SOLUBLE PEPTIDE TREATMENT REVERSES CD8 T CELL-INDUCED DISEASE IN A MOUSE MODEL OF SPONTANEOUS TISSUE-SELECTIVE AUTOIMMUNITY

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SOLUBLE PEPTIDE TREATMENT REVERSES CD8 T CELL-INDUCED DISEASE IN A MOUSE MODEL OF SPONTANEOUS TISSUE-SELECTIVE AUTOIMMUNITY

by

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ABSTRACT

SOLUBLE PEPTIDE TREATMENT REVERSES CD8 T CELL-INDUCED DISEASE IN A MOUSE MODEL OF SPONTANEOUS TISSUE-SELECTIVE AUTOIMMUNITY

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Autoimmunity is a complex process that involves recognition of self antigens by autoreactive T cells or tissue targeting by autoantibodies produced by B cells. Specific molecular targets have been identified in several autoimmune diseases but remain unknown in many others. Transgenic (Tg) mice have been utilized to express model antigens that can be recognized by T cells or by autoantibodies. To identify mechanisms by which CD8+ autoreactive T cells cause inflammation, we generated a double transgenic (DTg) murine model of autoimmunity by crossing K14-sOVA mice, which express soluble chicken ovalbumin (OVA), with OT-1 mice, whose CD8 T cells express Vα2/Vβ5 regions of the T cell receptor that are specific for SIINFEKL peptide (OVA 257-264) in association with class I MHC molecules. The K14-SOVA/OT-1 (#5 and #17) DTg mice develop normally, except that they undergo a destructive process that

selectively targets the external pinnae in the first 6 days of life. The purpose of this study was to elucidate the mechanism and attempt to obviate the resulting tissue-specific destruction. By light microscopy, the ear bud area displayed an intense inflammatory infiltrate of $V\alpha2/V\beta5^{+}CD8^{+}$ OT-1 cells when characterized by FACS. Administration of the TCR-recognized SIINFEKL peptide i.v. to pregnant F1 mice on days E16 and E18 in utero and i.p. to newborn pups on days 2 and 4 prevented the inflammatory response and resulted in development of normal-looking ears in 100% of pups. Treatment with the SIINFEKL peptide was shown to down-regulate the CD8 coreceptor and activate T-cells to differentiate into memory T-cells. This model can inform us about mechanisms of peripheral tolerance and potential therapies for autoimmune diseases in which specific molecular targets are known.

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PRIOR PUBLICATIONS & PRESENTATIONS

PUBLICATIONS

El-Guindy AS, **Paek SY,** Countryman J, Miller G (2006). Identification of constitutive phosphorylation sites on the Epstein-Barr virus ZEBRA protein. Journal of Biological Chemistry. 281(6):3085-95. PMID: 16321978.

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Paek SY, Nersesyan H, Blumenfeld H (2002). Functional MRI and Electrophysiologic Recordings of Absence Seizures in Rats. Abstract in STARS Summer Research Internship Program Symposium, Yale University. 364-372.

PRESENTATIONS

Poster at 59th Annual Montagna Symposium on the Biology of Skin, Salishan, Oregon, Oct 2010

- Title: Peptide protection of the pinna: How small molecules rescue ear phenotype in a mouse model of autoimmunity

Poster at Society for Investigative Dermatology Annual Meeting, Atlanta, Georgia, May 5-8, 2010

- Title: Peptide protection of the pinna: a model for studying the role of CD8+ T cells in mice

Poster at NIH Spring Research Festival, Bethesda, Maryland, May 2010

- Title: Peptide protection of the pinna: a model for studying the role of CD8+ T cells in mice

Poster at 7th Annual NICHQ (National Initiative for Children's Healthcare Quality) Forum, Miami, Florida, March 2008

- Title: Lifestyle Behaviors as Validated Measures of Pediatric Overweight and Obese Populations

Poster at UT Southwestern 46th Annual Medical Student Research Forum, Dallas, Texas, January 2008

- Title: Lifestyle Behaviors as Validated Measures of Pediatric Overweight and Obese Populations
- Awarded one of eight prizes for best poster

Oral Presentation at CRTP Scientific Presentations, NIH, Bethesda, Maryland, May 2010

- Title: Peptide protection of the pinna: a model for studying the role of CD8+ T cells in mice

Oral Presentation at STARS Summer Research Internship Program Symposium, Yale University, New Haven, Connecticut, August 2002

- Title: Functional MRI and Electrophysiologic Recordings of Absence Seizures in Rats

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LIST OF ABBREVIATIONS

Tg, transgenic
DTg, double transgenic
K14, keratin-14
mOVA, membrane-bound chicken ovalbumin
sOVA, soluble chicken ovalbumin
SIINFEKL/OVAp, chicken ovalbumin peptide 257-264
TCR, T-cell receptor
MHC, major histocompatibility complex
E16, embryonic day 16
F1, filial 1, first filial generation of animal offspring from cross mating two parental types

CHAPTER ONE

INTRODUCTION

Autoimmunity results when the immune system fails to control autoregulatory processes and cannot differentiate self from foreign antigens. Autoimmune diseases may be T-cell- or B-cell-mediated and may target specific tissue antigens directly through T-cell responses or antibodies. Identifying molecular target antigens has been a major challenge in studying autoimmune disorders, including those with known antigens, such as pemphigus vulgaris and bullous pemphigoid.[1] Ultimately, the difficult goal is to develop antigen-specific therapies.[2]

Autoreactive CD8+ T cells play a critical pathogenic role in organ-specific autoimmune diseases.[3, 4] Their mechanism of action has been studied in various diseases affecting the skin, including alopecia areata, psoriasis vulgaris, vitiligo, and cutaneous lupus erythematosus.[5] Previous attempts have been made to utilize self-peptides in the induction of antigen-specific tolerance[6-8] and to study how defective T-cell receptor (TCR) surface expression or signaling correlate with CD8+ T-cell tolerance.[9] Using a diabetic mouse model, Bercovici et al found that administration of an agonist peptide in the early neonatal period disrupted the progression of autoimmune diabetes due to down-regulation of autoreactive CD8+ T cells through apoptosis-induced cell death (AICD).[10] Injection of soluble hemagglutinin peptide into double transgenic (DTg) autoimmune diabetic mice on days 3-5 after birth resulted in prolongation of survival of the mice via a CD8-mediated process.

Transgenic mouse models are essential in evaluating epithelial-derived cognate antigens and their respective T cells and receptors. Autoimmune diseases that have been studied using mouse models include Goodpasture's, multiple sclerosis, experimental encephalomyelitis, and diabetes.[11] Self-antigen studies in mice have examined effector mechanisms of tissue destruction in these animals.[12]

To evaluate the function of CD8+ autoreactive T cells, our lab generated a double transgenic (DTg) mouse model of spontaneous autoimmunity by crossing K14-sOVA mice, which express soluble chicken ovalbumin (OVA) under control of the keratin-14 promoter, with OT-1 mice, whose CD8+ T cells express Vα2/Vβ5 chains of the T cell receptor (TCR) and are specific for the SIINFEKL peptide (OVA 257-264) in association with class I MHC antigens.[13, 14] The K14 promoter drives gene expression in thymic epithelial cells, as well as in basal cells of the epidermis, tongue, and esophagus.[15, 16] Chicken ovalbumin is a peptide commonly used in transgenic mice as a model self-antigen.

