# Differential induction of IgE-mediated anaphylaxis after soluble vs. cell-bound tolerogenic peptide therapy of autoimmune encephalomyelitis

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**The ability of different forms of myelin peptides to induce tolerance for the treatment of preestablished murine experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis, was evaluated. i.v. administration of myelin peptide-pulsed, ethylene carbodiimide-fixed syngeneic splenocytes, but not soluble myelin peptide monomers or oligomers, proved exceedingly effective at treating preestablished EAE, resulting in amelioration of disease progression. In addition to the lack of therapeutic efficacy of soluble peptide and peptide oligomer, administering them i.v. after the onset of clinical symptoms in many but not all peptide-induced EAE models led to a rapid-onset anaphylactic reaction characterized by respiratory distress, erythema, decreased body temperature, unresponsiveness, and, often, death. By using anti-IgE antibody treatments and mice with targeted mutations of the FcγRIII α-chain or the common γ-chain of FcεRI and FcγRI/III, we demonstrate that IgE crosslinking of FcεRI appears to be necessary and sufficient for myelin peptide-induced anaphylaxis. The implications of these findings to myelin peptide/protein tolerance strategies for the treatment of multiple sclerosis are discussed.**

myelin | tolerance

Multiple sclerosis (MS) is a CNS-demyelinating disease characterized by the perivascular infiltration of inflammatory mononuclear cells (1). Although the etiology of MS is unknown, evidence from human patients (2–4) and an animal model of MS, experimental autoimmune encephalomyelitis (EAE) (5–7), supports a major pathogenic role for autoreactive myelin-specific $CD4^+$ T cells, which are a logical target for clinical therapies. Two protocols that have shown promise in inducing antigen-specific immune tolerance for the prevention and/or treatment of EAE are the i.v. injection of soluble protein, peptide, or peptide oligomers (8–10) and the i.v. injection of myelin proteins or peptides chemically coupled to syngeneic splenocytes [antigen-coupled splenocytes (Ag-SP)] (11). We performed a side-by-side comparison of these two tolerance protocols in preventing EAE induction and ameliorating ongoing EAE. We found that i.v. injection of soluble peptide monomer was not particularly effective when administered at any time point tested, and a soluble peptide oligomer was only fully effective when administered after immunization (day +7) but before the onset of clinical symptoms of EAE. In contrast, i.v. administration of Ag-SP proved effective at preventing the onset of clinical symptoms and treating preestablished disease. In addition, an unexpected result of the administration of soluble peptide and peptide oligomer after the onset of clinical symptoms in many, but not all, peptide-induced EAE models was a rapid-onset anaphylactic reaction characterized by respiratory distress, erythema, decreased body temperature, unresponsiveness, and often death. This result is especially alarming given the proposed use of peptide-based tolerance therapies for the treatment of autoimmune diseases.

Anaphylaxis is an acute, life-threatening phenomenon that is usually but not always immune-mediated (12). The most comprehensively described mechanism of anaphylaxis involves the crosslinking of IgE bound to FcεRI on mast cells, leading to the release of preformed mediators, such as histamine, heparin, and tryptase; lipid-derived mediators, such as prostaglandins, leukotrienes, and platelet-activating factor; and cytokines. Collectively, these mediators initiate rapid vascular permeability, leading to plasma extravasation, tissue edema, bronchoconstriction, mucous overproduction, and leukocyte recruitment (13, 14). In rodents, mast cell degranulation, temperature loss, and mortality associated with active systemic anaphylaxis can also be mediated through the crosslinking of IgG1 bound to FcγRIII (15, 16). We show that the crosslinking of FcεRI appears to be necessary and sufficient in myelin peptide-induced anaphylaxis induced by the i.v. injection of soluble peptide or peptide oligomer on or after the peak of acute disease.

## Materials and Methods

**Mice.** Female mice (age, 5–7 weeks) were purchased from the following commercial sources: SJL mice were from Harlan Laboratories (Bethesda, MD); B10.PL, C57BL/6, and FcγRIIIα knockout (KO) mice on the C57BL/6 background were from The Jackson Laboratories; and FcRγ KO mice on the C57BL/6 background were from Taconic Farms. B10.S μMt mice were backcrossed five times to the SJL background. All mice were housed under specific pathogen-free conditions (viral antibody-free) in the Northwestern University Center for Comparative Medicine. Paralyzed animals were afforded easier access to food and water.

**Reagents.** Synthetic proteolipid protein (PLP) peptides $PLP_{139–151}$ (HSLGKWLGHPDKF), $PLP_{178–191}$ (NTWTTSQSIAFPSK), myelin basic protein $(MBP)_{84–104}$ (VHFFKNIVTPRTPPPSQGKGR), myelin oligodendrocyte protein $(MOG)_{35–55}$ (MEVGWYRSPFSRVVHLYRNGK), $MOG_{92–106}$ (DEGGYTCFFRDHSYQ), and $OVA_{323–339}$ (ISQAVHAAHAEINEAGR) were purchased from Genemed Biotechnologies, South San Francisco, CA. J5 (EKPKVEAYKAAAAPA 15mer) was synthesized as described in ref. 17. The H1 Rc antagonist, tripolidine, was purchased from Sigma. Anti-IgE antibody (R1E4) was the kind gift of Yang-Xin Fu (University of Chicago, Chicago).

