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IL-25 prevents T cell-mediated neurotoxicity by decreasing LFA-1 expression

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ABSTRACT

Autoimmune diseases such as multiple sclerosis (MS) are thought to develop due to a dysregulation in the normal T_H1–T_H17/T_H2 immune system balance, where pro-inflammatory responses with a T_H1/T_H17 prevalence develop. Some therapeutic treatments in MS promote a shift toward a T_H2-prevalent environment and this has been shown to be protective. However, not all patients respond to current immunomodulatory treatments in MS so that new immunomodulatory drugs that can promote a shift of the immune system into an anti-inflammatory T_H2 status are needed. IL-25 is a cytokine of the IL-17 family with powerful anti-inflammatory properties. This study demonstrates that IL-25 exerts neuroprotective functions by reducing T cell-mediated killing of human fetal neurons. The mechanism of action of this IL-25-mediated neuroprotective effect appears to be linked to reduction in the expression of the adhesion molecule LFA-1, which is relevant in stabilizing the immune synapse during cytotoxicity.

1. Introduction

Multiple sclerosis (MS) is an inflammatory, neurodegenerative and demyelinating autoimmune disease affecting the central nervous system (CNS) (Keegan and Noseworthy, 2002; Compston and Coles, 2008). This disease occurs early in life and is three times more common in women than in men (Vollmer, 2007; Tremlett et al., 2010). MS-affected CNS tissue is characterized by the presence of perivascular lesions containing sites of demyelination with axonal and oligodendrocytes loss (Giuliani and Yong, 2003; Lassmann et al., 2007; Korn, 2008). This process is hypothesized to be initiated by the activation of auto-reactive $CD4^+$ T helper (T_H) cells in the periphery (Giuliani and Yong, 2003; Korn, 2008; Fletcher, 2010). Once activated, naïve T_H cells differentiate into T_H1, T_H2 or the more recently identified population of T_H17 cells, depending on the immediate microenvironment (Laman et al., 1998; Steinman, 2007; Hemdan, 2010; Paul and Zhu, 2010). T_H1 cells have historically been implicated as the primary instigators of MS pathogenesis (Laman et al., 1998; Lassmann et al., 2007; Zidek et al., 2009). These cells are pro-inflammatory type of T helper cell involved in the clearance of bacteria and viruses (Tato et al., 2006; Wan, 2010). T_H1 cells promote the recruitment and growth of other inflammatory lymphocytes by secreting interferon gamma (IFN-γ) and interleukin-2 (IL-12) (Tato et al., 2006; Wan, 2010). These cytokines, along with other type-1 cytokines, are found at elevated levels in the CNS of MS patients (Schrempf and Ziemssen, 2007). The presence of these pro-inflammatory factors in the CNS can activate resident microglia and invading macrophages, which in turn have been implicated in demyelination and progression of the disease (Compston and Coles, 2008; Nair et al., 2008). Adoptive transfer of activated neuroantigen specific T_H1 cells has long been associated with the development of experimental autoimmune encephalomyelitis (EAE), an animal model of MS (Stinissen et al., 1997). In this model, MS is thought to be caused due to a dysregulation in the normal T_H1/T_H2 immune system balance (Hermans et al., 1997; Laman et al., 1998; Lassmann and Ransohoff, 2004; Bar-Or, 2008; Compston and Coles, 2008). In the normal course of events T_H1 and T_H2 regulate each other by the secretion of cytokine inhibitory to the development of the other cell type (Steinman, 2007).

However, this hypothesis is complicated by the recently discovered T helper cell subset, T_H17 cells (Batten et al., 2006; Steinman, 2007; Hemdan, 2010). T_H17 cells are pro-inflammatory T helper cells that secrete IL-17A and other pro-inflammatory cytokines (Segal, 2010). In inflammatory autoimmune diseases such as MS, T_H17 cells release IL-17 and promote the secretion of other inflammatory cytokines and chemokines. Consequently, other inflammatory cell types get recruited to the site of inflammation and play a devastating role in reinforcing and advancing the severity of the disease (Segal, 2010). Like T_H1 cells, adoptive transfer of T_H17 cells also induces EAE (Kroenke et al., 2008). This implicates both cell types as the key mediators of MS pathogenesis.

