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Human T cell expansion and experimental autoimmune encephalomyelitis inhibited by Lenaldekar, a small molecule discovered in a zebrafish screen

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ABSTRACT

Immune-mediated diseases [multiple sclerosis (MS), experimental autoimmune encephalomyelitis (EAE)] are driven by proliferating, highly activated autoreactive T-cells that are unresponsive to *in vivo* immunoregulatory mechanisms. The compound Lenaldekar (LDK) was identified in a zebrafish screen by inhibiting T-cell expansion. By monitoring mitogen- and antigen-driven proliferation, we found that LDK inhibited human and murine T-cell expansion in a non-cytolytic manner. This suppressive activity directly correlated with the degree of activation/proliferation of the T-cells. In testing LDK in an EAE model of MS, exacerbations were suppressed in treated animals. Therefore, LDK represents a novel therapeutic approach to T-cell-mediated autoimmune diseases.

1. Introduction

Approximately 5–8% of the United States population is afflicted with an autoimmune disease (Jacobson et al., 1997). While the etiology of autoimmune diseases is not fully elucidated, the causes are likely based on a combination of hereditary and environmental factors (von Herrath et al., 2003). Further, a common denominator between autoimmune diseases is dysregulation of the immune system that results in activated self-reactive T cells. A number of autoimmune diseases in humans such as type-1 diabetes, systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis (MS) are examples of antigen-specific activated T cells causing damage/immunopathology (Burns et al., 1983; Gregersen et al., 1987; Tisch and McDevitt, 1996; Mathis et al., 2001; Bertsias et al., 2010).

In MS, it is widely accepted that myelin-specific autoreactive T cells contribute to this inflammatory demyelinating disease of the central nervous system (Kawamura et al., 2008). In general, the activation of a T cell is dependent on the T cell receptor (TCR) binding to a specific peptide presented in the context of the major histocompatibility complex (MHC) molecules on the surface of an antigen presenting cell (APC). This recognition of peptide-MHC in combination with co-stimulatory molecules leads to expansion and cytokine release by the newly activated T cell (Davis et al., 1998). In MS patients, T cells recognize and are subsequently activated when their TCR binds to myelin peptide-MHC (Chou et al., 1989; Allegretta et al., 1990; Martin et al., 1990; Valli et al., 1993; Zhang et al., 1994). Among the events that lead to self recognition by autoreactive T cells and subsequently to immunopathogenesis are environmental cues such as infections that initiate and possibly drive the pro-inflammatory response (Libbey and Fujinami, 2010). Interestingly, myelin antigens incubated with human peripheral blood mononuclear cells (PBMCs) from both healthy and MS subjects resulted in similar levels of T cell proliferation; however, the affinity and duration of contact between TCR and myelin-antigen-containing APC were found to be higher in MS patients when compared to healthy subjects, indicating that the level of T cell activation is a factor in disease progression (Martin et al., 1990, 1993; Zhang et al., 1994; Tranquill et al., 2000; Montanaro et al., 2001; Bielekova et al., 2004).

Autoimmune diseases are due to the "breaching" of regulatory mechanisms employed by the immune system to prevent autoreactive T cells from causing disease (Mathis and Benoist, 2010). The mechanics that enable autoreactive T cells to subvert these effector mechanisms are still under investigation (Mathis and Benoist, 2010). Further, the nature and activation state of autoreactive T cells dictates an aggressive treatment course, and in some cases Tysabri® or natalizumab, interferons, and corticosteroids are used to limit exacerbations of disease. However, the available treatment courses for T cell-mediated autoimmune diseases, such as MS, can have broad and deleterious side effects, and the efficacy of these drugs might only be directed against a particular form of MS (Boster et al., 2008). Therefore, finding and developing new compounds that specifically modulate highly activated T cells, would be a beneficial therapeutic approach towards treating autoimmune diseases (Fujinami et al., 2006).

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In this study, the compound 1H-indole-3-carbaldehyde quinolin-8-yl-hydrazone, named Lenaldekar (LDK), was identified using a previously described *in vivo* T cell-reporting zebrafish system (Meeker et al., 2010). Further, LDK was found to be a potent inhibitor of immature normal and cMYC-transformed leukemic T cells in zebrafish and primary human leukemias (N.S.T., unpublished). Since leukemic T cells share many features with activated non-transformed T cells, we investigated LDK's efficacy to modulate T cell expansion by measuring human PBMC T cell proliferation. Using two different approaches to assess T cell proliferation, we found that LDK was able to significantly inhibit T cell proliferation to both mitogen and specific antigens *in vitro*. Further, the inhibition of T cell proliferation by LDK could not be accounted for by cytotoxicity. Interestingly, the potency of LDK increased in conjunction with T cell activation as was shown by escalating the concentration of three different antigens. This suggests that LDK has inhibitory effect(s) on T cell expansion. Using the experimental autoimmune encephalomyelitis (EAE) murine model system, which closely mirrors MS in humans, LDK was able to significantly suppress exacerbations of clinical disease. Thus, LDK was able to modulate highly activated T cells in a non-cytolytic manner and inhibit an autoimmune response *in vivo*, making LDK an attractive platform for development of novel therapeutics for autoimmune diseases, such as MS.