Abbreviations: Ag-SP, antigen-coupled splenocytes; CFA, complete Freund's adjuvant; EAE, experimental autoimmune encephalomyelitis; KO, knockout; MBP, myelin basic protein; MOG, myelin oligodendrocyte protein; MS, multiple sclerosis; PLP, proteolipid protein; wt, wild type.

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**Induction and Clinical Evaluation of EAE.** Female mice (age, 8–10 weeks) were immunized s.c. at three spots on the flank with 100 $\mu$l of an emulsion of peptide in complete Freund's adjuvant (CFA) containing 200 $\mu$g of *Mycobacterium tuberculosis* H37Ra (Difco) on day 0. In some experiments, mice also received another 100 $\mu$l of peptide/CFA emulsion on day 7 and/or 200 ng of *Bordetella pertussis* toxin (List Biological Laboratories, Campbell, CA) in 200 $\mu$l of PBS i.p. on days 0 and 2. Individual animals were observed every 1–3 days, and clinical scores were assessed on a scale of 0–4 as follows: 0, no abnormality; 1, limp tail or hind limb weakness; 2, limp tail and hind limb weakness; 3, partial hind limb paralysis; 4, complete hind limb paralysis. Data are reported as the mean clinical score.

**Soluble Peptide and Ag-SP Tolerances, Body Temperature Evaluation, and Analysis of Serum Histamine Levels.** Immunizing peptide, peptide oligomer, or an irrelevant peptide (50–200 $\mu$g) was given i.v. in 200 $\mu$l of PBS. Tolerance of Ag-SP was induced by the i.v. injection of $50 \times 10^6$ ethylene carbodiimide-treated peptide-pulsed Ag-SP, as described in ref. 11. Coupling efficiency has previously been determined to be ≈30%, yielding 24–55 $\mu$g of peptide per $50 \times 10^6$ splenocytes (18). Temperature was determined by using an implantable programmable temperature transponder pocket scanner and s.c. implanted temperature transponders (BioMedic Data Systems, Maywood, NJ). Serum histamine levels were quantitated by ELISA (ICN).

**Serum Peptide-Specific Ig Determination and Statistical Analyses.** Peptide-specific IgG1, IgG2b, and IgE levels in sera were determined by sandwich ELISA. Microtiter plates (96-well) were coated with 10 $\mu$g/ml peptide in PBS overnight at 4°C. The plates were blocked for 2 h at 37°C with 2% BSA in PBS. Serum was diluted in blocking buffer 1:1,000 for IgG1 and 1:10,000 for IgG2b and incubated in plates overnight at 4°C. Serum was not diluted for measurement of IgE. Antibodies were detected by isotype-specific biotinylated antibodies (Pharmingen) for 2 h at room temperature followed by streptavidin-horseradish peroxidase (Zymed) for 30 min at room temperature. ELISAs were developed with tetramethyl benzidine (BioFX Laboratories, Owings Mills, MD), and absorbance was measured at 450 nm with a VMax microplate reader (Molecular Devices). The percentage of animals showing clinical relapses or symptoms of anaphylaxis between any two groups of mice was analyzed by $\chi^2$ test using Fisher's exact probability. $P$ values $< 0.05$ were considered significant.

## Results

**Comparison of Soluble Myelin Peptide Versus Myelin Peptide-Coupled Splenocytes for the Prevention and Treatment of EAE.** To compare the relative efficiency of soluble peptide tolerance versus Ag-SP tolerance in the prevention of EAE, SJL mice were given soluble PLP peptide $PLP_{139–151}$, an oligomer containing 16 repeats of the $PLP_{139–151}$ epitope ($PLP_{139–151}$ 16-mer) (10), or $PLP_{139–151}$-coupled Ag-SP ($PLP_{139}$-SP) i.v. 7 days before priming with $PLP_{139–151}$ in CFA (Fig. 1*a*). At the doses used, only $PLP_{139–151}$-coupled cells proved to be effective at preventing the onset of EAE. In agreement with previous findings, the $PLP_{139–151}$16-mer (10) and the peptide-coupled cells (11, 19) completely protected against the onset of EAE when administered 7 days after immunization, whereas soluble $PLP_{139–151}$ peptide had no ameliorating effect on disease development (Fig. 1*b*). Lastly, we compared the efficiency of the tolerance induction protocols to ameliorate established EAE. $PLP_{139–151}$-SP treatment reduced disease severity and inhibited the subsequent relapse. Surprisingly, the administration of soluble peptide and soluble peptide oligomer at day 15 after immunization resulted in the death of 80% and 100% of the animals, respectively, within 30 min after treatment (Fig. 1*c*). The death-inducing reaction was characterized by ruffled fur, erythema, labored respiration, and unresponsiveness. The mouse receiving soluble $PLP_{139–151}$ that did not experience this adverse reaction went on to relapse in a manner similar to the mice receiving the control $OVA_{323–339}$ peptide.