Our lab previously reported that when K14-sOVA (#15) mice, which express high levels of OVA, were mated with OT-1 mice, 83% of the K14-sOVA/OT-1 F_1 pups died due to multi-organ inflammation.[17] Administration of soluble OVA peptide resulted in a dose-dependent increase in survival of the K14-sOVA/OT-1 (#15) DTg mice through down-regulation of peripheral CD8+ T cells, CD8+ coreceptor, and the Vα2 chain of the T-cell receptor. In the current study, we evaluate the K14-sOVA/OT-1 (#5) and K14-sOVA/OT-1 (#17) mice and investigate the role of antigen-specific immunotherapy in modifying the disease in these mice. The two strains exhibit high levels of OVA peptide in ear skin, with the #17 model demonstrating slightly higher levels. Otherwise, the K14-

sOVA/OT-1 (#5 and #17) strains can be considered equal, and both demonstrate selective tissue destruction of the external pinnae due to inflammatory infiltration of CD8+ T-cells during the first few days of life when left untreated. The aim of this study was to elucidate the mechanism of selective tissue destruction and to attempt to prevent that inflammation and subsequent loss of pinnae. We observed that administration of self-peptide at critical time points in the prenatal and neonatal period eliminated the inflammation-producing OT-1 cells, allowing for normal development of ears in the DTg mice.

CHAPTER TWO

MATERIALS AND METHODS

Mice

All mice used in this study were maintained on a 12-h light-dark cycle with free access to feed and water in a clean animal facility (mouse hepatitis virus- and pinworm-free). Animals were handled, bred, and used in accordance with institutional policies and with prior approval by the Animal Care and Use Committee of the National Institutes of Health. K14-sOVA Tg mice were generated similarly to the membrane-bound K14-mOVA mice previously described [13], but without the PDGF-receptor transmembrane domain, myc, and hemagglutinin sequences. The K14-sOVA (#5) and K14-sOVA (#17) were crossed with OT-1 mice ($OVA_{257-264}$-specific, class-I restricted TCR transgenic mice) to generate sOVA/OT-1 (#5 and #17) double transgenic mice. OT-1 mice were obtained from Dr. J. Kapp (Emory University, Atlanta, GA).

Timed pregnancies

Pregnancies were timed by limiting the period of mating to 24 h (classifed as day E0-E1) between K14-sOVA (#5 or #17) female and OT-1 male mice. Mice were separated by gender after that period and observed until first sign of pregnancy, roughly day E14. Birth was considered day 0.

Quantitative real-time PCR

Total RNA was extracted from various tissues using RNeasy Plus Kit (QIGEN, Clarita, CA) according to the manufacturer's instructions. Q-RT-PCR mixtures were assembled employing iScript One-Step RT-PCR Kit With SYBR Green, and Q-RT-PCR was performed in a Chromo4 RT-PCR machine (Bio-Rad, Hercules, CA). The following primer pairs were used: mGAPDH, forward primer 5' CGT GTT CCT ACC CCC AAT GT 3', reverse primer 5' TGT CAT CAT ACT TGG CAG GTT TCT 3'; OVA, forward primer 5' GGC ATC AAT GGC TTC TGA GAA 3', reverse primer 5' CCA ACA TGC TCA TTG TCC CA 3'.

CD8 T-cell depletion

250 μg of the anti-CD8 monoclonal antibody YTS169.4 (1 mg/mL, Serotec) was injected i.p. into pregnant K14-sOVA (17) mice on days E14 (embryonic day 14), E16, and E18. The 250 μg dose was determined in previous trials to sufficiently deplete CD8 T-cells.

Peptide treatment

SIINFEKL peptide (monomeric OVAp, composition Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu) was synthesized by PolyPeptide Group (San Diego, CA) and certified as 99.3% pure by HPLC. Initial studies involved i.p. injection of 100μg to 1 mg SIINFEKL peptide in 1x PBS (Life Technologies) into pregnant mice. In later studies, SIINFEKL peptide (1 mg/mL in PBS) was injected i.v. (200 μg) on days E14, E16, and E18 into pregnant K14-sOVA (#5) and K14-sOVA (#17) mice. Pups of these litters were subsequently injected i.p. (50 μg) on postnatal days 2 and 4.

Immunofluorescence microscopy

Ear buds from sOVA/OT-1 (#17) pups were harvested, frozen in Neg 50 medium (Thermo Scientific), and stored in -20° C. Cryosections were cut at 6 μm thickness and mounted onto silane-prep slides (Sigma). Prior to staining, slides were left in room temperature for 30 min to defrost. For immunofluorescence staining, slides were blocked with 3% skim milk-PBS + 5% goat serum, rinsed in PBS, and incubated with any of the following primary antibodies: anti-mouse rat CD8a (2 μg/mL), purified anti-mouse CD4 mAb (2 μg/mL), isotype rat IgG2a (2 μg/mL), or isotype mouse IgG2b (2 μg/mL). After three PBS washes, secondary antibody (Alexa Fluor 568 anti-rat goat IgG or Alexa 488 anti-mouse rabbit IgG) was added. The slides were again washed in PBS x 3 before being mounted with Prolong Gold with Dapi (Invitrogen). Images were viewed with a fluorescence microscope (Zeiss MicroImaging).

Flow cytometry

Single-cell suspensions were prepared from either pooled ear buds or pooled spleens of sOVA/OT-1 (#5 and #17) mice and resuspended in RPMI with 5% fetal bovine serum. RBC were lysed using ACK lysing buffer (Invitrogen). Cell counts were obtained manually with trypan blue exclusion. For cell surface staining, FITC-conjugated Vα2 (B20.1.1), PE (phycoerythrin)-conjugated Vβ5 (MR9.1), and APC (allophycocyanin)-conjugated anti-CD3, CD8, CD25, CD44, CD62L, CD69, and isotype control antibodies were used (BD Pharmingen). Stained cells were evaluated on a FACSCalibur (BD Biosciences, San Jose, CA) and analyzed using Flo Jo software (Tree Star Inc, Ashland, OR).

Histopathology

Embryonic mice and neonates were decapitated, and whole head samples were fixed in 10% neutral-buffered formalin. Paraffin-embedded tissues were sectioned and stained with H&E using standard techniques (American Histolabs).

Clinical score

Ear development of sOVA/OT-1 (#5 and #17) pups was assessed and put into the following categories by a blinded grader: no ears, partial ears, and fully formed ears. This procedure was standardized for all pups evaluated.

