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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

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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

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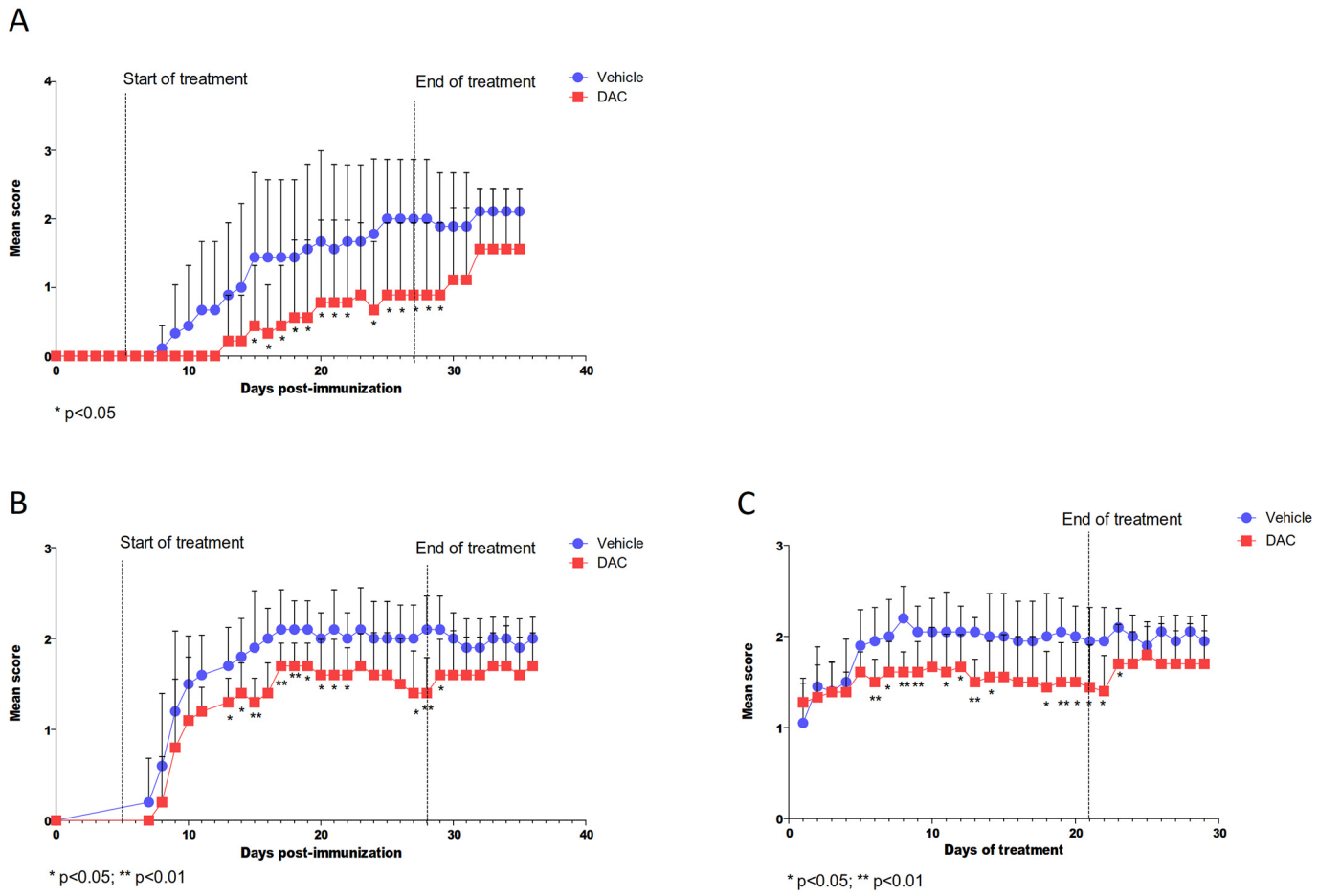

Fig. 1. Effect of DAC on clinical course of EAN. Mean clinical score of Lewis rats immunized with P0 peptide and treated with DAC 0.1 mg/Kg/day by i.p. injection, starting 5 days after immunization (A). Mean clinical score of C57Bl/6 mice immunized with P0 peptide and treated with DAC 0.1 mg/Kg/day by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

