H2A- and H2E-Derived CD4⁺CD25⁺ Regulatory T Cells: A Potential Role in Reciprocal Inhibition by Class II Genes in Autoimmune Thyroiditis

Gerald P. Morris, Yan Yan, Chella S. David and Yi-chi M. Kong

*J Immunol* 2005; 174:3111-3116; doi: 10.4049/jimmunol.174.5.3111

http://www.jimmunol.org/content/174/5/3111

---

**References**
This article cites 49 articles, 19 of which you can access for free at: http://www.jimmunol.org/content/174/5/3111.full#ref-list-1

**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
H2A- and H2E-Derived CD4\(^+\)CD25\(^+\) Regulatory T Cells: A Potential Role in Reciprocal Inhibition by Class II Genes in Autoimmune Thyroiditis\(^1\)

Gerald P. Morris,* Yan Yan,* Chella S. David,† and Yi-chi M. Kong\(^2\)\(^*\)

We recently described a novel H2E class II-transgenic model (A\(^-\)E\(^+\)) of experimental autoimmune thyroiditis (EAT) that permits disease induction with heterologous thyroglobulin (Tg), but unlike conventional susceptible strains, precludes self-reactivity to autologous mouse Tg. In transgenic E\(^+\)B10 (A\(^+\)E\(^+\)) mice, the presence of endogenous H2A genes is protective against H2E-mediated thyroiditis, inhibiting EAT development. The suppressive effect of H2A genes on H2E-mediated thyroiditis mirrors previous reports of H2E suppression on H2A-mediated autoimmune diseases, including EAT. The mechanism of the reciprocal-suppressive effect between class II genes is unclear, although the involvement of regulatory T cells has been proposed. We have recently reported that CD4\(^+\)CD25\(^+\) regulatory T cells mediate peripheral tolerance induced with mouse Tg in CBA mice. To determine whether these cells play a role in our E\(^+\)-transgenic model, we first confirmed the existence of CD4\(^+\)CD25\(^+\) T cells regulating thyroiditis in E\(^+\)B10.Ab\(^6\) (A\(^+\)E\(^+\)) and B10 (A\(^+\)E\(^+\)) mice by i.v. administration of CD25 mAb before EAT induction. The depletion of CD4\(^+\)CD25\(^+\) T cells enhanced thyroiditis induction in the context of either H2E or H2A. Moreover, reconstitution of CD4\(^+\)CD25\(^+\) T cells from naive B10 mice restored resistance to EAT. E\(^+\)B10 (A\(^+\)E\(^+\)) mice were also depleted of CD4\(^+\)CD25\(^+\) T cells before the challenge to determine their role in thyroiditis in the presence of both H2A and H2E genes. Depletion of CD4\(^+\)CD25\(^+\) regulatory T cells offset the suppression of H2E-mediated thyroiditis by H2A. Thus, these regulatory T cells may be involved in the reciprocal-suppressive effect between class II genes. The Journal of Immunology, 2005, 174: 3111–3116.

E xperimental autoimmune thyroiditis (EAT)\(^3\), the murine model for Hashimoto’s thyroiditis, is an organ-specific autoimmune disease characterized by mononuclear infiltrate in the thyroid, T cell-proliferative response, autoantibody production, and subsequent destruction of the thyroid follicles. EAT is inducible in susceptible mice by administration of mouse thyroglobulin (mTg), either in repeated doses (1) or in conjunction with an adjuvant such as CFA or LPS (2, 3). Susceptibility to EAT has long been linked to the H2 haplotype (4), paralleled by subsequent HLA associations with autoimmune thyroid disease (5). In particular, susceptibility to EAT has been mapped to the H2A locus (6). For example, mice bearing the A\(^b\) haplotype are susceptible to EAT induction, whereas mice bearing the A\(^b\) haplotype are resistant (6). The critical role of H2A genes in encoding susceptibility has been verified by the introduction of the Ab\(^b\) transgene into a resistant strain; the resultant expression of A\(^b\) molecules conferred EAT susceptibility (7). In contrast, involvement of the other murine MHC class II locus, H2E, in susceptibility to EAT is less clear; it is not genomic to every strain, and its presence is not required for susceptibility. However, functional H2E molecules arising from the presence of a H2Ea transgene have been shown to play a suppressive role in autoimmunity because induction of the E\(^a\) molecule suppressed the severity of myasthenia gravis in otherwise susceptible B10 mice (8) and inhibited diabetes development in NOD mice (9). Similarly, in EAT, H2E expression decreased thyroiditis severity after mTg immunization in otherwise susceptible B10.S (A\(^-\)E\(^+\)) mice (7).

