Basophil-Derived Amphiregulin Is Essential for UVB Irradiation–Induced Immune Suppression

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UVB irradiation (290–320 nm) is used to treat skin diseases like psoriasis and atopic dermatitis, and is known to suppress contact hypersensitivity (CHS) reactions in mouse models. Regulatory T cells (T_{reg} cells) have been shown to be responsible for this UVB-induced suppression of CHS. The epidermal growth factor (EGF)-like growth factor amphiregulin (AREG) engages EGFR on T_{reg} cells and, in different disease models, it was shown that mast cell–derived AREG is essential for optimal T_{reg} cell function *in vivo*. Here we determined whether AREG has a role in UVB-induced, T_{reg} cell–mediated suppression of CHS reactions in the skin. Our data show that AREG is essential for UVB-induced CHS suppression. In contrast to the general assumption, however, mast cells were dispensable for UVB-induced immune suppression, whereas basophil-derived AREG was essential. These data reveal, to our knowledge, a previously unreported function for basophils in the homeostasis of immune responses in the skin. Basophils thus fulfill a dual function: they contribute to the initiation of effective type 2 immune responses and, by enhancing the suppressive capacity of local T_{reg} cell populations, also to local immune regulation in the skin.

INTRODUCTION

UVB irradiation treatment is in wide clinical use for the treatment of skin disorders, such as psoriasis (Almutawa *et al.*, 2013). Depending on the intensity and the wavelength, UVB irradiation can induce a wide range of different physiological responses. At low dose, UVB irradiation induces immune suppression in mice (Toews *et al.*, 1980; Schwarz and Schwarz, 2011). FoxP3-expressing regulatory T cells (T_{reg} cells) have been shown before to have a central role in the process of local resolution of inflammation and the induction of peripheral tolerance (Cobbold *et al.*, 2006), and it was shown that this UVB irradiation–induced form of antigen-specific immune tolerance is T_{reg} cell–mediated and can adoptively be transferred into naive mice (Elmets *et al.*, 1983; Schwarz *et al.*, 2004). Although it is well established that inflammatory conditions can influence T_{reg} cell function, the precise signals that enhance or dampen T_{reg} cell function during different forms of inflammation remain poorly understood. We have recently shown that the functionality of local T_{reg} cells and their capacity to resolve local inflammation is determined by signals received via the EGFR (Zaiss *et al.*, 2013). EGFR is highly expressed in T_{reg} cells (Zaiss *et al.*, 2013), which is induced via STAT5 signaling (Beier *et al.*, 2012). The epidermal growth factor (EGF)-like growth factor amphiregulin (AREG) enhances the suppressive capacity of T_{reg} cells in *in vitro* suppression assays, and mast cell–derived AREG is essential for optimal T_{reg} cell function *in vivo* (Zaiss *et al.*, 2013).

Here we tested whether UVB-mediated immune suppression is dependent on AREG. Using UVB exposure–induced immune suppression in either C57BL/6 wild type (*wt*) or *AREG* gene–deficient mice, and transfer experiments, we show that UVB-induced immune suppression is AREG-dependent. In contrast to general assumption, however, we find that not mast cell–derived AREG but basophil-derived AREG is essential for UVB-induced suppression of contact hypersensitivity (CHS).

RESULTS

UVB exposure of 400 J m^{-2} per day leads to optimal suppression of CHS in C57BL/6 mice

Exposure to UVB irradiation can induce a wide range of different inflammatory reactions. Low levels of UVB irradiation induce immune suppression in mice (Toews *et al.*, 1980;

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Abbreviations: AREG, amphiregulin; BM, bone marrow; CHS, contact hypersensitivity; EDLN, ear-draining lymph node; SDLN, skin-draining lymph node; T_{reg} cells, regulatory T cells; wt, wild type

Schwarz and Schwarz, 2011). To determine the precise intensity at which UVB irradiation induces immune suppression in C57BL/6 mice, mice were exposed to UVB irradiation, ranging over different intensities, then sensitized with the hapten DNFB and, 10 days later, challenged with DNFB on the ear. In untreated mice, such a challenge induces a CHS reaction that leads to ear swelling, which is a typical form of delayed-type hypersensitivity. As shown in Figure 1a, UVB irradiation within a specific window of intensity induced immune suppression, reaching a maximum at a range of 400 J m^{-2} per day. UVB irradiation exposure of a higher intensity diminished this suppression (Figure 1a). Prior UVB irradiation treatment (400 J m^{-2} per day) enhanced the portion of FoxP3-expressing T cells in the ear-draining lymph nodes (EDLNs) of challenged mice (Figure 1b), which directly correlated with diminished IFNγ and IL-17 expression in the EDLNs (Figure 1c and d, respectively). These data show that UVB irradiation exposure at 400 J m^{-2} per day induces the most optimal suppression of CHS in C57BL/6 mice.

