Augmenting DAF levels *in vivo* ameliorates experimental autoimmune encephalomyelitis

Qing Li [a,b], Danping Huang [a], Kristine Nacion [a], Hong Bu [b], Feng Lin [a,*]

[a] *Institute of Pathology, Case Western Reserve University, Cleveland, OH, USA*
[b] *Department of Pathology, West China Hospital, Sichuan University, Chengdu, China*

ARTICLE INFO

ABSTRACT

Recent studies in experimental autoimmune encephalomyelitis (EAE) have found that CNS injury in $Daf1^{-/-}$ mice is much greater than in wild types (WTs), suggesting that upregulating DAF levels *in vivo* might ameliorate disease. To test this, we generated a *Daf1* transgenic (Tg) mouse which had elevated DAF levels on its cell surfaces. In by-stand C3b uptake assays, *Daf1* Tg mouse erythrocytes took up less C3b on their surfaces than WT erythrocytes. When co-cultured with OT-II CD4^{+} T cells together with OVA$_{323-339}$ peptide, *Daf1* Tg mouse bone marrow derived dendritic cells (BM-DCs) produced less C5a and C3a than WT BM-DCs and stimulated a lesser T cell response. In MOG$_{35-55}$ immunization induced EAE model, *Daf1* Tg mice exhibited delayed disease onset and decreased clinical scores compared to WTs. Histological analyses showed that there were less inflammation and demyelination in spinal cords in *Daf1* Tg mice than those in WTs. In accordance with these results, *Daf1* Tg mice had decreased MOG$_{35-55}$ specific Th1 and Th17 responses. These data provide further evidence that DAF suppresses autoreactive T cell responses in EAE, and indicate that augmenting its expression levels could be effective therapeutically in treating multiple sclerosis as well as other T cell mediated diseases.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Decay accelerating factor (DAF, CD55) is a cell surface complement regulator that dissociates C3/C5 convertases and thereby prevents the generation of C3a and C5a (Medof et al., 1984). In previous studies using mice targeted in *Daf1*, the murine homologue of human DAF, we (Liu et al., 2008) and others (Liu et al., 2005) showed that $Daf1^{-/-}$ mice exhibited greater central nervous system (CNS) inflammation and demyelination in experimental autoimmune encephalomyelitis (EAE), a myelin-specific T cell mediated disease model for multiple sclerosis (MS). We also found that during APC:T cell interactions, both partners locally synthesize C3, factor B, factor D and C5, components required for C5a and C3a generation via the alternative pathway complement activation (Heeger et al., 2005). The absence of DAF lowers restraint on junctional C3/C5 convertases formation which results in increased levels of local C5a and C3a, both of which promote Th1/Th17 cell generation (Liu et al., 2008; Strainic et al., 2008) and sustain effector T cell viabilities after

binding to their respective receptors (C5aR or C3aR) on APCs and/or T cells (Lalli et al., 2007, 2008).

Although much new information on T cell responses has been gained through the above and other studies on $Daf1^{-/-}$ mice, until now it has not been possible to study the effects of DAF overexpression *in vivo*. To address this issue, we generated a *Daf1* transgenic (Tg) mouse under the constitutive ROSA26 promotor (Zambrowicz et al., 1997) on the C57BL/6 background. To better characterize the distribution of the transgenic *Daf1* gene expression, we crossed *Daf1* Tg mice with $Daf1^{-/-}$ mice to generate the *Daf1* Tg/KO mice that only express the transgenic *Daf1* gene. Using these *Daf1* Tg/KO mice, we verified that the transgenic *Daf1* gene was expressed on all tissues and cells examined. By studying the *Daf1* Tg mice on C57BL/6 background, we found that they expressed elevated levels of DAF protein on all cells examined and their bone marrow derived dendritic cells (BM-DCs) generated 2-fold less C5a and C3a during antigen-specific APC:T cell interactions and stimulated lesser T cell responses *in vitro*. In *in vivo* studies on EAE, *Daf1* Tg mice exhibited lowered clinical scores, diminished MOG$_{35-55}$ specific Th1/Th17 responses, decreased central nervous system (CNS) inflammation and reduced demyelination. These data indicate that upregulating DAF levels *in vivo* suppresses T cell autoimmunity and ameliorates disease severity in EAE.

* Corresponding author at: Institute of Pathology, Case Western Reserve University School of Medicine, 2085 Adelbert Road, Room 306, Cleveland, OH 44106, USA. Tel.: +1 216 368 2118.
E-mail address: feng.lin@case.edu (F. Lin).

2. Materials and methods

2.1. Peptides and antibodies

Mouse MOG$_{35-55}$ peptide (MEVGWYRSPFSRVVHLYRNGK) was purchase from Anaspec (San Jose, CA), Complete Frunds' adjuvant (CFA) was from Sigma (St. Louis, MO), supplemental *Mycobacterium tuberculosis* was from BD Biosciences (Franklin Lakes, NJ). Rat anti-mouse IFN-γ mAbs were purchased from BD Biosciences. Rat anti-mouse DAF mAb 2C6 (Spiller et al., 1999) was kindly provided by Dr. B.P. Morgan, Cardiff University, U.K.

