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Cutaneous denervation of psoriasiform mouse skin improves acanthosis and inflammation in a sensory neuropeptide dependent manner

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Abstract

Nervous system involvement in psoriasis pathogenesis is supported by increases in nerve fiber numbers and neuropeptides in psoriatic skin and by reports detailing spontaneous plaque remission following nerve injury. Using the KC-Tie2 psoriasisform mouse model, we investigated the mechanisms by which nerve injury leads to inflammatory skin disease remission. Cutaneous nerves innervating dorsal skin of KC-Tie2 animals were surgically axotomized and beginning 1d following denervation, $CD11c^+$ cell numbers decreased by 40% followed by a 30% improvement in acanthosis at 7d and a 30% decrease in $CD4^+$ T cell numbers by 10d. Restoration of SP signaling in denervated KC-Tie2 skin prevented decreases in $CD11c^+$ and $CD4^+$ cells but had no affect on acanthosis; restoration of CGRP signaling reversed the improvement in acanthosis and prevented denervated-mediated decreases in $CD4^+$ cells. Under innervated conditions, small molecule inhibition of SP in KC-Tie2 animals resulted in similar decreases to those observed following surgical denervation for cutaneous $CD11c^+$ and $CD4^+$ cell numbers; whereas small molecule inhibition of CGRP resulted in significant reductions in $CD4^+$ cell numbers and acanthosis. These data demonstrate that sensory nerve-derived peptides mediate psoriasiform dendritic cell and T cell infiltration and acanthosis and introduce targeting nerve-immunocyte/keratinocyte interactions as potential psoriasis therapeutic treatment strategies.

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Conflict of Interest

The authors state no conflict of interest.

Introduction

Psoriasis is a complex, multifaceted disease whose pathogenesis has not been fully elucidated. Whereas great advances have been made in understanding the roles of the immune system and the epidermal components of this skin disease, the contribution of the cutaneous neural system still remains under explored. Numerous observations suggest that the nervous system may play a role in this disease including the symmetrical distribution of plaque on the body, likely reflective of peripheral sensory nerve-interactions with immunomodulatory networks (Farber *et al.*, 1986); the observation that emotional stress is often linked to either onset and/or exacerbation of psoriasis (Seville, 1977); the increase in nerve fibers and their secreted neuropeptides in involved psoriatic skin (for review (Saraceno *et al.*, 2006)), and the striking finding that psoriasis undergoes remission following nerve transection, nerve injury, or decreased nervous system functioning (Dewing, 1971; Farber *et al.*, 1990; Joseph *et al.*, 2005; Perlman, 1972; Stratigos *et al.*, 1998).

Published animal models of psoriasis have failed to report increases in cutaneous nerve numbers and/or density or changes in neuropeptide expression (Gudjonsson *et al.*, 2007) resulting in limited opportunity to study the role of nerves and their derived factors in preclinical models of psoriasis. We recently reported the generation and characterization of a KC-Tie2 murine model of psoriasisform skin disease, based upon keratinocyte-specific expression of the angiopoietin receptor Tie2 (Wolfram *et al.*, 2009). Although Tie2 is not normally expressed in human KCs, ectopic expression of Tie2 in KCs initiates cellular events resulting in a skin phenotype that resembles many characteristics of human psoriatic disease including the increased presence of $CD4^+$ and $CD8^+$ T cells, $CD11c^+$ and $F4/80^+$ myeloid derived cells, a psoriasis-like cytokine and chemokine profile, and responsiveness to clinically efficacious therapeutics including CsA (Wolfram *et al.*, 2009) and TNFα inhibition (Ward *et al.*, 2010b). Moreover, genomic expression changes in involved KC-Tie2 mouse skin highly correlate with genomic expression changes identified in involved human psoriatic skin (Swindell *et al.*, 2010). Recently, we reported the increased presence of cutaneous nerves in KC-Tie2 mouse skin (Ward *et al.*, 2010a), thereby providing a unique preclinical model to test the contributions and significance of the cutaneous neural cell population in sustaining psoriasisform skin inflammation.

Results

KC-Tie2 mice have increased neuropeptide expression in skin and dorsal root ganglia

We previously reported increases in cutaneous nerve fiber numbers in KC-Tie2 mouse skin (Ward *et al.*, 2010a). Using qRT-PCR, we examined neuropeptide mRNA expression levels in KC-Tie2 mouse skin and identified a 4.1-fold increase in SP ($p=0.07$) and a 9.2-fold increase in NPY ($p<0.001$; Figure 1a) and no differences in expression of VIP, somatostatin, or CGRP. However, skin-derived neuropeptide expression does not reflect nerve-specificity as other skin cells synthesize these molecules. Nerve-derived factors are synthesized in the neuronal cell body (located in the dorsal root ganglion, DRG) and are anterogradely transported to the skin where they are secreted and exert their effects; therefore DRGs from the thoracic region of KC-Tie2 and littermate control animals were dissected and mRNA expression of the same neuropeptides was examined. No statistically significant changes in

VIP, NPY or somatostatin expression were found, however expression of SP (3.7-fold, $p<0001$) and CGRP were significantly increased in KC-Tie2 mice (3.6-fold, $p<0.0002$; Figure 1b).

Surgical cutaneous denervation results in loss of PGP9.5$^+$ nerves and significant improvement of acanthosis in KC-Tie2 animals

We developed surgical denervation procedures transecting the thoracic-level cutaneous nerves at their entry site into back skin. Using this approach, one side of mouse back skin was denervated, while the contralateral side underwent sham surgery, leaving the nerves intact (Figure S1a–c), allowing the direct comparison of the psoriasiform phenotype in the presence or absence of nerves in the same animal. A complete loss of detectable PGP9.5$^+$ nerve fiber immunofluorescence staining was apparent beginning 7d post-denervation and remained apparent until 10d (Figure S1c). Analyses was limited to 10d post-denervation, as beyond 10d, PGP9.5$^+$ nerve staining reappeared, likely reflective of collateral reinnervation through sprouting of nerves from adjacent dermatomes (Kinnman, 1987), a finding consistent with what others have reported (Siebenhaar *et al.*, 2008). Western blotting confirmed the decrease in PGP9.5 protein levels 7–10d following denervation (Figure S1d) although small residual levels of PGP9.5 protein remained, most likely due to expression by non-neural tissues, including dendritic cells (DCs) (Stankovic *et al.*, 1999) and Merkel cells (Casasco *et al.*, 1990).