Lack of UVB irradiation–induced immune suppression in AREG gene-deficient mice

UVB-mediated suppression is transferrable and has been shown to be T_{reg} cell mediated (Elmets *et al.*, 1983; Schwarz *et al.*, 2004). As we have shown that T_{reg} cells, for optimal function, are dependent on AREG-induced EGFR signaling, we tested the role of AREG in UVB-induced immune suppression. To this end, we exposed both C57BL/6 *wt* and *AREG* gene–deficient mice to UVB irradiation, sensitized them, and challenged them 10 days later on the ear. In agreement with an earlier study that showed that AREG is not essential for the induction of CHS in mice (Yagami *et al.*, 2010), both non-UVB-treated *wt* and *AREG*-deficient control mice developed a clear CHS reaction upon challenge. Challenge of non-sensitized mice led to minor ear swelling only. UVB irradiation induced immune suppression of CHS in *wt* mice, whereas UVB irradiation treatment had no immune tolerizing effects in *AREG* gene–deficient mice (Figure 2a and b). These data show that UVB-mediated immune suppression is dependent on AREG expression.

Efficient suppression of CHS by T_{reg} cells is dependent on AREG expression

AREG enhances T_{reg} function *in vivo* and in *in vitro* suppression assays (Zaiss *et al.*, 2013). To determine whether this UVB-induced and AREG-dependent form of immune suppression is T_{reg} cell mediated, we performed cell transfer experiments from UVB-treated animals into animals that had not been exposed to UVB. First, we transferred cells isolated from the skin-draining lymph nodes (SDLNs, i.e., inguinal, axial, and brachial lymph nodes) of UVB-exposed C57BL/6 animals into *wt* C57BL/6 or *AREG* gene–deficient mice. Tracing of the transferred cells in the spleen of recipient mice, based on the expression of CD45.1 and CD45.2 congenic markers, showed that similar amounts of cells had been transferred (Figure 3a). These transferred cells conferred their suppressive capacity onto *wt* but not onto *AREG* gene–deficient recipient mice (Figure 3b). Because, after challenge, frequencies of transferred T_{reg} cells in the ear-draining lymph nodes of *wt* and AREG gene-deficient recipient mice were similar (Figure 3c), we concluded that AREG expression in recipient mice was essential for efficient UVB-mediated immune suppression.

To verify that T_{reg} cells mediated the observed immune suppression, T_{reg} cells were purified from UVB-exposed FoxP3-GFP transgenic mice by FACS sorting and were transferred into *wt* C57BL/6 or *AREG* gene–deficient mice. As shown in Figure 4a, transfer of T_{reg} cells was sufficient to induce suppression of CHS in *wt* but not in *AREG* gene–deficient mice. Thus, T_{reg} cells contribute to UVB-induced immune suppression.

To determine whether the requirement for AREG in this model was mediated through a direct effect on T_{reg} cells, we transferred similar amounts of cells isolated from the SDLNs of either UVB-exposed *wt* C57BL/6 or CD4cre × *EGFR*$^{fl/fl}$ mice (a mouse strain that lacks EGFR expression specifically on T cells) into *wt* C57BL/6 mice. Transferred cells derived from UVB-treated *wt* mice suppressed CHS significantly ($P = 0.007$) better in recipient mice compared with cells derived from UVB-treated mice that lacked EGFR expression in T cells (Figure 4b). These data show that AREG acts directly on T_{reg} cells and enhances their suppressive capacity in this hypersensitivity model.

