MHC-Independent Genetic Factors Control the Magnitude of CD4⁺ T Cell Responses to Amyloid-β Peptide in Mice through Regulatory T Cell-Mediated Inhibition

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*J Immunol* published online 26 September 2011
http://www.jimmunol.org/content/early/2011/09/25/jimmunol.1003953
MHC-Independent Genetic Factors Control the Magnitude of CD4+ T Cell Responses to Amyloid-β Peptide in Mice through Regulatory T Cell-Mediated Inhibition

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Accumulation of amyloid-β (Aβ) is considered the triggering factor of pathogenic lesions in Alzheimer’s disease (AD), and vaccines targeting Aβ are promising therapeutic options. However, the occurrence of meningoencephalitides attributed to T cell responses in 6% of Aβ-immunized patients underscores the need for a better understanding of T cell responses to Aβ. We characterized the parameters controlling the magnitude of Aβ-specific CD4+ T cell responses in mice. T cell responsiveness to Aβ1-42 was highly heterogeneous between mouse strains of different H-2 haplotypes, with SJL/J (H-2s) mice displaying a weak response, mainly specific for Aβ10-24, and C57BL/6 (H-2b) mice displaying a weak response to Aβ16-30. Surprisingly, C57BL/6 mice congenic for the H-2b haplotype (B6.H-2b), which display a “permissive” MHC class II allele for presentation of the immunodominant Aβ10-24 epitope, showed a very weak CD4+ T cell response to Aβ, suggesting that MHC-independent genes downmodulate Aβ-specific CD4+ T cell responses in C57BL/6 background. Vaccine-induced CD4+ T cell responses to Aβ were significantly enhanced in both C57BL/6 and B6.H-2b mice upon depletion of regulatory T cells (Tregs), whereas Treg-depleted SJL/J mice displayed unaltered Aβ-specific T cell responses. Finally, Treg depletion in C57BL/6 transgenic APPPS1 mice, a mouse model of AD, results in enhanced vaccine-induced CD4+ T cell responses in AD compared with wild-type animals. We concluded that the magnitude of Aβ-specific CD4+ T cell responses is critically controlled in both physiological and pathological settings by MHC-independent genetic factors that determine the overall potency of Aβ-specific Treg responses. The Journal of Immunology, 2011, 187: 000–000.

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lzheimer’s disease (AD) is a severe neurodegenerative disorder characterized by progressive loss of memory and cognitive functions. Accumulation of amyloid-β peptide (Aβ), both as soluble oligomers and as compact fibrillar aggregates in neuritic plaques and diffuse deposits, seems to play a key role in initiating the pathogenic events in AD. Active immunization against Aβ has emerged as a promising therapeutic strategy that can prevent Aβ deposition and reverse cognitive decline in murine models (1–3). However, an Aβ-supposedly related to inappropriate activation of Aβ-immunodominant Aβ10-24 peptide (AN1792) in human AD patients was prematurely interrupted due to the occurrence of meningoencephalitides in 6% of the cases, supposedly related to inappropriate activation of Aβ-specific T cells (4, 5). In contrast, although Aβ responses may control Aβ plaque burden (6), data suggest that Aβ-specific CD4+ T cell responses can reverse cognitive decline and synaptic loss in AD mice (7, 8). Altogether, these preclinical and clinical studies suggest that Aβ-specific T cell responses display multiple functions with complex outcomes, which could be either detrimental or beneficial, depending on the modulation of the reactivity and functionality of Aβ-specific Th cells.

T cell reactivity to Aβ varies significantly among individuals bearing different MHC haplotypes. Differing levels of Aβ-specific T cell responses have been observed in PBMCs of human subjects, and were specific for various Aβ-derived epitopes restricted to distinct HLA-DR alleles (9, 10). Similarly, distinct T cell epitopes have been identified in SJL (H-2b haplotype) and C57BL/6 mice (H-2b), which differ significantly in their propensity to develop Aβ-specific CD4+ T cell responses (11). Thus, both mouse and human studies suggest an association between MHC class II (MHC-II) alleles and Aβ immunogenicity. However, it is not known whether additional genetic parameters critically influence the magnitude of Aβ-specific T cell responses.

In this study, we examined whether non-MHC genetic factors modulate the strength of CD4+ T cell responses to Aβ. The comparative study of these responses in mice with different H-2 haplotypes or congenic at the H-2 locus indicated that the magnitude of Aβ-specific T cell responses induced by vaccination is genetically determined by both MHC-dependent and -independent factors. Among the latter, the intrinsic ability to develop Aβ-specific regulatory T cell (Treg) responses varies between individuals and is a crucial parameter modulating CD4+ T cell responses to Aβ in both nonpathological conditions and in the course of AD.

Materials and Methods

Mice

C57BL/6 (H-2b haplotype), BALB/c (H-2d), SJL/J (H-2s), DBA/1 (H-2b), and CBA/J mice (H-2k) were purchased from Elevage Janvier (Le Genest-Saint-Iréné, France, Université Pierre et Marie Curie, and INSERM.