Ten days following unilateral surgical denervation, KC-Tie2 mouse skin showed a 34% decrease ($p<0.0001$) in acanthosis on the denervated side compared to the contralateral innervated control side (Figure 2) consistent with published reports detailing the spontaneous remission of psoriasis in humans following loss of cutaneous nerve innervation (Dewing, 1971; Farber *et al.*, 1990; Joseph *et al.*, 2005). Moreover, a marked reduction in the cellular proliferation marker, Ki67 was observed in keratinocytes of denervated KC-Tie2 skin compared to sham operated skin (Figure 2e–f).

Immunocytes and IL-23 protein expression are reduced in denervated skin

To identify potential mechanisms underlying the improvement in acanthosis following denervation, we examined changes in immune cell presence and angiogenesis between innervated and denervated skin. Ten days following surgical denervation, the number of cutaneous macrophages and mast cells remained unchanged between sham operated and denervated skin (Figure S2a–b). However a decrease (16%, $p=0.01$) in the number of cutaneous CD4$^+$ T cells was identified (Figure 3a–d). Despite this decrease, IFNγ, IL-17 and IL-6 protein expression remained constant in denervated skin as compared to sham skin (Figure 3e–g).

The most striking observation was a 50% decrease in the number of dermal CD11c$^+$ cells in denervated skin, representing a return to levels comparable to innervated littermate (non-KC-Tie2) controls (Figure 4a–d). DCs are potent producers of IL-23, and ELISA analyses revealed a 33% decrease in IL-23 protein expression in whole skin lysates isolated from denervated KC-Tie2 mouse back skin compared to sham operated innervated skin (Figure 4e).

Examination of the dermal vasculature revealed no changes in the number of dermal blood vessel numbers (Figure S3a) or in IGF-1 or VEGF protein (Figure S3b–c).

CD11c$^+$ cell number decreases precede improvements in acanthosis and reductions in CD4$^+$ T cell numbers

To determine the temporal sequence of events following surgical denervation, parallel cohorts of KC-Tie2 animals underwent surgical denervation on the same day and changes to acanthosis, CD11c$^+$ and CD4$^+$ cell numbers were quantitated 1, 3, 5, 7, and 10d following surgery. CD11c$^+$ cell numbers decreased significantly beginning 1d following surgery ($p=0.011$) and this decrease was sustained through 10d ($p=0.016$). Acanthosis improvements did not become significant until 7d ($p=0.019$) whereas CD4$^+$ cell numbers were significantly decreased 10d following denervation ($p=0.029$; Table S1).

SP and CGRP receptor agonist administration into denervated skin reverses CD11c$^+$ and CD4$^+$ T cell improvements and acanthosis in a neuropeptide specific manner

To identify the significance of SP and CGRP contributions to the denervation mediated outcomes observed in KC-Tie2 skin, intradermal injections of CGRP peptide, the SP receptor agonist (GR73632; peptide agonist of NK-1R) or PBS were administered into the region of denervated skin daily for the 10d period following denervation. Doses and administration routes were chosen based on previous reports showing *in vivo* effectiveness in mediating biological responses in skin (Fuller *et al.*, 1987; Niizeki *et al.*, 1999). Introduction of PBS or the SP receptor agonist (GR73632) into denervated skin failed to alter the denervated mediated improvement in acanthosis ($p=0.04$ sham vs denervated + PBS; $p=0.05$ sham vs denervated + GR73632); however introduction of CGRP peptide into denervated skin markedly blunted the effect of denervation on acanthosis. CGRP treated denervated skin was significantly thicker compared to denervated + PBS ($p=0.012$) and denervated + GR73632 treated skin ($p=0.013$; Figure 5a), however the reversal of acanthosis following CGRP peptide treatment was not absolute, as CGRP treated denervated skin remained slightly thinner than control sham operated innervated skin ($p=0.03$). Examination of Ki67 expression showed similar staining between sham and denervated skin treated with CGRP peptide (Figure S4e–f) but remained decreased in denervated skin treated with PBS or GR73632 (Figure S4a–d) compared with sham operated skin.

Examination of CD4$^+$ T cell numbers in denervated skin treated with PBS confirmed the reduction in T cell numbers following denervation ($p=0.001$). Following intradermal injections of CGRP peptide or the SP receptor agonist (GR73632), CD4$^+$ cell numbers returned to sham operated, innervated skin levels (Figure 5b).

CD11c$^+$ cell numbers in denervated skin were not affected by treatment with PBS ($p=0.01$) or with CGRP peptide ($p=0.034$); however administration of the SP receptor agonist GR73632 returned CD11c$^+$ cell numbers to sham operated innervated skin levels (Figure 5c).

SP and CGRP receptor antagonist administration to innervated skin mimics denervation-mediated improvements in immune cell infiltration and acanthosis in a neuropeptide specific manner

To confirm key functions for SP and CGRP in maintaining the psoriasiform skin phenotype in KC-Tie2 animals, we examined acanthosis and immune cell infiltration following treatment with either RP67580, a well characterized SP receptor antagonist (Arck *et al.*, 2003) or with $CGRP_{8–37}$, a well characterized selective CGRP receptor antagonist (Costa *et al.*, 2008; Legat *et al.*, 2004). Innervated KC-Tie2 mice were treated with RP67580 every other day or with $CGRP_{8–37}$ or PBS daily for a period of 30d, a timepoint we previously showed was sufficient to reverse acanthosis and inflammation (Ward *et al.*, 2010b; Wolfram *et al.*, 2009). Examination of the skin revealed no changes in epidermal thickness or Ki67 immunostaining after treatment with PBS or RP67580 but a significant improvement in acanthosis and decreases in Ki67 staining in mice treated with $CGRP_{8–37}$, reminiscent of what was observed following cutaneous surgical denervation ($p=0.009$; Figure 6a; Figure S4g–i). Examination of $CD4^+$ cell numbers revealed significant decreases in dermal T cell numbers in KC-Tie2 mice that were treated with either RP67580 ($p=0.03$) or $CGRP_{8–37}$ ($p=0.01$) but not PBS (Figure 6b). Finally, significant reductions in the $CD11c^+$ cell population were only observed following treatment with the SP receptor antagonist (RP67580; $p=0.001$) and not PBS or $CGRP_{8–37}$ (Figure 6c).