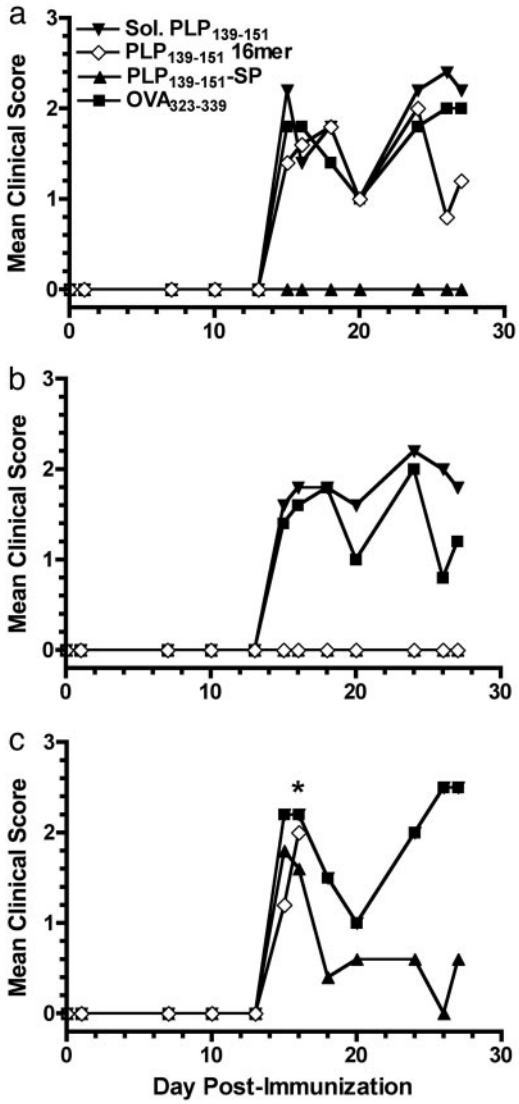


**Fig. 1.** Administration of peptide-coupled cells but not soluble (Sol.) peptide ameliorates established EAE. Five to six female SJL mice per group were immunized s.c. with 50 $\mu$g of $PLP_{139–151}$/CFA on day 0. Mice were given 200 $\mu$g of soluble $PLP_{139–151}$, 200 $\mu$g of soluble $PLP_{139–151}$ 16-mer, $50 \times 10^6$ $PLP_{139–151}$-SP, or 200 $\mu$g of soluble $OVA_{323–339}$ i.v. (*a*) Before immunization (day −7). (*b*) After immunization but before clinical onset (day +7). (*c*) At the peak of acute disease (day +15). Data are representative of two experiments. *, Four of five mice receiving soluble $PLP_{139–151}$ and five of five mice receiving the soluble $PLP_{139–151}$ 16-mer died within 30 min of injection.

**Soluble Peptide Tolerance Induces Histamine-Dependent Anaphylaxis in Myelin Peptide-Primed Mice.** Consistent with an anaphylactic response, body temperature dramatically and rapidly dropped upon administration of soluble $PLP_{139–151}$ to $PLP_{139–151}$/CFA-primed SJL mice in four of five treated animals (Fig. 2*a*). In addition, high levels of histamine were detected in the serum of mice within 10 min of injection of $PLP_{139–151}$ (Fig. 2*b*). Neither a decrease in body temperature nor histamine release were observed in primed mice treated with soluble $OVA_{323–339}$ peptide (data not shown) or $PLP_{139–151}$-coupled cells (Fig. 2 *a* and