CHAPTER THREE

RESULTS

K14-sOVA/OT-1 (#5 and #17) F_1 double transgenic pups develop normally, except for the bilateral loss of pinnae

The K14-sOVA (#5 and #17) strains were generated similarly to the Tg mice described in Shibaki et al, but without the PDGF-receptor transmembrane domain.[13] When these mice were crossed with OT-1 mice, the progeny developed normally but underwent a destructive process selectively targeting the outer ear. The gross and photomicrographic images of DTg mice on days 1-9 after birth are illustrated in Figure 1. At day 2, there was hyperemia of dermal vasculature, followed on day 3 by epidermal necrosis and pustular intraepidermal bullae formation. This progressed to involve both cranial and lateral pinnal epidermis, and underlying facial epidermis, over the following 2-3 days. By day 6 or 7, an inflammatory cleft had formed between the lateral surface of the pinna and adjacent skin, which adhered distally on days 7-8 and closed by dermal adhesion on day 9. The cranial epidermal surface regenerated on days 7-9, while the accompanying inflammatory dermal event resulted in dysplastic chondroplasia and permanent disfiguration of the external ear. Histology of tongue, esophagus, and trunk skin of K14-sOVA/OT-1 (#5 and #17) mice on days 4, 8, and 14 after birth indicated that the inflammatory process was limited to the ears (Figure 2).

Figure 1. Development of K14-sOVA/OT-1 (#5 and #17) DTg mouse ear on days 1-9 after birth. DTg mice developed normally until birth, after which the pinnae (external ear auricles) underwent degeneration, with bullous lesions at the dermal-epidermal junction associated with epidermal necrosis and leukocyte infiltration. This degenerative process progressed over days 3-6, resulting in loss of epidermis on the pinnal surface, and eventually, loss of external ears by day 10. Photomicrographs are of K14-sOVA/OT-1 (#17) mice, oriented with the mouse's crown to the right side of each image and ventral (anterior) aspect to the left, with one representative ear shown for each day of age. Day 1 was defined as the first day after birth. H&E stain. (Bars = 100 μm)

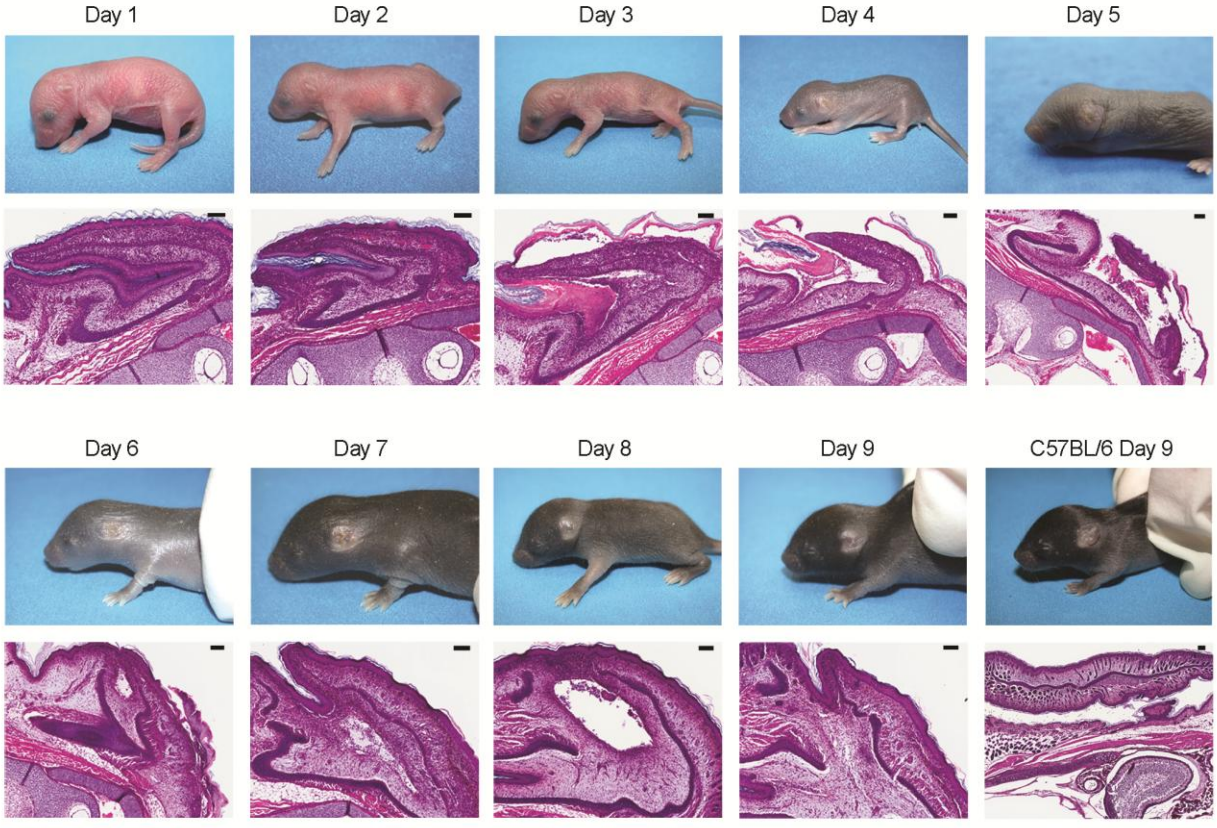
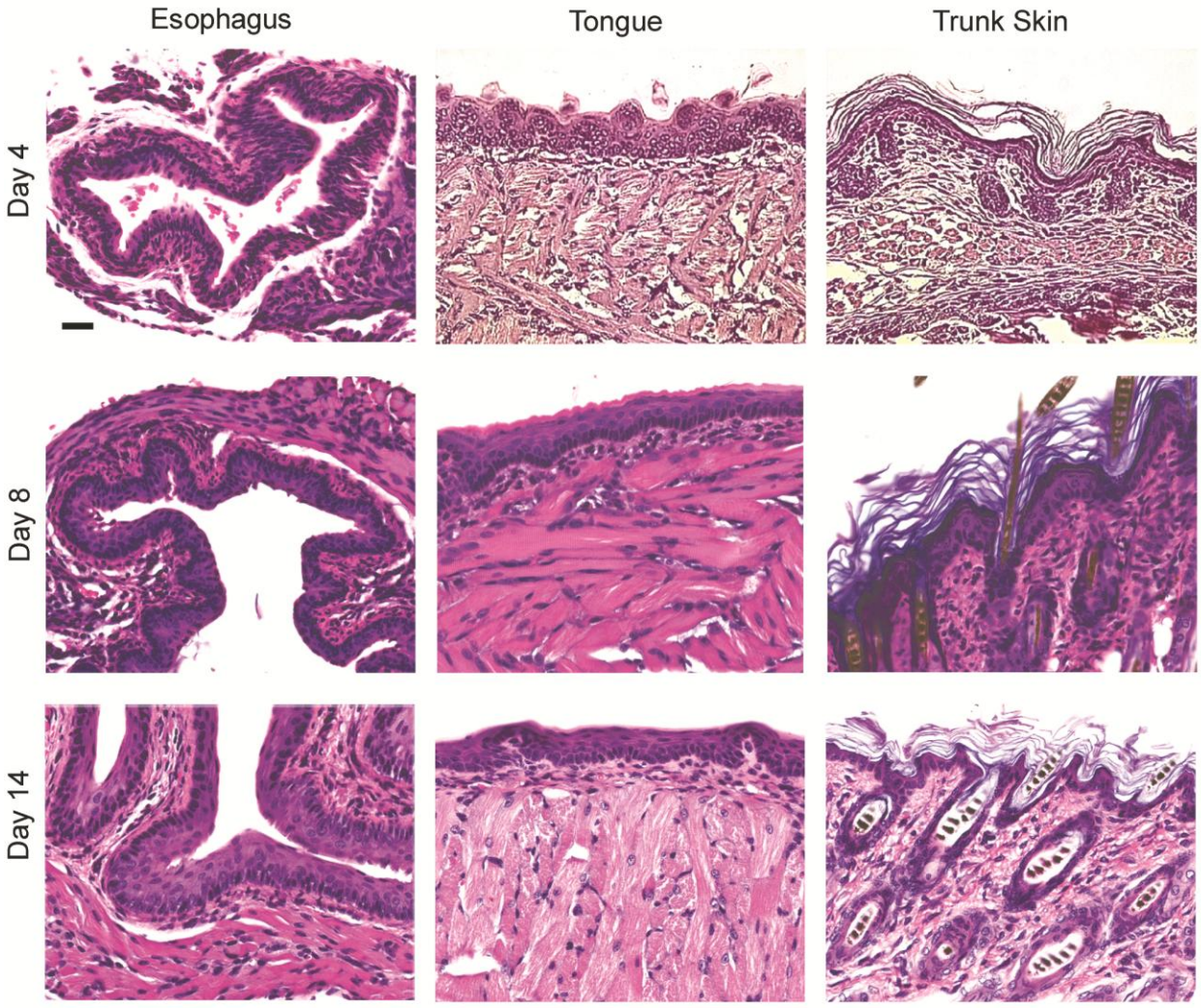