2. Materials and methods

2.1. Human subjects

Blood was collected in acid citrate dextrose, and PBMCs were isolated by Ficoll and preserved in liquid nitrogen, as previously described (Eckels et al., 1999a). Peripheral blood samples were obtained from healthy donors during routine donation and screened for antibodies against human immunodeficiency virus, hepatitis C virus (HCV), hepatitis B virus and human T-lymphotropic virus types I and II. All donors were free from chronic viral infection except Patient PB3019 who is chronically infected with HCV, as determined by PCR. These studies have been reviewed and approved by the University of Utah Institutional Review Board.

2.2. Zebrafish compound screening

Zebrafish compound screening was performed as previously described (Langenau et al., 2004). Briefly, the transgenic $p56^{lck}$:enhanced green fluorescent protein (EGFP) zebrafish line (lck:EGFP) was maintained and bred in E3 water. Eggs produced by mating lck:EGFP zebrafish were collected and larvae were raised to 5–6 days post fertilization. Subsequently, three larvae were added per well in a 96-well plate. Compounds to be tested were added to the 96-well plate at a final concentration of 10 μM per well. After treating larvae for 48 h with test compounds, zebrafish thymic GFP expression was assessed using a Nikon Eclipse E600 imaging system. Compounds that caused little to no larval toxicity and quenched thymic GFP fluorescence in all three larvae per well were considered candidate compounds. LDK was chosen due to the compound's low half maximal inhibitory concentration (IC_{50}) for proliferation of the Jurkat cell line and T cell leukemia (T-ALL) cell lines, in comparison to other candidate compounds (N.S.T. unpublished data).

2.3. Drug synthesis

The drugs 1H-indole-3-carbaldehyde quinolin-8-yl-hydrazone (LDK) and a similar compound 4-(1H-benzimidazol-2-yl) quinoline, named compound 6 (C6), were obtained from the Chembridge DIVERSet library (ChemBridge, San Diego, CA). C6 is used as a control in our studies since it has a very similar chemical structure. Compounds were dissolved in dimethyl sulfoxide (DMSO) (Sigma, St. Louis, MO) to a final concentration of 0.1% and resuspended in RPMI-1640 (BioWhittaker, Walkersville, ME).

2.4. Antigens and mitogens

Synthetic peptides representing the human leukocyte antigen DRB1*1501-restricted epitope surrounding HCV non-structural-3 (NS3) amino acids 358–375 (amino acids 1384–1401 of the HCV polyprotein) ($NS3_{358-375}$) and the myelin proteolipid protein (PLP) peptide $PLP_{139-151}$ were both synthesized using 9-fluorenylmethyloxycarbonyl chemistry and HPLC purified to >90% at the Blood Center of Wisconsin (Milwaukee, WI) and the University

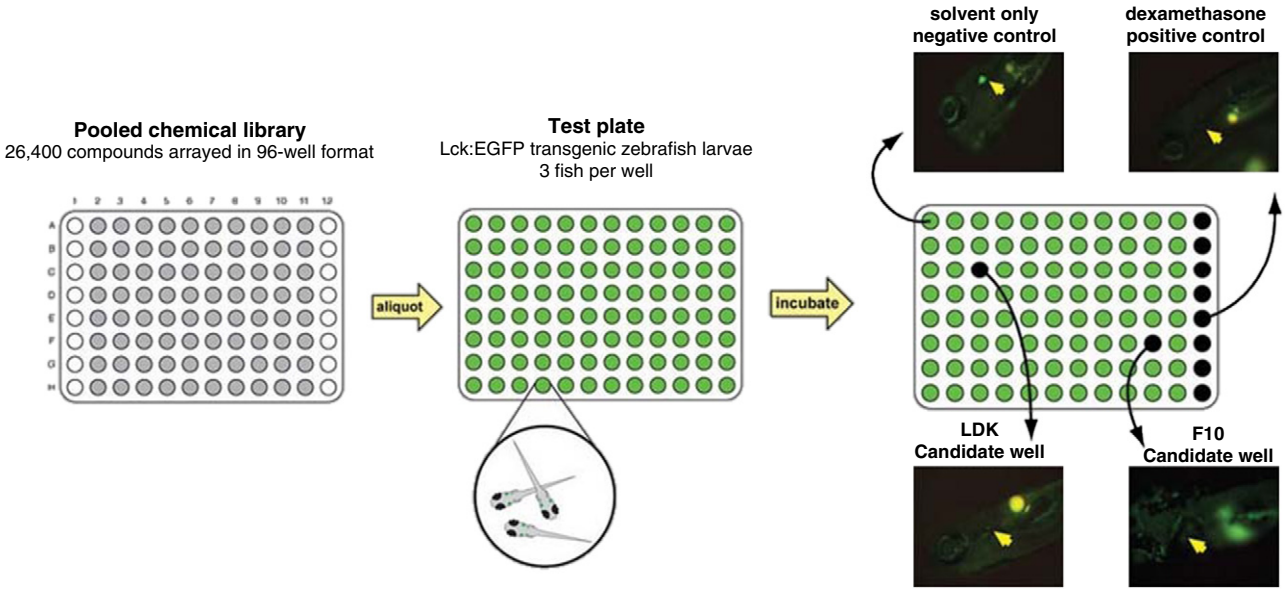

Fig. 1. LDK eliminates EGFP expressing T cells in transgenic zebrafish. Screening of a small molecule library was assessed using zebrafish larvae with the T cell-specific transgene lck:EGFP. Three larvae per well in 96-well plates were treated with indicated compounds or controls. EGFP emission was measured 48 h later: no effect/normal fluorescence (dark green); strong effect (individual black wells); positive control (far right black column). LDK had a similar effect as the positive control dexamethasone by eliminating thymic fluorescence in zebrafish.