Received for publication December 3, 2010. Accepted for publication August 25, 2011.

This work was supported by grants from Association France Alzheimer, Fondation de France, Université Pierre et Marie Curie, and INSERM.

Address correspondence and reprint requests to Dr. Guillaume Dorothée and Prof. Pierre Aucouturier, INSERM, Unité Mixte de Recherche S 938, Laboratoire Système Immunitaire et Maladies Conformationnelles, Hôpital Saint-Antoine, F-75012 Paris, France; and Université Pierre et Marie Curie, Université Paris 6, Centre de Recherche Saint-Antoine, Hôpital Saint-Antoine, F-75012 Paris, France. E-mail addresses: guillaume.dorothée@inserm.fr and pierre.aucouturier@insERM.fr

Abbreviations used in this article: AD, Alzheimer’s disease; Aβ, amyloid-β peptide; APP, amyloid precursor protein; DLN, draining lymph node; MHC-II, MHC class II; PLP, proteolipid protein; RT, room temperature; Teff, effector T cell; Treg, regulatory T cell; wt, wild-type.

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INSMR, Unité Mixte de Recherche S 938, Laboratoire Système Immunitaire et Maladies Conformationnelles, Hôpital Saint-Antoine, F-75012 Paris, France; and Université Pierre et Marie Curie, Université Paris 6, Centre de Recherche Saint-Antoine, Hôpital Saint-Antoine, F-75012 Paris, France

Pierre Aucouturier, and Guillaume Dorotheé

The Journal of Immunology

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1003953
Saint Isle, France). C57BL/6 mice congeneric for the H-2b haplotype (strain B6.SIL-H2b C57/Cy) were obtained from the Jackson Laboratory (Bar Harbor, ME). APPPS1 transgenic mice (Thy1-APPApoE706/706/JNL... Thy1-PSJL166P) on the C57BL/6 background (12) were kindly provided by Prof. Mathias Jucker (Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany). Animals were bred and maintained under strictly monitored specific-pathogen-free conditions. Experiments were conducted in compliance with the French legislation and European Union recommendations.

**Ages**

All peptides were purchased from Genecust (Dudelange, Luxembourg). Human Aβ1-42 peptide was dissolved at 40 mg/ml in DMSO and stored at −20°C. Overlapping Aβ-derived 15-mer peptides were dissolved at 3 mM in DMSO, before final dilution at 5 or 15 μM in RPMI 1640 medium for in vitro T cell stimulation. OVA (Sigma-Aldrich, St. Louis, MO), OVA peptides (ovap15-24, PFASTGTMSTMVLIDE and ovap226-313, ISQA VHAHAHEINEAGR), and proteolipid protein (PLP) peptides (PLP90-105, HCLGKWLHPDKX and PLP178-191, NTWTTTCQSIFAPPSK) were dissolved at 25, 1 and 2.5 mg/ml, respectively, in distilled water, and aliquots were stored at −20°C.

**Immunization**

Before immunization, Aβ1-42 and PLP peptides were extemporaneously diluted to 2 mg/ml in 1.25 mM Tris-HCl (pH 7.4). Six- to eight-week-old mice were immunized by footpad injections with 100 μg Aβ1-42, 50 μg PLP peptides, or 1.25 mg OVA emulsified in an equal volume of CFA. In some experiments, mice were boosted 2 wk later with 60 μg Aβ1-42 emulsified in an equal volume of IFA.

**In vivo depletion/inactivation of CD25+ Tregs**

At day 4 before immunization, mice received an i.p. injection of ~150 μg anti-CD25-depleting mAb (clone PC61). To assess depletion efficiency, Tregs were quantified in blood samples the day before immunization by a combination of intracellular and surface staining using PE-conjugated anti-Foxp3 (FJK-16S; eBioscience, San Diego, CA) and biotinylated anti-CD25 mAb (clone PC61). To assess depletion efficiency, mice were boosted 2 wk later with 60 μg Aβ1-42 emulsified in an equal volume of IFA.

**T cell isolation**

Ten days after the last immunization, popliteal and inguinal draining lymph nodes (DLNs) were harvested and mashed into single-cell suspensions. When indicated, CD4+ T cells were purified using the negative CD4+ T cell depletion cocktail (BD Biosciences) to avoid nonspecific staining. PE-Cy5–conjugated anti-TCR (H57-597), FITC-conjugated anti-CD4 (L3T4), biotinylated anti-CD25 mAb (clone PC61) and allophycocyanin-conjugated streptavidin (BD Biosciences) were used for cell-surface staining. For intranuclear staining, cells were fixed, permeabilized, and incubated with PE-conjugated anti-Foxp3 (FJK-16S; eBioscience), according to the manufacturer’s protocol. Stained cells were analyzed using a FACSCalibur (Becton Dickinson) flow cytometer, and data were processed with FlowJo software (Tree Star, Ashland, OR).