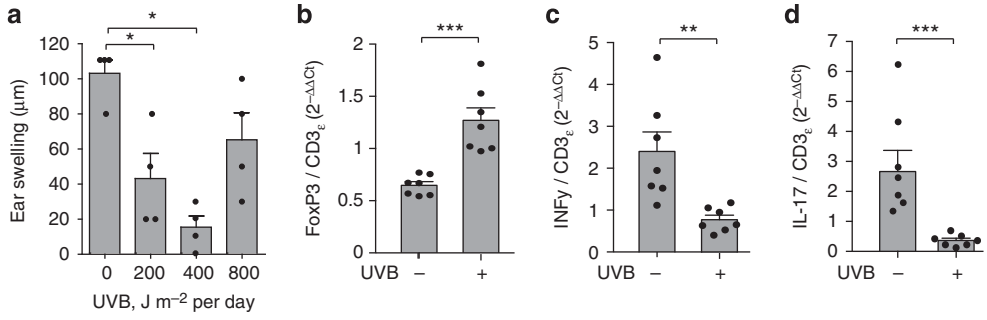

Figure 1. UVB irradiation suppresses contact hypersensitivity-induced ear swelling in mice. C57BL/6 *wt* mice were exposed to different intensities of UVB irradiation and were then sensitized with DNFB. Ten days later mice were challenged on both ears, and (**a**) ear swelling was measured after 48 hours of challenge. Ear-draining lymph nodes (EDLNs) were extracted for quantitative PCR analysis of CD3ε, FoxP3 (**b**), IFNγ (**c**), and IL-17 (**d**) mRNA expression. Bars represent average + SEM; $n = 4$–7 mice per group, and dots represent individual mice. wt, wild type.*$P = 0.0286$; **$P = 0.0012$; ***$P = 0.0006$.

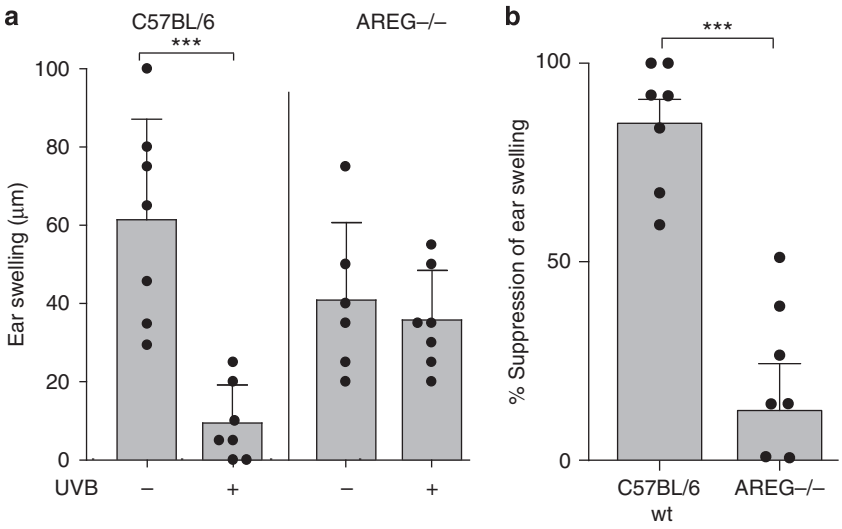

Figure 2. UVB irradiation suppresses ear swelling in *wt* but not in AREG$^{-/-}$ mice. C57BL/6 *wt* and AREG$^{-/-}$ mice were either irradiated or left untreated. All mice were sensitized with DNFB and challenged 10 days later. (**a**) Ear swelling was measured, and (**b**) the percentage of suppressed ear swelling in UVB-exposed versus unexposed control animals was determined. Bars represent average + SEM; $n = 7$ mice per group, and dots represent individual mice. AREG, amphiregulin; wt, wild type. ***$P = 0.0006$.