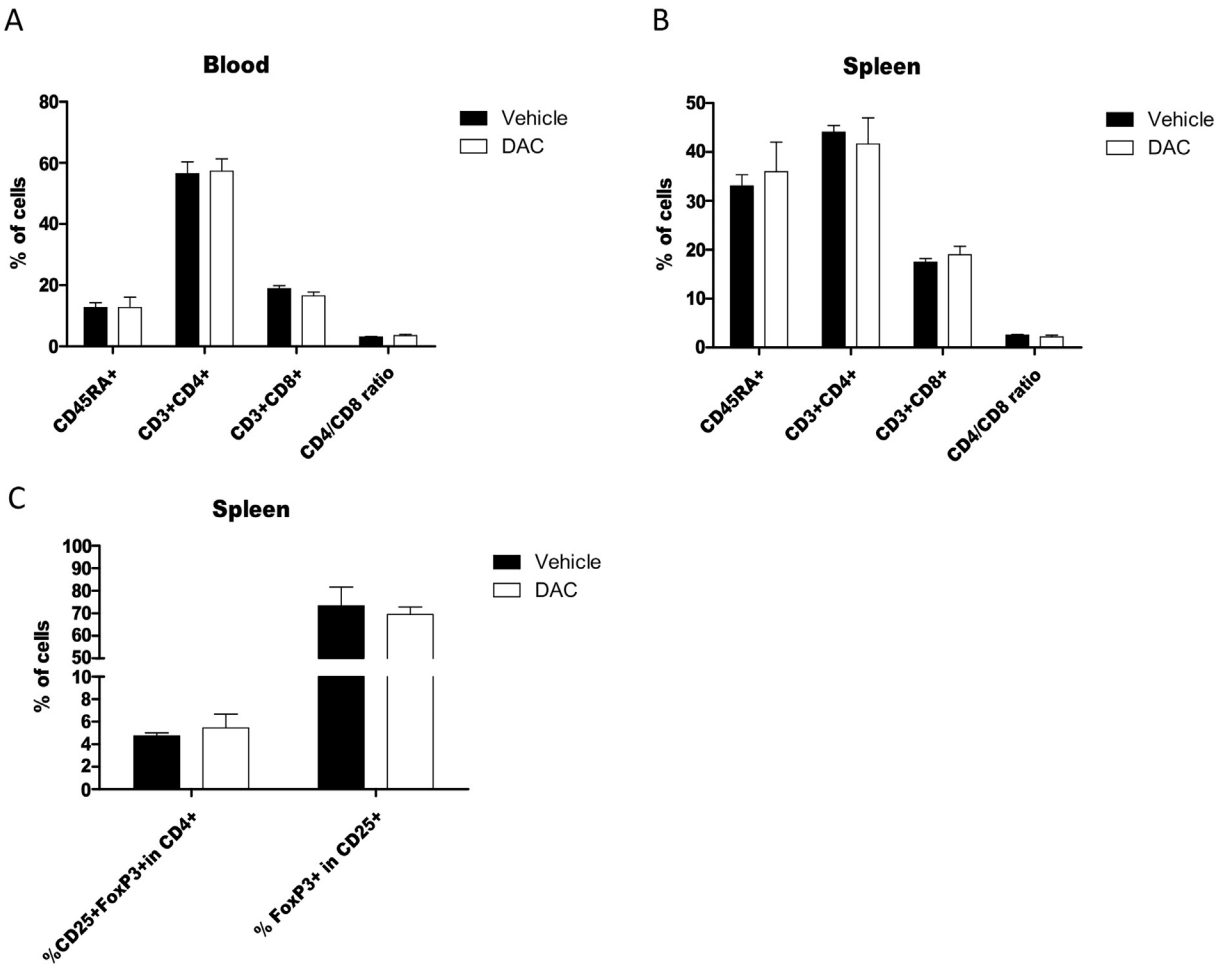

Fig. 2. Effect of DAC on peripheral T cell composition. On day 20th post-immunization, T cell composition was determined by flow cytometry in blood (A) and in spleen cells (B). Mean percentage of regulatory T cells in the spleen of satellite groups of rats (C).

purposes (Directive 86/609/EEC, enforced by the Italian D.L. No. 116 of January 27, 1992).