Discussion

Psoriasis has primarily been thought of as a disease driven by abnormal interactions between KCs and inflammatory immunocytes. However, clinical evidence suggests that the nervous system also plays a key role in psoriasis pathogenesis. Using the KC-Tie2 murine model of psoriasis, we confirmed significant increases in SP and CGRP mRNA in KC-Tie2 DRG, a finding supporting reports of increases in SP^+ and $CGRP^+$ sensory nerve numbers in involved skin of psoriasis patients (Saraceno *et al.*, 2006) and then developed an experimental approach resulting in loss of cutaneous innervation, therein allowing us to mimic several case reports describing spontaneous psoriasis remission following nerve injury (Dewing, 1971; Farber *et al.*, 1990; Joseph *et al.*, 2005). This loss of cutaneous innervation resulted in robust and reproducible improvements in acanthosis, decreases in $CD4^+$ T cells and an elimination of $CD11c^+$ cells concomitant with decreased IL-23 protein expression, thereby providing an experimental preclinical model mimicking case and anecdotal reports detailing spontaneous psoriasis remission following nerve injury.

The rapid decrease in $CD11c^+$ cells following denervation suggests that nerve derived factors signal directly to $CD11c^+$ cells in KC-Tie2 skin. Interestingly, in mouse models of neurogenic stress, reports indicate there are increased levels of SP and increased numbers of $CD11c^+$ DCs in the dermis (Joachim *et al.*, 2008). Thus it is tempting to speculate that signaling by SP in KC-Tie2 mouse skin contributes to cutaneous DC infiltration, and that denervation reduces $CD11c^+$ cell recruitment through downregulation of SP signaling. Supporting this idea were the findings that addition of the SP agonist (GR73632) under denervated conditions resulted in a restoration of the $CD11c^+$ population in the dermis and

that inhibition of SP signaling by treatment with the SP receptor-NK-1R antagonist (RP67580) lead to decreased $CD11c^+$ numbers in innervated KC-Tie2 mouse skin.

CGRP has well known effects on antigen presenting cells (APCs) and has been shown to inhibit antigen presentation through modulation of cAMP signaling, and to modulate cytokine production through inhibition of NFκB activation (Ding *et al.*, 2007; Hosoi *et al.*, 1993; Torii *et al.*, 1998). Our data demonstrate that introduction of exogenous CGRP to denervated KC-Tie2 skin failed to return $CD11c^+$ cell numbers back to levels observed under innervated conditions. Supporting this was the lack of effect on $CD11c^+$ DC numbers in innervated KC-Tie2 mouse skin following treatment with the CGRP receptor antagonist, $CGRP_{8–37}$, suggesting that in our model CGRP does not likely play a significant role in recruitment of $CD11c^+$ cells to the skin.

Interestingly while we observed significant decreases in acanthosis, we failed to see complete resolution of acanthosis, consistent with the idea that non-neural systems are significant contributors to sustaining disease. Alternatively it's plausible that with additional time beyond the 10d period, a more complete resolution in acanthosis could be observed; however analysis beyond the 10d time point was not feasible using the current model system due to collateral sprouting of adjacent nerves that re-innervate the skin. In both the SP inhibition and SP restoration experiments, SP had no effect on acanthosis, suggesting that while SP may serve as a key mediator of neural-DC interactions and sustainment of the psoriasiform phenotype in KC-Tie2 mice, other nerve-derived factors must contribute to resolution of acanthosis. Interestingly, animal experiments in which normal mouse skin was denervated have previously reported thinning of the epidermis and decreases in KC proliferation (Chiang *et al.*, 1998; Hsieh and Lin, 1999; Huang *et al.*, 1999) suggesting the potential for direct interaction between KC proliferation and nerve innervation, although contributions of the immune system were not examined. Direct interactions between nerves and KCs are supported by *in vitro* findings of others that demonstrate that CGRP is capable of directly influencing KC proliferation (Seike *et al.*, 2002; Yu *et al.*, 2009). This supports the idea that KC proliferation and epidermal hyperplasia present in psoriasis may occur as a direct effect of nerve-derived CGRP on the KCs themselves, and once the neural derived influences are lost following denervation, acanthosis improves as a result of less KC proliferation. The observation that exogenous CGRP introduction into denervated skin (and not SP) returned acanthosis and KC proliferation levels close to those observed in innervated KC-Tie2 skin provides support for this concept, and taken together with the receptor antagonist experimental findings demonstrating that CGRP (and not SP) inhibition in innervated skin resulted in significant decreases in acanthosis to the same degree as surgical denervation, identifies CGRP as a critical nerve-derived peptide capable of mediating KC proliferation and epidermal hyperplasia. While SP has been shown in some reports to stimulate proliferation of KCs in culture (Tanaka et al. 1988), we failed to see any SP-mediated effects on acanthosis or KC proliferation in our model. This may be due to a lack of SP effects on KC proliferation *in vivo*, or may reflect more complex interactions of SP with other peptides and cell types intrinsic to studying biological actions in an *in vivo* model system. While decreased acanthosis may be due to direct effects of nerve derived factors on keratinocytes, it also possible that acanthosis is secondary to immune modulation.

$CD4^+$ T cells decreased in number post axotomy only after decreases in $CD11c^+$ cell numbers and improvement in acanthosis were noted. Since both $CD11c^+$ cells and KCs produce pro-inflammatory cytokines that contribute to T cell infiltration and activation, such as IL-23 (Lee *et al.*, 2004; Zheng *et al.*, 2007) it's likely that both also contribute to the decrease in T cell numbers observed in denervated skin. Alternatively, nerve derived factors have the capacity to act directly on T cells; CGRP is capable of stimulating $CD4^+$ cell migration *in vitro* (Talme *et al.*, 2008), and SP can bind to its receptor, NK-1R which has been shown to be important for leukocyte accumulation in a model of TNFα induced cutaneous inflammation (Costa *et al.*, 2006). However, significant T cell decreases were not apparent until 10d following denervation, making a direct mechanism less likely in our model. The decrease in $CD4^+$ cells post denervation, albeit small was statistically different, however T cell-derived cytokines remained unchanged in their expression (IL-6, IFNγ, and IL-17), potentially explaining the lack of complete resolution of acanthosis in denervated skin. It remains possible that further decreases in T cell numbers and/or their derived cytokines would occur beyond the 10d period post-axotomy, resulting in further acanthosis resolution.