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These different subtypes of T helper cells work to actively suppress each other's expansion and dominance. IL-2 promotes T_H1 growth while preventing the activation and expansion of either T_H2 or T_H17 populations (Stockinger, 2007). T_H2 cells also play a role in inhibiting the differentiation of T_H17 cells; IL-4 produced by T_H2 cells inhibits the development of T_H1 and T_H17 cells alike, promoting an anti-inflammatory environment as well as blocking the infiltration of inflammatory cells into the CNS (Fort et al., 2001). This T_H2 induced anti-inflammatory environment is thought to be protective in MS (Fort et al., 2001). Pregnancy is a well known anti-inflammatory immune state where T_H2 cells dominate the immune response (Ehrlich et al., 2007). This attenuation also manifests physiologically as lesion growth is slowed during pregnancy (Ehrlich et al., 2007).

Similarly, helminthic parasite infections are another condition that can induce a strong T_H2 cell immune bias (Sewell et al., 2003). T_H2 cells are naturally involved with the clearance of helminthic parasites and defense at the body's mucosal sites by mediating B cell differentiation and a humoral type response to infection (Romagnani, 2006). Parasitic infection has been shown to modulate the body's response to other infections with a decreased inflammatory type response (Wickelgren, 2004). In the case of MS, parasitic infection has shown protective effect (Wickelgren, 2004). In mice models of MS, infection with parasites has been shown to reduce the severity and incidence of the disease (Walsh et al., 2009). Mirroring this effect, a decrease in the relapse rate and reduction in the relapse severity was observed in MS patients infected with helminthes (Correale and Farez, 2007). The epidemiology of MS supports the protective effects of parasitic infections that inflammatory autoimmune diseases are much more common in developed parts of the world, where hygiene is good and parasite infection is low. This suggests the involvement of an environmental factor in the pathogenesis of these diseases (Sewell et al., 2003; Willer et al., 2005; Fleming and Fabry, 2007). A factor that could be responsible for some of the protective effects seen in parasite infected MS patients is interleukin (IL)-25. IL-25 is a recently discovered cytokine that has been found to be upregulated in response to parasitic infection (Owyang et al., 2006). IL-25, also known as IL-17E, is an anti-inflammatory cytokine that has been shown to amplify T_H2 type responses through an increased secretion of type 2 cytokines, such as IL-13, IL-5 and IL-4 (Fort et al., 2001). Although IL-25 amplifies the secretion of these cytokines, it is not required for their secretion in response to parasitic infection (Owyang et al., 2006). It has been suggested that IL-25 is necessary for both the induction of innate and adaptive T_H2 mediated response (Barlow and McKenzie, 2009). Unlike other members of the IL-17 cytokine family which are secreted by T_H17 cells, IL-25 is secreted by activated T_H2 cells (Tamachi et al., 2006). Recently, activated mast cells and microglia have been shown to secrete IL-25 (Ikeda et al., 2003). The receptor for IL-25 is a 56 kDa single trans-membrane receptor that has been found in the lungs, liver, kidney, intestines, lymphoid organs and on activated dendritic cells (Sharkhuu et al., 2006). It appears that the receptor contains several subunits, known to include IL-17RA and IL-17RB (Rickel et al., 2008; Terashima et al., 2008).