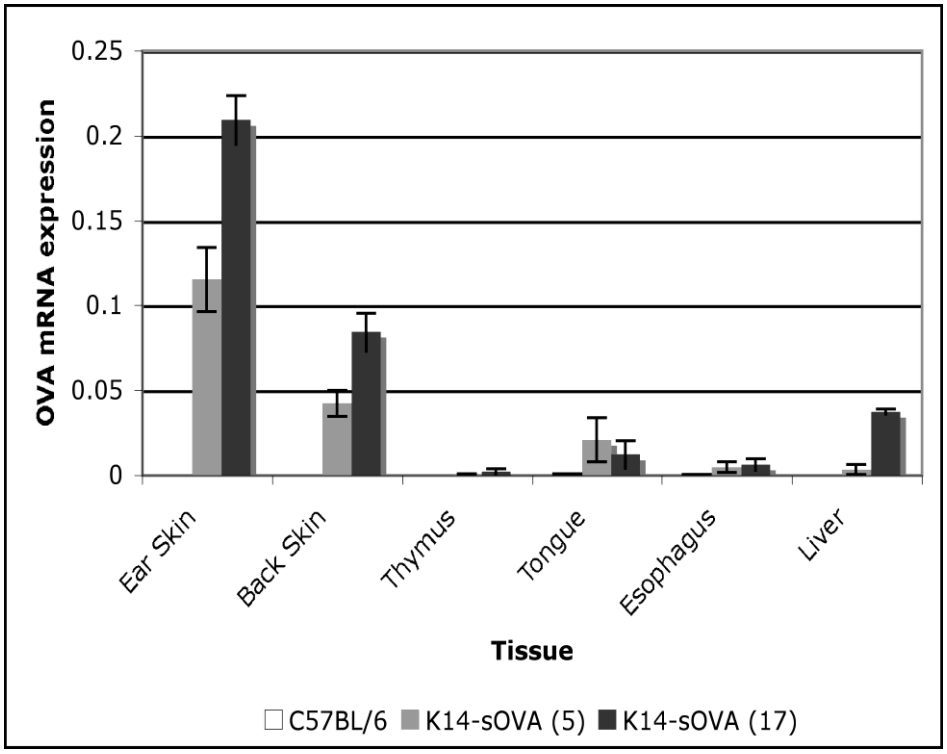
Figure 2. Histology of esophagus, tongue, and trunk skin of K14-sOVA/OT-1 (#5 and #17) mice. H&E sections obtained at day 4, 8, and 14 after birth revealed a lack of inflammation. Photomicrographs are of K14-sOVA/OT-1 (#5) mice. (Bar = 25 μm)



Ear skin of K14-sOVA (#5 and #17) expressed high levels of OVA mRNA

To determine why the inflammation was limited to the ears in K14-sOVA/OT-1 (#5 and #17) mice, the mRNA level of OVA in different tissues was determined. Cell suspensions were prepared from ear skin, back skin, thymus, tongue, esophagus, and liver of wild-type C57BL/6 and K14-sOVA (#5 and #17) Tg mice. OVA transgene mRNA expression levels were quantified by real-time PCR with mGAPDH as the housekeeping gene (Figure 3). Ear skin exhibited the highest levels of OVA mRNA expression compared to other tissues examined. Thus, the earless phenotype in the sOVA/OT-1 (#5 and #17) mice corresponded with the level of mRNA expression.

Figure 3. OVA mRNA expression levels in different tissues from C57BL/6 and K14-sOVA (#5 and #17) adult Tg mice. The OVA transgene mRNA expression levels were quantified by real-time PCR with mGAPDH as the housekeeping gene. The average of two mice from each group is shown.