**T cell proliferation**

For T cell-proliferation assays, splenocytes from nonimmunized syngeneic mice were used as APCs. RBCs were lysed in Tris-buffered ammonium chloride, and splenocytes were treated with mitomycin (50 μg/ml final) for 40 min at 37°C, followed by extensive washing. A total of 2 × 105 DLN-derived cells or 0.8–1 × 106 DLN-derived CD4+ T cells was stimulated with 1 × 104 syngeneic APCs/well, in the presence of 5 or 15 μg peptides. For measurement of Ag-induced T cell proliferation, cells were pulsed at day 3 with 1 μCi [3H]thymidine/well, and [3H]thymidine incorporation was measured 16 h later.

**ELISPOT**

Nitrocellulose-based 96-well plates (Millipore, Billerica, MA) were coated with 0.25 μg/50 μl/well of anti-mouse IFN-γ mAb (BD Biosciences) for 2 h at 37°C. Plates were washed and blocked with RPMI 1640 medium containing 10% FCS for ≥2 h at 37°C. DLN-derived cells or purified CD4+ T cells from individual mice were seeded at 2 × 104 or 0.8–1 × 105 cells/well, respectively, and stimulated with mitomycin-treated syngeneic spleenocytes (2 × 105 cells/well) in the presence of 5 or 15 μM antigenic peptides or with 0.4 μg/ml Con A as both a positive control and an internal reference of cell viability for normalization of data. Plates were incubated at 37°C (5% CO2) for 24 h, washed with PBS-0.05% Tween 20, and incubated for 2 h at 37°C with 0.05 μg/50 μl/well biotinylated anti-mouse IFN-γ mAb (BD Biosciences). After washing, alkaline phosphatase-conjugated streptavidin was added and incubated for 90 min (Roche, Basel, Switzerland) (75 μl/100 μl/well) and then washed, and IFN-γ–secreting cells were visualized using NBT/bromo-chloro-indolyl phosphate substrate (Promega). Spots were counted using an automated ELISPOT plate reader. Data were expressed as the number of IFN-γ+ CD4+ T cells per 106 Con A-reactive CD4+ T cells.

**ELISA**

To measure serum anti-Aβ Ab titers in immunized mice, flat-bottom Nunc MaxiSorp ELISA plates were coated overnight at 4°C with Aβ1-42 peptide (1 g/ml in 0.1 M NaHCO3, pH 8.5). Plates were then washed twice with PBS-0.05% Tween 20 and blocked with PBS-1% BSA for 2 h at room temperature (RT). After two washes, plates were incubated for 2 h at RT with serum samples diluted at 1:1,000, 1:3,000, 1:9,000, 1:27,000, 1:81,000, and 1:243,000 in blocking buffer. The Aβ1-17–specific mAb 6E10 (0.01 and 1 μg/ml) (Sigma-Aldrich) was used as a positive reference sample. Plates were washed and incubated for 90 min at RT with peroxidase-conjugated goat anti-mouse IgG Ab (Amersham, Little Chalfont, U.K.) and then revealed with O-phenylenediamine/H2O2 substrate (Sigma-Aldrich). Results were expressed as relative OD = experimental OD/ reference OD for 6E10 mAb in the same plate.

**FACS analysis**

Single-cell suspensions were prepared from inguinal and popliteal lymph nodes and incubated for 15 min with FcR-blocking Ab (2.4G2; BD Biosciences) to avoid nonspecific staining. PE-Cy5-conjugated anti-TCRβ (H57-597), FITC-conjugated anti-CD4 (L3T4), biotinylated anti-CD25 (7D4), and allophycocyanin-conjugated streptavidin (all from BD Biosciences) were used for cell-surface staining. For intranuclear staining, cells were fixed, permeabilized, and incubated with PE-conjugated anti-Foxp3 (FJK-16S; eBioscience), according to the manufacturer’s protocol. Stained cells were analyzed using a FACSCalibur (Becton Dickinson) flow cytometer, and data were processed with FlowJo software (Tree Star).

**Western blot analysis**

Murine thymus or brain samples were homogenized in 300 or 500 μl lysis buffer (250 mM NaCl, 5 mM EDTA acid, 0.1% Nonidet P-40, 50 mM HEPES supplemented with 10 mM DTT, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 50 μg/ml phenylmethyl sulfonyl fluoride, 2 mM sodium pyro phosphatase, 1 mM sodium orthovanadate), respectively, using Polytron 3100 homogenizer (Kinematica, Lucerne, Switzerland). After incubation for 30 min in lysis buffer, homogenates were centrifuged for 10 min at 4°C (14,000 rpm), supernatants containing the total protein extract were harvested, and protein concentrations were determined using the Bradford assay (BCA Protein Assay Kit; Thermo Scientific, Waltham, MA). One hundred microliters of total protein extracts was loaded on 10% SDS-PAGE gel, separated by electrophoresis, and transferred to nitrocellulose membrane (Amersham Hybond ECL, GE Healthcare, Little Chalfont, U.K). After blocking in PBS + 5% nonfat dry milk, membrane was incubated overnight with anti-amyloid precursor protein (APP) mouse Ab (22C11; Millipore). HRP-conjugated goat anti-mouse Ab (GE Healthcare) was used as a secondary Ab and incubated for 1 h at RT. Western blot was revealed by ECL using ultra-sensitive luminol substrate (SuperSignal West Femto Substrate; Thermo Scientific). Equal loadings were confirmed by stripping the membrane and reblotting with a specific Ab to β-actin (AC-15, Sigma-Aldrich). Densitometric quantification of bands was carried out using MultiGauge software (Fujifilm, Tokyo, Japan).

