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Decitabine induces regulatory T cells, inhibits the production of IFN-gamma and IL-17 and exerts preventive and therapeutic efficacy in rodent experimental autoimmune neuritis

Paolo Fagone[a], Emanuela Mazzon[b], Tinatin Chikovani[c], Andrea Saraceno[a], Santa Mammana[b], Giuseppe Colletti[a], Katia Mangano[a], Placido Bramanti[b], Ferdinando Nicoletti[a,*]

[a] Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy
[b] IRCCS Centro Neurolesi Bonino Pulejo, Stada Statale 113, C.da Casazza, 98124 Messina, Italy
[c] Department of Immunology, Tbilisi State Medical University, 0186 Tbilisi, Georgia

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ABSTRACT

Guillain-Barré syndrome (GBS) is an immune-mediated acute disorder of the peripheral nervous system. Despite treatment, there is an associated mortality and severe disability in 9 to 17% of the cases. Decitabine (DAC) is a hypomethylating drug used in myelodisplastic syndrome, that has been shown to exert immunomodulatory effects. We have evaluated the effects of DAC in two rodent models of GBS, the Experimental Allergic Neuritis (EAN). Both prophylactic and therapeutic treatment with DAC ameliorated the clinical course of EAN, increasing the numbers of thymic regulatory T cells and reducing the production of proinflammmatory cytokines. Our data suggest the possible use of decitabine for the treatment of GBS.

1. Introduction

Guillain-Barré syndrome (GBS) is a heterogeneous disorder of the peripheral nervous system (PNS), representing at least five different entities, including forms with predominant motor component, such as AIDP, AMSAN, and AMAN, and other variants, such as Fisher syndrome and acute pan-autonomic neuropathy. Most forms of GBS are characterized by immune-mediated demyelination and axonal damage of peripheral nerves that is clinically associated to progressive weakness of the limbs (Schafflick et al., 2017; Soliven, 2014).

From the immune-pathogenic point of view, unlike other autoimmune diseases, such as SLE and autoimmune hepatitis, where a simultaneous activation of Th1 and Th2 cytokines can be observed during the course of the disease (Barcellini et al., 1996; de Oliveira et al., 2015; Longhi et al., 2013), GBS is associated and seems to pathogenically depend on a selective increase of Th1 and Th17 proinflammatory cytokines, along with reduction of anti-inflammatory Th2 and Th3 cytokines, to infectious agents such as surface lipo-oligosaccharide components of Campylobacter and other microbial species mimicking PNS gangliosides (Nyati and Prasad, 2014; (Jasti et al., 2016). These responses may subsequently evoke epitope spreading to other putative myelin components antigens, such as P0, P2 and PMP22 (Khalili-Shirazi et al., 1993; Sinmaz et al., 2016; Soliven, 2014). Along with dysregulated balance of Th1 and Th17 T cell subsets, quantitative and qualitative defects of regulatory T cells have been described in GBS patients (Chi et al., 2007a; Harness and McCombe, 2008).

In spite of this deeper understanding of immune-pathogenic mechanisms operating in GBS, treatment of all the variants of GBS consists of either plasma exchange or intravenous immunoglobulin. GBS is the most frequent cause of acute flaccid paralysis and, despite treatment, there is an associated mortality and severe disability in 9 to 17% of the cases (Shahrizaila and Yuki, 2011). Therefore, new treatments are highly warranted for the treatment of GBS.