2.2. Generation of Daf1 Tg mice

The cDNA coding for mouse DAF1 protein was amplified by RT-PCR from mouse liver total RNA (Ambion, TX) using proof-reading Vent DNA polymerase (NEB, MA) and cloned into the expression vector pBroad3 under the ROSA26 promotor (Invivogen, CA). After sequencing, the expression construct was further verified by transfecting into CHO cells and transient DAF expression assayed by flow cytometry with 2C6 mAb. Linearized DAF expression cassette was injected into fertilized C57BL/6 mouse oocytes. Transgenic founders were identified by PCR (P1: 5′-GTC TCG TCG CTG ATT GGC TTC TTT-3′, P2: 5′-TGT ACC CTG GGT TGC ATG AGA AGT-3′) and bred with C57BL/6 mice. To generate the *Daf1* Tg mice without endogenous DAF expression (*Daf1* Tg/KO mice) for identifying the transgenic DAF expression pattern *in vivo*, *Daf1* Tg mice were bred with *Daf1*$^{-/-}$ mice, and the resulted *Daf1* Tg/KO mice were identified by PCR and verified by flow cytometry. All mice were housed in pathogen-free microisolator cages in the animal facility at Case Western Reserve University, all breeding and experiments were performed in accordance with the guidelines of the IACUC of Case Western Reserve University.

2.3. Reverse transcription PCR for transgenic Daf1 gene expression detection

Total RNAs were isolated from *Daf1* Tg/KO mice using Trizol (Invitrogen, CA). 2 μg total RNAs purified from brain, spinal cord, muscle, heart, kidney, stomach, liver, and spleen were treated with 2 units of RNase-free DNase I at 37 °C for 30 min before the reverse transcription to remove possible genomic DNA contamination. 1 μl of the reverse transcribed cDNA was used as template to amplify *Daf1* cDNA by PCR using primers on a thermal cycler (PTC-200, MJ Research, MA) using the following conditions: 94 °C 30 s, 60 °C 30 s and 72 °C 60 s, 35 cycles.

2.4. Flow cytometry analysis of transgenic Daf1 gene expression on cell surfaces

To verify transgenic *Daf1* gene expression on cell surfaces at the protein level, erythrocytes and leukocytes were isolated from WT, *Daf1*$^{-/-}$ or *Daf1* Tg/KO mice and stained with 5 μg/ml rat anti-mouse DAF mAb 2C6 or non-relevant rat IgG as control followed by analysis on a flow cytometer (LSR I, BD Biosciences, CA). For leukocytes isolation, 50 μl of blood was collected through tail vein from WT, *Daf1*$^{-/-}$ or *Daf1* Tg/KO mice into 1 ml of FACS buffer (1% BSA in PBS), then erythrocytes were lysed by incubating with RBC lysis buffer (eBioscience, CA) for 10 min. The lymphocytes, monocytes and neutrophils populations were gated by their characteristic distributions in a forward scatter-side scatter plot.

2.5. Mouse erythrocytes C3b uptake assay

To determine whether the *Daf1* transgene products are functional in inhibiting by-stand complement activation, C3b uptake assays using erythrocytes from WT or *Daf1* Tg mice were preformed. To do this, 2×10^6 erythrocytes were incubated with 1:10 dilution of normal mouse serum together with 400 μg/ml zymosan (Sigma, MO) in the GVB–EGTA Mg^{2+} buffer for 20 min. After incubation, erythrocytes were washed and stained with 5 μg/ml FITC conjugated rat anti-mouse C3 IgG or nonspecific IgG as a control. The samples were analyzed on a flow cytometer (LSR I). C3b deposition on erythrocyte surfaces was expressed as mean fluorescence intensity.

2.6. Western blot of locally produced C5a/C3a during APC:T cell interactions

BM-DCs from WT, *Daf1*$^{-/-}$ or *Daf1* Tg mice were generated by incubating BM cells with GM-CSF and IL-4 as described before (Heeger et al., 2005) and CD4$^+$ T cells were isolated by magnetic beads using negative selection following manufacturer provided protocol (Stemcell Tech., Vancouver, Canada). The BM-DCs were incubated with purified OT-II CD4$^+$ T cells together with 5 μg/ml OVA$_{323-339}$ peptide in serum-free HL-1 medium. Supernatants (equal amounts from each culture well) were collected after 3 days of culture and concentrated 10-fold using Microcon YM-10 centrifugal concentrators (Millipore, cutoff M.W. 3000). Equal volumes of supernatants were loaded and separated by SDS-PAGE and blotted on polyvinylidene difluoride membranes. Blots were probed with monoclonal rat anti-mouse C5a Ab or anti-mouse C3a Ab (BD Biosciences, CA) which only reacts with the C5a or C3a neoatigen and anti-rat HRP (Sigma, MO) Ab and visualized with ECL Western blotting detection kit (GE Healthcare, CA). The densities of the bands were semi-quantified using Metamorph Imaging software package (Molecular Devices, CA).

2.7. EAE induction and evaluation

8–10 weeks old female *Daf1* Tg and WT mice were immunized at base of tail and both thighs with 300 μg of mouse MOG$_{35-55}$ emulsified in CFA that had been supplemented with *M. tuberculosis* strain H37RA to 4 mg/ml. 0.4 μg of *Pertussis* toxin (List Biolab, CA) was injected i.p. right after immunization and the following day. Clinical severity was assessed daily with a 0–5 scoring system (0, no signs; 1, flaccid tail; 2, impaired righting reflex and/or gait; 3, partial hind limb paralysis; 4, total hind limb paralysis; 5, moribund or dead).

