

Treatment with anti-FcεRIα antibody exacerbates EAE and T-cell immunity against myelin

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ABSTRACT

Objective: To investigate the effects of targeting the high-affinity receptor for immunoglobulin E (FcεRI), that plays a central role in allergic responses and is constitutively expressed on mast cells and basophils, in clinical disease and autoimmune T-cell response in experimental MS.

Methods: Experimental autoimmune encephalomyelitis (EAE) was induced in C57BL/6 mice by immunization with myelin oligodendrocyte glycoprotein 35–55. Anti-FcεRI α-chain antibody was administered intraperitoneally. CNS immunohistochemistry, flow cytometry analysis of immune cell populations, IgE and histamine serum concentration, immune cell proliferation, and cytokine measurement were performed. In BALB/c mice, EAE was induced by immunization with myelin proteolipid protein 185–206.

Results: Treatment with anti-FcεRIα antibody resulted in exacerbation of EAE and increased CNS inflammation in C57BL/6 mice. Treated mice displayed long-lasting complete depletion of basophils in the blood stream and peripheral lymphoid organs and increased antigen-induced immune cell proliferation and production of interferon-γ, interleukin (IL)-17, IL-6, and granulocyte-macrophage colony-stimulating factor. In BALB/c mice, which are T-helper (Th) 2 prone and resistant to EAE, treatment with anti-FcεRIα antibody restored susceptibility to EAE.

Conclusion: Our observations that anti-FcεRIα antibody increases Th1 and Th17 responses against myelin antigen and exacerbates EAE suggest that FcεRI, basophils, and possibly other FcεRI-bearing cells that might be affected by this antibody play important roles in influencing the severity of CNS autoimmunity. *Neurol Neuroimmunol Neuroinflamm* 2017;4:e342; doi: 10.1212/NXI.0000000000000342

GLOSSARY

CFA = complete Freund adjuvant; **EAE** = experimental autoimmune encephalomyelitis; **FcεRI** = high-affinity receptor for immunoglobulin E; **GM-CSF** = granulocyte-macrophage colony-stimulating factor; **iDC** = inflammatory dendritic cell; **IFN** = interferon; **IL** = interleukin; **LNC** = lymph node cell; **MAR-1** = anti-FcεRIα monoclonal antibody; **MBP** = myelin basic protein; **MC** = mast cell; **MOG** = myelin oligodendrocyte glycoprotein; **PLP** = myelin proteolipid protein; **PTX** = pertussis toxin; **Th** = T helper.

MS is an autoimmune disease of the CNS, leading to demyelination and neurodegeneration.¹ CD4⁺ T cells reactive against myelin antigens and secreting T-helper (Th) 1 and Th17 cytokines are regarded as critical in MS and its mouse model experimental autoimmune encephalomyelitis (EAE).² Several lines of evidence suggest that immune components and mechanisms of Th2 immunity, associated with a set of cytokines such as interleukin (IL)-4, IL-5, and IL-13, can modulate the autoimmune response in EAE.^{3–5} Mast cells (MCs) and basophils are regarded as major effector cells in Th2 immunity and can contribute to the development of allergic

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disorders, clearance of parasites, and immune defense against venoms.⁶ These cells can also influence immune responses by releasing a plethora of mediators and cytokines.⁷

The high-affinity receptor for immunoglobulin E (FcεRI) plays a key role in IgE-mediated allergic reactions and is constitutively expressed on the surface of both MCs and basophils.⁸ Crosslinking of FcεRI-bound IgE by antigen induces in these cells the release of several cytokines and inflammatory mediators, such as histamine, heparin, and serotonin.⁸ The expression of FcεRI in mice can be induced during certain immune responses also in a subset of dendritic cells (inflammatory dendritic cells [iDCs])⁹ and in eosinophils.

The potential role of MCs in CNS autoimmunity has been extensively studied, although their exact function in this disorder is still controversial.^{10–15} Unlike MCs, the role of basophils in MS and EAE has never been directly investigated. Basophils are circulating granulocytes accounting for less than 1% of blood leukocytes. On activation, these cells can release several effector molecules, such as histamine, platelet-activating factor, leukotrienes, and Th2 cytokines IL-4 and IL-13.^{16–18} Basophils are an important innate source of Th2 cytokines required for Th2 immunity.^{18,7} In mouse models of autoimmune disorders such as lupus nephritis^{19,20} and collagen-induced arthritis, basophils have been suggested to play a detrimental role.^{21,22} Conversely, in a mouse model of autoimmune chronic colitis, basophils appeared to exert a beneficial role.²³

In this study, we attempted to evaluate the potential effects of targeting FcεRI in CNS autoimmunity using a monoclonal antibody against the α-chain of FcεRI (FcεRIα) in chronic EAE and in an EAE model induced in BALB/c mice, which are Th2 prone and are resistant against the disease.

METHODS **Mice, peptides, and EAE induction.** C57BL/6 (H-2^b) and BALB/c (H-2^d) female mice, 8–12 weeks old, were purchased from Charles River Laboratories Inc (Calco, Italy). FcεRI α-chain $-/-$ mice²⁴ (backcrossed to C57BL/6 for more than 8 generations) were kindly provided by Dr. SJ Galli (Stanford University, Stanford, CA). All mice were housed and maintained in specific pathogen-free conditions at the animal