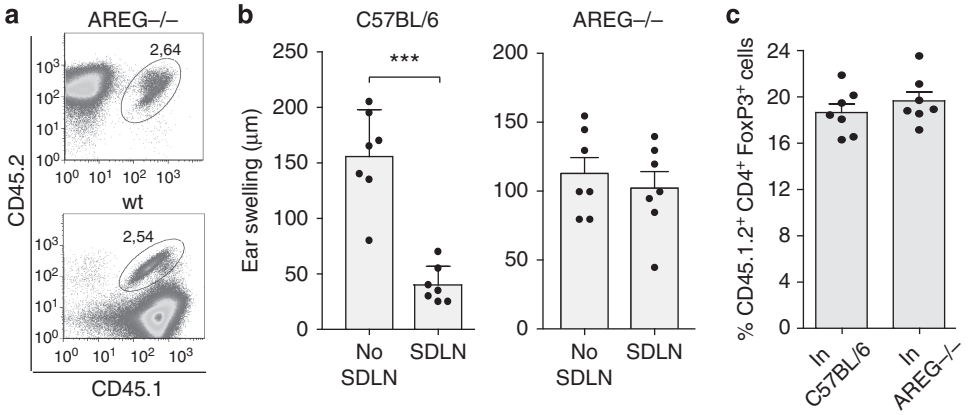

Figure 3. Transferred skin-draining lymph node cells confer the ability to suppress CHS onto *wt* but not AREG$^{-/-}$ mice. Naive CD45.1.2 BL/6 *wt* mice were irradiated and sensitized. Five days later cells from the skin-draining lymph nodes (SDLNs) were transferred into naive CD45.1 BL/6 *wt* or into naive AREG$^{-/-}$ mice (CD45.2). Recipient mice were sensitized and challenged 10 days later. (**a**) Frequency of transferred lymphocytes in the spleen of C57BL/6 and AREG gene-deficient recipient mice was determined, (**b**) ear swelling was measured in recipient mice and in control mice that had not received SDLN, and (**c**) the frequency of T_{reg} cells in the ear-draining lymph nodes of recipient mice was determined. Bars represent average + SEM; $n = 7$ mice per group, and dots represent individual mice. AREG, amphiregulin; CHS, contact hypersensitivity; wt, wild type. ***$P = 0.0006$.

Suppression of CHS is dependent on basophil-derived AREG

To determine the physiologically relevant source of AREG that enables T_{reg} cells to suppress CHS, we induced UVB-induced immune suppression in the mast cell–deficient mouse strain c-kit$^{w\text{-}sh}$. To our surprise, c-kit$^{w\text{-}sh}$ mice were perfectly capable of suppressing CHS upon UVB exposure (Figure 5a). These findings are in contrast to the findings of Hart *et al.* (1998), who reported that mast cells were essential for UVB-induced immune suppression. This group, however, used a mast cell–deficient mouse strain with a mutation in the c-kit receptor (W^f/W^f), which in addition to mast cell deficiency also displays basophil deficiency (Mancardi *et al.*, 2011). Not only mast cells but also human basophils (Qi *et al.*, 2010) as well as murine (Monticelli *et al.*, 2011) and human type-2 innate lymphoid cells (ILC-2) have been reported to produce AREG (Salimi *et al.*, 2013). All these cell types are known to have an important role in inflammatory conditions in the skin. We therefore first tested whether murine basophils also express AREG. To this end, we differentiated basophils *in vitro* and labeled them with FITC-labeled IgE antibodies. Basophils were then FACS sorted on the basis of IgE binding as well as CD49b and c-kit expression. IgE-bound cells that expressed CD49b but not c-kit (i.e., basophils) were then activated by antigen-specific cross-linking or via phorbol 12-myristate 13-acetate/