2.8. Histology and histochemical staining of spinal cords

At time of sacrifice, spinal cords were removed and fixed in 4% paraformaldehyde for 24 h, then embedded in paraffin. Sections were cut at 5 μm on a microtome and stained by haematoxylin and eosin (H&E) to reveal CNS inflammatory infiltrates and Luxol Fast Blue (LFB) to reveal demyelinated areas using standard protocols.

2.9. IFN-γ ELISA

IFN-γ levels in culture supernatants were measured by ELISA (eBioscience, CA) following the manufacturer provided protocol.

2.10. IFN-γ and IL-17 ELISPOT assays

23 days after immunization, mice were sacrificed and splenocytes were used for IFN-γ and IL-17 ELISPOT assays as has been done before in the lab (Heeger et al., 2005). Briefly, after lysis of the red blood cells with RBC lysis buffer (eBioscience, CA), 600,000 splenocytes were resuspended in 200 μl HL-1 media and added into the ELISPOT plate (Millipore, MA) pre-coated with rat anti-mouse IFN-γ mAb (clone 34, BD Pharmingen, CA) or rat anti-mouse IL-17 mAb (clone 50104, R&D systems, MN). 5 μg/ml of MOG$_{35-55}$ peptide was
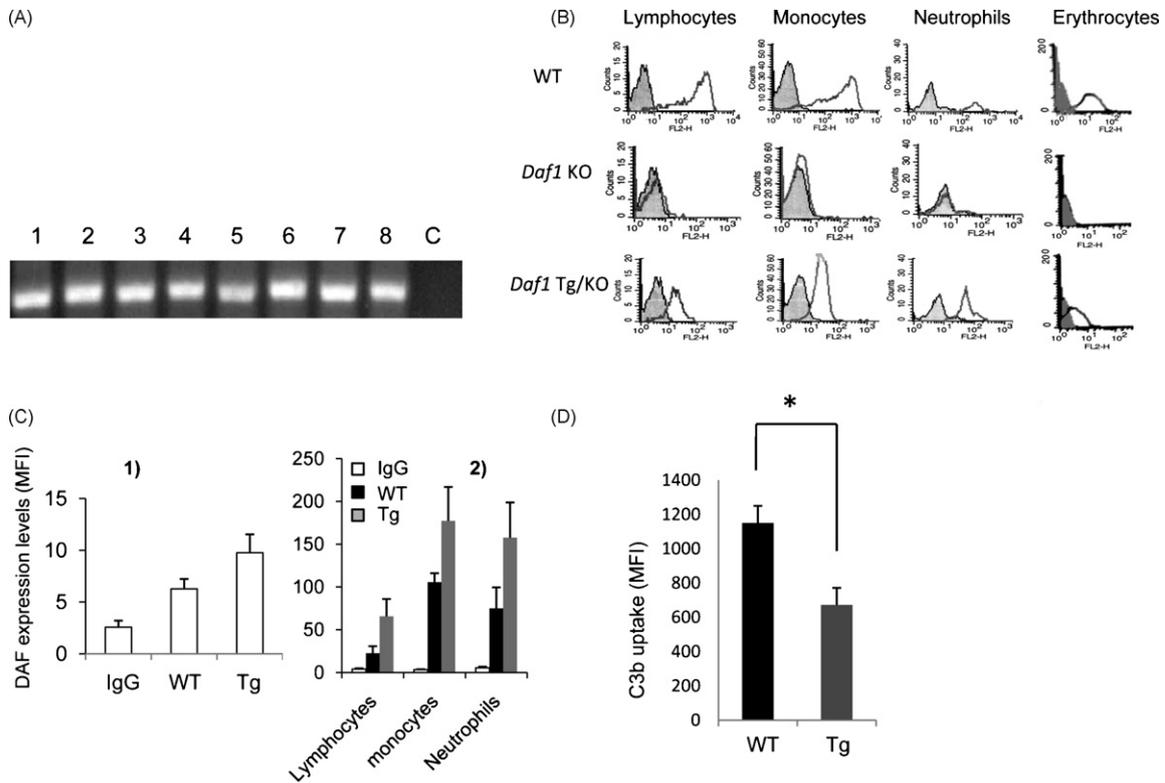
Fig. 1. Characterization of *Daf1* Tg mice. (A) RT-PCR of transgenic *Daf1* transcripts in *Daf1* Tg/KO mouse tissues. Brain (1), spinal cord (2), muscle (3), heart (4), kidney (5), stomach (6), liver (7) and spleen (8) were homogenized to isolate total RNA by Trizol. After Dnase I treatment to remove possible genomic DNA contamination, reserve transcripted RNA samples were used as PCR template to amplify a 200 bp fragment of *Daf1* cDNA. c, control; (B) DAF expression on leukocytes and erythrocytes of WT, *Daf1*$^{-/-}$ and *Daf1* Tg/KO mice. Shaded area, rat IgG control; open area, rat anti-mDAF mAb 2C6 staining; (C) DAF protein levels on *Daf1* Tg and WT mice cell surfaces. *Daf1* Tg and WT mice (*n* = 10) erythrocytes (1) and leukocytes (2) were stained with anti-mouse DAF mAb 2C6 and analyzed by flow cytometry. Averaged mean fluorescence intensities (MFI) were presented; (D) By-stand C3 uptake assay using *Daf1* Tg and WT erythrocytes. Complement activation was initiated by zymosan with 10% mouse serum in GVB Mg^{2+}–EGTA buffer and activation product C3b deposited on erythrocytes was detected by FITC-label anti-mouse C3 mAb and analyzed by flow cytometry. Averaged MFIs were presented (*$p < 0.05$).

added and incubated at 37 °C, 5% CO_2 overnight. After developing, spots were counted using an ELISpot anaylsis system (CTL, OH).