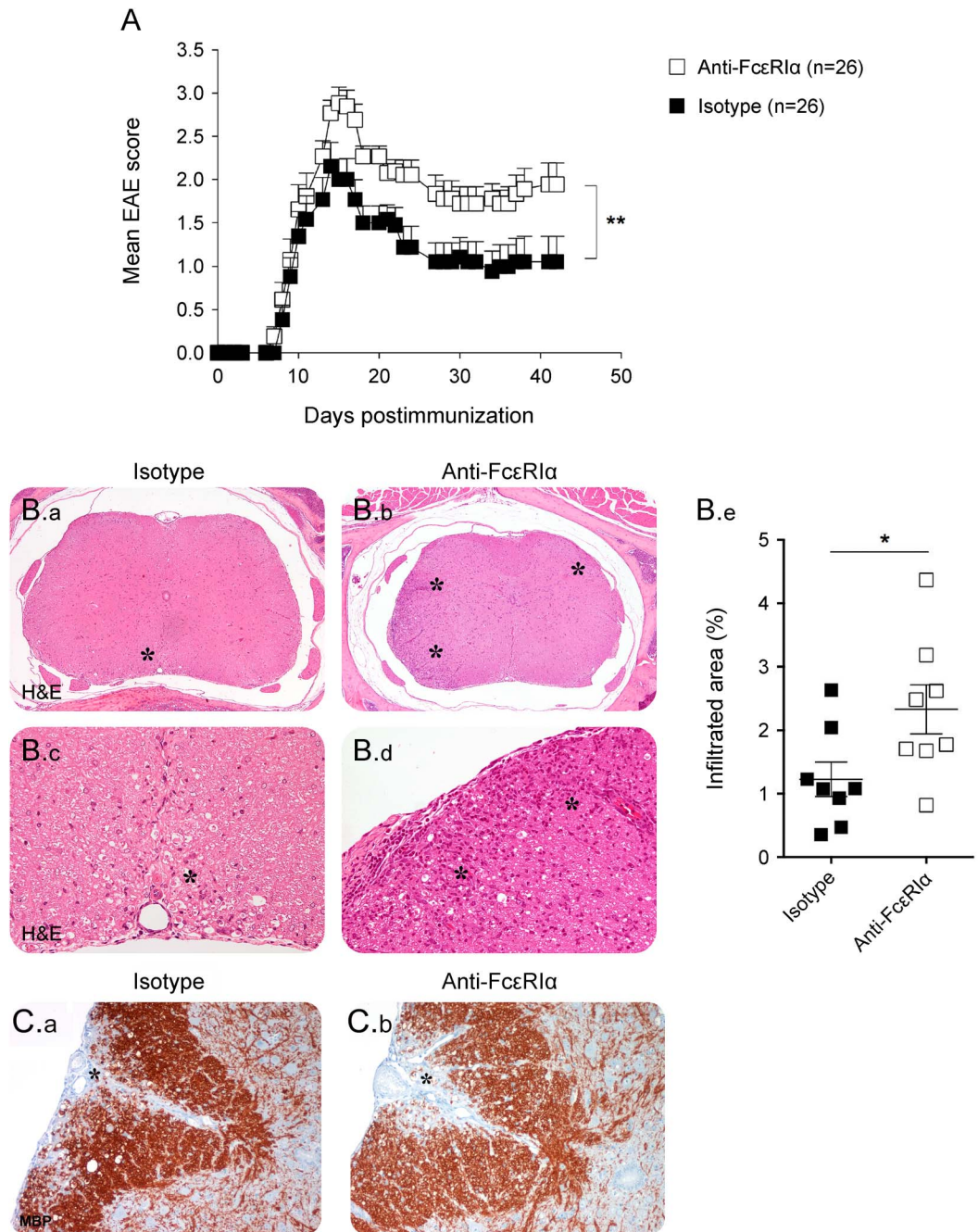
facility of the Foundation I.R.C.C.S. Neurological Institute Carlo Besta. Myelin oligodendrocyte glycoprotein (MOG) 35–55 (MEVGWYRSPFSRVVHLYRNGK) (synthesized by Dr. R. Longhi, Consiglio Nazionale delle Ricerche—CNR Milan, Italy) and myelin proteolipid protein (PLP) 186–204 (SIAFPSKTSASIGSLCADARMY) (Caslo ApS, Denmark) were synthesized using standard 9-fluorenylmethoxycarbonyl chemistry (Applied Biosystems, Foster City, CA) and purified as previously described.²⁵ EAE was induced in C57BL/6 mice with 100 μg of MOG 35–55 peptide in complete Freund adjuvant (CFA) (Difco; BD Bioscience, Buccinasco, Italy) subcutaneously and 50 ng of pertussis toxin (PTX; List Biological Lab, Campbell, CA) intravenously on day 0 and +2 postimmunization as described.²⁵ In BALB/c mice, EAE was induced with 200 μg of PLP 186–204 peptide in CFA containing 10 mg/mL of heat-killed *Mycobacterium tuberculosis* (Difco) subcutaneously and 500 ng of PTX intravenously on days 0 and 2 postimmunization.²⁶ Mice were assessed daily for clinical signs of EAE with a 5-point scale²⁵ by an observer masked to treatment assignment. All procedures involving animals were approved by the Ethical Committee of the Foundation Neurological Institute Carlo Besta and by the Italian General Direction for Animal Health at the Ministry of Health. Animal studies were performed in accordance with the institutional guidelines and national law (DL116/92), and carried out according to the Principles of Laboratory Animal Care (European Communities Council Directive 2010/63/EU).

Treatments. A monoclonal antibody against mouse α-chain of FcεRI (anti-FcεRIα; clone MAR-1; eBioscience, San Diego, CA) or isotype control antibody (clone eBio299Arm; eBioscience) was administered intraperitoneally at a dose of 5 μg/mouse twice daily for 3 consecutive days from day –7 to day –5 before EAE induction.