Interestingly, we have recently discovered a novel H2E-transgenic model that differs in EAT susceptibility from conventional strains. In this model, the conserved Ed\(^a\) transgene was introduced into traditionally resistant B10 (H2A\(^a\)) mice lacking expression of endogenous A\(^b\) molecules due to targeted deletion of the Ab gene (10). Unlike conventional strains, EAT induction in E\(^+\)B10.Ab\(^b\) (A\(^+\)) mice is limited to heterologous thyroglobulin (Tg), such as human or porcine, and restricted against induction with self-mTg (11). Moreover, when the endogenous A\(^b\) molecules were retained, a protective effect was observed. E\(^+\)B10 (A\(^+\)) mice demonstrated significantly decreased thyroiditis severity following EAT induction with human Tg (hTg) as compared with E\(^+\)B10.Ab\(^b\) mice (11), mirroring the suppressive effect of E\(^a\) molecules in the susceptible B10.S strain (7). The mechanism of the reciprocal-suppressive effect of H2E and H2A molecules remains to be characterized. Other investigations into the suppressive effect of H2E molecules on H2A-mediated responses indicate that it is an active mechanism of suppression that can be abrogated in vitro by the presence of blocking H2E mAbs, suggesting the possible involvement of suppressor T cells (12–14).

Investigations of suppressor T cells in other models of autoimmunity have characterized a small group of CD4\(^+\) T cells expressing CD25 (IL-2R\(\alpha\)) (\(~10\%) that exhibit regulatory activity. These CD4\(^+\)CD25\(^+\) T cells inhibited the development of autoimmunity, including thyroiditis, following reconstitution of nude mice with CD4\(^+\)CD25\(^+\) T cells (15), and in vivo depletion of CD4\(^+\)CD25\(^+\) T cells rendered autoimmune-resistant mouse strains susceptible to
induction of autoimmunity (16). We have also reported the influence of CD4+ CD25+ regulatory T cells in susceptibility to EAT in their role as mediators of induced peripheral tolerance in CBA mice bearing the susceptible A haplotype (17). Here, we examined the possibility that the suppressive effect of H2Aβ or H2Eβ molecules on the induction of EAT was mediated through CD4+ CD25+ regulatory T cells by using mouse strains expressing these molecules, either singly or together. Here, we report that CD4+ CD25+ regulatory T cells are generated in the context of either H2Eβ or H2Aβ, and these regulatory T cells inhibit the development of autoimmunity in the same context. We also demonstrate that reciprocal suppression of H2Eβ- or H2Aβ-mediated autoimmunity is reduced by depletion of CD4+ CD25+ T cells, suggesting that regulatory T cells may explain the suppressive effect observed by the addition of class II genes.

Materials and Methods

Mice

All mice were bred at the Immunogenetics Mouse Core Facility at the Mayo Clinic. The generation of congenic H2Eβ-transgenic mice in B10 and B10.Aβ mice was detailed previously (11). Briefly, MHC class II-deficient mice (Aββ) were derived by targeted deletion of the H2αβ gene (10) in B10 mice (lacking an endogenous E molecule) and screened by flow cytometry. Expression of the E molecule was introduced by intercrosses of B10 or B10.Aβ mice with Edα-transgenic mice and screened by flow cytometry (18). Progenies were backcrossed to obtain congenic E mice on the B10 or B10.Aβ background (11). Mice were kept on acidified, chlorinated water and used at 10–16 wk of age.