2.1.1. P0 induced EAN in Lewis rat

EAN was induced in rats by subcutaneous injection, at the base of the tail, of 200 µl of an emulsion containing 200 µg of P0 peptide 180–199 (Genemed Synthesis, Inc. USA) in 0.1 ml saline and 1.5 mg of Mycobacterium tuberculosis H37RA (Difco, Detroit, MI, USA) in 0.1 ml Freund's incomplete adjuvant (IFA, Sigma-Aldrich, Milan, Italy).

2.1.2. P0 induced EAN in C57Bl6 mice

C57Bl6 male mice were immunized twice with a one week interval by subcutaneous injection into the back with 120 µg of P0 peptide 180–199 (Genemed Synthesis) in 50 µl saline and 0.5 mg of *Mycobacterium tuberculosis* (strain H 37 RA; Difco, Detroit, MI, USA) in 50 µl Freund's incomplete adjuvant (FIA) (Sigma Aldrich, Milano, Italy). All mice received 400, 200, and 200 ng pertussis toxin (Sigma Aldrich, Milano, Italy) by i.p. injection on days −1, 0 and 3 post immunization (p.i.), respectively (Duan et al., 2004).

2.1.3. Experimental treatment

DAC (Sigma Aldrich) was administered at a concentration of 0.1 mg/Kg/day by i.p. injection. The injection volume was 1 ml/Kg. DAC was dissolved in DMSO to obtain a stock solution and diluted in saline to obtain the working solution. The treatments started 5 days after immunization (prophylactic regimen) or at disease onset (therapeutic regimen) and continued for 21 days. After interruption of the treatment, animals were observed for additional 9 days.

2.1.4. Clinical scoring

The animals were weighted on day of immunization and twice a week starting from day 7 until the end of the study. Starting from day 5, the animals were individually examined for the presence of paralysis by a clinical score as follows: 0 = no illness, 1 = flaccid tail, 2 = moderate paraparesis, 3 = severe paraparesis, 4 = moribund state, 5 = death (1–2). Clinical signs were monitored daily in each group of treatment in a blind fashion. Mean daily score, cumulative score, disease incidence were calculated.

2.2. Cell preparation

Satellite groups of rats were created and sacrificed on day 20th post immunization to study the immunopharmacological impact of the treatment. Blood was collected from vena cava and spleens and thymus harvested for subsequent ex vivo analysis. Cell suspensions were prepared by grinding the organs with the plunger of a 5 ml disposable syringe through a 40 um nylon mesh and by resuspending the cells in RPMI 1640 medium (Sigma, Milan, Italy) supplemented with 10% fetal calf serum, 2 mM glutamine, and 50 mg/ml of penicillin/streptomycin (complete medium). Splenocytes were treated with an ACK Lysis Buffer (Invitrogen, Life Technologies Italia, Monza, Italy) to remove red blood cells.