Experimental autoimmune neuritis (EAN) is a known and validated model of demyelination of the PNS, that can be induced in susceptible strains of rodents by immunization with myelin proteins P0, P2 and PMP22 (Schafflick et al., 2017). Clinical, histological and immune-pathogenic similarities between EAN and human GBS have made the myelin component (MC)-induced EAN a suitable and validated model of GBS. Both MC-induced EAN and GBS exhibit an acute onset with monophasic course of disease. In addition, both in MC-induced EAN and in certain forms of GBS, a predominant infiltration of the PNS from macrophages and T cells is observed (Schafflick et al., 2017, Soliven, 2014; Zhang et al., 2012). The contribution of T cells and macrophages to EAN is also proven by the ability of P2-specific T cell lines to transfer EAN to healthy animals (Soliven, 2014) and the possibility to prevent

* Corresponding author at: Via Santa Sofia n. 97, 95123 Catania, Italy.
E-mail addresses: ferdinic@hotmail.com, ferdinic@unict.it (F. Nicoletti).

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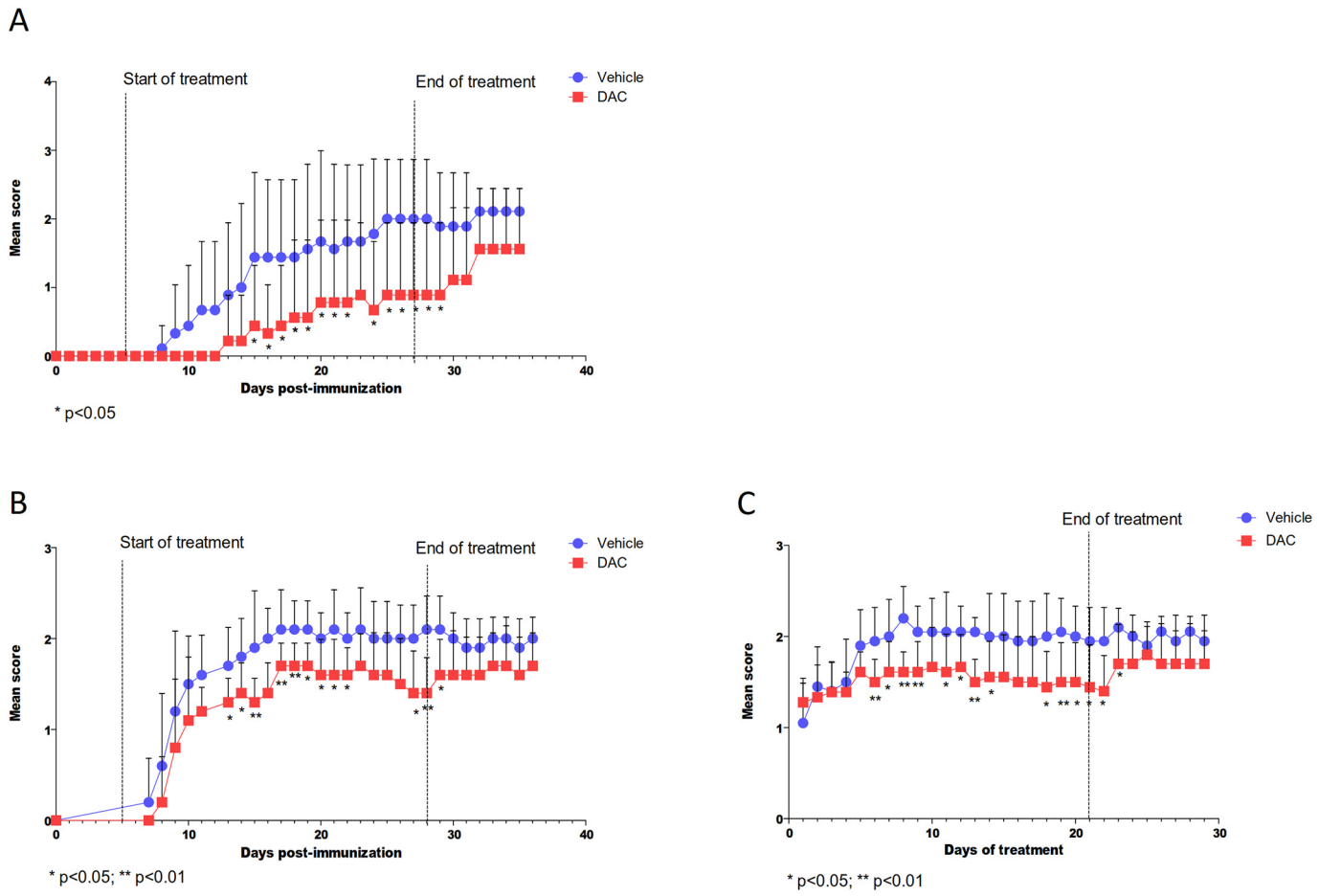