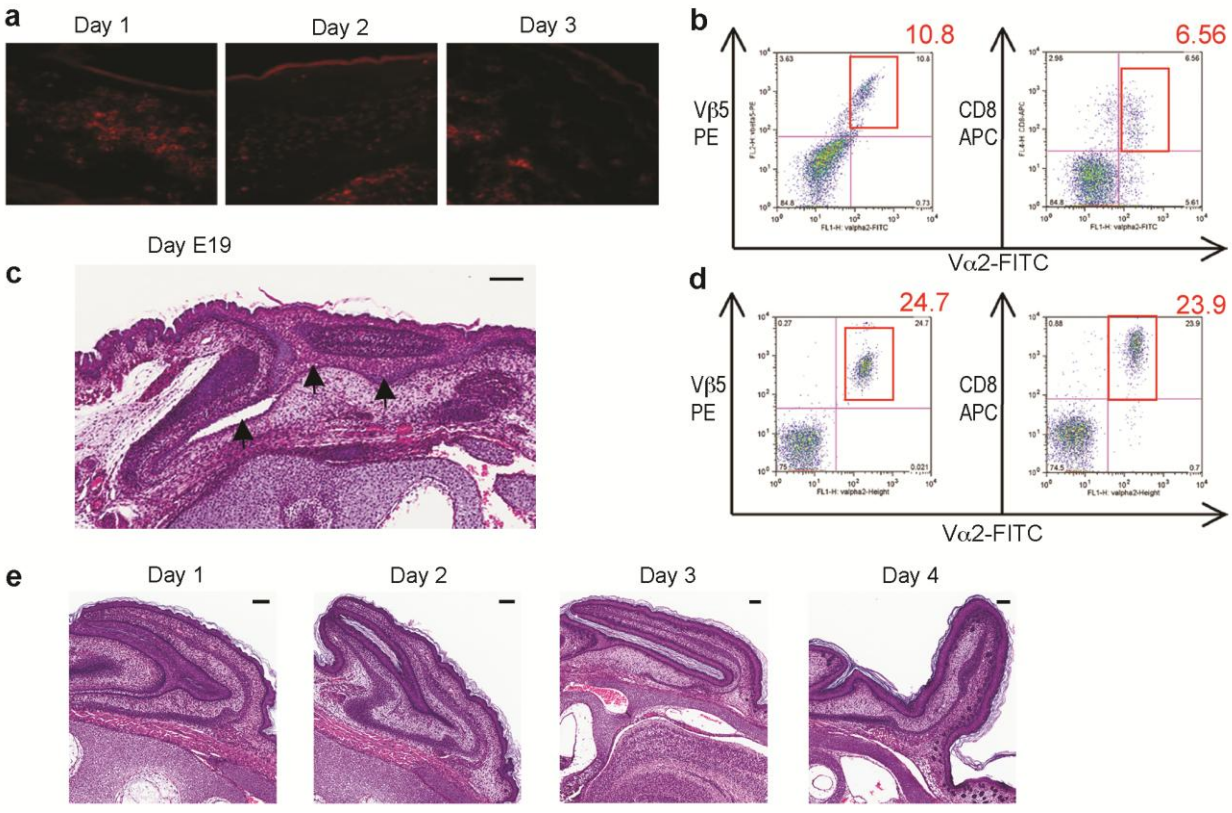
Inflammatory infiltrates in K14-sOVA/OT-1 DTg ear buds were comprised of CD8+ T-cells and were present before birth

To determine whether inflammation in the ear was caused by endogenous OT-1 cells, cryosections taken from ear buds of DTg pups on days 1, 2, and 3 after birth were stained for CD8 (Figure 4a). Immunofluorescence exhibited a collection of CD8-positive cells in the dermis on all three days. FACS analysis of cell suspensions from ears of day 5 K14-sOVA/OT-1 DTg pups demonstrated that these CD8-positive cells were also Vα2/Vβ5-positive (10.8%), indicating again that OT-1 cells infiltrated the ear (Figure 4b). The cryosections were also stained for CD4 but were negative for this cell type (data not shown).

Since inflammatory infiltrates were present in ear buds as early as day 1, we further evaluated K14-sOVA/OT-1 (#5 and #17) mice on days E18 (embryonic day 18) and E19, immediately prior to birth, to determine whether the process had begun *in utero*. A photomicrograph of the external ear taken during embryonic development day 19 (E19) revealed morphologic evidence of a zone of coordinate, epidermal keratinocyte programmed cell death (Figure 4c, middle and right arrows) that eventually led to the opening of the external auditory canal (Figure 4c, open space at left arrow) and the ultimate creation of lateral pinnal and facial skin surfaces. These surfaces re-epithelialized, freeing the pinna as an appendage from the rest of the head. The epidermal structure was the same topographical region of epidermolysis manifest by 3 days of age in K14-sOVA/OT-1 mice. Older mice expressed resolved re-epithelialized skin over misshapen chondrified cartilage and dilated external auditory canals (not shown). FACS analysis of spleens of K14-sOVA/OT-1 (#5 and #17) pups at day E18 demonstrated cells

that were positive for Vα2 and Vβ5 (11.4 to 24.7%), among which 10.8 to 23.9% were also positive for CD8 (Figure 4d). These Vα2/Vβ5^{+}CD8^{+} (OT-1) cells are likely responsible for eventual tissue destruction.

Figure 4. Inflammatory infiltrates in ears of K14-sOVA/OT-1 (#5 and #17) mice. (a) Immunohistochemical staining of ear buds of DTg mice at age day 1-3. Frozen sections were stained with anti-mouse rat CD8a and Alexa 568-goat anti-rat IgG at 1:200. (b) FACS analysis of cell suspensions from ears of day 5 DTg pups digested with liberase CI prior to staining and analysis. (c) Ear histology of DTg mice at day E19 (embryonic day 19). Arrows represent zone of epidermal keratinocyte programmed cell death; open space at left arrow is precursor to external auditory canal. (Bars = 50 μm) (d) FACS data of spleens of DTg mice at day E18 with Vα2/Vβ5-positive TCR (range 11.4-24.7%) and CD8+ T-cells (range 10.8-23.9%). (e) Histology of ears of DTg mice treated with anti-CD8+ T-cell depleting antibody. (Bars = 100 μm)



Administration of anti-CD8+ depleting antibody in utero resulted in normal ear development

To definitively prove that the CD8+ T cells caused the destruction of the pinna and that the destructive process in K14-sOVA/OT-1 (#5 and #17) DTg mice began before birth, three doses of the CD8+-depleting antibody (clone YTS169.4) were administered i.p. to pregnant K14-sOVA (#5 and #17) mice on days E14, E16, and E18 of gestation. All pups subsequently born to these treated mothers exhibited normal ear development and lacked the inflammatory infiltrates that were present in untreated K14-sOVA/OT-1 pups (Figure 4e, compare with Figure 1). This result indicates that $V\alpha2/V\beta5^{+}CD8^{+}$ OT-1 cells are responsible for tissue destruction of the ear.

SIINFEKL peptide treatment rescued the ear phenotype in K14-sOVA/OT-1 (#5 and #17) mice

Knowing that treating K14-sOVA/OT-1 (#5 and #17) mothers with anti-CD8+ depleting antibody was effective in preventing the destructive process, we determined whether similar results could be obtained using the TCR-recognized SIINFEKL peptide (OVAp) to target OT-1 cells specifically. Previous studies have shown that the SIINFEKL peptide induced a dose-dependent increase in survival of K14-sOVA/OT-1 (#15) mice with multi-organ inflammation.[17] Initial i.p. injections of pregnant K14-sOVA (#5 and #17) mice with 100 µg of peptide had no effect on ear inflammation and destruction, but repeated larger doses (300 µg to 1 mg) resulted in some pups being born with partial or full ears (Table 1 and Figure 5a). Since i.p.-injected peptide may have a variable distribution, we administered i.v. injections of 200 µg peptide on days E14, E16, and E18

and obtained a larger percentage of eared mice (Table 1, Figure 5b). The inconsistency of phenotype reversal appeared to be due to the persistence of selective ear tissue destruction after birth. Therefore, in addition to the i.v. dosing, we continued to administer SIINFEKL peptide to pups at 50 µg i.p. after birth. Reliably, 100% of pups that received SIINFEKL peptide i.v. in utero and i.p. after birth developed normal-looking ears (Table 1, Figure 5c). Complete prevention of tissue destruction in K14-sOVA/OT-1 (#5 and #17) mice was obtained with the optimal injection schedule shown in Figure 6: i.v. 200 µg SIINFEKL peptide on days E14, E16, and E18, followed by i.p. 50 µg peptide on days 2 and 4 after birth. These peptide-treated mice did not experience ear loss as adults, indicating that peptide treatment at a critical time period permanently reversed the ear destruction (data not shown).