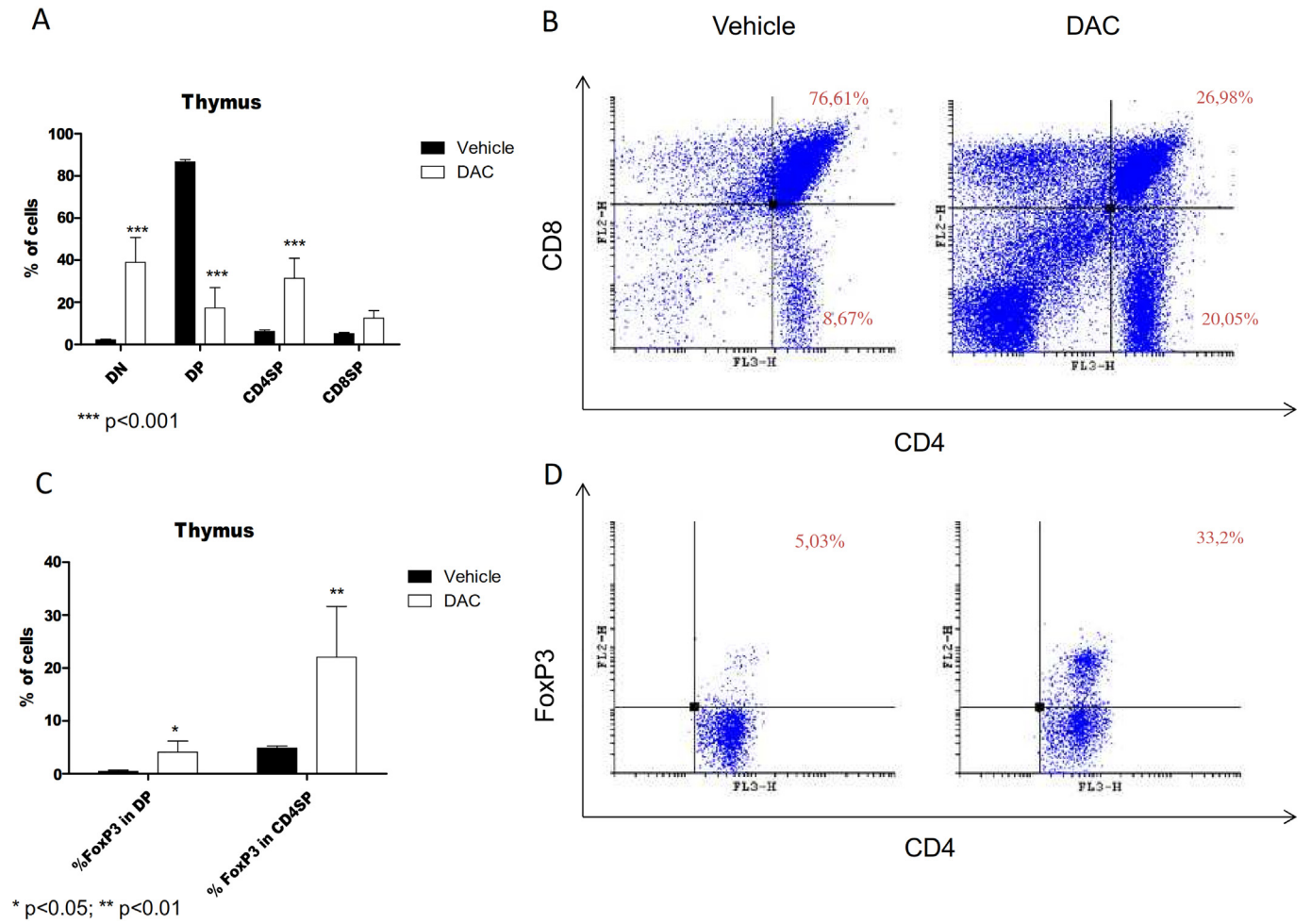

Fig. 3. Effect of DAC on thymus composition. On day 20th post-immunization, thymus was collected from satellite groups of rats for the determination of T cell composition by flow cytometry (A). Representative dot plots are shown (B). Mean percentage of FoxP3+ cells among thymocytes isolated from vehicle- and DAC-treated rats (C). Representative dot plots are shown (D).

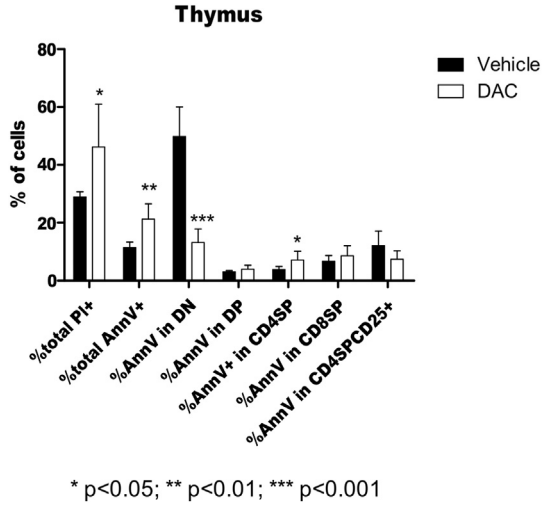

Fig. 4. Effect of DAC on thymocytes apoptosis. On day 20th post-immunization, thymus was collected from satellite groups of rats for the determination of apoptosis using Annexin V-FITC and Propidium Iodide staining.

2.2.1. Flow cytometry

Mouse anti-rat CD4-PE Cy5 (clone OX-35), Mouse anti-rat CD25-FITC (clone OX-39), anti-rat CD8-PE and Annexin V-APC were purchased from BD. Anti-rat Foxp3-PE (clone FJK-16s) and Rat Foxp3 Buffer Set were purchased from eBioscience (San Diego, CA, US). Flow cytometric analysis was performed on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA), and the data were analyzed using Cyflogic software (CyFlo Ltd., Turku, Finland). At least 50,000 cells were acquired per sample. The percentage of cells stained with a particular reagent was determined by subtracting the percentage of cells stained non-specifically with an isotype control antibody. Total cell count was determined by calibrating the instrument using cell counting beads (BD Biosciences).