Thyroglobulin

mTg and hTg were obtained from frozen thyroid fractions on a Sephadox G-200 column as described previously (19). Tg was checked for the presence of LPS by Limulus amebocyte assay (a 100-μg dose contained <1 ng of LPS) and diluted in nonpyrogenic saline.

mAbs and in vivo depletion of CD25+ T cells

CD25 mAb was produced by culture of 1 × 106 PC61 (rat IgG1) hybridoma cells (American Type Culture Collection; Ref. 20) in a Cell Max module (Cellico) and harvested according to manufacturer’s instructions. Harvested mAb was twice purified by ammonium sulfate precipitation, and specific Ab concentration was determined as described previously (17). For in vivo depletion, two 1-mg doses of CD25 mAb (containing 10–20% active Ab) or two 1-mg doses of polyclonal rat IgG control (Sigma-Aldrich) were given i.v. 4 days apart. Depletion was monitored in PBL by flow cytometry with PE-7D4 (rat IgM) (Southern Biotechnology Associates) 6 days later, in conjunction with anti-CD4 (YTA 3.1 and YTS 191.1, rat IgG2b) ascites fluid twice purified by ammonium sulfate precipitation and labeled with FITC-NORIG 7.16 (mouse anti-rat IgG2b; Refs. 21 and 22). Anti-CD25 mAb treatment resulted in a 70–90% reduction of CD4+ CD25+ T cells as indicated in the figure legends.

Adoptive transfer of CD4+ CD25+ T cells

As previously described (17), CD4+ CD25+ and CD4+ CD25+ T cell populations were enriched for adoptive transfer studies by magnetic separation of splenocytes from B10 mice first depleted in vivo of CD8+ T cells by administration of two 320-μg CD8 mAb (YTS 156.7 and YTS 169.4, rat IgG2b) doses (23, 24). CD4+ T cell populations were obtained by panning on anti-B cell mAb (187.1, rat IgG1; American Type Culture Collection) and anti-CD11b (18B.11, rat IgM)-coated petri dishes to remove B cells and macrophages. CD4+ CD25+ T cells were collected positively from the remaining cells by labeling with PE-7D4 and anti-PE magnetic microbeads (StemCell Technologies). Viability and purity of CD4+ CD25+ and CD4+ CD25+ fractions were assessed by trypan blue staining and flow cytometric analysis (average viability >95% at 75–90% purity). CD25 mAb-treated B10 mice were reconstituted i.v. with 1.5–3.0 × 107 enriched cells/mouse 12 days before challenge with 40 μg of mAb and 20 μg of LPS.

Induction of EAT

EAT was induced by injecting i.v. 40 μg of Tg, followed 3 h later with 20 μg of Salmonella enteritidis LPS on days 0 and 7. Animals were sacrificed on day 28, and EAT was assessed by histologic examination of thyroids, in vitro lymphocyte proliferation to Ag, and anti-Tg production.

Histologic evaluation of EAT

Thyroid specimens were sectioned vertically through both thyroid lobes (50–60 sections from 10–15 step levels). Mononuclear cell infiltration was scored on an index of 0–4.0: 0, no infiltration; 0.5, ≤0–10% thyroid involvement; 1.0, >10–20% involvement with follicular destruction; 2.0, >20–40% involvement; 3.0, >40–80% involvement; and 4.0, >80% involvement (25).

Measurement of autoantibody production

Mice were bled from the tail artery on day 28, and the sera was stored at −20°C. Anti-Tg titers were determined by ELISA using Tg-coated (1 μg/ml) Immulon I microtiter plates and alkaline phosphatase-labeled goat anti-mouse IgG (Sigma-Aldrich) as described previously (26).