2.11. Statistical analysis

All experiments were repeated at least twice. To determine whether groups were statistically different, the clinical scores were analyzed by the ANOVA test while other results were compared using the Student's *t*-test. A *p* value < 0.05 was considered significant.

3. Results

3.1. Generation of Daf1 Tg mice and Daf1 Tg/KO mice

For preparing the *Daf1* Tg mice, we cloned the murine *Daf1* cDNA into pBroad3 vector under the control of ROSA26 promoter which constitutively drives high levels of transgene expression *in vivo* (Farley et al., 2000; Kisseberth et al., 1999). Preliminary studies showed that this construct expressed high level of mDAF protein on the surface of CHO cells after transient transfection (data not shown). Following pronucleic injection of fertilized C57BL/6 oocytes, we screened 60 pups by PCR and identified 5 founders carrying the transgenic *Daf1* gene. After breeding with C57BL/6 mice, we selected the founder with the highest DAF expression, bred it with *Daf1*$^{-/-}$ mice and generated *Daf1* Tg/KO mice which lack the endogenous *Daf1* gene expression.

3.2. Tissue distribution of transgenic Daf1 expression

We employed the *Daf1* Tg/KO mice to determine the tissue distribution of the transgenic *Daf1* expression without the interference from the endogenous *Daf1* expression products. Briefly, we isolated total RNA from different tissues of *Daf1* Tg/KO mice and treated them with DNase I to remove any possible genomic DNA contamination. After this, we amplified *Daf1* cDNA transcripts by RT-PCR. This analysis showed that the transgenic *Daf1* gene transcript was expressed in all tissues that were examined (Fig. 1A).

To determine the *Daf1* transgene expression at the protein level on cell surfaces, we stained erythrocytes and leukocytes from WT, *Daf1*$^{-/-}$ and *Daf1* Tg/KO mice using rat anti-mDAF mAb 2C6 followed by flow cytometry analysis. These assays showed that transgenic DAF protein was present on surfaces of all tested cells (Fig. 1B).

3.3. Daf1 Tg mice possess higher level of DAF protein

Having verified that the *Daf1* transgene is expressed on all cells and tissues examined using the *Daf1* Tg/KO mice, we performed all our following studies using the original *Daf1* Tg mice on C57BL/6 background. We first assessed DAF levels on the *Daf1* Tg mouse erythrocytes and leukocytes by flow cytometry. These assays showed that these *Daf1* Tg mice possessed ~40% higher levels of DAF protein than WT mice on erythrocytes (Fig. 1C(1)), lymphocytes, monocytes and neutrophils (Fig. 1C(2)).
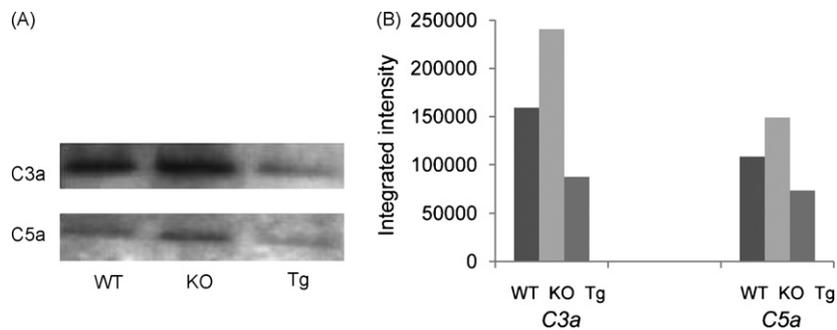
(A)

(B)

Fig. 2. *Daf1* Tg BM-DCs locally produced decreased levels of C5a and C3a when interacting with T cells. WT, *Daf1*$^{-/-}$ and *Daf1* Tg BM-DCs were incubated with purified OT-II CD4$^+$ T cells together with 5 μg/ml OVA$_{323-339}$ peptide for 3 days and supernatants were concentrated and blotted with anti-mouse C5a and C3a mAbs. The bands intensities were semi-quantitated by densitometry. Representative results from two individual experiments were shown.

3.4. Daf1 Tg mouse erythrocytes have stronger complement regulatory activity

The originally established role of DAF is to protect self-cells from autologous complement mediated injury by inhibiting C3/C5 convertases formation on their surfaces (Medof et al., 1984). To test whether *Daf1* Tg mice possess stronger complement regulatory activity because of higher DAF protein levels, we performed a C3b uptake assay on erythrocytes from *Daf1* Tg mice and WT mice using mouse serum as a complement source and zymosan as a complement activator. These assays showed that after incubation, *Daf1* Tg erythrocytes accumulated ∼half as much C3b on their surfaces as WT erythrocytes (Fig. 1D).