2.2.2. Determination of T cell population

Splenocytes, whole blood cells and thymocytes were incubated at 4 °C for 30 min with a combination of monoclonal anti-CD45RA, anti-CD8, anti-CD4 for the determination of naive T cells, CD4/CD8 ratio and T cell precursors. For the determination of Tregs, splenocytes and thymocytes were incubated with anti-CD4 and anti-CD25 fluorochrome-conjugated antibodies (BDBiosciences, Milan, Italy), fixed and permeabilized using the Rat Foxp3 Buffer Set (eBioscience) and incubated with an anti-rat Foxp3 monoclonal antibody. For the current analysis, Tregs were defined as CD4+, CD25hi, FOXP3+ cells (Aslam et al., 2012; Donia et al., 2009).

2.2.3. Determination of apoptosis in thymocytes

For the evaluation of apoptosis, thymocytes were washed with PBS, 100 ml of Annexin V-FITC binding buffer was added into the washed cell followed by the addition of 5 ml Annexin V-FITC and 10 ml PI and

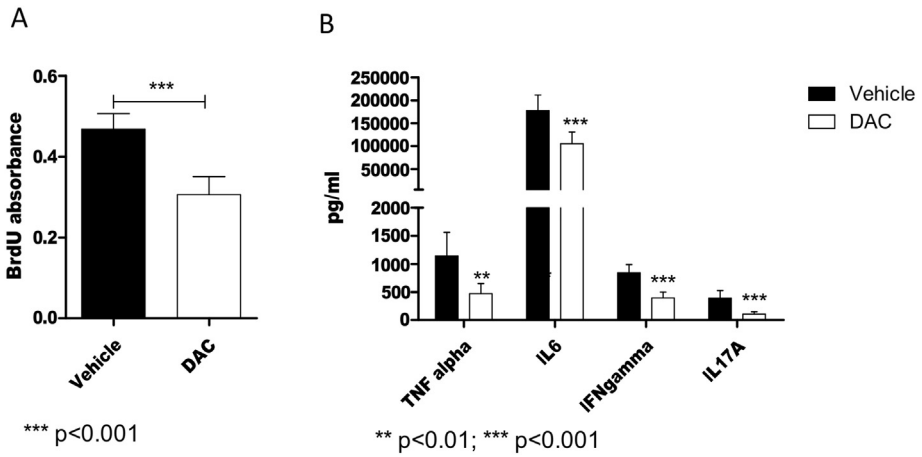

Fig. 5. Effect of DAC on antigen-specific proliferation and cytokine secretion. On day 20th post-immunization, spleen was collected from satellite groups of rats for the determination of ex vivo antigen-specific proliferation via BrdU incorporation assay (A) and of TNF alpha, IL6, IFNgamma and IL17A supernatant levels via ELISA (B).

incubated for 30 min. After incubation 400 ml PBS was added and analyzed.

2.3. Antigen-specific proliferation and cytokine release assays

Spleens were harvested from satellite rats at sacrifice, as indicated. Cell suspensions were prepared as previously described. Cells were pelleted and washed twice with PBS. Cells were then resuspended at 2×10^6 cells/ml in complete medium and re-restimulated with 10 µg/ml of P0 peptide (180–199) and incubated at 37 °C for 4 days. The quantification of the cell proliferation was evaluated by BrdU incorporation (10 µl/well), during DNA synthesis, 24 h before the end of the treatment. The ELISA assay (Roche, Germany) was performed according to manufacture instruction. The absorbance of the samples was measured in an ELISA reader at 450 nm. At the end of the incubation period, supernatants were also collected for subsequent determination of *TNF*- alpha, *IL-6*, IFN-gamma and IL-17A by ELISA (eBioscience).

2.4. Statistical analysis

The results are presented as means ± standard deviations (SD). Statistical analysis for significant differences on was performed with either two-tailed Student's *t*-test or the non-parametric Mann-Whitney *U* test. Values of $p < .05$ were considered statistically significant.

