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

Cécile Toly-Ndour, Gabrielle Lui, Maria Manuel Nunes, Martine Bruley-Rosset, Pierre Aucouturier and Guillaume Dorothée

*J Immunol* 2011; 187:4492-4500; Prepublished online 26 September 2011; doi: 10.4049/jimmunol.1003953

http://www.jimmunol.org/content/187/9/4492

**References**

This article cites 30 articles, 8 of which you can access for free at:

http://www.jimmunol.org/content/187/9/4492.full#ref-list-1

**Why *The JI*?** Submit online.

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*average

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
MHC-Independent Genetic Factors Control the Magnitude of CD4\(^+\) T Cell Responses to Amyloid-\(\beta\) Peptide in Mice through Regulatory T Cell-Mediated Inhibition

Cécile Toly-Ndour, Gabrielle Lui, Maria Manuel Nunes, Martine Bruley-Rosset, Pierre Aucouturier, and Guillaume Dorothée

Accumulation of amyloid-\(\beta\) peptide (A\(\beta\)) is considered the triggering factor of pathogenic lesions in Alzheimer’s disease (AD), and vaccines targeting A\(\beta\) are promising therapeutic options. However, the occurrence of meningoencephalitides attributed to T cell responses in 6% of A\(\beta\)-immunized patients underscores the need for a better understanding of T cell responses to A\(\beta\). We characterized the parameters controlling the magnitude of A\(\beta\)-specific CD4\(^+\) T cell responses in mice. T cell responsiveness to A\(\beta\)1-42 was highly heterogeneous between mouse strains of different H-2 haplotypes, with SJL/J (H-2\(^s\)) mice displaying a strong response, mainly specific for A\(\beta\)10-24, and C57BL/6 (H-2\(^b\)) mice displaying a weak response to A\(\beta\)16-30. Surprisingly, C57BL/6 mice congenic for the H-2\(^b\) haplotype (B6.H-2\(^b\)), which display a “permissive” MHC class II allele for presentation of the immunodominant A\(\beta\)10-24 epitope, showed a very weak CD4\(^+\) T cell response to A\(\beta\), suggesting that MHC-independent genes downmodulate A\(\beta\)-specific CD4\(^+\) T cell responses in C57BL/6 background. Vaccine-induced CD4\(^+\) T cell responses to A\(\beta\) were significantly enhanced in both C57BL/6 and B6.H-2\(^b\) mice upon depletion of regulatory T cells (Tregs), whereas Treg-depleted SJL/J mice displayed unaltered A\(\beta\)-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 A\(\beta\)-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 A\(\beta\)-specific Treg responses. The Journal of Immunology, 2011, 187: 4492–4500.

INSM, 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

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 reprints to Dr. Guillaume Dorotheé and Prof. Pierre Aucouturier, INSERM, Unité Mixte de Recherche S 938, Laboratoire Système Immunitaire et Maladies Conformationnelles, Hôpital Saint-Antoine, 184 Rue du Faubourg Saint-Antoine, F-75012 Paris, France. E-mail addresses: guillaume.dorothee@insERM.fr and pierre.aucouturier@insERM.fr

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

Copyright © 2011 by The American Association of Immunologists, Inc. 0022-1767/10/$16.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1003953
Saint Isle, France). C57BL/6 mice congenic for the H-2b haplotype (strain B6.SJL-H2b.C57BL/129ScSnJ) were obtained from the Jackson Laboratory (Bar Harbor, ME). APPPS1 transgenic mice (Thy1-APPM670/671NL, Thy1-PSL1668) 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.