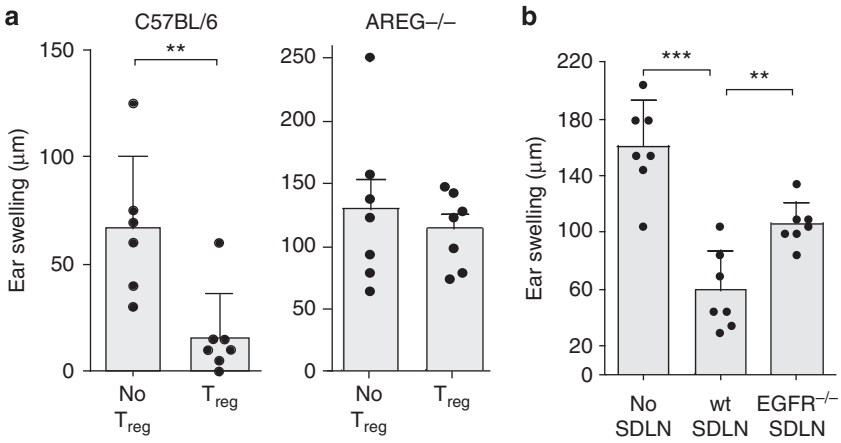

Figure 4. Suppression is transferred by T_{reg} cells whose function is dependent on AREG. (**a**) Naive FoxP3-GFP transgenic mice were irradiated and sensitized. Five days later T_{reg} cells were purified from the skin-draining lymph nodes (SDLNs) and spleen and transferred into naive C57BL/6 *wt* or AREG$^{-/-}$ mice. Recipient and control mice that had not received T_{reg} cells were sensitized and challenged 10 days later. Ear swelling was measured. $^{**}P = 0.0064$. (**b**) C57BL/6 *wt* or CD4cre × EGFR$^{fl/fl}$ mice were irradiated and sensitized. Five days later lymphocytes derived from the skin-draining lymph nodes (SDLNs) were transferred into naive C57BL/6 *wt* mice. Recipient mice were sensitized and then challenged 10 days later, and ear swelling in recipient mice versus control mice was determined. Bars represent average + SEM; $n = 7$ mice per group, and dots represent individual mice. AREG, amphiregulin; T_{reg}, regulatory T cells; wt, wild type. No SDLN vs. wt SDLN: $^{***}P = 0.0006$; wt SDLN vs. EGFR$^{-/-}$ SDLN: $^{**}P = 0.007$.

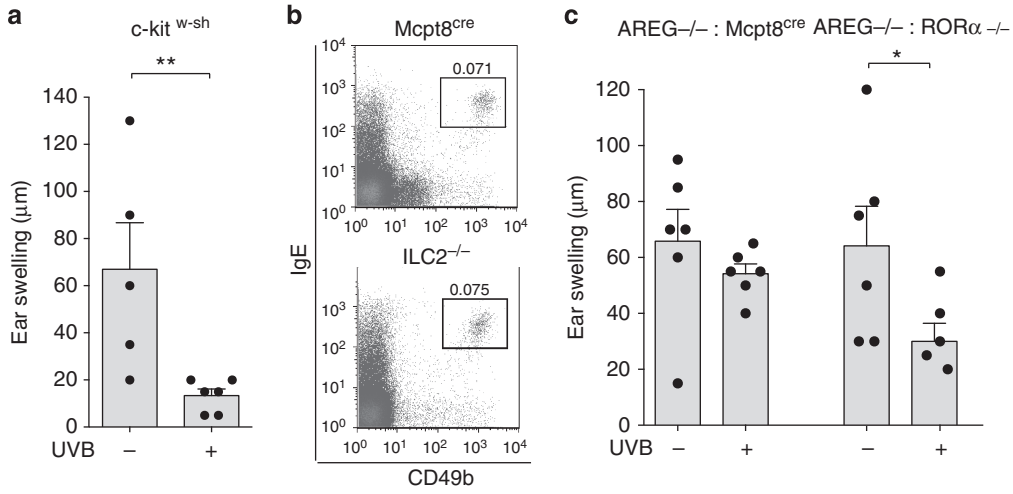

Figure 5. Basophil-derived AREG is essential for UVB irradiation–induced immune suppression. (**a**) Mast cell–deficient c-kit$^{w\text{-}sh}$ mice were irradiated and then sensitized with DNFB. Ten days later mice were challenged on both ears, and ear swelling was measured after 48 hours. (**b** and **c**) C57BL/6 mice were irradiated with 10 Gy gamma irradiation and reconstituted with a 1:1 mixture of bone marrow (BM) derived from AREG$^{-/-}$ and Mcpt8cre transgenic or derived from AREG$^{-/-}$ and RORα$^{-/-}$ mice. (**b**) Eight weeks after reconstitution, frequency of basophils in the spleen was measured by flow cytometry. (**c**) Reconstituted mice were irradiated with UVB light and then sensitized. Ten days later mice were challenged on both ears, and ear swelling was measured after 48 hours. Bars represent average + SEM; $n = 5$–6 mice per group, and dots represent individual mice. AREG, amphiregulin; ILC-2, type-2 innate lymphoid cells. $^{*}P = 0.0303$; $^{**}P = 0.0065$.

ionomycin activation. Either way of activation induced expression of AREG in basophils (Supplementary Figure S1 online). These data demonstrate that activated mouse basophils express AREG.