3.5. Daf1 Tg BM-DCs produce less C5a/C3a during T activation

We have previously shown that during antigen-specific APC:T cell interactions, *Daf1*$^{-/-}$ APCs generate more C5a/C3a than WT APCs which drives more robust T cell activation (Lalli et al., 2007; Strainic et al., 2008). To test whether the higher levels of DAF protein on *Daf1* Tg APCs inhibits local C5a/C3a generation, we mixed BM-DCs from WT, *Daf1*$^{-/-}$ or *Daf1* Tg mice with OT-II CD4$^+$ T cells together with 5 μg/ml OVA$_{323-339}$. After incubation for 72 h at 37 °C, 5% CO$_2$, we assayed supernatants for locally produced C5a/C3a by western blots and semi-quantitated their levels by densitometry. These assays (Fig. 2) showed that while *Daf1*$^{-/-}$ DCs generated higher levels of C5a/C3a than WT DCs, *Daf1* Tg DCs produced 2-fold less C5a/C3a.
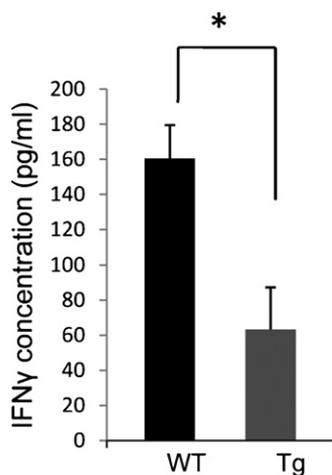
3.6. Daf1 Tg BM-DCs stimulate less T cell activation in vitro

Having verified that *Daf1* Tg BM-DCs locally generate less C5a/C3a during antigen-specific T cell activation, we next repeated the above experiment. After 48 h incubation, we assessed T cell activation by measuring IFN-γ released into the supernatants by ELISA. These assays (Fig. 3) showed that while OT-II CD4$^+$ T cells stimulated by WT DCs produced 160 ± 19 pg/ml of IFN-γ, OT-II CD4$^+$ T cells stimulated by *Daf1* Tg DCs produced 63 ± 24 pg/ml.

3.7. Daf1 Tg mice are protected against EAE

As indicated (Introduction), we (Liu et al., 2008) and others (Liu et al., 2005) previously found that DAF deficiency leads to markedly more severe EAE. To study the effect of *Daf1* overexpression on EAE, we immunized both *Daf1* Tg and WT mice with MOG$_{35-55}$ in CFA plus pertussis toxin. Daily evaluation showed that compared to WTs, *Daf1* Tg mice had delayed disease onset and decreased clinical scores. The average clinical score at the disease flare was 1.5 in the *Daf1* Tg mice as compared to 3.0 in the WT controls (Fig. 4).
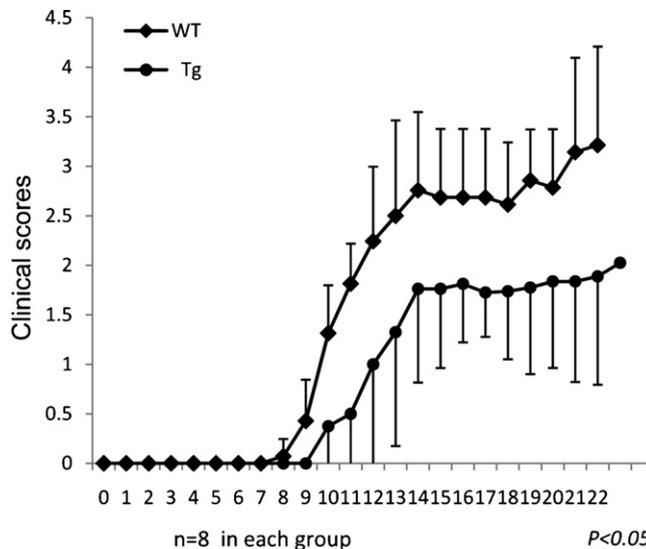

Fig. 3. *Daf1* Tg BM-DCs stimulated lesser T cell responses *in vitro*. *Daf1* Tg and WT BM-DCs were incubated with OT-II CD4$^+$ T cells together with 5 μg/ml OVA$_{323-339}$ peptide for 2 days and IFN-γ levels were measured in the supernatants by ELISA ($^*p < 0.05$). Results are representative of three individual experiments.

Fig. 4. *Daf1* Tg mice exhibited decreased disease severity in EAE. Both *Daf1* Tg and WT mice ($n = 8$ in each group) were immunized s.c. with 300 μg MOG$_{35-55}$ in CFA together with 400 ng of pertussis toxin to induce EAE. Clinical scores were recorded on daily basis ($^*p < 0.05$). Data were representative of three independent experiments.
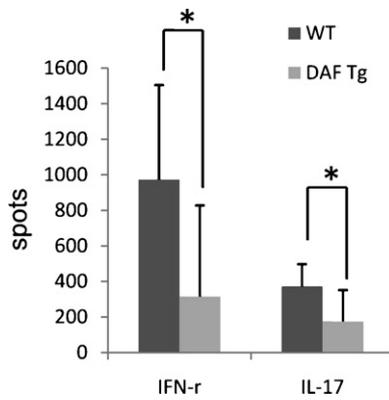
Fig. 5. *Daf1* Tg mice exhibited decreased MOG$_{35-55}$ specific Th1 and Th17 responses in EAE. 6×10^5 spleen cells were collected from each WT and *Daf1* Tg mice with EAE on day 22 and assayed for the numbers of IFN-γ and IL-17 producing T cells with the existence of 5 μg/ml of MOG$_{35-55}$ by ELISPOT assays (means plus SD, $n = 4$–8 in each group) ($^*p < 0.05$).