3. Results

3.1. DAC treatment improves clinical signs of EAN in rats and mice

In rats, clinical signs of EAN first appeared, in the vehicle-treated group, 8 days after immunization, and progressed to a moderate paraparesis, reaching a peak at day 25 (Fig. 1A). Late prophylactic treatment with DAC, starting on day 5 post immunization in rats that had not developed clinical signs of the disease, delayed the onset of the disease ($p = .0395$) as compared to vehicle-treated rats, and the animals started to show signs of EAN starting at day 13 post-immunization. The course of the disease in the DAC-treated group was significantly milder in comparison to the vehicle-treated group, as determined by a reduced cumulative score ($p = .0096$). After interruption of the treatment, the animals were observed for additional 9 days. An exacerbation of the disease was observed already in the mean score at day 2 of follow-up and the mean clinical score peaked to values not significantly different from those observed in the vehicle-treated animals by the end of the observation period (Fig. 1A).

In mice immunized with $P0_{180–199}$, clinical signs of disease were observed starting from day 7 post-immunization in the vehicle-treated group (Fig. 1B). DAC treatment did not delay the onset of the disease, however a significant reduction in the mean daily score was observed from day 13 post-immunization. Overall, DAC-treated mice showed a significant lower cumulative score as compared to control vehicle-treated mice (32.6 ± 4.2 vs. 24.6.4 ± 6.6, $p = .006$, in DAC- and vehicle-treated mice, respectively). For the therapeutic treatment, the mice first started treatment with DAC when they had developed a clinical score > 1 for 2 consecutive days. The mice were then randomly allocated in two different experimental groups and treatment was continued for 21 days. Therapeutic treatment with DAC of P0-immunized animals significantly ameliorated disease course, starting from day 6 after initiation of the therapy (Fig. 1C), entailing a significantly lower clinical cumulative score (39.6 ± 5.6 and 31.8 ± 5.1, in the vehicle- and DAC-treated groups of animals, respectively; $p = .006$) at the end of the experimental period. Both in the prophylactic- and therapeutic-DAC treated groups, an exacerbation of the disease was observed upon interruption of the treatment with a kinetic superimposable to that observed in the rats (Fig. 1B–C).

3.2. DAC treatment does not affect peripheral T cell population composition

Flow cytometry analysis of circulating T cells revealed that DAC administration was not associated to changes in the percentage of helper and cytotoxic T cells in the blood, as well as in the relative number of naïve (CD45RA+) T cells (Fig. 2A). Similarly, no changes in T cell composition and cell ratio were observed in splenocytes of DAC-treated rats as compared to vehicle-treated rats (Fig. 2B). Also, DAC treatment did not induce differences on the percentage of regulatory T cells (%CD25 + FoxP3 + in CD4) nor in the percentage of FoxP3+ cells among activated (CD25+) T cells in the spleen (Fig. 2C).

3.3. DAC affects thymocytes maturation and survival

Analysis of the cellular composition of thymi revealed that DAC-treated rats had a significantly increased percentage of Double Negative (DN) thymocytes ($p < .001$), as well as a reduction in the percentage of Double Positive (DP) cells ($p < .001$). Also, DAC-treated rats showed an increased relative number of CD4 Single Positive (SP) cells ($p < .001$). No variation in the percentage of CD8SP cells was observed in thymocytes from DAC-treated group as compared to vehicle-treated group (Fig. 3A-B). DAC administration was also associated to a significant increase in the percentage of cells expressing FoxP3 ($p < .05$ for the percentage of FoxP3 + DP and $p < .01$ for the percentage of FoxP3 + CD4SP cells) (Fig. 34C-D). In agreement with the observed increase in DN cells, a significant ($p < .001$) reduction of apoptotic cells among the DN cell population was found (Fig. 4). On the other hand, a significant increase in apoptotic CD4SP cells was observed in the DAC-treated group of rats ($p < .05$). Overall, DAC induced a significant increase in necrotic and apoptotic cells ($p < .05$) (Fig. 4).

3.4. DAC affects antigen-specific proliferation and cytokine production in rat EAN

The effect of DAC on antigen-specific proliferation was evaluated on splenocytes restimulated ex vivo with $P0_{180–199}$. Upon restimulation, splenocytes from DAC-treated animals showed a significantly reduced rate of proliferation as compared to splenocytes from vehicle-treated mice ($p < .001$) (Fig. 5A). In accordance, DAC-treated splenocytes showed a significant reduction in the secretion of the pro-inflammatory cytokines, TNF alpha ($p < .01$), IL6 ($p < .001$), IFNgamma ($p < .001$) and IL17A ($p < .001$), in comparison to splenocytes from vehicle-treated rats, as determined in the supernatant (Fig. 5B).