While SP and CGRP are some of the most well characterized nerve derived substances in psoriasis (Saraceno *et al.*, 2006) and in neurogenic inflammation (Arck *et al.*, 2006; Joachim *et al.*, 2008; Pavlovic *et al.*, 2008; Peters *et al.*, 2005; Peters *et al.*, 2007), other nerve derived compounds may also play roles. Norepinephrine, as one example, has been suggested to potentially play a role in skin inflammation (Saint-Mezard *et al.*, 2003), such that it can decrease differentiation of $CD4^+$ T cells and can regulate human DC and Langerhans cell functions in the skin (Goyarts *et al.*, 2008; Seiffert *et al.*, 2002). NGF, a keratinocyte derived growth factor is upregulated along with its receptors in human psoriatic skin (Raychaudhuri and Raychaudhuri, 2004), is critical at the early stages of plaque development, preceding T lymphocyte trafficking into the epidermis (Raychaudhuri *et al.*, 2008); and when inhibited can lead to resolution of skin inflammation in the SCID psoriasis mouse model (Raychaudhuri *et al.*, 2004). NGF can also increase the percentage of SP^+ and $CGRP^+$ nerve fibers in the DRG and skin (Joachim *et al.*, 2007). In established plaque from KC-Tie2 animals, NGF expression is unchanged compared to control animals (data not shown), despite increases in the number of cutaneous nerves and nerve-derived SP and CGRP. Future work examining the effects of denervation on the ability to initiate plaque formation using murine models of psoriasiform skin inflammation in which NGF is known to play a crucial role (such as the Imiquimod model (van der Fits *et al.*, 2009), may provide further insight into this bidirectional relationship.

In conclusion, we have demonstrated that the KC-Tie2 model contains neural alterations seen in psoriasis and that the epidermal and immunologic features of this model are dependent upon innervation of the skin. As a result of direct and indirect cellular interactions, ablation of nerve derived signals results in decreased DC numbers, IL-23 and T cell infiltration and improved acanthosis; similar to what we observed following Tie2 gene repression, CsA treatment, APC depletion and TNFα inhibition (Ward *et al.*, 2010b; Wolfram *et al.*, 2009), and what has been observed in human skin after efficacious treatment with TNFα inhibitors (Gottlieb *et al.*, 2005; Zaba *et al.*, 2007), anti-CD11a treatment

(Lowes *et al.*, 2005), anti-CD2 (Chamian *et al.*, 2005) and anti-IL12/23p40 (Griffiths *et al.*, 2010; Papp *et al.*, 2008) treatment. These pre-clinical findings provide evidence that the signals from the nervous system, specifically nerve derived SP and CGRP, sustain psoriasis, and that targeting the nervous system may be a therapeutic option.

Materials and Methods

Please refer to Supplementary Materials and Methods for a detailed and extensive description of experimental procedures used.

Transgenic mice and surgical axotomy of thoracic cutaneous nerves

All animal protocols were approved by the Case Western Reserve University institutional animal care and use committee and conformed to the American Association for Accreditation of Laboratory Animal Care guidelines. KC-Tie2 mice were generated as described previously (Wolfram *et al.*, 2009) and in detail in the Supplemental Methods. Cutaneous denervation surgery was adapted from previously described techniques (Siebenhaar *et al.*, 2008) and is described in detail in the Supplemental Methods.

In vivo neuropeptide inhibitor and agonist experimental approach

For SP and CGRP inhibitor experiments under innervated conditions and SP and CGRP agonist experiments under denervated conditions, two month old KC-Tie2 mice were used, and dosing and administration details were completed as detailed in the Supplemental Materials and Methods. For agonist and antagonist experiments, dosing and administration routes were chosen based on previous reports showing in vivo effectiveness in mediating biological responses in skin (Arck *et al.*, 2003; Costa *et al.*, 2008; Fuller *et al.*, 1987; Legat *et al.*, 2004; Niizeki *et al.*, 1999). For each animal, a pretreatment biopsy was taken, thus each animal served as its own control.

Tissue collection and histological and morphometric analyses

Tissue collection, H&E and toluidine blue staining and morphometric analyses were completed as previously (Wolfram *et al.*, 2009) and are described in detail in the Supplemental Methods.

Antibody detection, visualization and quantitation of $CD4^+$, $CD11c^+$, $F4/80^+$, MECA-32^+ and $Ki67^+$ cells was completed as before (Ward *et al.*, 2010b) and is described in detail in the Supplemental Methods.

Immunofluorescence against PGP9.5 was performed and quantitated as before (Ward *et al.*, 2010a) and is described in detail in the Supplemental Methods.

Protein isolation and analyses and RNA isolation and quantitation

Analyses of protein and RNA from back skin taken from adjacent areas of innervated and denervated skin from the same mice as used for histology and immunohistochemistry and immunofluorescence was completed as previously described (Wolfram *et al.*, 2009) and is described in detail in the Supplemental Methods.

Statistical analysis

All data are presented as mean ± standard error of the mean (SEM). Control vs KC-Tie2 animal data and comparison between different treatment groups was analyzed using an unpaired Student's t-test, whereas innervated vs denervated comparisons within the same animal were analyzed using a paired Student's t-test. Statistical significance was defined as $p<0.05$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

KC	keratinocyte
CsA	cyclosporin A
TNF	tumor necrosis factor
SP	substance P
CGRP	calcitonin gene related peptide
PGP9.5	protein gene product 9.5
NGF	nerve growth factor
VIP	vasoactive intestinal peptide
NPY	neuropeptide Y
DRG	dorsal root ganglion
mRNA	messenger ribonucleic acid
Tie2	Tunica interna endothelial cell kinase
d	days
IFN	interferon
IL	interleukin
DC	dendritic cell
ELISA	enzyme linked immunosorbant assay
NK-1R	neurokinin receptor 1
PBS	phosphate buffered saline

TNF	tumor necrosis factor
iNOS	inducible nitric oxide synthase
cAMP	cyclic adenosine monophosphate
NFkB	nuclear factor kappa B
tTA	tetracycline transactivator
Tet^{os}	tetracycline operator sequence