**Statistical analysis**

Statistical analyses were carried out with Graphpad Prism software (San Diego, CA), using the Mann–Whitney U test.

**Results**

Heterogeneity of Aβ-specific CD4+ T cell responses between mouse strains relates to the variety and strength of Aβ-derived epitopes restricted to MHC-II alleles

To assess CD4+ T cell reactivity to Aβ in different MHC contexts and genetic backgrounds, C57BL/6, BALB/c, DBA/1, CBA/J, and SJL/J mice, expressing H-2b, H-2d, H-2s, H-2h-2, and H-2h-2 haplotypes, respectively, were immunized with Aβ1-42 in CFA, and DLN-derived cells were analyzed for in vitro T cell proliferation induced by Aβ1-42. Responses were highly heterogeneous, with C57BL/6 mice (H-2b) displaying the lowest response and SJL/J mice (H-2h-2) exhibiting the strongest response (Fig. 1A). Of note,
all five mouse strains raised comparable ELISA titers of anti-Åβ Abs, indicating that differences in T cell responses did not have a significant impact on the levels of Åβ-specific B cell responses (Fig. 1B).

To identify the Åβ-derived CD4⁺ T cell epitopes in each of these H-2 haplotypes, we analyzed T cell proliferation induced by nine overlapping Åβ-derived 15-mer peptides. Although responses to 15-mer peptides were relatively low in most mouse strains, class II-restricted epitopes could be identified in four of the five tested strains. C57BL/6 mice (H-2b) displayed a weak, but reproducible, T cell proliferation to Åβ16-30 (Fig. 2), as previously described (11). In contrast, lymphocytes derived from SIL mice (H-2b) displayed a strong proliferative response to Åβ10-24 and the flanking peptides Åβ7-21 and Åβ13-27 (Fig. 2), in accordance with a previous report (11). Lymphocytes from immunized CBA/J (H-2k) and DBA/1 (H-2q) mice, expressing haplotypes H-2b, H-2d, H-2q, and H-2b, respectively, were immunized by footpad injection with CFA or CFA + Åβ1-42 and then boosted 2 wk later with IFA with or without Åβ1-42. DLN-derived cells were harvested on day 10 after boost and stimulated with mitomycin-treated syngeneic splenocytes in the presence of 15 μM Åβ1-42. T cell proliferation was measured 72 h later by [³H]thymidine incorporation. Results are from one representative experiment of two (mean ± SD; three mice/group). B, Åβ responses to Åβ1-42 were determined by ELISA at day 21 after boost, as described in Materials and Methods. Comparison of Åβ titers, corresponding to the dilution yielding a relative OD of 2, using the Kruskal–Wallis test, did not reveal significant differences (p > 0.05). Data are expressed as normalized OD (mean ± SD; three mice/group). Results are from one representative experiment of two.

MHC-independent genetic factors dominantly inhibit CD4⁺ T cell responses to Åβ in C57BL/6 mice

To determine whether the weak CD4⁺ T cell responsiveness to Åβ in C57BL/6 mice is exclusively due to poor immunogenicity of I-Åβ′-restricted epitope(s), we compared Åβ-specific CD4⁺ T cell responses in C57BL/6 (H-2b), SIL/J (H-2p), and congenic B6.H-2q mice. The B6.H-2p mice allow the presentation, on the C57BL/6 background, of the I-Åb′-restricted epitope Åβ10-24 of known high immunogenicity in SIL/J mice. Unexpectedly, CD4⁺ T cells from Åβ-immunized B6.H-2q mice proliferated only slightly in response to Åβ1-42, much more weakly than did SIL/J-derived CD4⁺ T cells, and with a similar magnitude as C57BL/6-derived lymphocytes. In addition, very little significant proliferation could be reproducibly measured in B6.H-2q-derived CD4⁺ T cells in response to the I-Åb′-restricted epitope Åβ10-24 (Fig. 3A). Thus, C57BL/6 mice expressing an H-2 haplotype permissive for the presentation of the potentially immunogenic epitope Åβ10-24 remain unable to develop a robust CD4⁺ T cell proliferative response to Åβ.