Flow cytometry. Single-cell suspensions were incubated with blocking monoclonal antibody anti-CD16/CD32 (2.4G2) (1:100) and then stained with APC anti-CD200R3, FITC anti-CD200R3, PE/Cy7 anti-CD49b, PE anti-CD49b, PerCP anti-CD45, PE-Vio770 anti-CD11b, FITC anti-CD3, APC-Vio770 anti-CD4, PE-vio770 anti-CD45/B220, PE anti-CD19, PE anti-CD115, and APC anti-FcεRIα (MAR1) (all from Miltenyi, Bergisch Gladbach, Germany). Cells were analyzed by flow cytometry on a FACSCanto (BD Biosciences), and analyses were performed using FlowJo software (Treestar Inc., Ashland, OR).

Tissue pathology and immunohistochemistry studies. Brains and spinal cords were removed and fixed in 10% formalin. Paraffin-embedded sections (4 μm) were used for hematoxylin and eosin staining. Sections were blindly and independently analyzed by 2 pathologists (E.F. and P.L.P.). The degree of inflammation was expressed by measuring the percentage of area infiltrated with immune cells over the total spinal cord section. For immunohistochemistry studies, sections were stained with rat anti-myelin basic protein (MBP) antibody (1/50 dilution; Millipore, Temecula, CA) for myelin, rabbit anti-CD3 monoclonal antibody (SP-7, 1/100 dilution; Thermo Scientific, Fremont, CA) for T cells, rat anti-B220 antibody (supernatant) for B cells, and rabbit anti-IBA1 antibody (1/300 dilution; Wako Chemicals, Neuss, Germany) for microglia cells and macrophages. CD3 and IBA-1 were revealed with EnVision+System-HRP labeled polymer anti-rabbit (Dako, Glostrup, Denmark), and B220 and MBP with rat HRP-Polymer Kit (Biocare Medical). 3,3'-diaminobenzidine was used as a chromogen according to the manufacturer's protocol (Dako, Glostrup, Denmark). Sections were counterstained with hematoxylin. Giemsa staining was performed for highlighting MCs.

Figure 1 Treatment with antibody against the α -chain of the high affinity receptor for immunoglobulin E in C57BL/6 mice exacerbates experimental autoimmune encephalomyelitis



(A) Clinical experimental autoimmune encephalomyelitis (EAE) scores of myelin oligodendrocyte glycoprotein 35-55-immunized mice treated with antibody against the α -chain of the high affinity receptor for immunoglobulin E (Fc ϵ R1 α) (white squares) or isotype control antibody (black squares) ($n = 26$ mice per group). Data (mean \pm SEM) are pooled from 2 consecutive experiments that gave similar results. $**p < 0.01$ by analysis of variance test. (B) Representative hematoxylin and eosin (H&E) staining of spinal cord sections from EAE mice treated with isotype control (B.a and B.c) or anti-Fc ϵ R1 α (B.b and B.d) 6 weeks after disease induction. Asterisks highlight immune cell infiltration. Original magnification 20 \times (B.a and B.b) and 40 \times (B.c and B.d). Graph (B.e) shows comparative analysis of infiltrated area (% of total area) in the spinal cord. Each dot represents an individual mouse. Mean \pm SEM are also depicted. $*p < 0.05$ Student t test, 2 tailed. (C) Representative anti-myelin basic protein staining of spinal cord sections from EAE mice treated with isotype control (C.a) or anti-Fc ϵ R1 α (C.b) 6 weeks after disease induction as in B. Asterisks highlight demyelinated areas. Magnification 60 \times .

T-cell proliferation. Axillary and inguinal lymph node cells (LNCs) were isolated from mice and cultured in vitro as described²⁵ in 96-well plates (5×10^5 cells/well) in 200 μ L enRPMI 1640 (EuroClone; Pero, Milan, Italy) supplemented

with 10% fetal bovine serum (Gibco Thermo Fisher, Waltham, MA). Cells were stimulated for 72 hours with MOG 35-55, Con A (2μ g/mL) or medium alone and pulsed for 18 hours with [³H]-thymidine before harvesting. Proliferation was

measured from triplicate wells on a beta counter (Perkin-Elmer, Waltham, MA).

Cytokine analysis. LNCs were cultured in the same conditions as described above²⁵ and cytokines analyzed in cells supernatants by sandwich ELISA (anti-mouse OptEIA ELISA Set for interferon [IFN]- γ , IL-6, IL-10, and IL-4, BD Pharmingen, San Jose, CA; Mouse IL-17 DuoSet and Mouse granulocyte-macrophage colony-stimulating factor [GM-CSF] DuoSet; R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

Serum histamine and IgE measurement. Serum samples were obtained from the tail vein and stored at -80°C until tested. Total IgE antibody concentration was measured on serum samples at a 1:50 dilution by ELISA (IgE OptEIA kit; BD Pharmingen).²⁵ Histamine concentration was measured with an enzyme immunoassay kit (ImmunoTech; Beckman Coulter, Brea, CA) according to the manufacturer's instructions.²⁵

Statistical analysis. Two-way analysis of variance followed by the Bonferroni post hoc test was used for comparison of EAE scores between groups. For all other analyses, unpaired Student *t* test, 2 tailed, was used. Analysis was performed using SPSS software (IBM SPSS Statistics, Armonk, NY). In all tests, *p* values <0.05 were considered statistically significant.