In vitro proliferation

Splenocytes were cultured in triplicate in RPMI 1640 + 1% normal mouse serum in flat-bottom 96-well plates at 6 × 105 cells/well, either with or without 40 ng of mTg, followed 3 h later with 20 ng of LPS. Splenocytes from CD25-depleted mice did not proliferate in response to mTg. A stimulation index was calculated as the mean cpm ± SE with Ags divided by mean cpm without Ags.

Statistical analysis

Individual pathology indices between groups were analyzed nonparametrically using the Mann-Whitney U test. A value of p < 0.05 was considered significant statistically.

Results

Depletion of CD4+ CD25+ T cells in E B10.Aβ (A′ E′) mice enhances EAT induced with hTg without altering the restriction against mTg.

We have recently reported that CD4+ CD25+ regulatory T cells are responsible for mediating induced tolerance to Tg. After depletion of CD4+ CD25+ T cells, tolerance induced by mTg pretreatment in CBA mice was abrogated, and their susceptibility to EAT induction was restored (17). To determine whether CD4+ CD25+ T cells also play a role in influencing thyroiditis development in the unique A′ E′-transgenic model that is permissive for thyroiditis induction only with heterologous Tg, but not self-mTg (11), CD4+ CD25+ T cells in E′ B10.Aβ mice were depleted in vivo. Two 1-mg doses of a depleting CD25 mAb or a polyclonal rat IgG control Ab were administered i.v. 4 days apart (days −14 and −10) as described previously (17). A reduction of 70–90% of CD4+ CD25+ T cells was observed by analysis of PBL on day −4, 6 days after the second dose of anti-CD25 (data not shown). Mice were then challenged on days 0 and 7 with the EAT induction protocol of 40 μg of either hTg or mTg, followed 3 h later by 20 μg of LPS. As illustrated in Fig. 1A, depletion of CD4+ CD25+ T cells in E′ B10.Aβ (A′ E′) mice resulted in greatly increased mononuclear infiltration of the thyroid following immunization with hTg; 69% (9 of 13) of the CD25-depleted mice exhibited 40–80% thyroid involvement, compared with 23% (3 of 13) in control rat IgG-treated mice. However, the depletion of CD4+ CD25+ T cells did not enable induction of thyroiditis with mTg in E′ B10.Aβ mice because none displayed thyroid destruction, and only 8% (1 of 12) showed mild infiltration.

Fig. 1B illustrates representative thyroid pathology from each group. Fig. 1Ba shows normal thyroid histology. Fig. 1Bb is a thyroid section from a hTg-immunized E′ B10.Aβ mouse, with mononuclear cell infiltration involving ~20% of the thyroid. In vivo depletion of CD4+ CD25+ T cells enhanced thyroiditis induced with hTg, resulting in more severe inflammation involving ~60% of the thyroid (Fig. 1Bd). In contrast, the resistance of
hTg-immunized mice pretreated with anti-CD25 compared with antibody production or in vitro-proliferative responses to Ags in that from a normal mouse. No differences were observed in auto/H11011
clear cells

Representative thyroid histology from experimental groups (original mag-
mification, 100): normal thyroid architecture (a); infiltration of mononu-
clear cells ≈20% following induction of EAT with hTg (b); lack of thy-
roiditis induction and mononuclear cell infiltration after mTg immunization
(c); and CD4+CD25+ T cells depleted before immunization with hTg,
infiltration, and destruction ≈60% (d).

FIGURE 1. In vivo depletion of CD4+CD25+ T cells enhances EAT induction with hTg but does not alter restriction against mTg in B10.E'Ab6 (A'E) mice. B10.E'Ab6 mice were given two 1-mg doses of anti-CD25 or rat IgG i.v. on days −14 and −10. Reduction of 75–90% of CD4+CD25+ T cells was observed by FACS analysis of PBL on day −4. Mice were challenged with 40 μg of either hTg or mTg, followed 3 h later by 20 μg of LPS on days 0 and 7, and the mice were killed on day 28. A. The graph represents mononuclear cell infiltration of thyroids of individual mice; results are pooled from two independent experiments. D, day. B, Representative thyroid histology from experimental groups (original magnification, ×100): normal thyroid architecture (a); infiltration of mononuclear
cells ≈20% following induction of EAT with hTg (b); lack of thy-
roiditis induction and mononuclear cell infiltration after mTg immunization
(c); and CD4+CD25+ T cells depleted before immunization with hTg,
infiltration, and destruction ≈60% (d).