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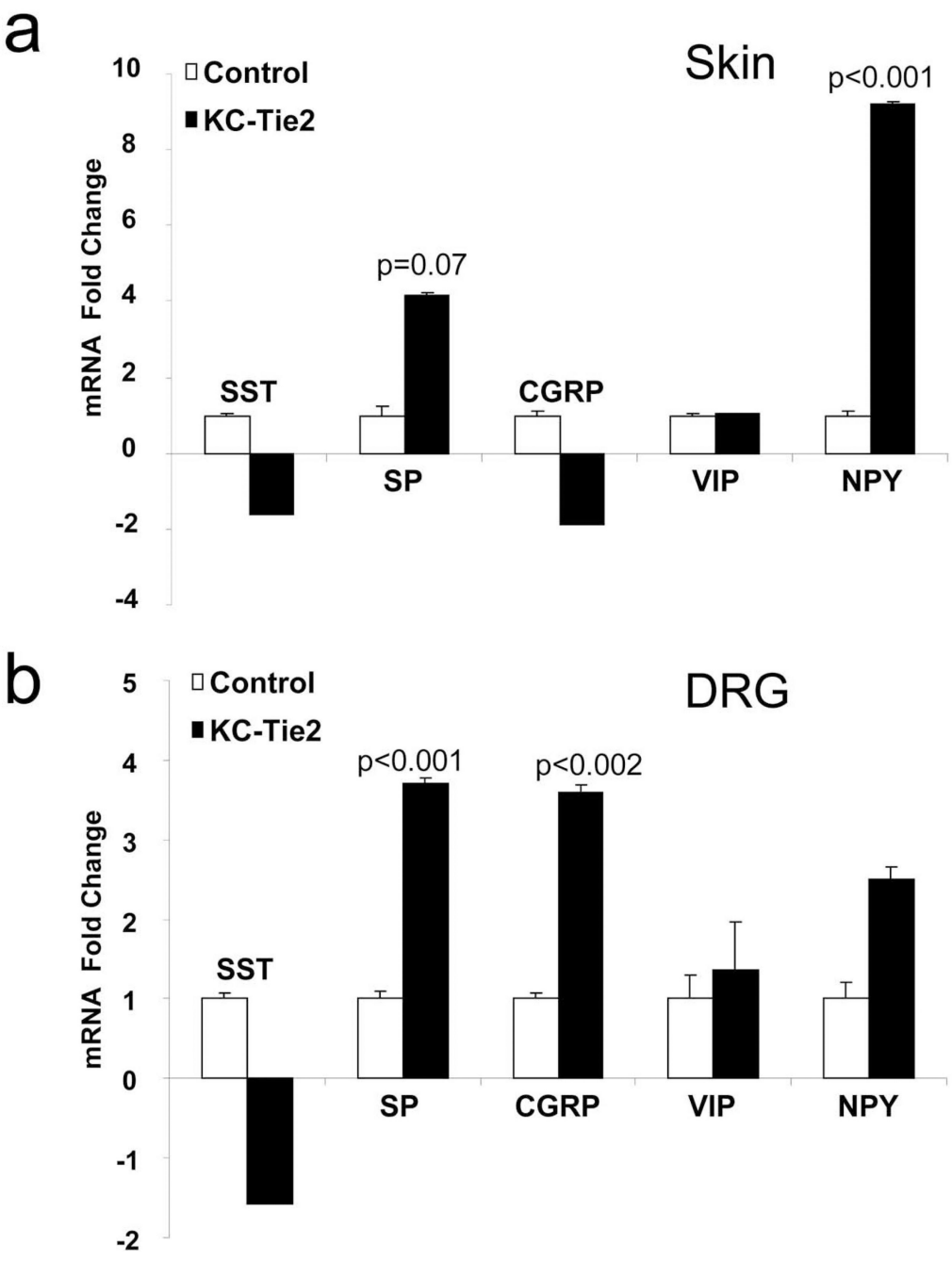

Figure 1. KC-Tie2 mice have increased expression of neuropeptides in skin and DRG than control mice

Real time PCR was performed using primers targeting the neuropeptides somatostatin (SST), SP (SP), calcitonin gene related peptide (CGRP), vasoactive intestinal protein (VIP) and neuropeptide Y (NPY). (a) KC-Tie2 animals (n=4) have increased levels of SP and NPY expression in whole back skin as compared to control animals (n=4) and (b) increased level of SP and CGRP expression in their dorsal root ganglia (DRGs) as compared to control animals. p values are as indicated.