To determine whether different in vitro secondary proliferative responses correlate with actual differences in the in vivo expansion of Åβ-specific effector T cells (Teffs), the latter were directly quantified ex vivo by ELISPOT assay. In line with proliferation data, immunized SIL/J mice displayed twice as many DLN-derived Åβ-specific IFN-γ-producing CD4⁺ T cells than did C57BL/6 mice (Fig. 3B). All of these effectors were specific for Åβ10-24 in SIL/J mice, whereas Åβ16-30-specific effectors from immunized C57BL/6 mice were barely detectable by ELISPOT. Finally, Åβ-specific IFN-γ⁺ CD4⁺ T cells from immunized C57BL/6 × SIL/J F1 mice were mostly specific for Åβ10-24, but the overall number of effectors specific for Åβ was reduced to the same level as in parental C57BL/6 mice.

Altogether, these data suggested that MHC-independent genetic factors dominantly downmodulate Åβ-specific CD4⁺ T cell responses in C57BL/6 mice.

Preferential development of Åβ-specific Treg responses in C57BL/6 genetic background

Because Tregs play a key role in controlling immunological responsiveness to self-Ags, we analyzed their potential implication in the differential regulation of Åβ-specific CD4⁺ T cell responses in C57BL/6 and SIL/J genetic backgrounds. We compared the impact of Treg depletion on the magnitude of CD4⁺ T cell responses to Åβ. To achieve transient depletion/inactivation of CD4⁺ CD25⁺ Tregs, mice were injected i.p. with anti-CD25 mAb (clone PC61) and immunized 4 d later with Åβ1-42, as described above. Flow cytometry analysis of popliteal and inguinal lymph node cells indicated that PC61 treatment induced the depletion of CD4⁺ CD25⁺ Foxp3⁺ Tregs and CD4⁺ CD25⁺ Foxp3⁻ Teffs in both C57BL/6 and SIL/J genetic backgrounds in naive animals (i.e., before vaccination) (Fig. 4A, 4B). Without PC61 treatment, the frequency of both Tregs and Teffs significantly increased in both mouse strains upon vaccination with CFA + Åβ. PC61 treatment before immunization impaired vaccination-induced expansion of Tregs, but it did not alter the expansion of Teffs in both C57BL/6 and SIL/J genetic backgrounds (Fig. 4A, 4B). The extent of Åβ-specific CD4⁺ T cell responses in DLNs was quantified by
ELISPOT 10 d after immunization. Transient Treg depletion in C57BL/6 mice induced a 2.2-fold increase in the number of Aβ1-42–specific IFN-γ+ CD4+ Teffs induced by vaccination. Similarly, Aβ16-30–specific IFN-γ+ CD4+ T cells increased 2.8-fold following Treg depletion (Fig. 4C). Similarly to C57BL/6 mice, Treg depletion in B6.H-2b mice induced 3.4- and 2.6-fold increases in the numbers of vaccine-induced IFN-γ+ CD4+ effectors responding to Aβ1-42 and Aβ10-24, respectively. Interestingly, in contrast to C57BL/6 and B6.H-2b mice, SJL/J mice showed no increase in the numbers of Aβ1-42– or Aβ10-24–specific IFN-γ+ CD4+ effectors following Treg depletion (Fig. 4C). These data suggested that although Aβ–specific CD4+ T cell responses are naturally downmodulated by Tregs in the C57BL/6 background, SJL/J mice do not develop a measurable Treg-mediated spontaneous inhibition of CD4+ T cell responses to Aβ.

To determine whether the differential Treg-mediated inhibition in C57BL/6 and SJL/J backgrounds applies to any T cell response to self-Ags, we analyzed the impact of Treg depletion on PLP-specific CD4+ T cell responses in C57BL/6, SJL/J, and B6.H-2b mice. Transient Treg depletion before immunization with either H-2b–restricted (PLP178-191) or H-2s–restricted (PLP139-151) peptides significantly increased PLP-specific CD4+ T cell responses in C57BL/6 and SJL/J backgrounds, respectively (Fig. 5A). To further assess the pattern of Treg-mediated inhibition in C57BL/6 and SJL/J backgrounds, we also analyzed the impact of Treg depletion on CD4+ T cell responses to OVA, used as a model non-self Ag, in C57BL/6, SJL/J, and B6.H-2b mice. Similarly to PLP, transient Treg depletion before immunization with OVA increased the numbers of IFN-γ+ CD4+ Teffs detected by ELISPOT in response to full-length OVA in all three strains, the I-A^b–restricted epitope OVA232-239 in C57BL/6 mice, and to the I-A^s–restricted epitope OVA233-248 in SJL/J and B6.H-2b mice (Fig. 5B). Of note, although Treg depletion enhanced OVA-specific CD4+ T cell responses in all three strains, the increase was less in SJL/J background than C57BL/6 background. Thus, the differential Treg-mediated inhibition of Aβ–specific T cell responses in C57BL/6 and SJL/J genetic backgrounds is not general to any self or non-self Ag and is restricted, at least in part, to certain antigenic specificities. Because Aβ is generated by proteolytic cleavage of APP, APP protein expression in the thymus of C57BL/6 and SJL/J mice was analyzed by Western blot. No difference in APP expression was observed between C57BL/6 and SJL/J mice, although the expression was very faint in the thymus (Fig. 5C, SD). As expected, brain samples used as positive controls displayed a very strong expression of the lowest m.w. isoform of APP (Fig. 5C). Altogether, these data suggested that MHC-independent genetic factors modulating Aβ–specific CD4+ T cell responses include the propensity to develop Treg-mediated inhibitory responses to Aβ, which varies critically between individuals of different genetic backgrounds.