To test whether basophils and/or ILC-2 are involved in the suppression of CHS in mice that have been tolerized by UVB exposure, we tested mice that either lack basophils, i.e., Mcpt8cre transgenic mice (Ohnmacht *et al.*, 2010), or lack ILC-2, i.e., *RORα* gene–deficient mice (Wong *et al.*, 2012), for their involvement in UVB irradiation–induced immune suppression. As both cell types also have a central role in the initiation of immune responses and in the attraction of other leukocytes to the site of inflammation, and as *RORα* gene–deficient mice have a lethal phenotype that is not connected to the immune system (so-called ''staggerer''), we established mixed bone marrow chimeric (BMX) mice with a 1:1 mixture of bone marrow (BM) derived from *AREG* gene–deficient mice and either Mcpt8cre transgenic or *RORα* gene–deficient mice. Because of homeostatic expansion, frequencies of basophils and ILC-2 in these newly established BMX mice will be the same at 8 weeks after reconstitution. However, in BMX mice that received Mcpt8cre transgenic BM all basophils will be

derived from the AREG gene-deficient donor, whereas in mice that received *RORα* gene–deficient BM all ILC-2 will be derived from AREG gene-deficient donors. Eight weeks after reconstitution, we detected similar frequencies of basophils in the spleen of all BMX mice ($CD49b^+$ cells, Figure 5b), which demonstrates that all basophils in the spleen of BMX mice that received a mix of AREG gene-deficient and Mcpt8cre transgenic BM were derived from the *AREG* gene–deficient BM donor.

Eight weeks after reconstitution, mice were tested for UVB-induced suppression of CHS. BMX mice that lacked AREG expression in ILC-2 showed a high variability of responses but an overall significant suppression of CHS (Figure 5c). However, BMX mice that lacked AREG expression in basophils did not show any substantial suppression of CHS (Figure 5c).

Taken together, these data show that, in the skin, basophil-derived AREG is essential for T_{reg} cell–mediated suppression of CHS in mice.

DISCUSSION

In contrast to published data (Hart *et al.,* 1998), our data reveal that not mast cells but basophils have an essential role in UVB-induced immune suppression in mice. These results reveal a, to our knowledge, previously unreported function of basophils in the regulation of immune responses in the skin. So far, basophils have been best known for their early production of IL-4 (Sokol *et al.*, 2009), for their contribution to Th2 cell differentiation (Perrigoue *et al.*, 2009; Sokol *et al.*, 2009; Yoshimoto *et al.*, 2009), for their association with the pathogenesis of allergic (Dvorak and Mihm, 1972; Ohnmacht *et al.*, 2010) and atopic dermatitis (Ito *et al.*, 2011), and with the induction of anaphylaxis in specific mouse models (Tsujimura *et al.*, 2008). Also their unique contribution to protective immunity against helminth infections (Ohnmacht and Voehringer, 2010) and ticks (Wada *et al.*, 2010) is well established. Recently, however, it became apparent that basophils are of high importance in the regulation of skin homeostasis also (Egawa *et al.*, 2013). For instance, basophils mediate the differentiation of inflammatory monocytes into alternatively activated, so-called M2 macrophages at the site of inflammation. This differentiation of inflammatory monocytes into M2 macrophages within allergic skin lesions significantly contributes to the alleviation of allergic skin inflammation (Egawa *et al.*, 2013). Here we reveal a, to our knowledge, previously unreported function of basophils during skin inflammation—i.e., to enable local T_{reg} cells to suppress CHS. The unexpected discovery of this immune regulatory function for basophils is in line with a number of other findings that indicate a substantial contribution of type 2 immune responses to immune suppression and tissue repair (Allen and Wynn, 2011; Gause *et al.*, 2013).