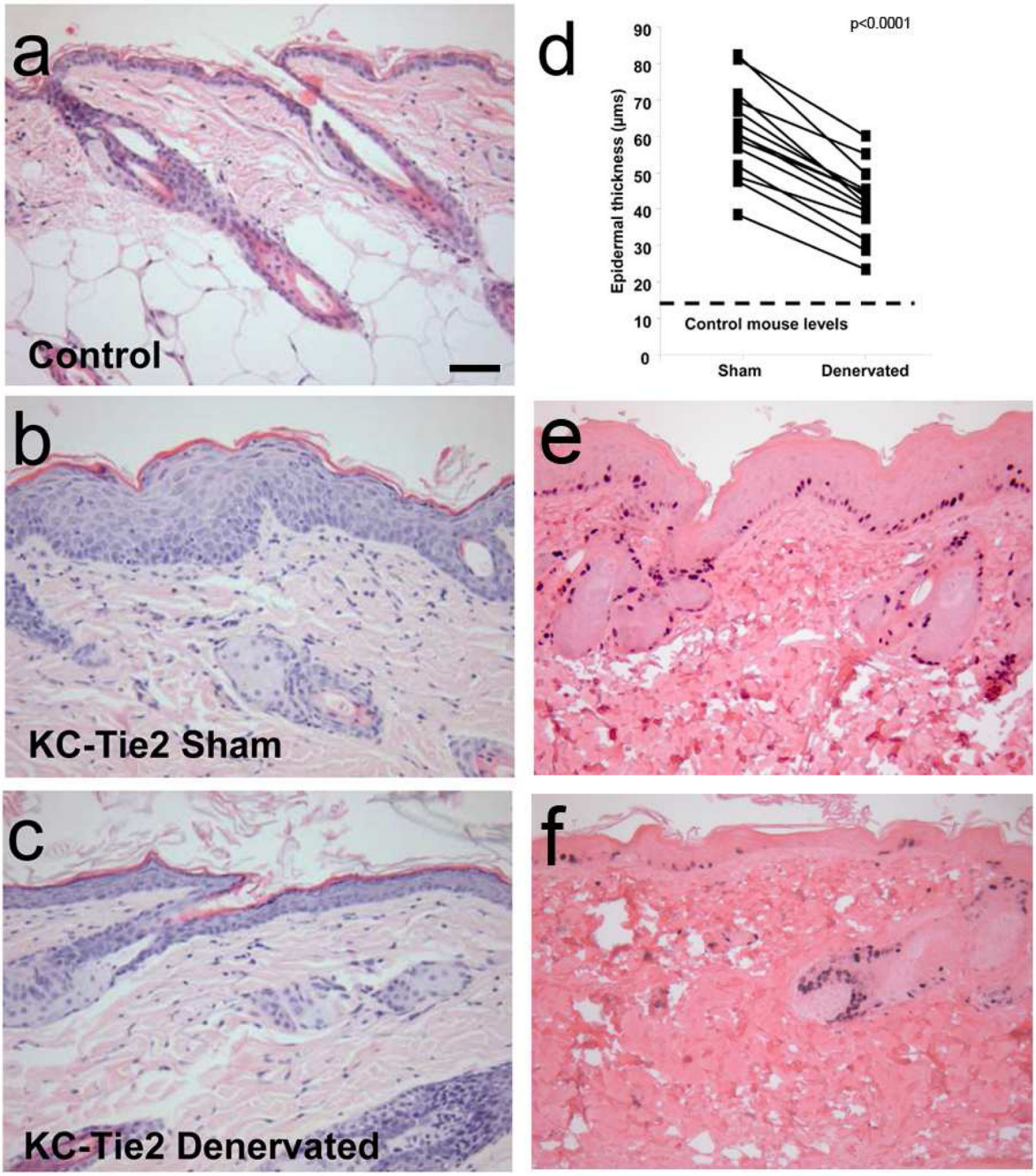

Figure 2. Surgical denervation of KC-Tie2 mouse skin results in decreased acanthosis

Representative images of H&E staining of sections of (a) control, (b) KC-Tie2 sham operated and (c) KC-Tie2 denervated back skin. (d) Epidermal thickness (in µM) of the sham operated side and the denervated side of back skin is presented for individual animals (n=13). The hatched line represents average epidermal thickness levels in innervated control mouse back skin. Representative images of Ki67 stained sections of (e) KC-Tie2 sham operated and (f) KC-Tie2 denervated back skin. p values are as indicated. Scale bar = 50µM.

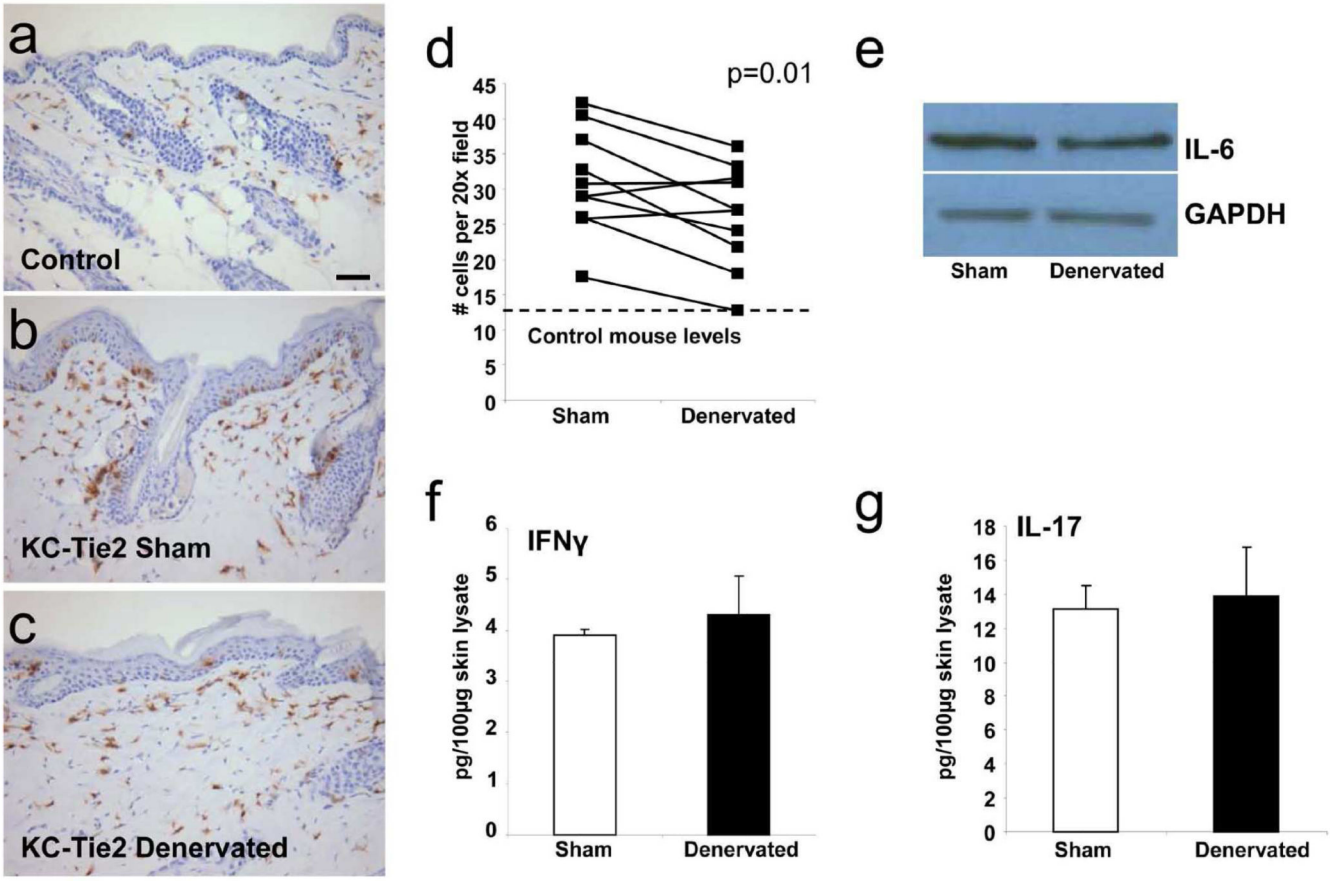

Figure 3. CD4$^+$ T cell numbers decrease in denervated skin while T cell derived cytokines remain unchanged

(a–c) Representative images of CD4 immunohistochemical staining in back skin sections of (a) control, (b) KC-Tie2 sham operated and (c) KC-Tie2 denervated back skin. (d) CD4$^+$ T cell numbers in sham operated skin and denervated skin are presented for individual animals and are significantly decreased in KC-Tie2 denervated mouse skin compared to sham operated skin (n=10). The hatched line represents average number of CD4$^+$ T cells in back skin of control mice. Western blotting (e) and ELISA (f–g) demonstrate no changes in IFNγ, IL-17 and IL-6 between sham operated and denervated KC-Tie2 mouse skin. p values are as indicated. Scale bar = 50μM.

