1998) and is even higher in the cerebrospinal fluid (CSF) of patients with HAM/TSP (Nagai et al., 2001). However, there are some carriers who have higher proviral loads in the peripheral blood than some HAM/TSP patients (Nagai et al., 1998), suggesting that a high proviral load is not sufficient to cause HAM/TSP. While the proviral load cannot be used to distinguish HAM/TSP patients from carriers, a highly activated immune response is the trademark of HAM/TSP. For example, in HAM/TSP patients, there is high production of inflammatory cytokines and chemokines in the

* Corresponding author at: Department of Rare Diseases Research, Institute of Medical Science, St. Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki, Kanagawa 216-8512, Japan.
E-mail address: yyamano@marianna-u.ac.jp (Y. Yamano).

http://dx.doi.org/10.1016/j.jneuroim.2016.08.012
0165-5728/

CSF, but this is not observed in ACs (Bangham et al., 2015), which suggests there is a unique pathway responsible for initiating and maintaining chronic inflammation in HAM/TSP. Importantly, HTLV-1 is observed in spinal cord lesions only in the T-cell infiltrates, and there is no convincing evidence that cells normally residing in the CNS become infected (Matsuoka et al., 1998). As for possibility of autoimmune involvement, there is insufficient evidence to make a case for antigen mimicry as a factor in HAM/TSP pathogenesis (Bangham et al., 2015).

Thus, HAM/TSP is neither a simple infection of the nervous system nor an autoimmune disease. It is now widely accepted that HTLV-1-infected T cells invade the spinal cord and trigger a strong virus-specific immune response, stimulating the production of proinflammatory cytokines and chemokines, leading to chronic inflammation, which causes tissue damage. This is known as bystander damage (Bangham et al., 2015). However, the precise mechanism by which HTLV-1 activates this kind of aggressive immune response has not yet been established.

3. HTLV-1 causes imbalance in Th cell lineages in HAM/TSP

$CD4^+$ helper T cells, which are central players of the adaptive immune response, are the predominant reservoir of HTLV-1 (Richardson et al., 1990). There are various Th cell subsets: Th1, Th2, Th17, and T-regulatory (Treg) cells. These subsets work together to maintain balance in the immune system, and "imbalance" is known to cause dangerous immune abnormalities (Murphy and Stockinger, 2010). To understand the effects of HTLV-1 infection on the functioning of Th cells, we investigated how these individual subsets are targeted by HTLV-1 in HAM/TSP. Recently, we showed that $CD4^+$ cells that have the CCR4 receptor are much more likely to be infected by HTLV-1 (Yamano et al., 2009; Araya et al., 2014). Although CCR4 is known to be selectively expressed on Treg and Th2 cells in healthy individuals, more detailed flow cytometric analysis of Foxp3 expression in $CD4^+CD25^+CCR4^+$ T cells of HAM/TSP patients demonstrated that the expression level of Foxp3 was low and that the frequency of the $Foxp3^{low}$ population was very high in $CD4^+CD25^+CCR4^+$ T cells. Moreover, analysis of cytokine expression in this $Foxp3^{low}$ $CD4^+CD25^+CCR4^+$ T cell population of HAM/TSP patients revealed that these cells had excessive levels of IFN-γ. Importantly, the frequency of these IFN-γ-producing $CD4^+$-$CD25^+CCR4^+$ T cells in HAM/TSP patients was correlated with disease severity (Yamano et al., 2009).

Human $FoxP3^+CD4^+$ T cells can be segregated into three subpopulations on the basis of FoxP3 and CD45RA expression levels: (i) $FoxP3^{high}CD45RA^-$ cells, designated as effector Treg (eTreg) cells, which are terminally differentiated and highly suppressive; (ii) $FoxP3^{low}CD45RA^+$ cells, designated naïve Treg cells, which differentiate into eTreg cells upon antigenic stimulation; and (iii) $FoxP3^{low}$ $CD45RA^-$ non-Treg cells, which do not possess suppressive activity but secrete proinflammatory cytokines and exacerbate inflammation (Sugiyama et al., 2013). Since CCR4 is known to be expressed on memory ($CD45RA^-$) cells, IFN-γ-overproducing $Foxp3^{low}CD4^+CD25^+CCR4^+$ T cells observed in HAM/TSP patients are similar to the abovementioned $FOXP3^{low}$ $CD45RA^-$ non-Treg cells. Thus, in healthy donors, the $CD4^+CD25^+CCR4^+$ T cell population primarily consists of suppressor T cell subsets such as Treg and Th2, whereas that of HAM/TSP patients consists of a high number of IFN-γ-producing $Foxp3^{low}$ cells, which may result in the collapse of immune system balance. This imbalance may at least partly account for the excessive immune response observed in HAM/TSP (Fig. 1).