Treg responses downmodulate the magnitude of Aβ–specific CD4+ T cell responses in C57BL/6 APPPS1 mice

To assess the impact of Treg-mediated inhibition on CD4+ T cell responses to Aβ in the context of AD, we analyzed the effect of Treg depletion in 4-mo-old Aβ–depositing double-transgenic APPPS1 C57BL/6 mice. Similarly to results in C57BL/6 and SJL/J mice (Fig. 4A, 4B), flow cytometry analysis of DLN-derived cells indicated that PC61 induced the depletion of both CD4+ CD25+Foxp3+ Tregs and CD4+CD25+Foxp3+ Teffs in naive APPPS1 mice and wild-type (wt) littermates. PC61 treatment before immunization impaired vaccination-induced expansion of Tregs, but did not alter the expansion of Teffs, in both wt and APPPS1 mice (Fig. 6A). Control PBS-injected APPPS1 mice and
Materials and Methods
SJL/J, and (C57BL/6 by injection with CFA or CFA + A. Mice were immunized by footpad injection with CFA or CFA + A producing cells between Treg-depleted wt and APPPS1 mice did not reach statistical significance, a trend (p = 0.08) was observed toward enhanced Aβ-specific CD4+ T cell responses in APPPS1 mice compared with wt mice. In contrast, transient Treg depletion before immunization with either H-2b–restricted epitope PLP178-191 or full-length OVA increased PLP- or OVA-specific CD4+ T cell responses similarly in both wt and APPPS1 mice, respectively (Fig. 6C, 6D). Altogether, these data suggested that APPPS1 C57BL/6 mice spontaneously develop CD4+ T cell responses to Aβ, the expansion of which is prevented through Treg-mediated inhibition.

Discussion
The present study demonstrated that the magnitude of CD4+ T cell responses to Aβ vary according to genetic factors that include non-MHC elements. Our data indicated that the heterogeneity of Aβ-specific CD4+ T cell responses is related, at least in part, to qualitative and quantitative differences in Aβ-derived epitopes restricted to various MHC-II alleles. Importantly, MHC-independent genetic factors that determine the overall potency of individuals to develop Aβ-specific Treg responses play a critical role in the modulation of CD4+ T cell responses to Aβ. Such Treg-mediated inhibitory responses completely downmodulate Aβ-specific CD4+ T cell responses that spontaneously arise in the course of AD in a mouse model and prevent their expansion upon Aβ vaccination.