E'B10.Ab6 mice to thyroiditis induction with mTg was unaltered by
depleting CD4+CD25+ T cells; the thyroid section from a CD4+CD25+
T cell-depleted, mTg-challenged mouse shown in
FIG. 1b demonstrates no mononuclear cell infiltration, similar to
that from a normal mouse. No differences were observed in auto-
antibody production or in vitro-proliferative responses to Ags in
hTg-immunized mice pretreated with anti-CD25 compared with
mice pretreated with rat IgG, because both remained strongly posi-
tive (data not shown). As previously reported (11), splenocytes
from hTg-immunized mice did not respond to mTg (data not shown).
The increase in thyroiditis severity following anti-CD25
administration indicates that CD4+CD25+ regulatory T cells in-
fluencing thyroiditis development can be generated in the context
of H2E. The inability of regulatory T cell depletion to alter the
resistance to mTg-induced EAT shows that the unique property of
the E'B10.Ab6 mouse to respond only to hTg is a function of
restricted presentation of autoantigen by the Eβ molecule and is not
a result of CD25+ T cell regulation.

Depletion of CD4+CD25+ T cells enables susceptibility to EAT
induction with mTg in traditionally resistant B10 (H2b) mice
Previous investigation of this unique Eβ-transgenic mouse model of
EAT revealed a suppressive effect of the endogenous Aβ genes;
E'B10 (A'βE') mice developed less severe thyroiditis following
immunization with hTg, compared with E'B10.Ab6 (A'E') mice
(11). This suppressive effect of the endogenous Aβ genes might be
mediated through CD4+CD25+ regulatory T cells, particularly
those activated by self-peptides presented by the endogenous Aβ
molecule. To determine whether CD4+CD25+ T cells exert a reg-
ulatory influence on thyroiditis development by self-Tg epitopes in
the context of Aβ, we selected traditionally EAT-resistant congenic
B10 (A'E') mice devoid of H2E molecules. Their CD4+CD25+
T cells were depleted before challenge with the EAT induction
protocol. As illustrated in Fig. 2, depletion of CD4+CD25+ T cells
enabled the induction of thyroiditis with mTg because eight of nine
(89%) CD25-depleted mice developed mononuclear cell infil-
tration of the thyroid, compared with two of eight (25%) controls.
Thus, EAT resistance in B10 mice is mediated by CD4+CD25+
regulatory T cells in the normal context of Aβ. However, depletion
of CD4+CD25+ T cells did not enable thyroiditis induction with
40-μg doses of hTg (Fig. 2), a dose sufficient to induce EAT in
A'E' mice (Fig. 1A).

Reconstitution of CD4+CD25+ T cells restores EAT resistance
in CD25 mAb-treated B10 mice
To determine whether adoptive transfer of CD4+CD25+ T cells
would restore EAT resistance in B10 mice, B10 mice were de-
peled of CD4+CD25+ T cells with two 1-mg doses of anti-CD25
mAb on days −26 and −22 (75–90% reduction as measured by
flow cytometric analysis of PBL 6 days later). On day −12, 1.5–
3.0 × 106 CD4+CD25+ or CD4+CD25− T cells from naive B10
mice were transferred. Recipient mice were then challenged with
40 μg of mTg and 20 μg of LPS on days 0 and 7, and thyroiditis
severity was assessed on day 28. Fig. 3 illustrates that 70% of
CD25+ T cell-depleted mice receiving CD4+CD25+ T cells had
no thyroiditis; the remaining 3 of 10 (30%) mice had mononuclear
cell infiltration of 5–20%. In contrast, more consistent and exten-
sive mononuclear cell infiltration and destruction of up to 40% of
the thyroid was observed in CD4+CD25− T cell-reconstituted