RESULTS Anti-Fc ϵ RI α antibody treatment increased the severity of EAE. To evaluate the potential effects of targeting Fc ϵ RI in CNS autoimmunity, we treated C57BL/6 female mice with a monoclonal antibody against Fc ϵ RI α (MAR-1) or an isotype control antibody. Treatment (5 μg) was administered to mice intraperitoneally twice daily for 3 consecutive days. One week after treatment, EAE was induced by active immunization with MOG 35–55 and PTX. In 2 consecutive experiments, treatment with anti-Fc ϵ RI α antibody resulted in higher incidence of EAE and significant exacerbation of disease severity compared with isotype control-treated mice (figure 1A and table). In line with these clinical findings, neuropathologic analysis of the spinal cord obtained from mice 6 weeks after EAE induction revealed significantly increased inflammation in the white matter of anti-Fc ϵ RI α antibody-treated mice compared with isotype control-treated mice (figure 1B). In both groups, infiltrating cells were mainly composed of macrophages, with a minority of cells represented by T cells and very rare B cells (data not shown). Granulocytes were barely detected in both control

and treated mice. In Giemsa-stained sections of the spinal cord, rare cells with the morphological appearance of MCs were observed in perivascular areas in both treatment groups (data not shown). In parallel with the higher extent of inflammation observed in anti-Fc ϵ RI α antibody-treated mice, in spinal cord sections of these mice, demyelination was more prominent compared with that observed in sections of isotype control-treated mice (figure 1C).

We then analyzed by flow cytometry the effects of anti-Fc ϵ RI α antibody treatment on the frequency of immune cell subsets in blood and peripheral lymphoid organs. Because an experimental group was treated with anti-Fc ϵ RI α antibody, we could not use the same fluorochrome-labeled monoclonal antibody clone MAR-1 for the identification of Fc ϵ RI-expressing cells. As previously reported,^{27,28} we observed that anti-Fc ϵ RI α antibody induced complete depletion of basophils identified as CD200R3+, CD49b+, CD3 $^{-}$, CD45/B220 $^{-}$ cells in the blood, lymph node, and spleen (figure 2A). In these treated mice, basophils were completely depleted at the time of EAE induction, during priming and acute phase of EAE, and began to reappear in blood circulation 3 weeks after immunization (figure 2C). The frequency of other leukocytes known to play important roles in EAE, including T cells, B cells, and monocytes, was not significantly modified by the treatment (figure 2B). Anti-Fc ϵ RI α antibody (MAR-1) staining of leukocytes disappeared in Fc ϵ RI α -chain $-/-$ mice, confirming that this antibody binds uniquely to its target, the α -chain of the receptor (figure 2D).

Anti-Fc ϵ RI α antibody-treated mice exhibited enhanced Th1 and Th17 responses against myelin antigen. We next evaluated whether the exacerbated EAE phenotype observed in mice treated with anti-Fc ϵ RI α antibody was associated with an alteration of T-cell activation and Th regulation. We isolated LNCs from anti-Fc ϵ RI α antibody-treated and isotype control-treated mice with EAE during the priming phase of the disease (7–10 days after immunization) and examined the recall response to peptide stimulation in vitro. LNCs from anti-Fc ϵ RI α antibody-treated mice displayed increased proliferation in response to MOG 35–55 compared with isotype control-treated mice (figure 3A). Moreover, in LNCs of anti-Fc ϵ RI α antibody-treated mice, the secretion of proinflammatory cytokines IFN- γ , IL-17, and IL-6, known to play a critical role in EAE, was significantly increased (figure 3A). GM-CSF, considered essential in CNS autoimmune inflammation,²⁹ was also increased in the supernatant of antigen-stimulated LNCs from anti-Fc ϵ RI α antibody-treated mice compared to that from isotype control-treated mice (figure 3A). We did not observe

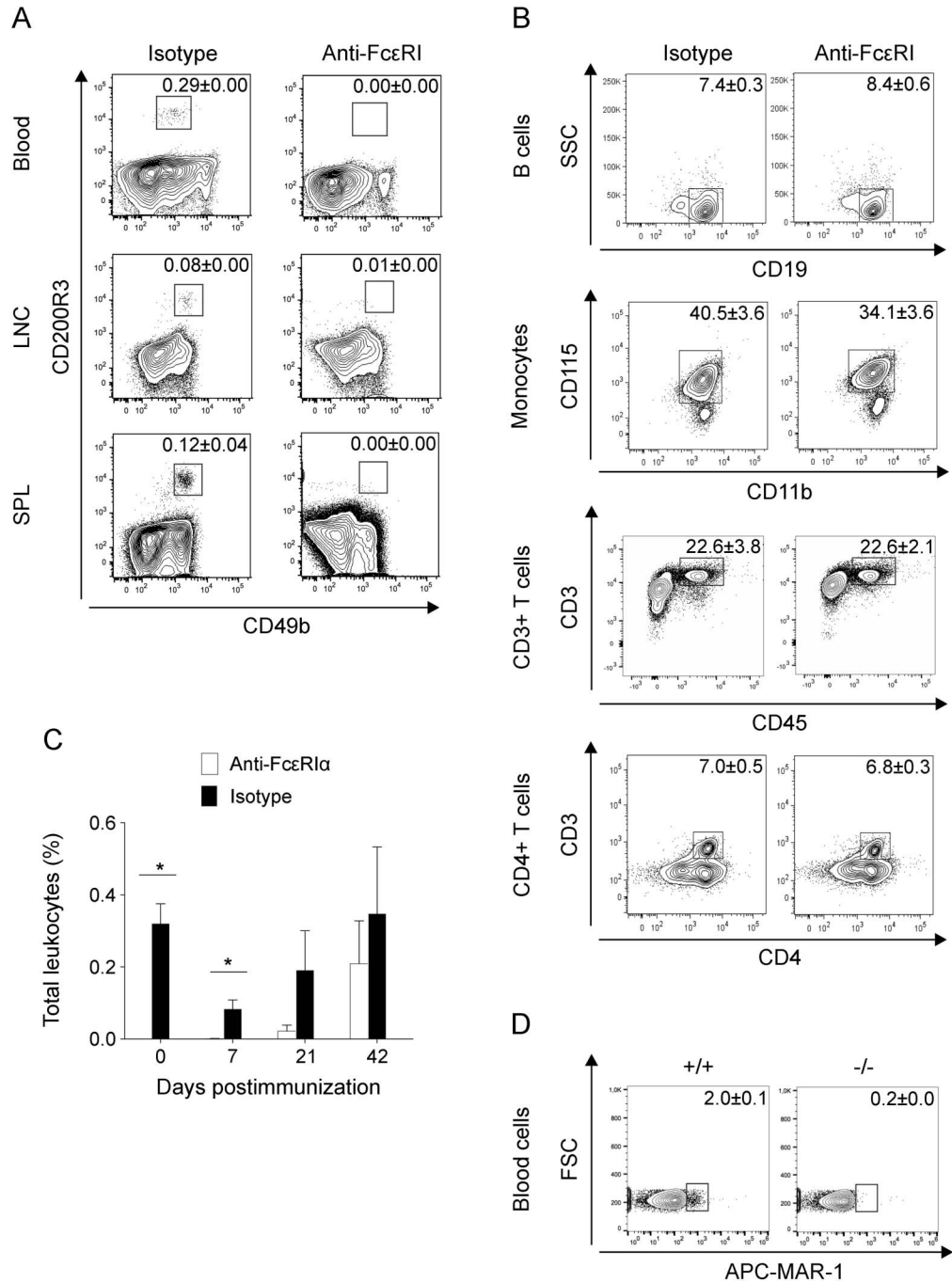
Table Experimental autoimmune encephalomyelitis features in C57BL/6 mice treated with antibody against the α -chain of the high affinity receptor for immunoglobulin E