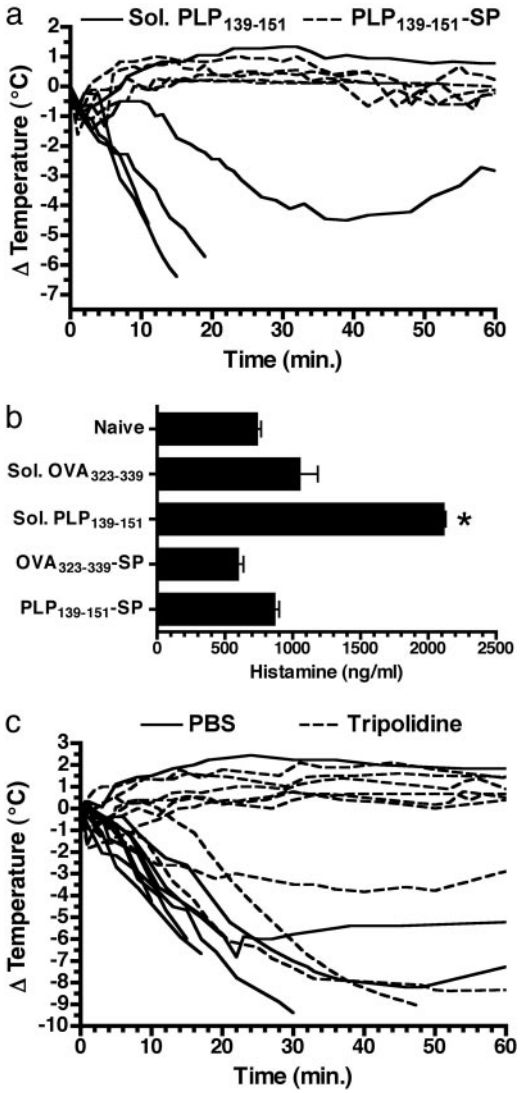


**Fig. 2.** Administration of soluble (Sol.) $PLP_{139-151}$ but not $PLP_{139-151}$-coupled cells after the peak of acute EAE results in anaphylaxis preventable by pretreatment with an H1 Rc antagonist. Groups of five to nine female SJL mice were immunized s.c. with 50 $\mu$g of $PLP_{139-151}$/CFA on day 0. (*a* and *b*) At day +20, shortly after the peak of acute disease, mice were injected i.v. with 200 $\mu$g of soluble $PLP_{139-151}$, 200 $\mu$g of soluble $OVA_{323-339}$, $50 \times 10^6$ $PLP_{139-151}$-SP, or $50 \times 10^6$ $OVA_{323-339}$-SP. Mice were then monitored for change in body temperature for 60 min. (*a*) Severe loss of body temperature was observed in four of five mice receiving soluble $PLP_{139-151}$ vs. none of the five mice receiving $PLP_{139-151}$-SP ($P = 0.048$). (*b*) Alternatively, serum histamine levels were determined by ELISA 10 min after treatment. *, Serum histamine levels in $PLP_{139-151}$-injected mice were significantly greater than in naïve mice and mice injected i.v. with $OVA_{323-339}$ peptide, $P < 0.01$. (*c*) At day +20, mice received 200 $\mu$l of PBS i.p. or 400 $\mu$g of tripolidine. All mice were given 200 $\mu$g of soluble $PLP_{139-151}$ i.v. 30 min later and monitored for change in body temperature. Tripolidine reduced the incidence of anaphylaxis from 89% (eight of nine mice) in PBS-treated controls to 33% (three of nine mice), $P = 0.05$. All results are representative of two experiments.

*b*). Moreover, treatment with the H1 receptor antagonist tripolidine 30 min before soluble peptide challenge significantly protected the mice from the adverse clinical reaction (Fig. 2*c*).

To determine the range of peptides and mouse strains susceptible to induction of anaphylaxis after i.v. peptide tolerance, EAE was induced in SJL, C57BL/6, and B10.PL mice primed with a variety of known encephalitogenic myelin peptides and mice were treated i.v. with soluble peptides at or shortly after the peak of acute disease

**Table 1. Differential induction of soluble myelin peptide-induced anaphylaxis in different strains of mice primed with various myelin peptides**

| Mouse groups (priming peptide) | i.v. soluble peptide treatment | Anaphylactic response, *n*/total |
|---|---|---|
| SJL | | |
| A ($PLP_{139-151}$) | $PLP_{139-151}$ | 9/10* |
| | $PLP_{178-191}$ | 0/7 |
| | $OVA_{323-339}$ | 0/5 |
| | J5† | 0/5 |
| B ($PLP_{178-191}$) | $PLP_{178-191}$ | 0/8 |
| | $OVA_{323-339}$ | 0/7 |
| C ($MBP_{84-104}$) | $MBP_{84-104}$ | 6/6* |
| | $OVA_{323-339}$ | 0/4 |
| | J5† | 0/5 |
| D ($MOG_{92-106}$) | $MOG_{92-106}$ | 0/6 |
| | $OVA_{323-339}$ | 0/4 |
| E ($OVA_{323-339}$) | $OVA_{323-339}$ | 0/8 |
| | $PLP_{139-151}$ | 0/7 |
| C57BL/6 | | |
| F ($MOG_{35-55}$) | $MOG_{35-55}$ | 8/10* |
| | $OVA_{323-339}$ | 0/7 |
| | J5† | 0/5 |
| G ($PLP_{178-191}$) | $PLP_{178-191}$ | 0/5 |
| | $OVA_{323-339}$ | 0/5 |
| B10.PL | | |
| H ($MBP_{Ac1-11}$) | $MBP_{Ac1-11}$ | 6/6* |
| | $OVA_{323-339}$ | 0/4 |

Mice were primed with the various myelin peptides or $OVA_{323-339}$ in CFA on day 0. Mice were injected i.v. with 200 $\mu$g of the indicated soluble peptides on the day of peak acute disease. Incidence of anaphylactic responses were judged by the evidence of severe respiratory distress.
*$P < 0.005$ vs. mice injected i.v. with 200 $\mu$g of the non-self $OVA_{323-339}$ peptide.
†J5 is a synthetic 15-mer demonstrated to suppress EAE induced by $PLP_{139-151}$ in SJL mice (20).