Ag

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 ml 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 (OVA 233–248, OVA 174–181, ISQAVHAAAEINEAGR), and proteolipid protein (PLP) peptides (PLP130–145, HCLGKWLPDKKFKF and PLP 176–191, NTWTQQSIALFPKSK) 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 12.5 mM Tris-HCl (pH 7.4). Six- to eight-week-old mice were immunized by footpad injections with 100 μg Aβ1-42 peptide (1 μg/ml in 0.1 M NaHCO3, pH 8.3). 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 1:1000, 1:3000, 1:9000, 1:27000, 1:81000, and 1:243000 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 Ab 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 pyrophosphate, 1 mM sodium orthovanadate), respectively, using Polytron 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 micrograms 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 incu- bated 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 α-tubulin (1:50,000; 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-2k, and H-2s 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-2d) exhibiting the strongest response (Fig. 1A). Of note,
all five mouse strains raised comparable ELISA titers of anti-\( \alpha \)-\( \beta \) Abs, indicating that differences in T cell responses did not have a significant impact on the levels of \( \alpha \)-\( \beta \)-specific B cell responses (Fig. 1B).

To identify the \( \alpha \)-\( \beta \)-derived CD4\(^+\) T cell epitopes in each of these H-2 haplotypes, we analyzed T cell proliferation induced by nine overlapping \( \alpha \)-\( \beta \)-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-2\( ^b \)) displayed a weak, but reproducible, T cell proliferation to \( \alpha \)-\( \beta \)1-42 in SJL/J mice, whereas A\( \beta \)16-30–specific effectors from immunized SJL/J mice were mostly specific for A\( \beta \)10-24, but the overall number of effectors specific for A\( \beta \) was reduced to the same level as in parental C57BL/6 mice. Altogether, these data suggested that MHC-independent genetic factors dominantly downmodulate \( \alpha \)-\( \beta \)-specific CD4\(^+\) T cell responses in C57BL/6 mice.

Preferential development of \( \alpha \)-\( \beta \)-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 \( \alpha \)-\( \beta \)-specific CD4\(^+\) T cell responses in C57BL/6 and SJL/J genetic backgrounds. We compared the impact of Treg depletion on the magnitude of CD4\(^+\) T cell responses to A\( \beta \). 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 A\( \beta \)1-42, 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 SJL/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 + A\( \beta \). PC61 treatment before immunization impaired vaccination-induced expansion of Tregs, but it did not alter the expansion of Teffs in both C57BL/6 and SJL/J genetic backgrounds (Fig. 4A, 4B). The extent of \( \alpha \)-\( \beta \)-specific CD4\(^+\) T cell responses in DLNs was quantified by

MHC-independent genetic factors dominantly inhibit CD4\(^+\) T cell responses to A\( \beta \) in C57BL/6 mice

To determine whether the weak CD4\(^+\) T cell responsiveness to A\( \beta \) in C57BL/6 mice is exclusively due to poor immunogenicity of I-A\( \alpha \)-restricted epitope(s), we compared \( \alpha \)-\( \beta \)-specific CD4\(^+\) T cell responses in C57BL/6 (H-2\( ^b \)), SJL/J (H-2\( ^s \)), and congenic B6.H-2\( ^k \) mice. The B6.H-2\( ^b \) mice allowed the presentation, on the C57BL/6 background, of the I-A\( \alpha \)-restricted epitope A\( \beta \)10-24 of known high immunogenicity in SJL/J mice. Unexpectedly, CD4\(^+\) T cells from \( \alpha \)-\( \beta \)-immunized B6.H-2\( ^k \) mice proliferated only slightly in response to A\( \beta \)1-42, much more weakly than did SJL/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-2\( ^k \)-derived CD4\(^+\) T cells in response to the I-A\( \alpha \)-restricted epitope A\( \beta \)10-24 (Fig. 3A). Thus, C57BL/6 mice expressing an H-2 haplotype permissive for the presentation of the potentially immunogenic epitope A\( \beta \)10-24 remain unable to develop a robust CD4\(^+\) T cell proliferative response to A\( \beta \).