3.8. Daf1 Tg mice have diminished T cell responses against MOG$_{35-55}$

To assess MOG$_{35-55}$ specific T cells responses in *Daf1* Tg and WT mice with EAE, we collected splenocytes and performed IFN-γ and IL-17 ELISPOT assays. These studies showed that consistent with the reduced clinical scores, *Daf1* Tg mice had ~3-fold less MOG$_{35-55}$ specific Th1 and Th17 responses than WT controls (Fig. 5).

3.9. Daf1 Tg mice exhibit decreased CNS inflammation and reduced demyelination

To compare CNS inflammation and demyelination, we collected spinal cords from *Daf1* Tg and WT mice with EAE 23 days after immunization, processed them for H&E and myelin staining. These analyses (Fig. 6) showed that in accordance with lowered clinical scores and diminished MOG specific Th1/Th17 responses, *Daf1* Tg mice exhibited decreased leukocytes infiltration and reduced demyelination in spinal cords.

4. Discussion

In this report, we described the generation of a murine *Daf1* Tg mouse on C57BL/6 background and its derivative *Daf1* Tg/KO
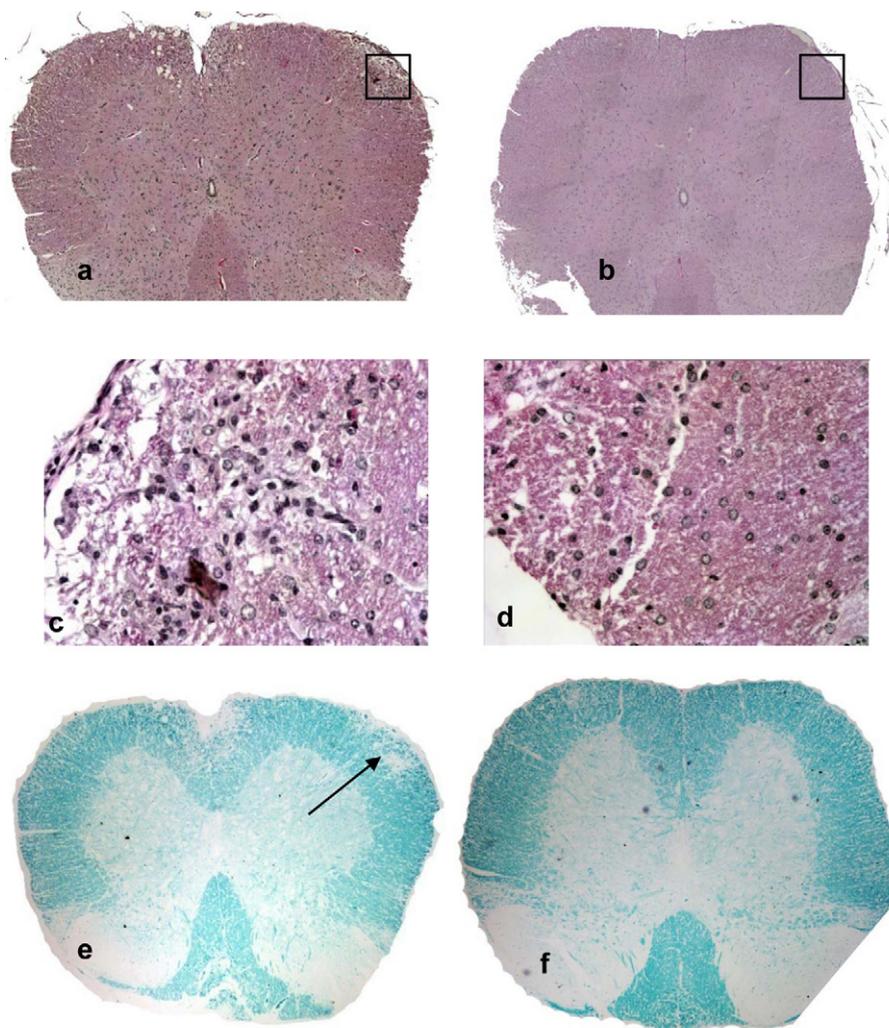

Fig. 6. *Daf1* Tg mice exhibited decreased inflammatory cell infiltration and demyelination in spinal cords. Spinal cord sections from WT and *Daf1* Tg mice with EAE were stained with H&E to reveal inflammatory cell infiltration and Luxol Fast Blue to show demyelination. Representative pictures were shown here. (A and C) WT H&E staining (20×) (A) and (400×) (C) showing infiltrated leukocytes; (B and D) *Daf1* Tg H&E staining (20×) (B) and (400×) (D), little if any infiltrated cell was seen; (E) WT luxol fast blue staining, showing demyelination in the infiltrated area (arrow); (F) *Daf1* Tg luxol fast blue staining, showing intact myelin sheath.

mice which only expressed the transgenic *Daf1* gene. Using the *Daf1* Tg/KO mice, we found that the transgenic *Daf1* gene is ubiquitously expressed. Consequently, *Daf1* Tg mice had ~40% higher DAF protein levels on examined cell surfaces and exhibited stronger complement regulatory activity against by-stand complement activation. During antigen-specific APC:T cell interactions, *Daf1* Tg BM-DCs produced less C5a/C3a than WTs and stimulated less T cell activation. Contrary to $Daf1^{-/-}$ mice which exhibited more severe CNS injury in EAE, *Daf1* Tg mice were protected. They had lower clinical scores, less CNS inflammation/demyelination and fewer MOG_{35-55} specific IFN-γ and IL-17-producing effector T cells than WT mice.