(Table 1). Interestingly, anaphylaxis was only induced in some of the strain/myelin peptide combinations examined. SJL mice developed anaphylactic reactions in response to treatment with soluble $PLP_{139-151}$ or $MBP_{84-104}$ but not in response to $PLP_{178-191}$ or $MOG_{92-106}$. Anaphylaxis was observed in C57BL/6 mice undergoing $MOG_{35-55}$-induced EAE but not $PLP_{178-191}$-induced EAE and in B10.PL mice undergoing $MBP_{Ac1-11}$-induced EAE after administration of the soluble homologous peptide at the peak of acute disease. $OVA_{323-339}$ neither elicited anaphylaxis in any of the myelin peptide-primed mice nor induced anaphylaxis in SJL mice primed with this immunogenic epitope. Interestingly, J5, a synthetic peptide 15-mer that inhibits $MBP_{85-99}$-induced EAE in humanized mice and $PLP_{139-151}$-induced EAE in SJL mice (20), did not result in anaphylaxis when administered after the peak of acute disease in SJL mice primed with $PLP_{139-151}$ or $MBP_{84-104}$ or in $MOG_{35-55}$-primed C57BL/6 mice.

**Peptide-Induced Anaphylaxis Is Antibody- and FcR-Dependent.** Because of the possible clinical consequences of such a detrimental response to soluble peptide administration in ongoing and proposed autoimmune disease clinical trials, we further examined the possible mechanism(s) underlying the induction of peptide-induced anaphylaxis. To determine whether functional antibody production was necessary for this reaction to occur, B cell-deficient SJL $\mu$Mt mice were primed with $PLP_{139-151}$/CFA and injected i.v. with 200 $\mu$g of soluble $PLP_{139-151}$ after the peak of acute disease. In contrast to wild-type (wt) SJL mice, B cell-deficient SJLs did not undergo peptide-induced anaphylaxis as determined by clinical signs and loss of body temperature (data not shown) or the release of histamine in the serum (Fig.

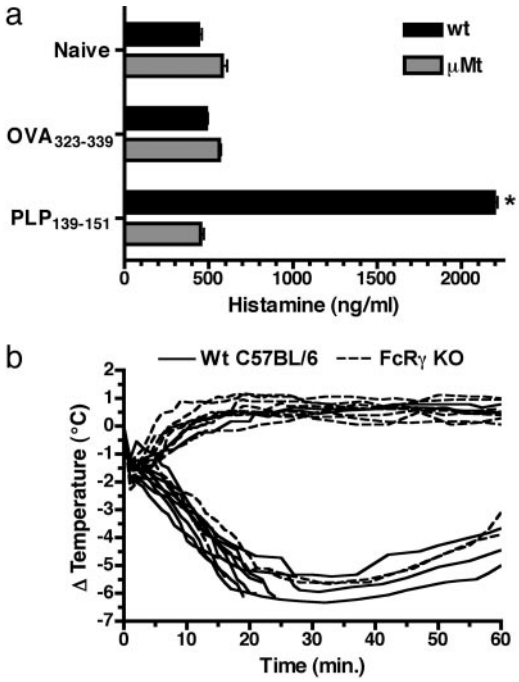


**Fig. 3.** Peptide-induced anaphylaxis is antibody- and FcR-dependent. (*a*) wt SJL and SJL $\mu$Mt mice were immunized s.c. with 50 $\mu$g of $PLP_{139-151}$/CFA on day 0. At day +20, all mice received 200 $\mu$g of soluble $PLP_{139-151}$ or soluble $OVA_{323-339}$ and were monitored for serum histamine levels 10 min thereafter. *, Serum histamine levels in $PLP_{139-151}$-injected wt SJL mice were significantly greater than in the appropriate controls, $P < 0.01$. (*b*) Groups of nine to 10 wt C57BL/6 or FcR$\gamma$ KO mice were immunized s.c. with 200 $\mu$g of $MOG_{35-55}$/CFA on day 0. In addition, all mice were given 200 ng of *B. pertussis* toxin i.p. on days 0 and 2. At day +20, all mice received 50 $\mu$g of soluble $MOG_{35-55}$ i.v. and were monitored for changes in body temperature. Incidence of anaphylaxis was 78% (seven of nine mice) in wt mice and 20% (two of 10 mice) in FcR$\gamma$ KO mice, $P = 0.023$. Results are representative of two experiments.