4. Discussion

DAC, a synthetic analogue of cytidine, already approved by FDA for the treatment of myelodysplastic syndrome, is a drug that acts as an hypomethylating agent by irreversibly binding DNA methyltransferases, thus inhibiting DNA methylation (Plimack et al., 2007; Saba, 2007). DAC is a derivative of azacytidine, used to treat patients with acute myeloid leukemia and myelodysplastic syndrome. In these patients, azacytidine has been described to increase Treg cell number in peripheral blood (Costantini et al., 2013; Goodyear et al., 2012; Schroeder et al., 2013).

Increasing line of evidence proves that DAC exerts a powerful immunopharmacological action both in vitro and in vivo and is effective in models of autoimmune and allo-immune responses (Chan et al., 2014; Mangano et al., 2014b; Wang et al., 2017; Zheng et al., 2009). The beneficial clinical effects have been associated with profound modulation of the immune responses, including increased numbers of regulatory T cells in NOD mice (Zheng et al., 2009) and inhibitory effects on the proinflammatory cytokines in murine EAE (Wang et al., 2017).

Our present study supports these data, as it demonstrates that DAC ameliorates the course of EAN both in mouse and rat models and that this beneficial action was associated to a significant reduction in the number of Th1/Th17 cells and in the production of pro-inflammatory cytokines, including TNF-alpha, IFN-gamma and IL-17, that are thought to play a key role in the pathogenesis of EAN, as shown by in vivo studies with specific inhibitors of these cytokines (Wang et al., 2014; Zhang et al., 2012; Zhu et al., 2001). The significance of the inhibition of IL-6 secretion by DAC in the context of EAN remains to be established, as conflicting data have been reported on the role of this cytokine in the development of the disease, with apparent evidence of a pathogenic role of endogenous IL-6 vs a protective role of exogenously administered IL-6 (Deretzi et al., 1999; Zhu et al., 1997). If so, inhibition of endogenous IL-6 production by DAC could have contributed to the beneficial clinical effects of the drug in EAN development and maintenance.

In addition, DAC treatment influenced the composition of thymus in the rats, as it increased the percentage of Double Negative (DN) thymocytes and reduced the percentage of Double Positive (DP) cells, as compared to vehicle-treated rats. DAC-treated rats also exhibited an increased relative number of CD4 Single Positive (SP) cells and of Foxp3 + CD4SP cells. In agreement with the observed increase in DN cells, a significant reduction of apoptotic cells among the DN cell population was found. On the other hand, a slight although significant increase in the percentage of apoptotic CD4SP was observed. On the basis of these data and as also propsed by Zhang et al. (2007), we hypothesize that Tregs may be more resistant to apoptosis than effector cells. The fact that DAC induces apoptosis in CD4SP but not in their precursor DN T cells indicate that during the maturation process, this progeny of cells acquire factors that switch anti-apoptotic effects of DAC in early maturation at DN stage, to proapototic effecs at SP stage. Although the mechanisms responsible for this maturation-dependent dicothomic effects of DAC remains to be studied, the apoptosis-inducing effects of DAC in CD4SP cells may represent another important immunopharmacological tool of DAC, as manipulation of thymus apoptosis is envisaged as a novel and promising approach for the treatment of autoimmune diseases, since defective negative selection of T cells in thymus may contributeo to development of autoimmune diseases (Delfino et al., 2011).

Our observation that DAC significantly increased the percentage of thymic Foxp3 + CD4SP cells in rat EAN is in line with previous data (Zheng et al., 2009) demonstrating no changes in the number of Tregs among blood and spleen cells. This is somehow surprising as, in vitro treatment of thymocytes and splenocytes with DAC shows overlapping effects on FoxP3 induction (Zheng et al., 2009). The reason for this discrepant in vitro vs in vivo responsiveness of thymocytes and peripheral immune cells to DAC requires additional studies. The observation that recurrence of EAN was observed in the animals as soon as after 2 days after interruption of the treatment indicates that the immunomodulatory effects of the drug whether on regulatory T reg in the thymus and/or on the production of proinflammatory cytokines, are rapidly reversible upon interruption of the treatment. Of note, the recurrence of EAN occurred more rapidly than we have observed in EAE models (Mangano et al., 2014a, 2014b). The reasons for these different kinetic of disease recurrence probably rely on the different nature of the models and different immunopathogenetic aspects. Regardless of this, these data anticipate that continuous treatment with DAC of GBS patients would be necessary to secure long lasting beneficial effects.