In line with previous reports identifying Aβ-derived T cell epitopes (11, 13–15), dominant CD4+ T cell epitopes in SJL/J, C57BL/6, and BALB/c mice were found in Aβ10-24, Aβ16-30, and Aβ1-30 peptides, respectively. Although Aβ10-24 elicited a strong I-Aq–restricted CD4+ T cell response in SJL/J mice, the same epitope induced a much weaker response when presented in the H-2b haplotype (DBA/1 mice), although the overall magnitude of CD4+ T cell responses to full-length OVA was similar in both strains. Thus, similar Aβ-derived epitopes display qualitative differences in various MHC contexts. Quantitative differences in the number of Aβ-derived epitopes restricted to various MHC-II alleles were also observed. Although Aβ-specific CD4+ T cell responses in SJL/J, C57BL/6, and DBA/1 mice were each targeted to a unique epitope, located between residues 10 and 24, 16 and 30, and 10 and 27, respectively, CD4+ T cells proliferated in response to both Aβ10-24 and Aβ19-33 in CBA/J mice. In humans, differing levels of CD4+ T cell responses to Aβ have been observed in PBMCs and were specific for various Aβ-derived epitopes restricted to different HLA-DR alleles, with the DRB1*1501 allele being highly immunogenic (9, 10). Altogether, these studies indicated that the MHC genotype is an important parameter modulating CD4+ T cell reactivity to Aβ. Importantly, the impact of MHC on T cell-related immunogenicity may be more stringent for small Ags compared with large-protein Ags. Indeed, the reduced spectrum of epitopes processed from short antigenic peptides, such as Aβ1-42, likely decreases the probability of gener-
ating diverse immunodominant epitopes restricted to various MHC alleles. Hence, MHC genotype has to be taken into particular consideration when evaluating the pathophysiological impact of T cell responses induced by Ab vaccination. In addition to the impact of MHC, our study demonstrated that MHC-independent genes control the magnitude of Ab-specific CD4+ T cell responses in mice through Treg-mediated inhibition. H-2s–restricted CD4+ T cell responses of similar Ab specificities were differentially regulated in C57BL/6 and SJL/J backgrounds. The very mild T cell response to Ab observed in B6.H-2s mice, which display the potentially highly Ab-immunogenic H-2s allele in the context of C57BL/6 genetic background, indicated that MHC-independent genetic features in C57BL/6 background prevent the expansion of Ab-specific T cell responses. Analysis of (C57BL/6 × SJL/J) F1 mice, which display the H-2s allele in the context of a 1/1 mix genetic background, further suggested that MHC-independent genes that downmodulate Ab-specific CD4+ T cell responses in C57BL/6 mice are dominant over SJL/J-derived genetic factors. Conversely, a report by Monsonego et al. (11) showed that although NOD mice display an Ab-immunogenic MHC context and genetic background, NOD.H-2b congenic mice are unable to mount T cell responses to Ab, highlighting the poor Ab immunogenicity of the H-2b haplotype. Hence, both MHC-independent genetic features and MHC haplotype critically control the magnitude of Ab-specific CD4+ T cell responses. The MHC-independent dominant inhibitory effect in C57BL/6 mice is related to the propensity for developing Treg-mediated inhibitory responses to Ab, which varies critically between individuals of different genetic backgrounds. Such Treg-mediated inhibitory responses may have an important implication in the context of AD, because they strongly downmodulate the expansion of Ab-specific CD4+ T cell responses that spontaneously arise in the course of AD in APPPS1 mice. Interestingly, increased suppressive activity of CD4+CD25+ Tregs has been described in AD patients, but its pathophysiological significance remains to be assessed (16). Genetic background- and sex-related differences in the numbers and functional properties of Tregs were previously reported in mice. Increased frequency of Tregs in male SJL mice compared with female SJL mice, as well as their preferential secretion of IL-10, may contribute to skewed development of Th2-type immune responses (17). Of note, C57BL/6 male and female mice did not show differences in Treg numbers (17). Other studies indicated that BALB/c mice displayed increased frequency of CD4+CD25+ Tregs compared with C57BL/6 mice, although Tregs...
specific or OVA-specific CD4+ T cells in both C57BL/6 and SJL/J mice. Treg depletion increases the number of vaccination-induced PLP-specific T cell responses. C57BL/6, SJL/J, and B6.H-2b mice were injected i.p. with anti-CD25–depleting mAb (PC61) or PBS 4 d before footpad immunization with CFA, CFA + PLP178–191 (C57BL/6 mice), or CFA + PLP136–151 (SJL/J and B6.H-2b mice). DLN-derived CD4+ T cells were isolated on day 10, and the numbers of IFN-γ–producing Teffs specific for either H-2b–restricted epitope PLP178–191 or H-2s–restricted epitope PLP136–151 were quantified ex vivo by ELISPOT assay. A, Impact of transient Treg depletion on the magnitude of OVA-specific CD4+ T cell responses. Mice were treated as described in A, before footpad immunization with CFA or CFA + OVA. Numbers of IFN-γ–producing Teffs specific for OVA, H-2b–restricted epitope Ova323–339, or H-2s–restricted epitope Ova233–248 were quantified in DLN-derived CD4+ T cells on day 10 by ELISPOT assay. Data in A and B are expressed as numbers of IFN-γ+ CD4+ T cells/10^6 Con A-reactive CD4+ T cells. Results are from one representative experiment of two (mean ± SD; three mice/group). *p < 0.05, **p < 0.01, Mann–Whitney U test.

C. Western blot analysis of APP protein expression in the thymus of C57BL/6 and SJL/J mice. Whole brain protein extracts were used as a positive control, and β-actin served as a loading control. D. Densitometric quantification, relative to β-actin, of gel shown in C. Values are mean ± SD.

from both strains are phenotypically and functionally similar (18). However, CD4+CD25+ T cells from BALB/c and C57BL/6 mice differ in their susceptibility to Treg-mediated inhibition (18). Interestingly, transient Treg depletion in BALB/c mice induced a notable increase in the numbers of vaccine-induced Aβ1-42– or Aβ7-21–specific IFN-γ+ CD4+ effectors, suggesting that Treg-mediated inhibition of CD4+ T cell responses to Aβ is not restricted to C57BL/6 mice (data not shown). Whether similar quantitative and qualitative differences in Treg responses are also genetically regulated in humans remains to be determined. Functional polymorphisms in the FOXP3/Scurfen gene have been associated with altered susceptibility to autoimmune diseases, including type 1 diabetes (19), systemic lupus erythematosus (20), psoriasis (21), autoimmune thyroid diseases (22), and primary biliary cirrhosis (23). Thus, genetic polymorphisms in genes involved in Treg development and function may translate into altered susceptibilities to autoimmune diseases.