Predominantly, mast cells have so far been associated with immune suppressive functions in the skin. So is, for instance, in a skin transplant model the collaboration between T_{reg} cells and mast cells essential for the induction of immune tolerance (Lu *et al.*, 2006). Also in a chronic UVB irradiation model, it has been shown that mast cell–derived IL-10 has an important role in the dampening of sunburn-associated inflammation in the skin (Grimbaldeston *et al.*, 2007). In addition, we have shown that mast cell–derived AREG is essential for optimal T_{reg} cell function in BM transplantation–induced skin rejection, in T-cell–mediated colitis, and in a tumor vaccination model (Zaiss *et al.*, 2013). All these examples are forms of slowly developing, chronic inflammation. Thus, it is tempting to speculate that a chronic form of inflammation allows mast cells to immigrate, proliferate, and differentiate at the site of inflammation. In CHS, however, antigen-specific T_{reg} cells are induced by UVB exposure on the back of a mouse, whereas acute inflammation is induced in the ear of a mouse. Basophils are one of the first cells that infiltrate the site of inflammation but, with a half-life of about 2 days, are also relatively short-lived (Ohnmacht and Voehringer, 2009). Remarkably, our data indicate that AREG derived from ILC-2 appears to have a less strong influence on T_{reg} cell function in UVB-induced immune suppression of CHS than that derived from basophils (Figure 4a). Substantial numbers of ILC-2 can be found in non-inflamed skin (Roediger *et al.*, 2013), and it will be interesting to investigate in future studies why basophils contribute significantly better to the T_{reg} cell–mediated suppression of CHS than do ILC-2.

Given these observed differences in the different types of inflammation, it is tempting to speculate that mast cells and basophils fulfill similar functions at different stages of inflammation. Although mast cells carry type 2 responses during later phases of inflammation, basophils may have a more important role during early phases of local inflammatory responses. In this regard it is interesting to note that mast cells can not only enhance T_{reg} cell function (Zaiss *et al.*, 2013) but can also suppress it. For instance, degranulation of mast cells temporarily abolishes T_{reg} cell function *in vivo* (de Vries *et al.*, 2009), a process possibly mediated via histamine-mediated suppression of T_{reg} cell function (Forward *et al.*, 2009). Similarly, exposure of basophils to T-cell–derived IL-3 induces AREG expression (Qi *et al.*, 2010). The amounts of AREG expressed by basophils are much higher when stimulated by IL-3 than by IgE cross-linking, which was in direct negative correlation to IL-4 expression and histamine release by basophils (Qi *et al.*, 2010). Thus, it is attractive to speculate that, in the case of basophils as well, the type of inflammation determines whether basophils enhance or diminish the suppressive capacity of local T_{reg} cell populations. In this regard, it is also very interesting to note that a developmental and functional heterogeneity within basophil populations has been revealed recently (Siracusa *et al.*, 2011). One type of basophils, the so-called IL-3-elicited basophils, is highly sensitive to IgE cross-linking and degranulates and releases histamine upon IgE-mediated activation. The other type of basophils, the so-called thymic stromal lymphopoietin-elicited basophils, is less sensitive to IgE-mediated cross-linking but readily produces cytokines upon exposure to IL-3 or IL-33 (Siracusa *et al.*, 2011). Thymic stromal lymphopoietin is a predominant epithelial cell–derived cytokine expressed in the skin. Therefore, it would be interesting to see whether these two different basophil populations might have opposing roles in T_{reg} cell–mediated regulation of local inflammation in the skin, which would further emphasize the special role the skin has in immune regulation.

MATERIALS AND METHODS

Mice

C57BL/6 mice were purchased from Charles River and Harlan. C57BL/6 FoxP3-GFP transgenic, c-kit$^{w\text{-}sh}$, CD4cre × *EGFR*$^{flox/flox}$, *AREG* gene–deficient mice, C57BL/6.SJL (CD45.1), and CD45.1.2 mice (F1 of C57BL/6 × C57BL/6.SJL) were bred in-house under specific pathogen-free conditions. *AREG* gene–deficient mice had been backcrossed at least 14 times onto C57BL/6 (Zaiss *et al.*, 2006). Mice were used between 7 and 17 weeks of age. All animal experiments had been approved by the Committee on Animal experiments of Utrecht University prior to their performance.