FIGURE 2. In vivo depletion of CD4+CD25+ T cells enables EAT induction with mTg, but not hTg, in B10 (A'E') mice. Two 1-mg doses of anti-CD25 or polyclonal rat IgG i.v. were administered to B10 mice on
days −14 and −10. Reduction of 75–85% of CD4+CD25+ T cells was observed by FACS analysis of PBL on day −4. Mice were challenged on
days 0 and 7 with 40 μg of either hTg or mTg, followed 3 h later by 20 μg of
LPS and killed on day 28. The graph represents mononuclear cell in-
filtration of thyroids of individual mice; results are pooled from two inde-
pendent experiments. D, Day.
mice. The specific requirement for CD4⁺CD25⁺ T cells to restore resistance to thyroiditis induction in CD25⁻T cell-depleted B10 mice reinforces the role of CD4⁺CD25⁺ T cells in regulating susceptibility.

**Depletion of CD4⁺CD25⁺ T cells reverses the suppressive effect of endogenous A² molecule in E²B10 (A⁺E⁺) mice**

The findings presented above demonstrate that CD4⁺CD25⁺ T cells are generated in the presence of either H2A or H2E molecules because their removal enhanced thyroiditis severity in both A⁻E⁻ and A⁺E⁺ mice. It was then of interest to examine the role of CD4⁺CD25⁺ T cells in E²B10 (A⁺E⁺) mice, in which the presence of A² genes resulted in reduced thyroiditis severity after hTg induction (11). After depleting CD4⁺CD25⁺ T cells, E²B10 mice were challenged with either hTg or mTg. As shown in Fig. 4, both hTg- and mTg-induced EAT were increased significantly (p < 0.01) by the elimination of CD4⁺CD25⁺ T cells. E²B10 mice immunized with hTg demonstrated strong autoantibody production and in vitro-proliferative response, irrespective of CD4⁺CD25⁺ T cell depletion (data not shown). Mice depleted of CD4⁺CD25⁺ T cells demonstrated a slight increase in anti-mTg Ab production but no difference in in vitro-proliferative response of splenocytes, compared with polyclonal rat IgG-treated controls (data not shown). The increase in the severity of thyroiditis induced with either hTg or mTg seen in anti-CD25-treated mice indicates that CD4⁺CD25⁺ T cells also regulate thyroiditis development in E⁺B10 (A⁻E⁺) mice.

**Discussion**

We have previously observed reciprocal-suppressive effects of both H2E and H2A genes on thyroiditis. H2E⁺ genes reduced the severity of thyroiditis encoded by H2A⁺ in B10.S mice (7), and H2A⁺ genes suppressed H2E⁺-mediated thyroiditis in E⁺B10,Ab⁺ mice (11). Although such suppressive effects of H2E on other autoimmune responses encoded by H2A molecules has also been previously described (8, 9), the reciprocal effect of H2A genes on H2E-mediated thyroiditis, to our knowledge, has not been demonstrated in other systems. The generation of regulatory T cells responding to Ags presented in the context of H2E has been proposed as a potential suppressor mechanism (8, 9, 12–14).

CD4⁺CD25⁺ regulatory T cells, a subset of T lymphocytes essential in maintaining peripheral immunoregulation inhibiting the development of autoimmunity (15, 28, 29), are derived from high-affinity interactions with self-peptide, both during ontogeny (30–32) and in the periphery (33). We have recently described a regulatory role for peripherally amplified CD4⁺CD25⁺ T cells in EAT-susceptible CBA mice, which mediate mTg-induced tolerance to EAT (17). The influence of CD4⁺CD25⁺ T cells in regulating susceptibility to EAT and the potential role of class II molecules in generating these cells led to an examination of their potential influence in class II-transgenic strains.