Table 1. SIINFEKL peptide treatment of K14-sOVA/OT-1 (#5 and #17) mice. DTg pups treated with self-peptide only in utero inconsistently developed ears, whereas pups treated both i.v. in utero and i.p. after birth with OVAp developed normal-looking ears 100% of the time.

Method	Total pups	Full / Partial ears	No ears
i.p. in utero (100 μg-1mg)	62	14 (23%)	48 (77%)
i.v. in utero (200 μg)	36	14 (39%)	22 (61%)
i.v. in utero (200 μg) + i.p. after birth (50 μg)	29	29 (100%)	0 (0%)
i.p. after birth (50 μg)	30	16 (53%)	14 (47%)

Figure 5. Photos of peptide-treated K14-sOVA/OT-1 (#5 and #17) DTg mice with partial and full ears. Mice received SIINFEKL peptide as follows:

(a) i.p. only

(left panel) day 11 littermates: 500 μg in utero

(right panel) day 11 pup: 50 μg on day 2 and 4;

(b) i.v. only

(left panel) day 17 littermates: 200 μg in utero

(right panel) day 24 mouse: 200 μg in utero;

(c) i.v. + i.p.

(left panel) day 14 pups: i.v. 200 μg in utero + i.p. 50 μg on days 2, 5, 8

(right panel) day 33 mice: i.v. 200 μg in utero + i.p. 50 μg on days 2 and 4

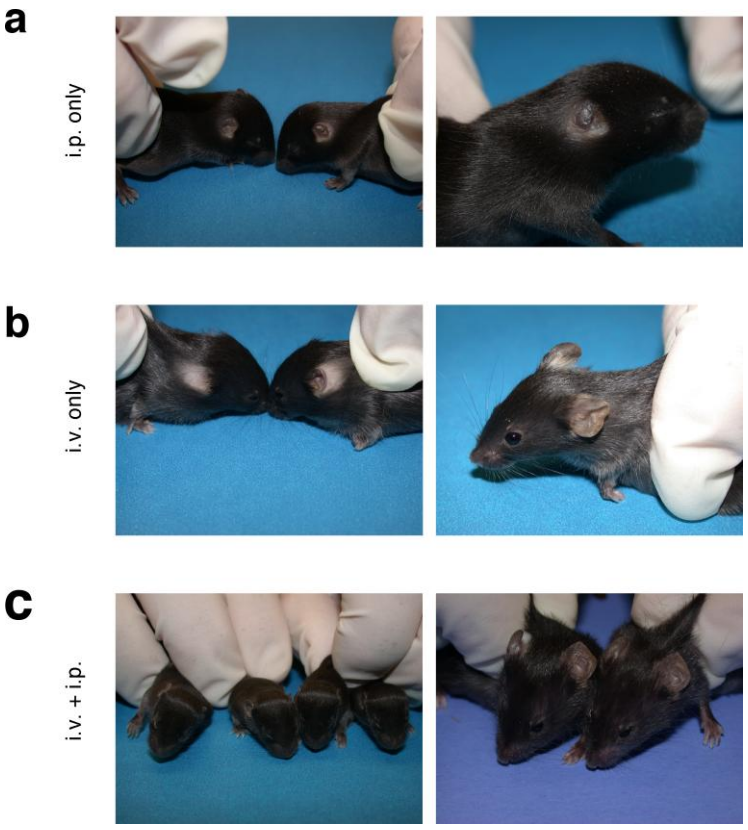
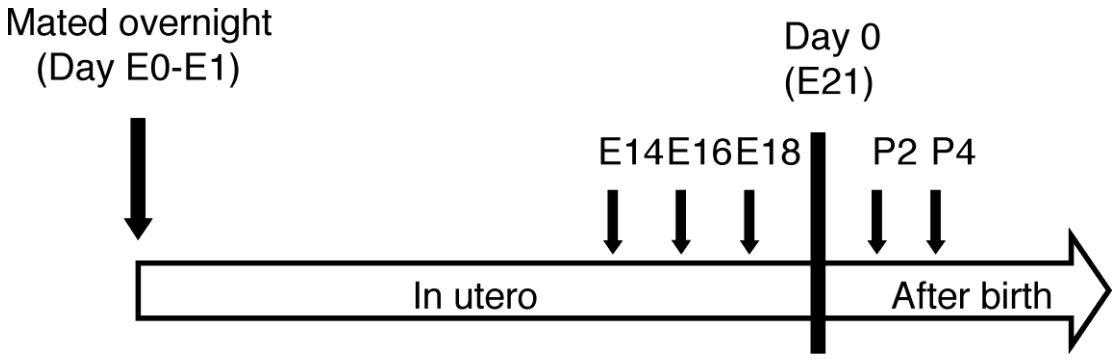


Figure 6. Optimal schedule of peptide injections into K14-sOVA/OT-1 (#5 and #17) DTg mice. Following a 24 h mating period, females were separated and observed for pregnancy until day E14. Pregnant mice were then injected i.v. with SIINFEKL peptide (200 μg) on days E14, E16, and E18. Pups of these litters were also injected i.p. with SIINFEKL (50 μg) on days 2 and 4 after birth.