Group	Incidence (%)	Onset, d	Mean maximum score	Cumulative disease score
Anti-Fc ϵ RI α	26/26 (100)	10.1 \pm 0.4	3.3 \pm 0.2 ^a	46.2 \pm 4.2 ^a
Isotype	22/26 (84.6)	10.3 \pm 0.4	2.5 \pm 0.3	30.7 \pm 4.2

Data are shown as mean \pm SEM of pooled data from 2 consecutive independent experiments, each with 13 mice per group that gave similar results.

^a*p* <0.05 .

Figure 2 Flow cytometry analysis of basophils and immune cell subsets in C57BL/6 mice treated with antibody against the α -chain of the high affinity receptor for immunoglobulin E



(A and B) Lymph nodes, spleens, and blood were obtained at the time of experimental autoimmune encephalomyelitis (EAE) induction, 7 days after treatment with antibody against the α -chain of the high affinity receptor for immunoglobulin E or isotype control antibody and analyzed by flow cytometry. (A) Representative flow cytometry plots of basophils in blood, lymph node cells (LNCs), and splenocytes (SPL) of treated mice. Live cells were gated on CD3⁻ and CD45/B220⁺ cells. Numbers represent mean percentage of total leukocytes \pm SEM (n = 5 mice per group) from 1 of 3 independent experiments that gave similar results. (B) Representative flow cytometry plots of B cells, monocytes, T cells, and CD4⁺ T cells in blood cells of antibody treated mice. Live cells were gated on expression of CD45. Data represent mean percentage \pm SEM (n = 5 mice per group) and are representative of 2 independent experiments that gave similar results. (C) Quantification of basophils in the blood of C57BL/6 mice treated with anti-Fc ϵ RI α (white bars) or isotype control antibody (black bars) before EAE induction (day 0) and at different time points during disease. Data represent mean \pm SEM of 3-6 animals per group for each time point and are representative of 2 consecutive experiments that gave similar results. (D) Representative flow cytometry plots of APC-conjugated anti-Fc ϵ RI α (MAR-1) antibody staining in blood leukocytes of naive Fc ϵ RI α -chain +/+ and -/- mice. Data represent mean percentage \pm SEM of 3 mice per group and are representative of 2 independent experiments. Similar data were obtained by staining with APC-MAR-1 antibody LNCs and splenocytes of Fc ϵ RI α -chain +/+ and -/- mice. *p < 0.05 Student t test, 2 tailed. FSC = forward-scattered light; SSC = side-scattered light.

significant differences in IL-10 production in antigen-stimulated LNCs from anti-FcεRIα antibody-treated mice vs those from isotype control-treated mice (figure 3A), while IL-4 was below the detection limit (data not shown). We next repeated the analysis of the recall response in LNCs from mice 6 weeks after EAE induction, at a time where the frequency of basophils in the blood of anti-FcεRIα antibody-treated mice had returned almost to normal values (figure 2C). No differences were observed at this time point in either proliferation (figure 3B) or cytokine production between anti-FcεRIα antibody-treated mice and isotype control-treated mice, with the exception of the suppressor cytokine IL-10, which was significantly higher in anti-FcεRIα antibody-treated mice (figure 3B).

Effects of anti-FcεRIα treatment on serum concentration of histamine and total IgE. Histamine has been shown to modulate the immune response in EAE,^{30–32} and histidine decarboxylase-deficient mice (–/– mice), that are histamine deficient, displayed exacerbated EAE, and increased Th1 responses.^{25,33} Because basophils, which represent an important source of histamine, were completely depleted by anti-FcεRIα antibody in our model, we measured the concentration of histamine in mouse sera during EAE. Both anti-FcεRIα antibody and isotype control-treated groups displayed similar levels of serum histamine concentrations (figure 3C). Of note, histamine levels did not change significantly in either group during the course of EAE (figure 3C). These findings suggest that the increased severity of EAE that we observed in anti-FcεRIα antibody-treated mice is unlikely to be related with an effect of the treatment on blood histamine levels. These findings also suggest that there are substantial sources of blood histamine in these mice in addition to basophils.