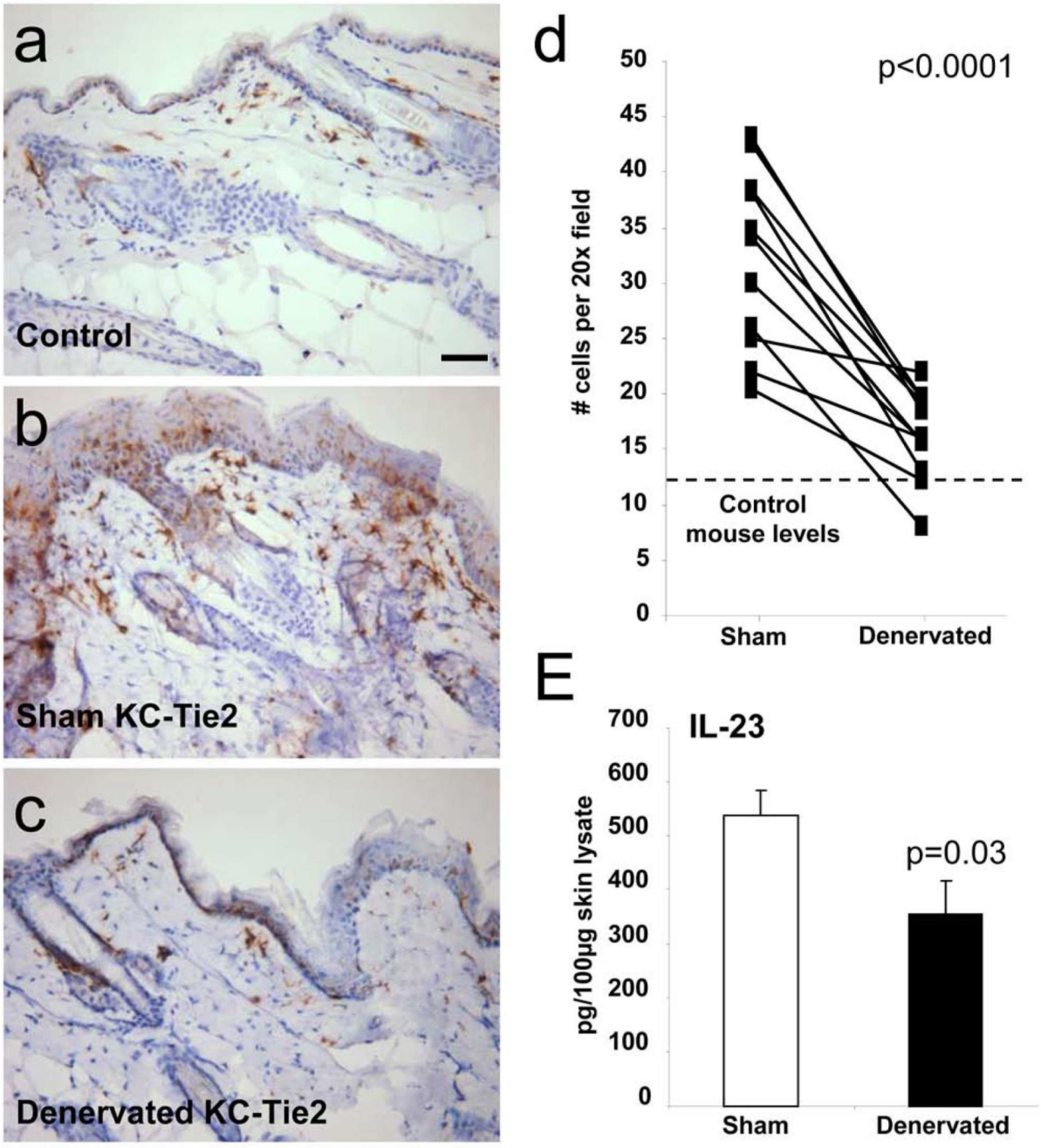

Figure 4. Surgical denervation of KC-Tie2 mouse skin reduces CD11c$^+$ dendritic cell numbers back to control mouse levels and decreases IL-23 protein expression

(a–c) Representative images of CD11c$^+$ immunohistochemical staining in back skin sections of (a) control, (b) KC-Tie2 sham operated and (c) KC-Tie2 denervated back skin. (d) CD11c$^+$ cell numbers in sham operated skin and denervated skin are presented for individual animals (n=11). The hatched line represents average number of CD11c$^+$ cells in back skin of control mice. (e) ELISA analysis of IL-23 protein expression demonstrates a significant decrease in denervated skin compared to sham operated skin in KC-Tie2 mice. p values are as indicated. p values are as indicated. Scale bar = 50µM.