Although severe complications observed in a limited proportion of AN1792-vaccinated patients were attributed to proinflammatory T cell responses, preclinical murine models did not show evidence of T cell-related side effects. The results of our study shed new light on the retrospective understanding of this discrepancy between preclinical and clinical settings. The critical impact of both the MHC and the dominant background-related Treg-mediated inhibition of CD4+ T cell responses to Aβ may explain, at least in part, the occurrence of meningoencephalitis in a fraction of AN1792 patients and underscores the need to develop appropriate preclinical murine models to accurately evaluate the potential impact of vaccine-induced Aβ-specific T cell responses. Transgenic AD mice on the SJL background, which would allow the presentation of the I-A2–restricted strong T cell epitope Aβ10-24 without significant Aβ-specific Treg-mediated inhibition, may constitute such a relevant preclinical model. The yet unexplained complications of AN1792 led pharmaceutical companies to totally dismiss T cell responses to Aβ and design strategies focused exclusively on Aβ-specific Abs. However, experimental results in mouse models, as well as recent clinical trial data, challenge the efficiency of such approaches and highlight possible associated...
side effects (24–26). A follow-up study on a group of AN1792 patients suggested that the level of vaccine-induced Abs correlated with the clearance of amyloid plaques but not with clinical re-

response (27). In our study, it is worth noting that Aβ-specific Ab responses were only mildly affected by genetic background, as opposed to T cell responses to Aβ. In parallel, several reports sugges-
ted that CD4^+ T cells may mediate neuroprotective re-
sponses in various neurodegenerative conditions, including AD (7, 8, 28–30). Interestingly, previous reports in an optic nerve-
injury model suggested that the ability to spontaneously mani-
ifest such T cell-dependent protective responses under neurode-
egenerative conditions may be restricted by naturally occurring CD4^+CD25^+ Tregs. Furthermore, this effect of Tregs was not uniform, and their expression in different individuals seemed to be genetically determined (31), reminiscent of our findings.

In conclusion, our study demonstrated that Aβ-specific CD4^+ T cell responses are critically modulated by MHC-independent genetic factors that determine the propensity to develop Treg-

mediated inhibitory responses to Aβ. These data underscore the need to reassess the roles, both pathogenic and/or beneficial, of different T cell subsets in both the pathophysiology and immu-
notherapy of AD. In this context, extensive analyses aimed at

FIGURE 6. Treg responses strongly downmodulate Aβ-specific CD4^+ T cell responses in C57BL/6 APPPS1 mice. A, PC61 treatment selectively inhibits vaccination-induced expansion of CD4^+CD25^+Foxp3^+ Tregs but not CD4^+CD25^-Foxp3^- Teffs in both WT and APPPS1 mice. Four-month-old animals were injected i.p. with anti-CD25-depleting mAb (PC61) or PBS and then left untreated or immunized on day 4 with CFA + Aβ1-42. DLN-derived CD4^+ T cells were isolated 10 d later, and the frequency of CD4^+CD25^+Foxp3^+ and CD4^+CD25^-Foxp3^- cells was determined by flow cytometry. Mean ± SD values for three to five animals/group. Gated on TCR^β^CD4^+ cells. *p < 0.05, Mann–Whitney U test. The p values are for differences between PBS- and PC61-treated animals. B, Four-month-old C57BL/6 APPPS1 mice or littermate controls were injected i.p. with anti-CD25-depleting mAb (PC61) or PBS 4 d before footpad immunization with CFA or CFA + Aβ1-42. DLN-derived CD4^+ T cells were isolated on day 10, and the numbers of IFN-γ-producing Teffs specific for Aβ1-42, I-A^b^-restricted epitope Aβ16-30, or Aβ1-15 peptide used as a negative control were quantified ex vivo by ELISPOT assay. C, Impact of transient Treg depletion on the magnitude of PLP-specific CD4^+ T cell responses. Four-month-old APPPS1 mice or littermate controls were injected i.p. with PC61 or PBS and then immunized 4 d later with CFA or CFA + PLP^178–191_. DLN-derived CD4^+ T cells were isolated on day 10, and the numbers of IFN-γ-producing Teffs specific for H-2^d^-restricted epitope PLP^178–191_ were quantified ex vivo by ELISPOT assay. H-2^d^-restricted epitope PLP^139–151_ was used as a negative control. D, Impact of transient Treg depletion on the magnitude of OVA-specific CD4^+ T cell responses. Mice were treated as described in C, before footpad immunization with CFA or CFA + OVA. Numbers of IFN-γ-producing Teffs specific for OVA, H-2^d^-restricted epitope Ova^323–339_, or H-2^d^-restricted epitope Ova^233–248_ were quantified in DLN-derived CD4^+ T cells on day 10 by ELISPOT assay. B–D, Data are expressed as numbers of IFN-γ^+ CD4^+ T cells/2 × 10^5 CD4^+ T cells. Results are from one representative experiment of two (mean ± SD; three mice/group). *p < 0.05, **p < 0.01, Mann–Whitney U test.
deciphering the impact of Tregs on the neuropathological and clinical parameters of AD in APPPS1 mice are underway.

Acknowledgments
We thank Mathias Jucker for generously providing the APPPS1 mice and for helpful discussions, Claude Carnaud for helpful comments and discussion and for critical reading of the manuscript, Thomas Chaigneau for technical support, and Delphine Muller and Tatiana Ledent for assistance with mouse husbandry.

Disclosures
The authors have no financial conflicts of interest.

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