Basophils are a predominant source of IL-4 in allergen and parasite-activated blood leukocytes.³⁴ This cytokine induces in B-cell antibody class switch toward IgE and IgG1. We previously reported that IgE increases in mouse serum during EAE,²⁵ although the biological significance of the increase of antibodies of this class is yet to be defined. We therefore measured IgE concentrations in serum and found that IgE concentrations were significantly decreased in anti-FcεRIα antibody-treated mice during the priming phase of EAE (day 7) compared with isotype control-treated mice. These differences in IgE concentrations disappeared 6 weeks after EAE induction, at the time when basophil frequency had returned to almost normal levels (figure 3D).

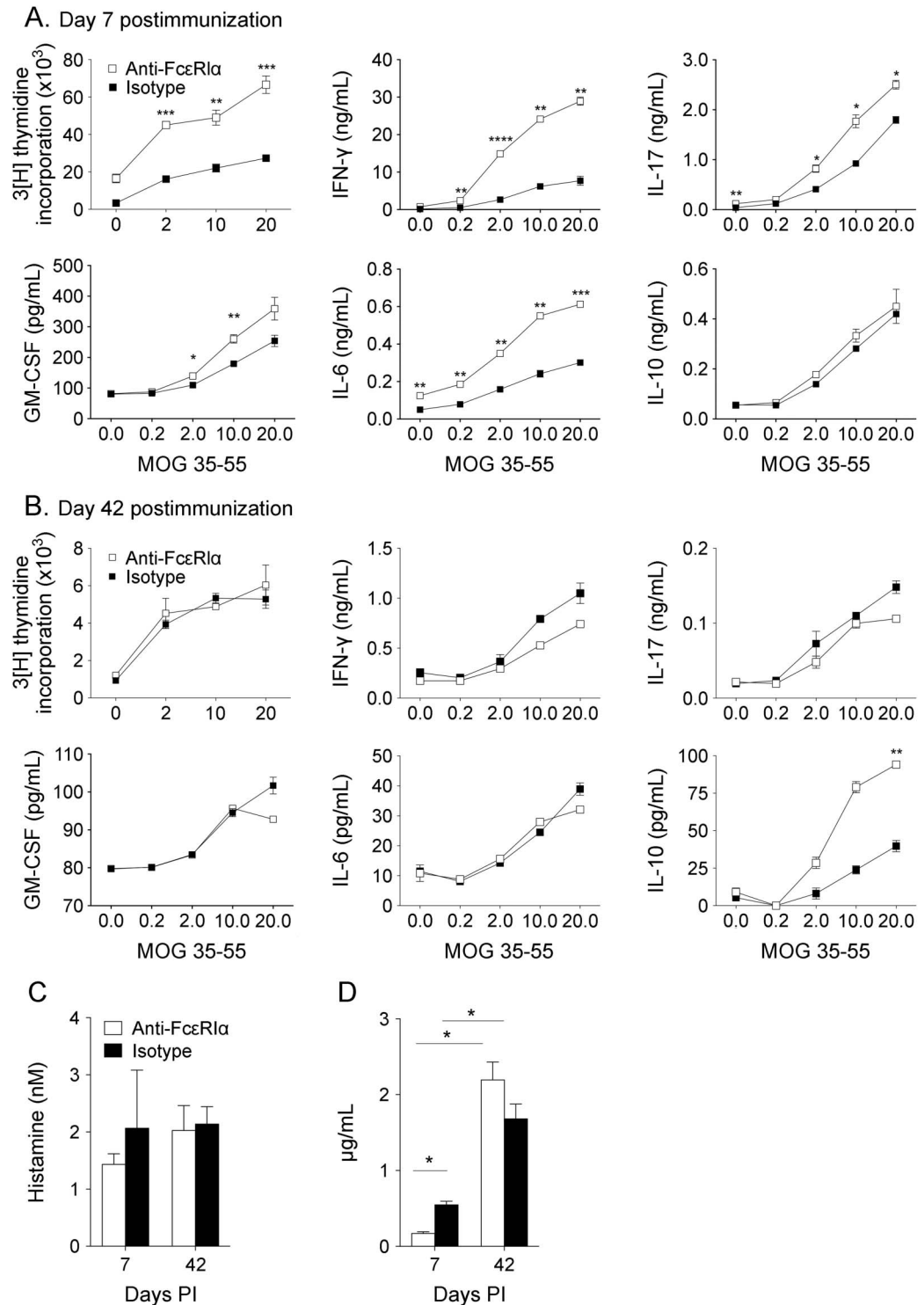
Treatment with anti-FcεRIα induced susceptibility to EAE in resistant BALB/c mice. The results reported above show that anti-FcεRIα antibody treatment both exacerbated EAE in C57BL/6 mice and depleted

basophils, suggesting that depletion of these cells and perhaps additional effects of anti-FcεRIα antibody treatment contributed to increase the severity of EAE in this model. To further explore this hypothesis, we used anti-FcεRIα antibody in another model of EAE obtained in BALB/c mice, which are Th2 prone and resistant against EAE.³⁵ We attempted to induce EAE in this strain by immunization with the self-antigen PLP 186–204 in CFA and PTX.²⁶ Saline-treated mice were completely resistant to EAE (none of 5 mice immunized developed the disease), and among isotype control-treated mice, only 2 of 5 (40%) displayed minimal signs of EAE (score 1 = tail paralysis) that lasted only 1 day (figure 4A). Conversely, the majority of BALB/c mice (4 of 5; 80%) treated with anti-FcεRIα antibody developed EAE (figure 4A), with maximum scores of 1, 2 (hind-limb paralysis), and 5 (death). Similar to what we observed in C57BL/6 mice, flow cytometry analysis conducted on blood cells of anti-FcεRIα antibody and isotype control-treated BALB/c mice revealed that this treatment induced complete depletion of basophils (figure 4B).