Accumulating studies in recent years have shown that in addition to their conventional roles of protecting self-tissues from autologous complement mediated attack, cell surface complement regulators moderate T cell activity through different mechanisms (Kemper and Atkinson, 2007). Cross-linking membrane cofactor protein (MCP, CD46), a cofactor of factor I to inactivate C3b fragments during complement activation, induces the generation of IL-10 producing regulatory T cells which inhibits by-stander T cell responses (Barchet et al., 2006; Kemper et al., 2003). Knocking out genes of CD59, whose conventional role is to inhibit the formation of membrane attack complex (MAC) in the terminal complement activation pathway, appears to augment T cell activity in a complement independent manner (Longhi et al., 2005). In addition, cross-linking Crry, a rodent specific cell surface complement regulator which possesses activities similar to DAF and MCP, triggers co-stimulating signals that induce T cell proliferation and IL-4 production (Jimenez-Perianez et al., 2005).

By studying $Daf1^{-/-}$ mice independently developed using different strategies (Lin et al., 2001; Sun et al., 1999), we and others have shown that DAF deficiency leads to markedly more severe tissue injury mediated by systemic complement activation in different disease models including experimental autoimmune myasthenia gravis (Kaminski et al., 2006; Lin et al., 2002b), acute and chronic nephrotoxic serum induced nephritis (Lin et al., 2002a; Sogabe et al., 2001), documenting the important roles of DAF in protecting self-tissue from autologous complement mediated injury. Recently we and others also found that in addition to its traditional role of protecting self-cells from autologous complement mediated attack, DAF protects mice from T cell mediated injury by inhibiting local C5a/C3a productions during antigen-specific APC:T cell interactions (Heeger et al., 2005; Liu et al., 2005). $Daf1^{-/-}$ mice exhibit heightened central nervous system injury in EAE (Liu et al., 2008) and worsened retinal damage in experimental autoimmune uveitis (EAU) (An et al., 2009), a model for human autoimmune posterior uveitis, both of which are mediated by augmented autoantigen specific Th1 and Th17 responses. In addition, $Daf1^{-/-}$ mice also develop exacerbated lymphadenopathy, splenomegaly and dermatitis when bred onto the MRL/lpr background (Miwa et al., 2002, 2007). All these data strongly argue that upregulating DAF levels *in vivo* could protect mice from T cell mediated autoimmunity.

Although systemic administration of soluble recombinant DAF protein could partially test the above hypothesis, because DAF is a cell surface bound protein, it is more appropriate to use the DAF overexpressing Tg mice to study the long term effects of DAF upregulation *in vivo*. It is unclear why the DAF transgenic construct delivered high levels of DAF expression *in vitro* but only moderate levels of DAF expression *in vivo* in the transgenic mice. One explanation could be that no generic intron was included within the construct (Choi et al., 1991). Nevertheless, in our newly generated *Daf1* Tg mice, although expression levels of the *Daf1* transgene are not as high as we expected, the effects of DAF upregulation on T cell responses are significant in our experimental settings. The upregualted DAF levels effectively inhibit local C5a/C3a generations during antigen-specific T cell activations which result in less intense

T cell responses both *in vitro* and *in vivo*, thereby protect Daf1 Tg mice against CNS injury in EAE. This report not only provide further evidence that DAF is important in modulating T cell responses in EAE, but also suggest that upregulating DAF levels by pharmaceutics or gene therapy could suppress myelin-specific T cell responses and ameliorate disease severity in multiple sclerosis.

Acknowledgements

References

An, F., Li, Q., Tu, Z., Bu, H., Chan, C.C., Caspi, R.R., Lin, F., 2009. DAF protects against T cell autoreactivity that leads to experimental autoimmune uveitis. Invest. Ophthalmol. Vis. Sci..

Barchet, W., Price, J.D., Cella, M., Colonna, M., MacMillan, S.K., Cobb, J.P., Thompson, P.A., Murphy, K.M., Atkinson, J.P., Kemper, C., 2006. Complement-induced regulatory T cells suppress T-cell responses but allow for dendritic-cell maturation. Blood 107, 1497–1504.

Choi, T., Huang, M., Gorman, C., Jaenisch, R., 1991. A generic intron increases gene expression in transgenic mice. Mol. Cell. Biol. 11, 3070–3074.

Farley, F.W., Soriano, P., Steffen, L.S., Dymecki, S.M., 2000. Widespread recombinase expression using FLPeR (flipper) mice. Genesis 28, 106–110.

Heeger, P.S., Lalli, P.N., Lin, F., Valujskikh, A., Liu, J., Muqim, N., Xu, Y., Medof, M.E., 2005. Decay-accelerating factor modulates induction of T cell immunity. J. Exp. Med. 201, 1523–1530.

Jimenez-Perianez, A., Ojeda, G., Criado, G., Sanchez, A., Pini, E., Madrenas, J., Rojo, J.M., Portoles, P., 2005. Complement regulatory protein Crry/p65-mediated signaling in T lymphocytes: role of its cytoplasmic domain and partitioning into lipid rafts. J. Leukoc. Biol. 78, 1386–1396.

Kaminski, H.J., Kusner, L.L., Richmonds, C., Medof, M.E., Lin, F., 2006. Deficiency of decay accelerating factor and CD59 leads to crisis in experimental myasthenia. Exp. Neurol. 202, 287–293.

Kemper, C., Atkinson, J.P., 2007. T-cell regulation: with complements from innate immunity. Nat. Rev. Immunol. 7, 9–18.