Our first step to identify a potential role for CD4⁺CD25⁺ regulatory T cells in mediating the reciprocal inhibition by H2E⁺ and H2A⁺ was to verify their existence in each individual MHC context. For CD4⁺CD25⁺ T cells derived from the E⁻ mouse in E⁺B10,Ab⁺ (A⁺E⁺) mice, we depleted them in vivo before EAT induction. This transgenic mouse strain is susceptible to thyroiditis induced with heterologous Tg but uniquely resistant to thyroiditis induction with self mTg (11). We observed an increase in thyroiditis severity after hTg immunization (Fig. 1), indicating that CD4⁺CD25⁺ regulatory T cells are generated in the context of E⁻ and the absence of A². Of interest is the observation that depletion of CD4⁺CD25⁺ T cells did not enable B10.E⁺Ab⁺ (A⁺E⁺) mice to shed the restriction against mTg-induced thyroiditis (Fig. 1). Because mTg, possessing both unique and shared epitopes (34), is more thyroidogenic than hTg, the inability of A⁺E⁺ mice to develop thyroiditis following immunization with mTg reinforces the absolute requirement for the correct class II presentation of Tg in EAT induction and indicates that the removal of peripheral inhibition of autoimmunity does not supersede this requirement.

Having demonstrated the existence of CD4⁺CD25⁺ T cells that inhibit EAT in the context of E⁻, we next examined the development of such cells in the context of A², a traditionally EAT-resistant haplotype. After depleting CD4⁺CD25⁺ T cells in B10 (A⁻E⁺) mice, we immunized the mice with either mTg or hTg. The elimination of resistance to EAT induction with mTg in B10 mice following CD4⁺CD25⁺ T cell depletion (Fig. 2) indicates that CD4⁺CD25⁺ regulatory T cells that influence thyroiditis development also exist in the context of A², and their removal leads to an increase in susceptibility. These data are reminiscent of an earlier report describing increased susceptibility to mTg-induced thyroiditis in EAT-resistant C57BL/6 (H2A⁺) mice after administration of cyclophosphamide, an agent known to inactivate preferentially regulatory T cells (35). The data also agree with the
finding that elimination of CD4+CD25+ regulatory T cells reduced resistance against the induction of gastritis in traditionally resistant mice (16). The specific role of CD4+CD25+ T cells in influencing susceptibility in traditionally EAT-resistant mice is reinforced additionally by the restoration of resistance to EAT induction following the transfer of CD4+CD25+ T cells in CD25 mAb-treated B10 mice (Fig. 3). Thus, resistance to autoimmunity may, at least in some instances, reflect greater peripheral regulation rather than a poor response to autoimmune. The ability of class II molecules to generate regulatory T cells effective in inhibiting autoimmunity likely varies between haplotypes and may explain the genetic predisposition of certain haplotypes to the development of autoimmunity. In contrast, the inability of hTg to induce thyroiditis in B10 mice, even after depletion of CD4+CD25+ regulatory T cells (Fig. 2), may be related to its poor thyroiditogenicity in traditionally susceptible strains (36, 37) or to its involvement of regulatory T cells other than those expressing CD25 (38). It may also indicate the existence of Aβ specificity for epitopes not shared between mTg and hTg, similar to the Eβ molecule for nonconserved hTg epitopes (Fig. 1; Ref. 11).