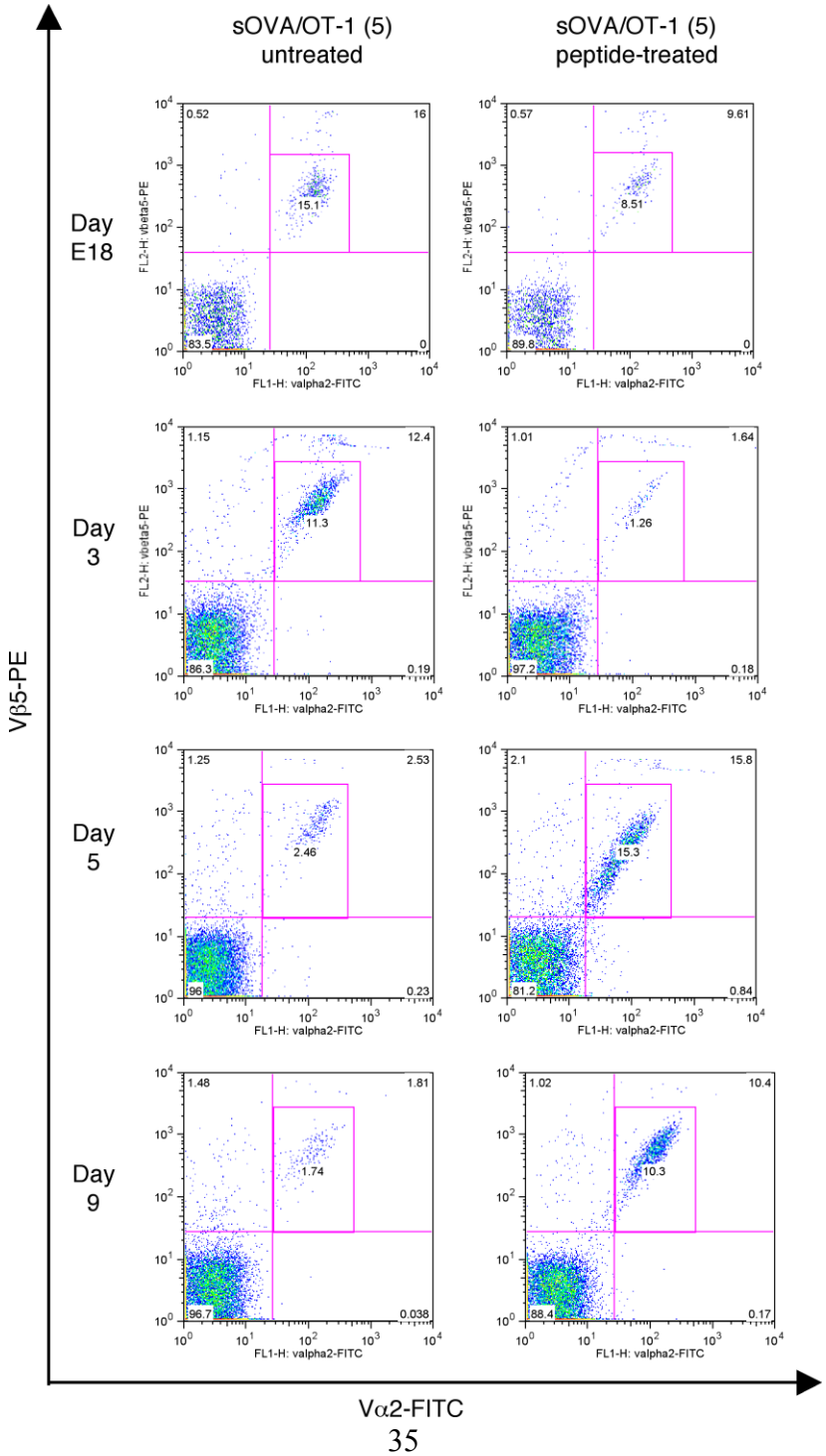
Peptide treatment markedly reduced OT-1 cell numbers at early time points

To determine the mechanism of phenotype reversal in our mouse model of tissue-specific autoimmunity, we analyzed the effect of peptide treatment in the spleen as a secondary lymphoid organ. Spleens from K14-sOVA/OT-1 (#5) untreated and K14-sOVA/OT-1 (#5 and #17) pups treated with the optimal peptide dosing schedule (Figure 6) were harvested on days E18, 3, 5, and 9 after birth, pooled into single cell suspensions, and stained with Vα2, Vβ5, and CD3. Mice evaluated at days 5 and 9 continued to receive i.p. 50 μg of peptide on days 4 and 6.

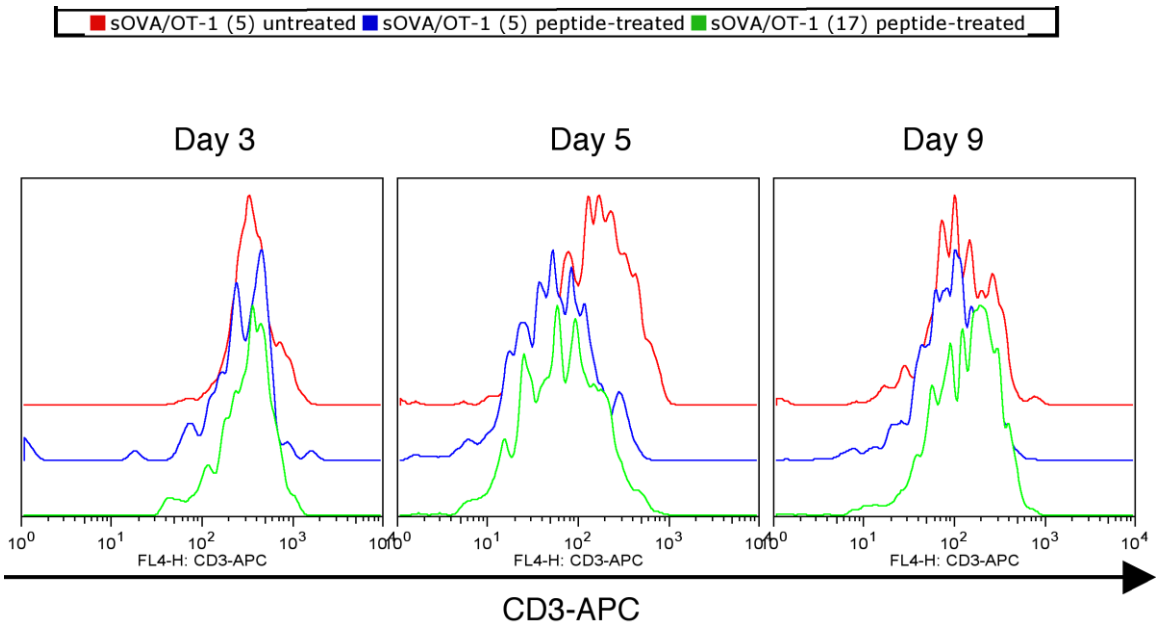
A decrease in the number of Vα2/Vβ5-positive OT-1 cells in spleens of peptide-treated K14-sOVA/OT-1 (#5) mice compared to untreated mice on day E18 and day 3 after birth was observed, indicating that peptide injection markedly decreased OT-1 cell numbers in the spleen. Interestingly, the percentage of OT-1 cells in peptide-treated mice increased on days 5 and 9, whereas it decreased in untreated K14-sOVA/OT-1 (#5) mice (Figure 7a). These changes were not associated with differences in total number of splenocytes (data not shown). The increase in OT-1 cells at days 5 and 9 may be due to the expansion of peripheral CD8+ T-cells following peptide injection, as observed in Bercovici et al.[10] However, this proliferation does not result in immune inflammatory changes or ear destruction.

Figure 7. Elimination of OT-1 cells at earlier time points following peptide treatment. FACS analysis of spleens of untreated and peptide-treated K14-sOVA/OT-1 (#5 and #17) pups harvested on days E18, 3, 5, and 9 and stained for (a) Vα2/Vβ5 (OT-1 cells) and (b) CD3. Pups were treated i.v. with 200 μg SIINFEKL peptide on days E14, E16, and E18 and i.p. 50 μg peptide on days 2, 4, and 6, as appropriate.