Kemper, C., Chan, A.C., Green, J.M., Brett, K.A., Murphy, K.M., Atkinson, J.P., 2003. Activation of human CD4+ cells with CD3 and CD46 induces a T-regulatory cell 1 phenotype. Nature 421, 388–392.

Kisseberth, W.C., Brettingen, N.T., Lohse, J.K., Sandgren, E.P., 1999. Ubiquitous expression of marker transgenes in mice and rats. Dev. Biol. 214, 128–138.

Lalli, P.N., Strainic, M.G., Lin, F., Medof, M.E., Heeger, P.S., 2007. Decay accelerating factor can control T cell differentiation into IFN-gamma-producing effector cells via regulating local C5a-induced IL-12 production. J. Immunol. 179, 5793–5802.

Lalli, P.N., Strainic, M.G., Yang, M., Lin, F., Medof, M.E., Heeger, P.S., 2008. Locally produced C5a binds to T cell-expressed C5aR to enhance effector T-cell expansion by limiting antigen-induced apoptosis. Blood 112, 1759–1766.

Lin, F., Emancipator, S.N., Salant, D.J., Medof, M.E., 2002a. Decay-accelerating factor confers protection against complement-mediated podocyte injury in acute nephrotoxic nephritis. Lab. Invest. 82, 563–569.

Lin, F., Fukuoka, Y., Spicer, A., Ohta, R., Okada, N., Harris, C.L., Emancipator, S.N., Medof, M.E., 2001. Tissue distribution of products of the mouse decay-accelerating factor (DAF) genes. Exploitation of a Daf1 knock-out mouse and site-specific monoclonal antibodies. Immunology 104, 215–225.

Lin, F., Kaminski, H.J., Conti-Fine, B.M., Wang, W., Richmonds, C., Medof, M.E., 2002b. Markedly enhanced susceptibility to experimental autoimmune myasthenia gravis in the absence of decay-accelerating factor protection. J. Clin. Invest. 110, 1269–1274.

Liu, J., Lin, F., Strainic, M.G., An, F., Miller, R.H., Altuntas, C.Z., Heeger, P.S., Tuohy, V.K., Medof, M.E., 2008. IFN-gamma and IL-17 production in experimental autoimmune encephalomyelitis depends on local APC–T cell complement production. J. Immunol. 180, 5882–5889.

Liu, J., Miwa, T., Hilliard, B., Chen, Y., Lambris, J.D., Wells, A.D., Song, W.C., 2005. The complement inhibitory protein DAF (CD55) suppresses T cell immunity in vivo. J. Exp. Med. 201, 567–577.

Longhi, M.P., Sivasankar, B., Omidvar, N., Morgan, B.P., Gallimore, A., 2005. Cutting edge: murine CD59a modulates antiviral CD4+ T cell activity in a complement-independent manner. J. Immunol. 175, 7098–7102.

Medof, M.E., Kinoshita, T., Nussenzweig, V., 1984. Inhibition of complement activation on the surface of cells after incorporation of decay-accelerating factor (DAF) into their membranes. J. Exp. Med. 160, 1558–1578.

Miwa, T., Maldonado, M.A., Zhou, L., Sun, X., Luo, H.Y., Cai, D., Werth, V.P., Madaio, M.P., Eisenberg, R.A., Song, W.C., 2002. Deletion of decay-accelerating factor (CD55)

exacerbates autoimmune disease development in MRL/lpr mice. Am. J. Pathol. 161, 1077–1086.

Miwa, T., Maldonado, M.A., Zhou, L., Yamada, K., Gilkeson, G.S., Eisenberg, R.A., Song, W.C., 2007. Decay-accelerating factor ameliorates systemic autoimmune disease in MRL/lpr mice via both complement-dependent and -independent mechanisms. Am. J. Pathol. 170, 1258–1266.

Sogabe, H., Nangaku, M., Ishibashi, Y., Wada, T., Fujita, T., Sun, X., Miwa, T., Madaio, M.P., Song, W.C., 2001. Increased susceptibility of decay-accelerating factor deficient mice to anti-glomerular basement membrane glomerulonephritis. J. Immunol. 167, 2791–2797.

Spiller, O.B., Harris, C.L., Morgan, B.P., 1999. Efficient generation of monoclonal antibodies against surface-expressed proteins by hyperexpression in rodent cells. J. Immunol. Methods 224, 51–60.

Strainic, M.G., Liu, J., Huang, D., An, F., Lalli, P.N., Muqim, N., Shapiro, V.S., Dubyak, G.R., Heeger, P.S., Medof, M.E., 2008. Locally produced complement fragments C5a and C3a provide both costimulatory and survival signals to naive CD4+ T cells. Immunity 28, 425–435.

Sun, X., Funk, C.D., Deng, C., Sahu, A., Lambris, J.D., Song, W.C., 1999. Role of decay-accelerating factor in regulating complement activation on the erythrocyte surface as revealed by gene targeting. Proc. Natl. Acad. Sci. U.S.A. 96, 628–633.

Zambrowicz, B.P., Imamoto, A., Fiering, S., Herzenberg, L.A., Kerr, W.G., Soriano, P., 1997. Disruption of overlapping transcripts in the ROSA beta geo 26 gene trap strain leads to widespread expression of beta-galactosidase in mouse embryos and hematopoietic cells. Proc. Natl. Acad. Sci. U.S.A. 94, 3789–3794.