The existence of CD4+CD25+ T cells influencing thyroiditis induction in both the context of Aβ and Eβ supports the idea that these cells may be responsible for the reciprocal-suppressive effect between the two molecules observed previously (8, 11). Suppression of H2A-dependent autoimmune responses by introducing H2E molecules has been described in other models (8, 9, 39). Using our unique Ag-restricted, Eα-transgenic model, we were able to examine the influence of CD4+CD25+ regulatory T cells on Aβ- and Eα-mediated thyroiditis when both Eβ and Aβ genes were present. The increase in thyroiditis severity in CD25+ T cell-depleted Eα B10 (Aα Eα) mice immunized with either hTg (mediated by Eα) or mTg (mediated by Aβ; Fig. 4) indicates that CD4+CD25+ regulatory T cells influencing thyroiditis exist when both H2A and H2E molecules are present. The reduction in the suppressive effect of the Aβ genes on Eα-mediated thyroiditis after depleting CD4+CD25+ T cells suggests that these cells may be responsible for the inhibitory effect due to the presence of the second class II allele. We cannot point definitively to CD4+CD25+ regulatory T cells as mediators of the suppressive effect of H2A genes on H2E-mediated EAT observed in Eβ B10 mice at present because we demonstrated the existence of CD4+CD25+ regulatory T cells acting independently in the context of both Eβ and Aβ (Figs. 1 and 2). We have not discriminated between regulatory T cells generated by either H2A or H2E when eliminating regulatory T cells in vivo, and the observed increase in thyroiditis severity following CD4+CD25+ T cell depletion may be a result of decreased function of Eβ- or Aβ-derived regulatory T cells that act independently to inhibit thyroiditis mediated in the same context. Moreover, several other possibilities exist to explain the suppressive effect of the H2A genes in this model, such as alteration of the T cell repertoire, potentially including the deletion of autoreactive cells, and qualitative changes in T cell responses due to a combination of T cell epitopes presented in the context of Aβ and Eβ. Another interesting possibility arises from previous observations that presentation of an epitope from the Eα-chain by H2A molecules can inhibit collagen-induced arthritis in mice (39), and presentation of a HLA-DR-derived peptide by HLA-DQ can suppress collagen-induced arthritis in transgenic mice (40). A similar event may be occurring in the Eβ B10 mice, although we have not examined whether the Eβ molecule can present Aβ-derived peptides, nor whether Aβ molecules can present Eβ-derived peptides in B10.S mice (7). Nevertheless, our data demonstrate the existence of CD4+CD25+ regulatory T cells generated by both Aβ and Eβ molecules and that these regulatory T cells may reciprocally suppress the development of autoimmune thyroiditis induced in either context.

The increased susceptibility of Eβ B10 mice to mTg-induced EAT as compared with congenic B10 mice (Figs. 2 and 4) is also of interest and may relate to regulatory T cell function. The loss of resistance to mTg-induced thyroiditis cannot be attributed to the induction of a responding class II molecule because the resultant Eβ is not able to support mTg-induced EAT (11, 41). Possibly, this increase in susceptibility results from the alteration of the T cell repertoire because expression of the H2E molecule results in deletion of T cells expressing TCR VB5 and VB11 (42). Although the protective effect of Eβ genes in NOD mice cannot be associated with the deletion of commonly used TCR VB subsets (43), qualitative changes in regulatory T cells could potentially affect the T cell repertoire, and their deletion could lead to increased susceptibility of Eβ B10 mice to mTg-induced EAT.

Here, our findings indicate that CD4+CD25+ regulatory T cells generated in both the context of H2A and H2E play an important role in influencing the development of thyroiditis. These data also suggest that CD4+CD25+ regulatory T cells may be involved in the reciprocal-suppressive effect exerted between H2A and H2E. Susceptibility to autoimmunity has long been described as a function of class II genes in animal models (4), an association mirrored by human studies (5). More recently, human autoimmune disease has been linked to CTLA-4 (44) and FOXP3 (45), genes associated with CD4+CD25+ regulatory T cell activity (46–50). Our data suggest that the influence of class II genes and regulatory T cell-associated genes may not be independent and that class II genes may influence the development of autoimmunity both by their presentation of autoantigens as well as by their generation of CD4+CD25+ regulatory T cells.

Acknowledgments

We thank Julie Hanson and staff for breeding and maintaining the transgenic mice, A. M. Mazurco for preparation of histology sections, and Dr. A. A. Giraldo for assistance in evaluation of thyroid pathology. We also thank Dr. C. Jeffries for providing S. enteritidis LPS and Dr. H. Waldmann for the CD4, CD8, and CD11b mAbs.

Disclosures

The authors have no financial conflict of interest.

References

from nonself in susceptibility to heterologous thyroglobulins in autoimmune thyroditis. Immunogenetics 50:22.