Fig. 1. Effect of DAC on clinical course of EAN. Mean clinical score of Lewis rats immunized with P0 peptide and treated with DAC 0.1 mg/Kg/day by i.p. injection, starting 5 days after immunization (A). Mean clinical score of C57Bl/6 mice immunized with P0 peptide and treated with DAC 0.1 mg/Kg/day by i.p. injection, starting 5 days after immunization (B) or at disease onset (C).

EAN by depletion of macrophages by administration of silica dust or by blocking macrophage function with cyclooxygenase inhibitors (Soliven, 2014). Like in human GBS, MC-induced EAN is associated to dysregulated production of Th1/Th17 cytokines and impaired function and numbers of regulatory T cells, along with upregulated expression of Toll like receptors during the course of the diseases (Jung et al., 2004; Schafflick et al., 2017).

Nonetheless, like with most models of human autoimmune diseases, caveats should be kept in mind when the rodent EAN model is used as model of GBS, as this model fails to fully mirror the heterogenic complexity of the human disease counterpart. For example, auto-reactivity against myelin proteins has only been observed in a small proportion of GBS patients and the infiltration of mononuclear cells and immune cell-mediated myelin destruction in the PNS is known to occur only in a proportion of GBS patients who exhibit demyelinating disease phenotype (Schafflick et al., 2017).

MC-induced EAN may therefore represent a useful preclinical tool for the in vivo identification of lead compounds and drugs with an in vitro immunopharmacological profile suitable for counteracting immunoinflammatory demyelinating events of the PNS and for their further evaluation in the clinical setting.

Decitabine (DAC, 5-aza-2′-deoxycytidine) is a hypomethylating drug currently used for the treatment of myelodisplastic syndrome and is currently gaining much attention for its use in other forms of blood cancer, including acute myeloblastic leukemia (Gardin and Dombret, 2017; Sato et al., 2017), as well as solid cancer (Linnekamp et al., 2017). Recent studies have also shown that DAC possesses potent immunomodulatory properties through different pharmacological mechanisms, that entail induction of regulatory T cells and shift of the Th1-Th17/Th2 cytokine balance, in favor of the latter, with consequential promotion of an anti-inflammatory mileau. This immunopharmacological profile of DAC may be related to its capacity to exert the beneficial action reported by ourselves and others in rodent models of type 1 diabetes, multiple sclerosis and allograft rejection (Chan et al., 2014; Mangano et al., 2014a; Wang et al., 2017; Zheng et al., 2009).

Along this line, we have evaluated the clinical and immunopharmacological efficay of DAC in mouse and rat model of EAN. The data indicate that prophylactic and even therapeutic treatment with DAC markedly ameliorated the clinical course. The effect was associated with profound immunological modification of the so-treated rats, that included augmented numbers of regulatory T cells, and reduced production of the proinflammmatory cytokines, IFN-gamma and IL-17.

2. Materials and methods

2.1. Animals and EAN induction

Lewis male rats and C57Bl6 male mice (Envigo, San Pietro al Natisone, UD, Italy) weighing between 200 and 220 g and 20–22 g, respectively were housed within a limited access rodent facility and kept in groups of maximum 3 rats and 5 mice, in polycarbonate isolator cages with a filter top and external air supply and free access to food and water. Animal care was in compliance with local regulations on the protection of animals used for experimental and other scientific