To determine whether different in vitro secondary proliferative responses correlate with actual differences in the in vivo expansion of \( \alpha \)-\( \beta \)-specific effector T cell populations (Teffs), the latter were directly quantified ex vivo by ELISPOT assay. In line with proliferation data, immunized SJL/J mice displayed twice as many DLN-derived \( \alpha \)-\( \beta \)-specific IFN-\( \gamma \)-producing CD4\(^+\) T cells than did C57BL/6 mice (Fig. 3B). All of these effectors were specific for A\( \beta \)10-24 in SJL/J mice, whereas A\( \beta \)16-30–specific effectors from immunized C57BL/6 mice were barely detectable by ELISPOT. Finally, \( \alpha \)-\( \beta \)-specific IFN-\( \gamma \)-producing CD4\(^+\) T cells from immunized C57BL/6 \( \times \) SJL/J F1 mice were mostly specific for A\( \beta \)10-24, but the overall number of effectors specific for A\( \beta \) was reduced to the same level as in parental C57BL/6 mice.

Preferential development of \( \alpha \)-\( \beta \)-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 \( \alpha \)-\( \beta \)-specific CD4\(^+\) T cell responses in C57BL/6 and SJL/J genetic backgrounds. We compared the impact of Treg depletion on the magnitude of CD4\(^+\) T cell responses to A\( \beta \). 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 A\( \beta \)1-42, 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 SJL/J genetic backgrounds in naive animals (Fig. 4A, 4B). Without PC61 treatment, the frequency of both Tregs and Teffs significantly increased in both mouse strains upon vaccination with CFA + A\( \beta \). PC61 treatment before immunization impaired vaccination-induced expansion of Tregs, but it did not alter the expansion of Teffs in both C57BL/6 and SJL/J genetic backgrounds (Fig. 4A, 4B). The extent of \( \alpha \)-\( \beta \)-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 background, 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-Aβ–restricted epitope OVA323–339 in C57BL/6 mice, and to the I-Aβ–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 to 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 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

FIGURE 2. CD4+ T cell epitope mapping of Aβ1–42 in different H-2 haplotypes. C57BL/6 (H-2b), BALB/c (H-2d), DBA/1 (H-2q), CBA/J (H-2k), and SJL/J (H-2s) mice were immunized by footpad injection with CFA or CFA + Aβ1–42 and then boosted 2 wk later with IFA with or without Aβ1–42. DLN-derived cells were harvested on day 10 after boost and stimulated with mitomycin-treated syngeneic splenocytes in the presence of 15 μM of Aβ1–42 or a library of nine overlapping Aβ-derived 15-mer peptides. 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). The y-axis scale for each mouse strain was defined considering Aβ1–42–induced proliferation as a maximum.

The Journal of Immunology 4495

Downloaded from http://www.jimmunol.org/ by guest on April 22, 2022
wt littermates displayed similar numbers of Aβ1-42– or Aβ16-30–specific IFN-γ+ CD4+ Teffs in response to Aβ vaccination (Fig. 6B). As expected, transient Treg depletion before immunization in wt littermates induced a 2.9-fold increase in the number of Aβ1-42–specific IFN-γ+ CD4+ T cells, but no significant difference in the number of Aβ16-30–specific IFN-γ+ CD4+ T cells was observed. Interestingly, Treg depletion in APPPS1 mice induced a stronger enhancement of Aβ-specific CD4+ T cell responses, with 5.9- and 3.1-fold increases in the numbers of Aβ1-42– and Aβ16-30–specific IFN-γ+ CD4+ effectors, respectively (Fig. 6B). Although the difference in Aβ1-42–specific IFN-γ–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-Aβ–restricted CD4+ T cell response in SJL/J mice, the same epitope induced a much weaker response when presented in the H-2q haplotype (DBA/1 mice), although the overall magnitude of CD4+ T cell responses to full-length Aβ1-42 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 Aβ vaccination.