IL-25 has been considered a candidate for therapy in other T_H1/T_H2 mediated disorders such as Crohn's disease, inflammatory bowel disease (IBD) and allergic reactions but the extent of its involvement is yet to be examined (Rioux et al., 2001; Podolsky, 2002; Kleinschek et al., 2007). More recently, it has been shown that IL-25 inhibits the production of pro-inflammatory cytokines in IBD $CD4^+$ T cells and it suppresses the differentiation of these cells into Th1 and Th17 cells (Su et al., 2013). The role of IL-25 in MS is still unclear; however, evidence suggests that it may contribute in the amelioration of EAE (Kleinschek et al., 2007). Kleinscheck and colleagues demonstrated that exogenous IL-25 treatment of mice prior to EAE induction reduced both duration and severity of EAE symptoms (Kleinschek et al., 2007). Indeed, the administration of IL-25 increases the secretion of IL-13, reinforcing a T_H2 environment within the CNS (Kleinschek et al., 2007). However, the effect of IL-25 treatment at a cellular level and its mechanisms of action are yet to be explored.

Clinical trials with disease modifying agents or anti-inflammatory drugs are achieving promising results especially in relapse reduction, however, the search for drugs that have neuroprotective and enhanced repair mechanisms is still ongoing (Marta and Giovannoni, 2012). Therefore, in this study, we investigated the potential neuroprotective role of IL-25 in the human system. Using an *in vitro* model of inflammatory-mediated neurodegeneration with human cells, we examined the effect of IL-25 directly on some of the cells immediately involved in MS pathogenesis such as T lymphocytes and neurons. We found that IL-25 reduces T cell-mediated neuronal killing through a mechanism that affects the expression of lymphocyte function-associated antigen-1 (LFA-1; CD11a/CD18).

2. Materials and methods

2.1. T cell culture

Collection of blood samples and isolation of human peripheral blood mononuclear cells (HPBMCs) from healthy volunteer donors were approved by the University of Alberta Biomedical Ethics Committee (UABEC). Informed verbal consent was obtained by the donors and their names were properly documented in a blood donor registry before participating in the study. PBMCs were separated from venous blood by Ficoll–Hypaque (GE Health Care) density gradient centrifugation as previously described (Giuliani et al., 2003). Cells were re-suspended in AIM-V serum free lymphocyte culture medium (Invitrogen) and plated at a density of 1×10^6 cells/ml in 200 μl into 96 well flat-bottomed tissue culture. To generate activated T cells, the plates were pre-treated with anti-human CD3 antibody (BD PharMingen), at a concentration of 5 μg/ml, and the cells were allowed 72 h of incubation, at 37 °C and 5% CO_2, before experimentation. Control unactivated T cells were plated into uncoated plates. IL-25 (R & D Systems) was added to the appropriate cell cultures at a concentration of 50 ng/ml at the same time the cells were cultured into the anti-CD3 antibody immobilized plates, unless otherwise specified.

2.2. Human fetal neurons culture

The protocol for collection of human brain tissue from therapeutic abortions of 15–20 week fetuses was approved by UABEC. The procedures were performed in agreement with the guidelines approved by UABEC and following the recommendations of the Royal Commission on New Reproductive Technologies; and the donor's mother provided informed consent in writing before the procedure. Human cortical fetal neurons (HFNs) were isolated as previously described (Giuliani et al., 2003). Briefly, brain specimens were washed in phosphate buffered saline (PBS) followed by removing the meninges and blood clots. The fragmented brain pieces were transferred into a 50 ml tube and digested in 4 ml 2.5% trypsin (Gibco) and 6–8 ml DNase I (Roche) for 30 min in a 37 °C water bath. The activity of trypsin was inhibited by the addition of 4 ml FBS. Cells were filtered in 125 μm mesh and centrifuged for 5 min at 1200 rpm. Following the final washing step, cells were suspended in culture medium (described below) and plated on 10 μg/ml polyornithine coated T75 flasks at a density of at least 70×10^6 cells/flask in 25 ml medium. The cell culture was supported with MEM medium supplemented with 10% FBS, 1% L-glutamine, 1% essential amino acids, 1% sodium pyruvate, 0.1% dextrose, as well as 1% antibiotics. Unless otherwise indicated, all medium components were obtained from Life Technologies. The cell culture was incubated at 37 °C with 5% CO_2. The cultures were enriched for neurons by the addition of B-D-arabinofuranoside (Ara-C; Sigma-Aldrich) every second time the media on the cells was changed.