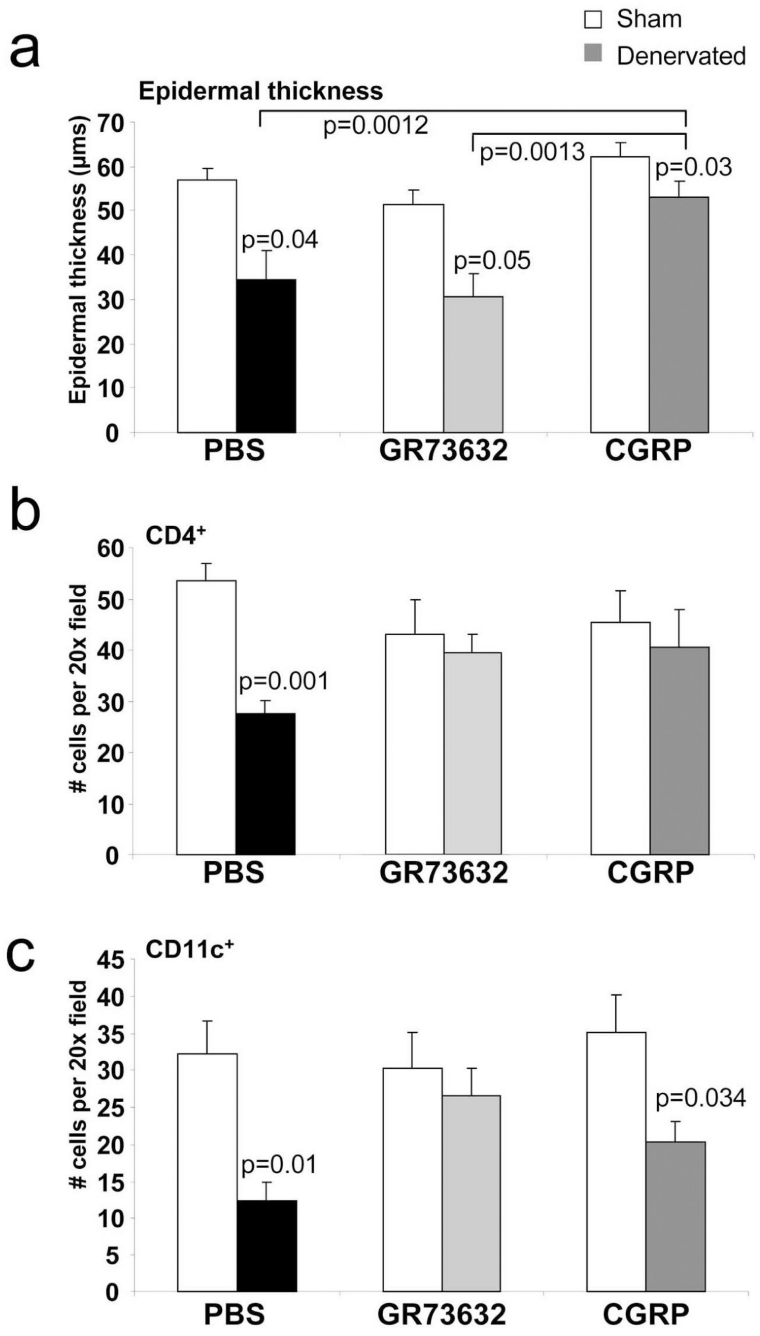

Figure 5. Restoration of neuropeptide signaling under denervated conditions returns CD11c$^+$ and CD4$^+$ cell numbers and acanthosis back to innervated conditions in a neuropeptide specific manner

Epidermal thickness (a), CD4$^+$ cell number (b), and CD11c$^+$ numbers (c) are presented for cohorts of animals (n=4 per group) that had PBS, SP receptor agonist (GR73632), or CGRP peptide injected intradermally into the denervated side of the back skin daily beginning one day after surgery. p values are as indicated.

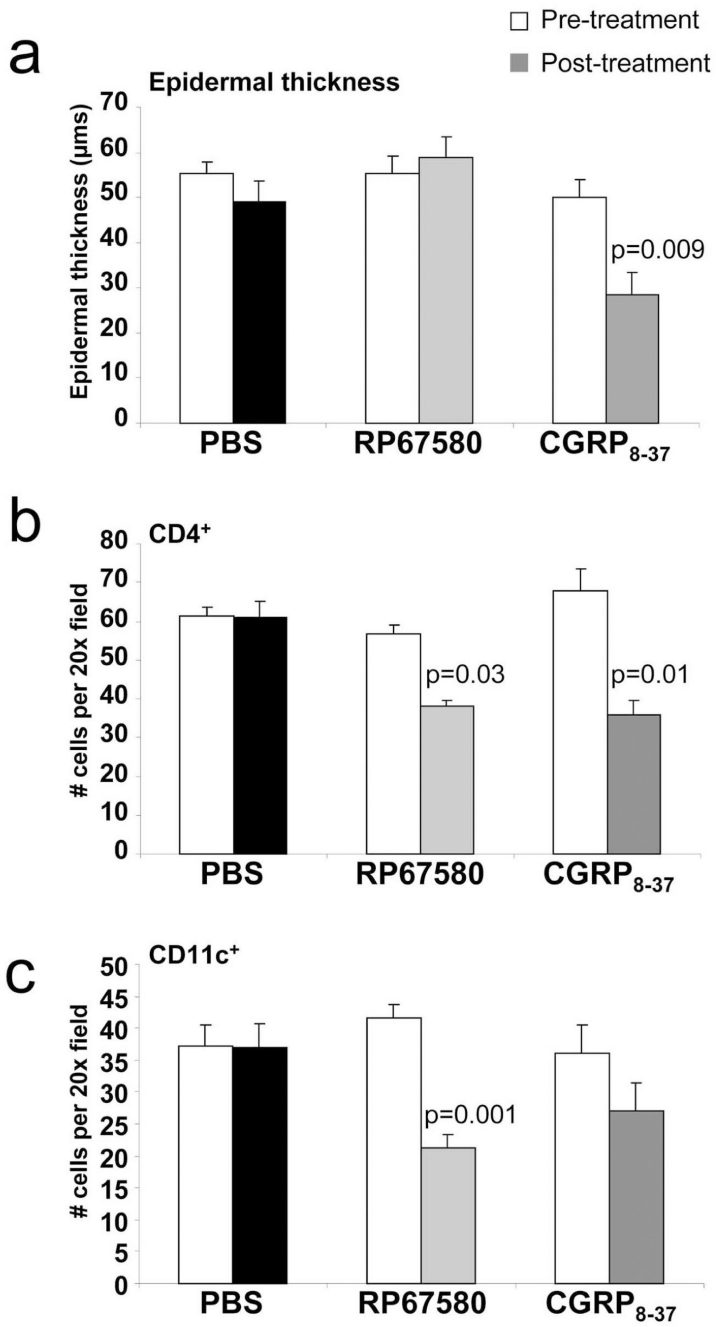

Figure 6. Inhibition of SP or CGRP activity under innervated conditions mimics denervated-mediated changes to acanthosis and CD11c$^+$ and CD4$^+$ cell numbers in a neuropeptide specific manner

Epidermal thickness (a), CD4$^+$ cell number (b), and CD11c$^+$ T cell number data (c) are presented for cohorts of animals (n=4–6 per group) prior to (pre-treatment) and after (post-treatment) 30 days of treatment with either PBS, the selective SP receptor NK-1R antagonist (RP67580), or the CGRP antagonist, $CGRP_{8–37}$. p values are as indicated.