The repeatedly confirmed immunopharmacological profile of DAC, that seems to act in models of autoimmune diseases by upregulation of FoxP3 population, is in apparent conflict with an in vitro study (Kehrmann et al., 2014), that has evaluated the impact of DAC treatment in vitro on Treg polarization of naïve T cells. The results indicated that while the treatment of CD4 + CD25- cells with DAC reduced the global DNA methylation by 60%, the FOXP3 Treg-specific demethylated region (FOXP3-TSDR) underwent an approximate reduction in methylation of only 20%. This is sharp contrast with what is observed in Treg cells where the FOXP3-TSDR is almost completely unmethylated. DAC-treated cells exhibited increased levels of FoxP3 and of the receptor for latent TFG-beta, GARP, and expressed the Th1-and Th17-specific transcription factors, TBX21 and RORγt, and of the proinflammatory cytokines, IFNγ and IL-17. Accordingly, DAC-induced Tregs showed a lack of immune-suppressive function. Whole-genome transcriptomic analysis also revealed that the gene expression profile of DAC-treated cells only partially overlapped that of Treg cells (Kehrmann et al., 2014). The reason for the discrepancy with the data obtained upon ex vivo and in vivo conditions in models of autoimmune and allo-immune responses is not known. Nonetheless, the strong therapeutic efficacy of DAC in these models of immunoinflammatory disorders clearly supports the immunopharmacological profile of DAC. Possible differences in cell donors and of the in vitro vs in vivo study conditions may also be involved.

The present clinical and immunopharmacological observation, along with literature data, points to DAC as a drug with an immunopharmacological profile capable of counteracting the pathogenic mechanisms of GBS. An impaired suppressive function of Treg cells in CIDP as compared to healthy controls have been reported (Sanvito et al., 2009). It is of interest that the abnormalities in circulating cells seem to respond differently to IVIg therapy with no increase in CD4 + CD25+ cells in mixed-variant GBS (Pritchard et al., 2007) and an increase in CD4 + CD25+ cells and FoxP3+ cells in AIDP and AMAN (Chi et al., 2007b) and AIDP (Maddur et al., 2014), respectively.

Regardless of these discrepancies that remain to be clarified, our findings have important translational implications as they indicate that, as in rodent EAN, DAC may efficiently modulate the immunological abnormalities that have been linked to the pathogenesis of GBS, including the augmented production of IFN-gamma and IL-17 (Li et al., 2014) and the reduced number and function or regulatory T cells (Chi et al., 2007b; Harness and McCombe, 2008; Maddur et al., 2014).

Future studies are in progress to evaluate the influence of DAC on the production of Th22 cells and its secretory product, IL-22, that has also been reported to be upregulated in the course of GBS and to correlate with disability score (Li et al., 2014).

The potential translation of these proofs of concepts data to the clinical setting and the predicitivity of the beneficial effects of DAC to human GBS remains to be proved. Rodent EAN can indeed be prevented or cured by different compounds in discovery phase or that are in clinical use for different indications, including cyclophosphamide, fusidic acid, FK506, specific antagonist of MIF and TNF-alpha and specific autoantigen tolerization, administered by systemic or oral route (Adachi et al., 1992; Di Marco et al., 1999; Mangano et al., 2008; Nicoletti et al., 2005; Zhang et al., 2012; Gaupp et al., 1997). However the efficacy of these compounds in GBS patients has been hampered by the limited number of clinical studies carried out with compounds and drugs that have been successfully used in the preclinical setting. It is however of interest that as much as human GBS benefit from IvIg, rat EAN can also be ameliorated by IvIg (Kajii et al., 2014). In addition, anecdotal reports by ourselves and others have demonstrated a rapid and clear response of GBS patients to fusidic acid, that ameliorated EAN (Nicoletti et al., 1998).

Additional relevance warranting studies aimed at determining the role of DAC in human GBS come from the fact that DAC is already used in the clinical setting, and could be immediately studied in Phase II proof of concept studies at least in those patients that are poor responsive to standard of care treatment.

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