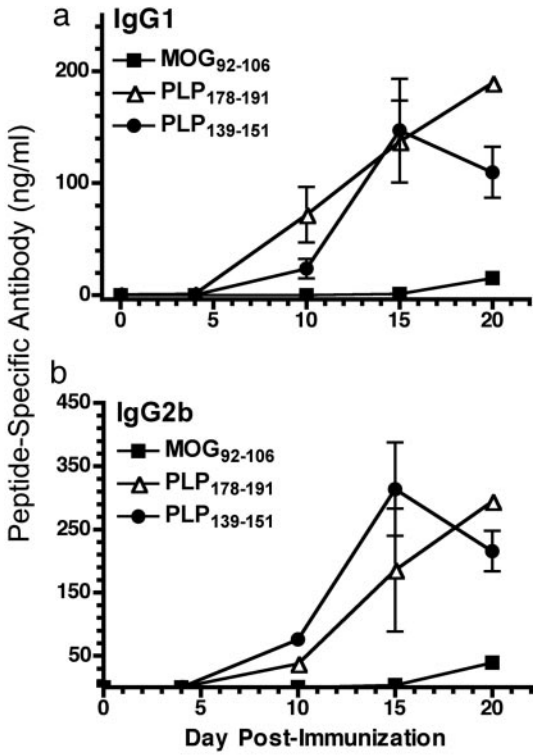


**Fig. 4.** Antigen-specific antibody production after immunization with different myelin peptides. Groups of 10–12 female SJL mice were immunized s.c. with 50 $\mu$g of $PLP_{139-151}$/CFA, 100 $\mu$g of $PLP_{178-191}$/CFA, or 200 $\mu$g of $MOG_{92-106}$/CFA on day 0. Mice primed with $MOG_{92-106}$ were given a booster of 200 $\mu$g of $MOG_{92-106}$/CFA on day 7 and 200 ng of *B. pertussis* toxin i.p. on days 7 and 9. Serum was collected on days 0, 4, 10, 15, and 20 after immunization from representative animals for analysis of peptide-specific antibody by ELISA. Serum was diluted 1:1,000 for IgG1 (*a*) and 1:10,000 for IgG2b (*b*). Results are shown as the mean ± SEM of two to four animals.

3*a*). Similar results were obtained with $MBP_{Ac1-11}$ in B10.PL $\mu$Mt mice (data not shown). Antibody-dependent anaphylaxis in mice can be mediated by the crosslinking of IgE bound to Fc$\varepsilon$RI on mast cells or by the crosslinking of IgG1 bound to Fc$\gamma$RIII on macrophages, mast cells, and/or other cell types. To confirm that the necessity for functional antibody was due to binding to Fc receptors, FcR$\gamma$ KO mice, lacking the shared common FcR$\gamma$ chain, were assessed for their ability to undergo peptide-induced anaphylaxis. $MOG_{35-55}$-primed wt C57BL/6 mice displayed severe anaphylaxis following administration of soluble $MOG_{35-55}$ after the peak of acute disease (Fig. 3*b*). However, FcR$\gamma$ KO mice, lacking Fc$\varepsilon$RI and Fc$\gamma$RIII, displayed a significant decrease in the incidence of anaphylaxis after the administration of soluble $MOG_{35-55}$ (Fig. 3*b*). The two cases of anaphylaxis observed in the FcR$\gamma$ KO mice possibly could be due to a direct effect of $MOG_{35-55}$ on the mast cells because it has been shown that whole MBP (21), whole MOG (22) and $MOG_{35-55}$ (M. Brown, personal communication) can cause mast cell degranulation in an antibody-independent manner. Because of the antibody-independent effect of $MOG_{35-55}$, this peptide was used at a dose of 50 $\mu$g per mouse, which led to anaphylaxis in $MOG_{35-55}$-primed mice but not in unprimed wt and KO C57BL/6 mice (data not shown).

**Peptide-Specific IgG1 Is Not Sufficient for Peptide-Induced Anaphylaxis.** To determine whether anaphylactic responses correlated with the degree of antibody production to the different myelin peptides and to begin to differentiate between the roles of IgE and IgG1 in myelin peptide-induced anaphylaxis, serum levels of peptide-specific antibodies throughout the EAE disease course were determined in SJL mice primed with $PLP_{139-151}$, $PLP_{178-191}$, or $MOG_{92-106}$. Although $MOG_{92-106}$ did not prime a potent antibody response, priming with $PLP_{139-151}$ or $PLP_{178-191}$ induced large amounts of specific IgG1 (Fig. 4*a*) and IgG2b (Fig. 4*b*), indicating that the inability of $PLP_{178-191}$ to prime for anaphylaxis is not due to a failure to stimulate class switching and antibody production by B cells and that the presence of antigen-specific IgG1 is not sufficient for peptide-induced anaphylaxis. C57BL/6 mice primed with $PLP_{178-191}$ also experience clinical EAE and produce large amounts of $PLP_{178-191}$-specific IgG1 (data not shown) and yet do not succumb to anaphylaxis upon i.v. challenge with soluble peptide (Table 1). Serum peptide-specific IgE was below the limit of detection in all peptide/strain combinations assayed (data not shown).