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

Differences between ATLL and HAM/TSP patients provide clues for solving the mysteries of HAM/TSP pathogenesis. While the $CD4^+$-$CD25^+CCR4^+$ T cells of ATLL patients tend to include many $Foxp3^{high}$ cells, those of HAM/TSP patients include many IFN-γ-producing $Foxp3^{low}$ cells (Yamano et al., 2009; Karube et al., 2004). In addition, the expression of the HTLV-1 protein product Tax in $CD4^+CD25^+CCR4^+$ T cells is higher in HAM/TSP versus ATLL patients despite similar proviral loads (Araya et al., 2011). Therefore, we hypothesized that HTLV-1, via intracellular Tax expression and subsequent transcriptional alterations, causes chronic inflammation by infecting $CD4^+CCR4^+$ T cells and inducing their transformation into Th1-like, IFN-γ-producing proinflammatory cells.

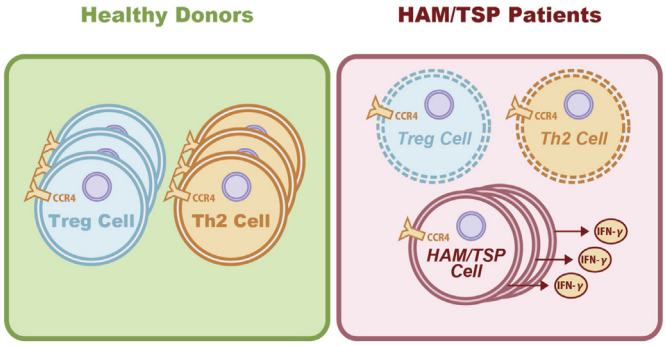

Fig. 1. Differences in the composition of $CD4^+CD25^+CCR4^+$ T cells in healthy donors and HAM/TSP patients. In healthy donors, the $CD4^+CD25^+CCR4^+$ T cell population consists of suppressor T cell subsets such as Treg and Th2; by contrast, in HAM/TSP patients, there are a high number of IFN-γ producing $Foxp3^{low}$ cells, here referred to as HAM/TSP cells. The abnormal composition of T cells illustrated here is thought to deregulate the immune system and cause an excessive inflammatory response.

Recently, we performed an in-depth analysis of the mechanism by which Tax influences the function of $CD4^+CD25^+CCR4^+$ T cells (Araya et al., 2014). Using DNA microarray analysis of $CD4^+CD25^+$-$CCR4^+$ T cells from HAM/TSP patients, we identified T-bet, known as a master transcriptional factor in Th1 differentiation, as a key intermediary between Tax expression and IFN-γ production. We demonstrated that Tax, in complex with specificity protein 1 (Sp1), amplifies T-bet transcription and, subsequently, IFN-γ production. We also showed that T-bet expression is elevated in the $CD4^+CD25^+CCR4^+$ cells of HAM/TSP patients but not ATLL patients, suggesting that this trait is specific to HAM/TSP pathogenesis.

5. High levels of Th1-like $CD4^+CCR4^+$ T cells are present in the CNS

T-cell infiltrates in the CNS, a phenomenon indicative of spinal cord inflammation, is a well-known feature of HAM/TSP. Researchers have worked to characterize these cells over the years, and 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 (Umehara et al., 1994; Furuya et al., 1997; Moritoyo et al., 1999). Recently, we found Th1-like $CD4^+CCR4^+$ T-cells in the CSF and spinal cord lesions of HAM/TSP patients (Araya et al., 2014). Most of these $CD4^+CCR4^+$ T-cells in the CNS co-expressed Th1 markers such as CXCR3, T-bet, and IFN-γ. While cells isolated from the CSF of HAM/TSP patients contained a high proportion of these $CD4^+CCR4^+CXCR3^+$ cells, they were virtually nonexistent in the peripheral blood of both HAM/TSP patients and healthy subjects. We inferred that the cells had migrated to the CNS, possibly due to some chemotactic factor, leaving few cells behind in the peripheral blood of the HAM/TSP patients. The surprising observation that the Ki67 marker for cell proliferation was absent in the overwhelming majority of $CD4^+CCR4^+CXCR3^+$ cells in the CSF signified that the cells were indeed proliferating elsewhere and subsequently migrating to the CNS (Araya et al., 2014).

6. CXCL10–CXCR3 interaction plays a crucial role in cell migration

Due to the high proportion of $CCR4^+$ HTLV-1 infected cells, the high proviral load in the CSF of HAM/TSP patients, and the elevated levels of CCL22 in the peripheral blood of HAM/TSP patients (Toulza et al., 2010), it might be hypothesized that the infected cells migrate across the

Please cite this article as: Yamano, Y., Coler-Reilly, A., HTLV-1 induces a Th1-like state in CD4+CCR4+ T cells that produces an inflammatory positive feedback loop via astrocytes in HAM/TS..., J. Neuroimmunol. (2016), http://dx.doi.org/10.1016/j.jneuroim.2016.08.012