In addition to the impact of MHC, our study demonstrated that MHC-independent genes control the magnitude of Aβ-specific CD4+ T cell responses. C57BL/6 (H-2b), SJL/J (H-2s), and B6.H-2s mice 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-Aδ-restricted epitope Aβ10-24, I-Ab-restricted epitope Aβ16-30, or Aβ1-15 peptide used as a negative control were quantified ex vivo by ELISPOT assay. Data are expressed as numbers of IFN-γ+ CD4+ T cells/10^3 Con A-reactive CD4+ T cells. Results are from one representative experiment of three (mean ± SD; three to five mice/group). *p < 0.05, Mann–Whitney U test.

FIGURE 4. Preferential development of Aβ-specific Treg responses in C57BL/6 genetic background. A and B, PC61 treatment selectively inhibits vaccination-induced expansion of CD4+CD25+Foxp3+ Tregs but not CD4+CD25−Foxp3− Teffs in both C57BL/6 and SJL/J genetic backgrounds. 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. A, Dot plots for one representative animal in each experimental group. B, Mean ± SD for three to five animals/group. Gated on TCRβ+CD4+ cells. *p < 0.05, **p < 0.01, Mann–Whitney U test. The p values are for differences between PBS- and PC61-treated animals. C, Impact of transient Treg depletion on the magnitude of Aβ-specific CD4+ T cell responses. C57BL/6 (H-2b), SJL/J (H-2s), and B6.H-2s mice 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-Aδ-restricted epitope Aβ10-24, I-Ab-restricted epitope Aβ16-30, or Aβ1-15 peptide used as a negative control were quantified ex vivo by ELISPOT assay. Data are expressed as numbers of IFN-γ+ CD4+ T cells/10^3 Con A-reactive CD4+ T cells. Results are from one representative experiment of three (mean ± SD; three to five mice/group). *p < 0.05, Mann–Whitney U test.
**FIGURE 5.** Preferential Treg-mediated inhibition of Aβ-specific T cell responses in C57BL/6 genetic background is not general to any Ag but does not rely on altered APP expression in the thymus. A, Impact of transient Treg depletion on the magnitude of PLP-specific CD4+ T cell responses. C57BL/6, SJL/J, and B6.H-2 mice were injected ip. with anti-CD25-depleting mAb (PC61) or PBS 4 d before footpad immunization with CFA, CFA + PLP178-191 (C57BL/6 mice), or CFA + PLP139-151 (SJL/J and B6.H-2 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 PLP139-151 or H-2s-restricted epitope Ova139-151 were quantified ex vivo by ELISPOT assay. B, 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 Ova123-139, or H-2s-restricted epitope Ova243-258 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/10^3 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/Scurfin 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 potencies of developing Treg responses in humans. Finally, in contrast to its effect on Aβ-specific T cell responses, transient Treg depletion increases the number of vaccination-induced PLP-specific or OVA-specific CD4+ Teffs in both C57BL/6 and SJL/J genetic backgrounds. Hence, background-related differential Treg-mediated inhibition is not general to any self or non-self Ag, but instead is restricted, at least in part, to certain antigenic specificities, including Aβ. The differential effect of Tregs on Aβ-reactive T cells was not related to differences in thymic expression of APP in C57BL/6 and SJL/J mice. Further experiments will be needed to decipher the mechanistic explanation of this phenomenon. 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-A^d-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 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 suggested that CD4+ T cells may mediate neuroprotective responses 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 manifest such T cell-dependent protective responses under neurodegenerative 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 immunotherapy 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− cells was determined by flow cytometry. Mean ± SD values for three to five animals/group. Gated on TCRβ+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, 1-Aβ2-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 + PLP178–191. DLN-derived CD4+ T cells were isolated on day 10, and the numbers of IFN-γ-producing Teffs specific for H-2b-restricted epitope PLP178–191 were quantified ex vivo by ELISPOT assay. H-2b-restricted epitope PLP139–151 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-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. B–D, Data are expressed as numbers of IFN-γ+ CD4+ T cells × 105 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 helpful discussions, Claude Carnaud for helpful comments and deciphering the impact of Tregs on the neuropathological and clinical parameters of AD in mice with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 400: 173–177.