**Peptide-Induced Anaphylaxis Depends on IgE/Fc$\varepsilon$RI but Not IgG1/Fc$\gamma$RIII.** Lastly, we attempted to define the potential roles of the IgG1/Fc$\gamma$RIII and IgE/Fc$\varepsilon$RI pathways in this model of anaphylaxis. To assess whether functional Fc$\gamma$RIII is necessary for anaphylaxis induction, wt C57BL/6 and Fc$\gamma$RIII$\alpha$ KOs, which have a targeted mutation in the $\alpha$-chain responsible for binding to the Fc portion of IgG1 but which have functional Fc$\varepsilon$RI, were primed with $MOG_{35-55}$/CFA, challenged with soluble peptide shortly after the peak of acute disease, and monitored for changes in body temperature. Fc$\gamma$RIII$\alpha$ KO mice underwent anaphylaxis at a similar incidence and severity as wt C57BL/6 mice (Fig. 5*a*), indicating that the presence of Fc$\gamma$RIII is not

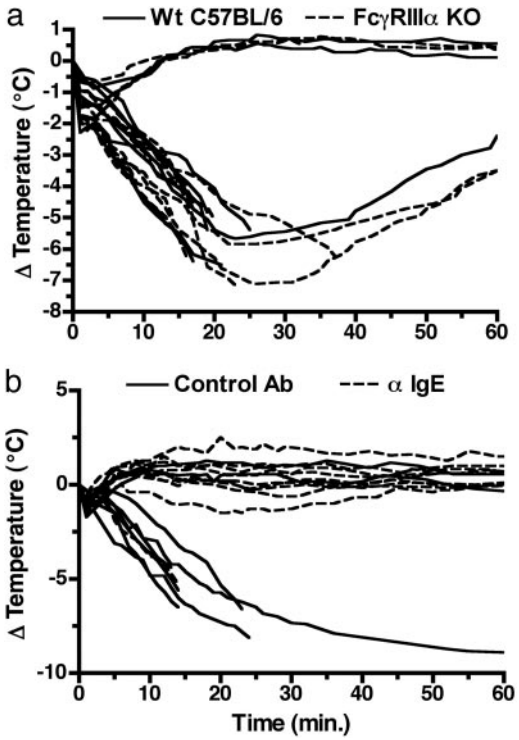


**Fig. 5.** Peptide-induced anaphylaxis depends on IgE/FcεRI but not IgG1/FcγRIII. (*a*) Groups of 10 female wt C57BL/6 or FcγRIIIα KO mice were immunized s.c. with 200 μg of $MOG_{35–55}$/CFA on day 0 and given 200 ng of *B. pertussis* toxin i.p. on days 0 and 2. On day 20, all mice were given 50 μg of soluble $MOG_{35–55}$ i.v. and monitored for change in body temperature. Incidence of anaphylaxis was 80% (eight of 10 mice) in both groups. (*b*) Groups of eight to 10 female SJL mice were immunized s.c. with 50 μg of $PLP_{139–151}$/CFA on day 0. On days 0, 7, and 14, mice were given 30 μg of control antibody or monoclonal anti-IgE (hybridoma clone R1E4) i.p. in 200 μl of PBS. On day 20, all mice were given 200 μg of soluble $PLP_{139–151}$ i.v. and monitored for change in body temperature. Anti-IgE reduced the incidence of anaphylaxis from 75% (six of eight mice) in control-treated mice to 10% (one of 10 mice), $P = 0.013$. Results are representative of two experiments.

required for myelin peptide-induced anaphylaxis. To definitively investigate the role of the IgE/FcεRI pathway in the anaphylactic response in the absence of commercially available FcεRI KO mice, we treated $PLP_{139–151}$/CFA-primed SJL mice with a control antibody or the R1E4 anti-IgE monoclonal antibody (Fig. 5*b*), which binds the Fc region of murine IgE, effectively blocking interaction between IgE and FcεRI (23), at 7-day intervals starting on the day of priming. Anti-IgE treatment prevented the induction of anaphylaxis upon administration of soluble $PLP_{139–151}$. This result along with the normal induction of anaphylaxis in FcγRIIIα KOs strongly indicates that the IgE/FcεRI pathway is necessary and sufficient for induction of anaphylaxis following soluble peptide tolerance.

## Discussion

Current therapies for autoimmune diseases use various antigen-nonspecific immunosuppressive and/or antiinflammatory strategies. Currently, glatiramer acetate (GA), a random polymer of L-alanine, L-glutamic acid, L-lysine, and L-tyrosine, is the only approved therapy for MS that is purported to act in a semiantigen-specific manner (24). GA is thought to induce T cells that produce T helper 2 cytokines, such as IL-4, IL-5, IL-10, and IL-13, which suppress inflammatory cells (25, 26). GA treatment requires daily s.c. injections and is beneficial to only a minority of relapsing-remitting MS patients (27), and 10% of patients experience a transient systemic postinjection reaction characterized by flushing, chest tightness, palpitations, dyspnea, and anxiety (28). Thus, there is need for new antigen-specific therapies for MS and other autoimmune diseases. Approaches, including oral tolerance and altered peptide ligands, have shown promise in EAE but were not efficacious in subsequent clinical trials (29–31). The current results clearly demonstrate enhanced efficacy and safety of tolerance induced by Ag-SP vs. soluble peptide monomers and oligomers to safely inhibit progression of established EAE.