DISCUSSION Th1 and Th17 cells play key roles in the pathogenesis of EAE and MS, and it is widely accepted that Th2 responses hinder the development and pathobiology of these diseases. However, emerging evidence suggests that, in addition to Th2 polarized cells that counteract Th1 and Th17 responses, also effector cells and mediators of the Th2 immunity can limit the severity of EAE. For example, we and others have shown that EAE is more severe in the absence of histamine,^{25,33} and similar findings were observed in MC-deficient *c-kit* mutant mice under certain experimental conditions.^{14,15} In this report, to further test the hypothesis that mechanisms of the Th2 immunity can influence the development in EAE, we targeted with a monoclonal antibody FcεRI, that plays a crucial role in IgE-mediated allergic reactions and is constitutively expressed on MCs and basophils. We found that treatment with this antibody before the induction of the disease exacerbated MOG 35–55 EAE in C57BL/6 mice and increased CNS neuroinflammation. Moreover, anti-FcεRIα antibody increased Th1 and Th17 responses against myelin antigen. Last, treatment induced susceptibility to EAE in BALB/c mice, which are Th2 prone and resistant to the development of EAE.

The mechanisms involved in disease exacerbation caused by anti-FcεRIα antibody treatment are yet to be fully understood. Complete depletion of basophils and/or alteration of the frequency and function of other FcεRI-bearing cells associated with the treatment can be postulated as one potential mechanism. Indeed, anti-FcεRIα antibody has been previously shown to deplete basophils *in vivo* and to reduce

Figure 3 Effects of antibody against the α -chain of the high affinity receptor for immunoglobulin E treatment on antigen-induced T-cell response and on serum concentration of histamine and IgE antibody



(A and B) Draining lymph nodes were obtained from antibody against the α -chain of the high affinity receptor for immunoglobulin E (Fc ϵ RI α) or isotype control antibody-treated mice ($n = 5$ mice per group) 7 days (A) and 42 days (B) after experimental autoimmune encephalomyelitis (EAE) induction. Proliferation (mean \pm SEM from triplicate wells) and cytokine production (mean \pm SEM from duplicate wells) were analyzed in lymph node cells stimulated *in vitro* with serial concentrations of myelin oligodendrocyte glycoprotein (MOG) 35-55 or medium alone. Data are representative of 2 independent experiments that gave similar results. (C and D) serum samples were obtained from mice treated with anti-Fc ϵ RI α (white bars) or isotype control antibody (black bars) 7 and 42 days after the induction of EAE. Histamine was measured by enzyme immunoassay in duplicated wells ($n = 3$ to 6 mice per group in each time point) (C), and total IgE was measured by ELISA in duplicated wells ($n = 8$ to 16 mice per group in each time point) (D). Data represent mean \pm SEM from 2 different experiments. PI = postimmunization. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ by Student *t* test, 2 tailed. GM-CSF = granulocyte-macrophage colony-stimulating factor; IFN = interferon; IL = interleukin.

the frequency of peritoneal MCs.²⁷ In addition to the potential effects of anti-FcεRIα antibody on basophils and MCs, this antibody can also deplete a population of dendritic cells, namely iDC, that can express FcεRI after immunization with an allergen.⁹ In this regard, in the model of CNS autoimmunity that we used, we failed to detect by flow cytometry such immune cells (non-T and non-B cells expressing FcεRI and lacking CD49b) in lymph nodes, spleens, or blood of mice during the course of EAE (data not shown). It is possible that the generation of these cells critically depends on the type of antigen and/or immunization protocol used, and that such FcεRI + iDCs might play a marginal or no role in the development of EAE. Thus, it is unlikely that depletion of iDCs contributed to the results that we observed.

We found that anti-FcεRIα antibody-treated mice exhibited enhanced recall responses against MOG 35–55 antigen in their LNCs compared with isotype control-treated mice, with increased T-cell proliferation and secretion of Th1 and Th17 cytokines, such as IFN-γ, IL-17, IL-6, and GM-CSF. These cytokines are known to play important roles in the development of EAE.^{1,2,29} Thus, immune modulation might represent an important mechanism by which anti-FcεRIα antibody treatment induces EAE exacerbation. In line with our results, in another model of T-cell autoimmunity, Gomez et al. have shown that depletion of basophils by monoclonal antibodies in a mouse model of autoimmune colitis resulted in enhanced production of Th1 and Th17 cytokines and exacerbated colitis.²³

Among the mechanisms potentially contributing to disease exacerbation observed in anti-FcεRIα antibody-treated mice, depletion of basophils and possibly MCs as important sources of mediators and cytokines that can counteract Th1/Th17 autoimmunity can be postulated. For example, IL-4, of which these cells represent important sources, plays a key role in counteracting CNS autoimmunity and limits the severity of EAE.² Of interest, in anti-FcεRIα antibody-treated mice, serum concentration of IgE, whose production depends on IL-4, was significantly lower during the priming phase of EAE, at a time when basophils were completely depleted. This difference disappeared at a later time point, when basophil frequency had returned to normal level.