(a)



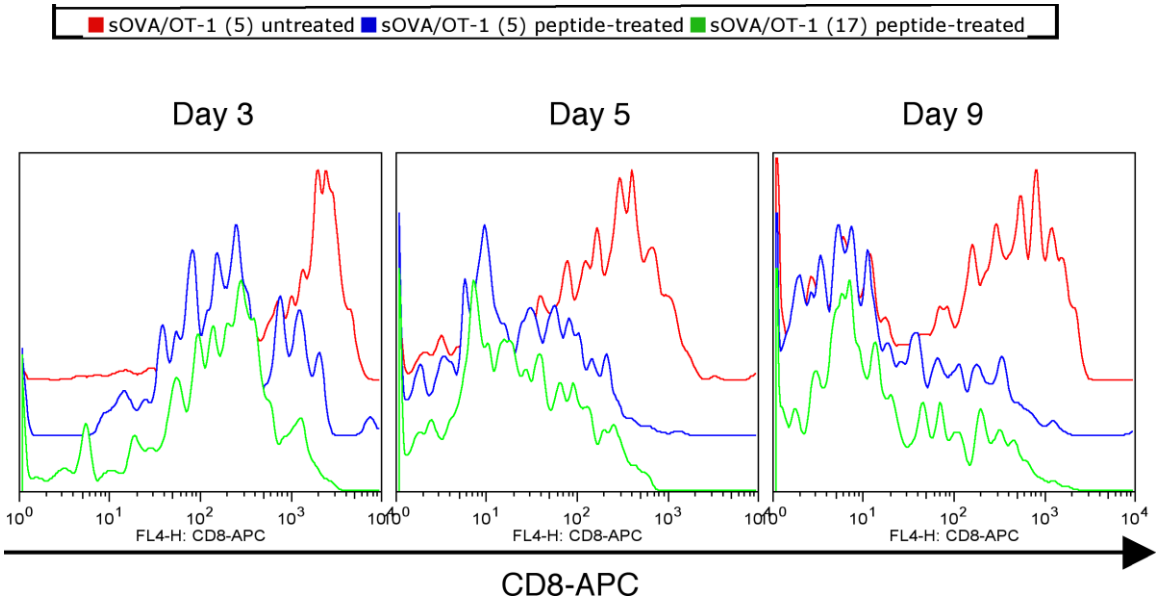
(b)



Peptide treatment induced down-regulation of the CD8 coreceptor

Spleens of untreated K14-sOVA/OT-1 (#5) and peptide-treated K14-sOVA/OT-1 (#5 and #17) mice were harvested on days 3, 5, and 9 and stained for CD8. The mean fluorescence intensity (MFI) of CD8 at all time points in OT-1 cells of peptide-treated DTg mice was lower than that in untreated mice, suggesting that CD8 was down-modulated upon peptide injection (Figure 8).

Figure 8. Down-regulation of the CD8 coreceptor after peptide treatment. Spleens harvested and pooled from untreated K14-sOVA/OT-1 (#5) and peptide-treated K14-sOVA/OT-1 (#5 and #17) mice on days 3, 5, and 9 were stained for CD8. Mean fluorescence intensity of CD8 in OT-1 cells was lower at all time points in peptide-treated mice compared to untreated mice.



CHAPTER FOUR

DISCUSSION

In this report, the development and evaluation of a mouse model of CD8+ T cell-mediated autoimmunity and the potential for antigen-specific immunotherapy are presented. Destruction of ears of K14-sOVA/OT-1 (#5 and #17) double transgenic pups was obviated by self-peptide treatment, which eliminated $V\alpha2/V\beta5^{+}CD8^{+}$ OT-1 cells that participate in tissue destruction and down-regulated the CD8 coreceptor at early time points in the postnatal period.

Although we initially thought that Vα2Vβ5 CD8+ T cells in our double transgenic (DTg) mouse model would be tolerized in utero, we were surprised to find that spontaneous autoimmunity ensued in these mice. There are examples in the literature that support the findings herein described.[10, 18] DTg systems are preferred models to study peripheral immune tolerance of activated CD8+ T cells that have escaped thymic deletion and are involved in ongoing disease processes. Mechanisms of peripheral tolerance include clonal anergy, reduced surface expression of TCRs and CD8 T-cell deletion, immune deviation, and T-cell receptor editing.[19, 20] In clonal anergy, after encountering antigen, T cells rapidly enter the cell cycle, express early activation markers, and enter a state of hyporesponsiveness with decreased proliferation. CD8-specific T cells can further undergo peripheral clonal deletion after antigen administration, resulting in either complete or partial loss of memory T cells. Autoreactive T cells have been stimulated to undergo deletion mediated by apoptosis in peptide treatment of animal models of

experimental autoimmune neuritis and encephalomyelitis.[21, 22] Finally, a shift from Th1 to Th2 response occurs in tolerance due to mechanisms of immune deviation.

In our peptide-treated DTg mice, down-regulation of Vα2/Vβ5+ T-cells and the CD8 coreceptor appear to be two mechanisms by which soluble peptide therapy exerts its protective effects. Without the CD8 coreceptor, the corresponding autoreactive CD8 T-cells are unable to engage their targets. Additionally, OT-I cells were activated by peptide treatment, which may be important for tolerance induction, and CD3 and CD8 TCR were down-regulated as well.

Targeted therapy utilizing soluble peptides against T cell populations has become a topic of interest in immunology.[23] This transgenic mouse model is informative with regard to peripheral tolerance mechanisms and may be used to study localized CD8-mediated skin diseases where there is intense superficial dermal infiltration by T cells as in lichen planus and fixed drug eruptions. The pathogenesis of both these conditions is unclear, but studies have demonstrated involvement of intraepidermal CD8 T-cells.[24-27] Our model may also provide insight into potential therapeutic vaccines for children at high-risk for development of tissue-selective autoimmune diseases, including diabetes and multiple sclerosis.

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VITAE

So Yeon Paek was born in South Korea on May 17, 1984, the oldest of two children, to Ki Son and Dul Nyon Paek. Her family immigrated to Brownsville, Texas, when she was four years old. She attended Gonzalez Elementary (where she skipped the 4th grade), Vela Middle School, and South Texas High School for Health Professions magnet high school in Mercedes, Texas, where she graduated valedictorian in May 2001. She then attended Yale University and graduated with a B.S. in Molecular Biophysics and Biochemistry in 2005. After college, she decided to gain some life experience and consequently became an ESL (English as a Second Language) teacher in her hometown adult literacy center for one year before matriculating at UT Southwestern Medical School as a M.D. candidate in 2006. To gain knowledge of translational research and confirm her desire to enter academic medicine, So Yeon committed a year after third-year clerkships for a research fellowship in the Clinical Research Training Program (CRTP) at the National Institutes of Health in Bethesda, Maryland. She worked under the guidance of Dr. Stephen I. Katz in the Dermatology Branch of the National Cancer Institute where she gained valuable skills and insight into academic careers. She returned to UT Southwestern in 2010 to finish medical school and will graduate with a Doctorate of Medicine with Distinction in Research in June 2011. So Yeon plans to combine her passion for research, teaching, and patient care by pursuing a career as an academic dermatologist.