The i.v. injection of soluble peptide led to severe and often fatal anaphylaxis when administered to various mouse strains with established EAE induced by myelin peptides including $PLP_{139–151}$, $MBP_{84–104}$, $MOG_{35–55}$, and $MBP_{Ac1–11}$. Myelin-peptide induced anaphylaxis occurred by means of an IgE-dependent mechanism as anti-IgE antibody administration blocked the induction of anaphylaxis. Anaphylaxis was not observed in Ag-SP-treated mice likely because the cell-bound peptide is unavailable to crosslink antibody bound to Fc receptors on tissue-resident mast cells. IgG1/FcγRIII interactions were neither necessary (anaphylaxis was normally induced in FcγRIIIα KOs) nor sufficient (peptides that do not induce anaphylaxis, e.g., $PLP_{178–191}$, elicit high levels of peptide-specific IgG1 serum antibody). A possible explanation for the failure of certain peptides inducing high levels of specific IgG1 (e.g., $PLP_{178–191}$) to elicit anaphylaxis after i.v. challenge with the homologous soluble peptide is the recent finding that the type of IgG1 induced by priming with peptides in CFA is nonanaphylactic in comparison with the anaphylactic type of IgG1 induced by priming with peptides in alum (32).

Anaphylaxis has also been observed in murine models in which soluble self-peptide was injected i.p. to induce tolerance during ongoing EAE (33) or by repeated s.c. injections in prediabetic nonobese diabetic (NOD) mice (34). Pedotti *et al.* (33) reported that myelin peptide-specific IgG1 antibodies accounted for the ability of i.p. administered soluble peptide to cause anaphylaxis during EAE based on the demonstration of increasing amounts of $PLP_{139–151}$-specific IgG1, but not IgE, in the serum of mice with ongoing EAE. However, those data do not directly demonstrate a role for IgG1, nor do they exclude a role for IgE. In the NOD model, Liu *et al.* (34) have reported that IgG1 and IgE play a role in s.c. peptide-induced anaphylaxis based on the finding that only coadministration of antibodies blocking FcγRII and FcγRIII (hybridoma clone 2.4G2) and IgE (hybridoma clone EM95), or coadministration of an H1 Rc antagonist (tripolidine) and a platelet-activating factor antagonist, CV-6209, completely blocked the induction of anaphylaxis. The fact that administration of tripolidine, previously shown to prevent murine IgE-mediated anaphylaxis (16), alone prevented myelin peptide-induced anaphylaxis in our model lends strong support to the essential role of the IgE/FcεRI pathway. The differences between our findings and those of Liu *et al.* (34) may be due to the different mouse strains used, spontaneous NOD disease vs. adjuvant-induced EAE, and/or the different routes of soluble peptide administration.

It is unclear why some encephalitogenic myelin peptides do not trigger anaphylaxis upon i.v. challenge of primed mice with soluble peptide. It has been suggested that self-peptides expressed in the thymus and, therefore, involved in central tolerance induction do not induce anaphylaxis (33). This hypothesis is not supported by our study demonstrating the equal anaphylactic ability of $PLP_{139–151}$ and $MOG_{35–55}$, which are not (35, 36), and $MBP_{Ac1–11}$ and $MBP_{84–104}$, which are expressed in the rodent thymus (37). In addition, the ability of certain myelin peptides to trigger anaphylactic response is clearly not due to the fact that only certain peptides serve as efficient B cell epitopes, because priming with $PLP_{178–191}$ induces large amounts of antigen-specific IgG1 and IgG2b in SJL and C57BL/6 mice but does not result in anaphylactic shock upon i.v. challenge with soluble

peptide. Because the levels of peptide-specific IgE in the serum were below the limit of detection, there may be a quantitative difference in the amount of IgE produced in response to priming with different peptides. Peptide-specific IgE is likely very quickly and avidly bound to FcεRI on mast cells, thereby reducing the unbound amount in the serum to undetectable levels. Long-term maintenance of cell surface-bound IgE on mast cells has been described even after serum levels of IgE became undetectable (38) because of IgE-mediated stabilization and up-regulation of FcεRI (39) and the slow kinetics of IgE disassociation (40).

The clinical implications of these findings raise questions about the future use of soluble peptide tolerance therapies to treat autoimmune disease in humans. The potential danger of soluble peptide therapy is illustrated by a recent clinical trial using an $MBP_{85–99}$ altered peptide ligand to regulate MS, which was terminated because of systemic hypersensitivity reactions (31). However, as previously mentioned, glatiramer acetate appears to be well tolerated (38). In addition, we have shown that the J5 peptide, a peptide inhibitor designed to compete with $MBP_{85–99}$ for binding to HLA-DR2 with the ability to also suppress $PLP_{139–151}$-induced disease in SJL mice (20), has no harmful effects on mice when administered i.v. during ongoing disease (Table 1). Obviously, the mechanisms of self-peptide-induced anaphylaxis will need to be further investigated before proceeding with soluble peptide therapies. Significantly, tolerance induction with Ag-SP appears to safely and efficiently prevent and ameliorate established EAE and is not complicated by the induction of anaphylactic shock.

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