Among other mediators secreted by basophils and MCs that may potentially induce immune modulation, histamine, which is synthesized and secreted by these cells in high amounts, is also considered to play a role in limiting the severity of EAE. However, we did not find significant differences in histamine serum concentrations between mice treated with anti-FcεRIα antibody and those treated with isotype control antibody, and thus, it is

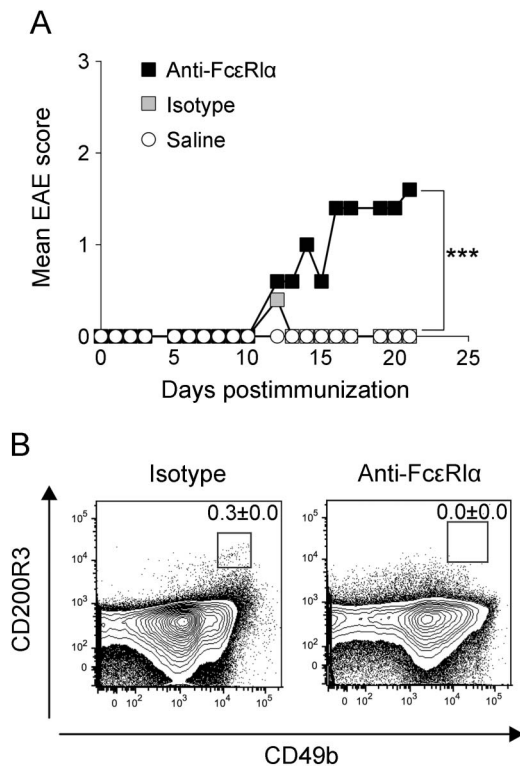
unlikely that the effects of this treatment are related to histamine.

Although it is generally accepted that immune deviation toward Th2 protects against EAE, Lafaille and colleagues reported that transfer of Th2 lymphocytes specific for MBP failed to protect against Th1 cell-mediated EAE. Moreover, these Th2 lymphocytes can cause themselves EAE when transferred into immune-deficient RAG-1 knockout recipient mice that lack mature T and B cells.³ An unusually high number of polymorphonuclear cells and MCs were observed in CNS inflammatory infiltrates of Th2 cell-induced EAE.³ In our study, we have used an active EAE model obtained in immune competent mice to evaluate the potential effects of targeting FcεRI, constitutively expressed on mouse MCs and basophils. We show that treatment with anti-FcεRIα antibody induced an exacerbation of Th1 and Th17 responses, with mechanisms likely involving immune cells such as basophils and MCs, both important sources of IL-4 and other key cytokines and involved in Th2 immunity. Understanding how Th2 lymphocytes and effector cells of Th2 immunity, such as basophils and MCs, intervene in the complex pathobiology of CNS autoimmunity remains an open field of investigation.

Of interest, in the context of CNS autoimmunity, it has been recently reported that subacute infection with intestinal parasites is associated with reduced disease flares in relapsing-remitting patients with MS, with reduced disability progression and fewer MRI changes compared with uninfected patients with MS.^{36,37} Although an increased production of IL-10 from B cells and an induction of regulatory T cells were observed in these parasite-infected patients with MS, it is also possible that activation of basophils occurring during helminth infection could have induced beneficial effects and reduced encephalitogenic responses in these patients. Of note, increasing evidence suggests that basophil activation can contribute to the induction or intensity of Th2 responses.³⁸ Such effects might be beneficial in the context of EAE and MS.

The findings that we report here are associated with the effects of a treatment with a monoclonal antibody. We confirmed that MAR-1 antibody binds uniquely to its target, the α-chain of FcεRI (figure 2D), and that it completely depletes basophils; however, this antibody may also reduce the numbers and/or affect the functions of other FcεRI-bearing cells. Alternatively, it is possible that MAR-1 antibody results in some activation of basophils (and/or other FcεRI-bearing cells) at the time of treatment administration, as well as depleting this population. In that case, such MAR-1-dependent cell activation (instead of or in addition to MAR-1-dependent cell

Figure 4 Treatment with antibody against the α -chain of the high affinity receptor for immunoglobulin E induces susceptibility to experimental autoimmune encephalomyelitis in resistant BALB/c mice



(A) Clinical experimental autoimmune encephalomyelitis (EAE) scores of myelin proteolipid protein 186-204-immunized mice treated before EAE induction with antibody against the α -chain of the high affinity receptor for immunoglobulin E (Fc ϵ RI α) (black squares), isotype control antibody (gray squares), or saline (white circles) ($n = 5$ mice per group). *** $p < 0.001$ by analysis of variance test. (B) Basophils were analyzed by flow cytometry in the blood of BALB/c mice 7 days after treatment with anti-Fc ϵ RI α or isotype control antibody, at the time of EAE induction. Representative dot plots. Numbers represent mean percentage of total leukocytes \pm SEM ($n = 5$ mice per group).

depletion) might influence the course of EAE in this setting. Understanding how these cells of the Th2 immunity intervene in the complex autoimmune response leading to CNS inflammation and demyelination might help defining potential new targets of treatment for this disease.

Our work demonstrates that treatment with anti-Fc ϵ RI α antibody plays a detrimental role in EAE and suggests that targeting of Fc ϵ RI and/or of cells expressing this receptor can influence CNS autoimmunity.

AUTHOR CONTRIBUTIONS

Silvia Musio designed and performed the experiments, acquired and analyzed data, performed statistical analysis, and wrote the manuscript. Massimo Costanza helped performing in vitro experiments, discussed results, and revised the manuscript. Pietro Luigi Poliani, Elena Fontana, and Manuela Cominelli performed histopathologic and immunohistochemistry

analysis and revised the manuscript. Gabriella Abolafio helped with flow cytometry experiments. Lawrence Steinman discussed the results at all stages and contributed to write the manuscript. Rosetta Pedotti initiated the study, designed research, interpreted data, supervised the study, and wrote the manuscript.

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DISCLOSURE

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