UVB irradiation and induction of CHS

TL20W/12 lamps (Philips, Eindhoven, The Netherlands) were used for the UVB irradiation. This lamp has about 60% output in the UVB range and an emission spectrum ranging from 280 to 350 nm with a maximum of around 310 nm. Relative spectral distribution measurement of the UVB source was ascertained with a calibrated standard UV-visible spectrometer (model 752, Optronic Laboratories, Gooch & Housego, Berlin, Germany). Throughout the experiments irradiation of the mice was monitored with a UVB detector device of Waldmann (Waldmann, Villingen-Schwenningen, Germany), which was calibrated with the above-mentioned spectrometer. A 4 × 2.5 cm field on the backs of mice was shaved. Mice were then either irradiated with the broadband UVB irradiation for 4 consecutive days or left untreated. The UVB intensity was measured before each experiment. Twenty-four hours after the last UVB irradiation, all mice were sensitized to DNFB by painting 25 µl 0.3% DNFB, dissolved in a 4:1 acetone/olive oil mixture, onto their shaved backs. Ten days after sensitization, mice were challenged on the ear with 20 µl 0.1% DNFB, dissolved in a 4:1 acetone/olive oil mixture. The challenge was repeated 24 hours later and ear thickness was measured 24 hours later, using a digital micrometer (''Quick Mini'', Mitutoyo, Kawasaki, Japan). Ear swelling was calculated by subtracting the thickness of the ear prior to the challenge from the thickness of the ear 48 hours after challenge. Average swelling of both ears was used for further analysis.

Adoptive transfer of immune response

Five days after UVB irradiation and DNFB sensitization, donor wt C57BL/6 or FoxP3-GFP transgenic mice were sacrificed by cervical dislocation, the skin-draining lymph nodes (inguinal, axillary, and brachial lymph nodes) were excised, and 1×10^7 SDLN cells or 4×10^5 FACS sorted T_{reg} cells were transferred into naive hosts via intravenous injection. Induction of contact hypersensitivity was performed as described above. FACS-based cell sorting was performed on a BD Influx Cell sorter.

Antigen-specific activation of *in vitro*-differentiated basophils

BM was isolated from C57BL/6 mice and differentiated in the presence of WEHI cell supernatant containing recombinant murine IL-3 for 7 days. Cells were then incubated with anti-CD49-PE, anti-c-kit-APC antibodies, and fluorescein isothiocyanate-conjugated IgE antibody specific for dinitrophenol (H1 dinitrophenyl-epsilon 26.82) (Liu *et al.* 1980). Fluorescein isothiocyanate-labeled cells (Fc-ε-R positive) were FACS sorted on the basis of CD45b expression (basophils) and lack of c-kit expression (to exclude mast cells). Purified basophils were activated in the presence of 100 ng ml^{-1} dinitrophenyl-BSA or phorbol 12-myristate 13-acetate/ionomycin, or were left untreated.

Generation of mixed BM chimeras

Mixed BMX mice were generated as previously described (Zaiss *et al.* 2008). In short, BM donor cells from C57BL/6 *wt* and RORα gene–deficient or from C57BL/6 *wt* and Mcpt8cre transgenic mice were mixed 1:1, and 10^7 cells were transferred intravenously. into lethally irradiated (10 Gy) C57BL/6 *wt* acceptor mice. Eight weeks after BM transfer, reconstituted mice were subjected to UVB-induced immune suppression as described above.

Sample collection and flow cytometry

Cells were isolated from spleen and EDLNs, red blood cells were removed, and cell surface staining of CD4 was performed. Staining for CD45.1 and CD45.2 was performed for at least 20 mins at 4 °C in the presence of Fc-block (2.4G2). Intercellular staining for FoxP3 was performed according to the manufacturer's specifications using a FoxP3 intracellular staining kit (eBioscience, Vienna, Austria). Samples were measured using a FACS CantoII (BD Biosciences, Breda, The Netherlands) and analyzed with FlowJo software (Tree star, Ashland, OR).

Quantitative real-time reverse transcription-PCR

Total mRNA was extracted from the EDLNs after challenge using TRIzol (Invitrogen, Paisley, UK) and further treated as previously described (Meulenbroeks *et al.* 2013). reverse transcription-PCR for IL-4, IFNγ, FoxP3, CD3ε, and 18 S was performed according to the manufacturer's instructions using TaqMan Gene Expression Assays (Applied BioSystems, Paisley, UK) Mm00445259, Mm00801778, Mm00475162, Mm00599684, and 4352930E, respectively.

Statistics

Statistical analysis between two groups was performed with a two-tailed Mann–Whitney *U*-test using statistical software (GraphPad Prism 6.0, La Jolla, CA). *P*-values <0.05 were considered significant. In the Figures, * depicts a *P*-value <0.05, ** a *P*-value <0.01, and *** a *P*-value <0.001.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Supplementary Figure 1:

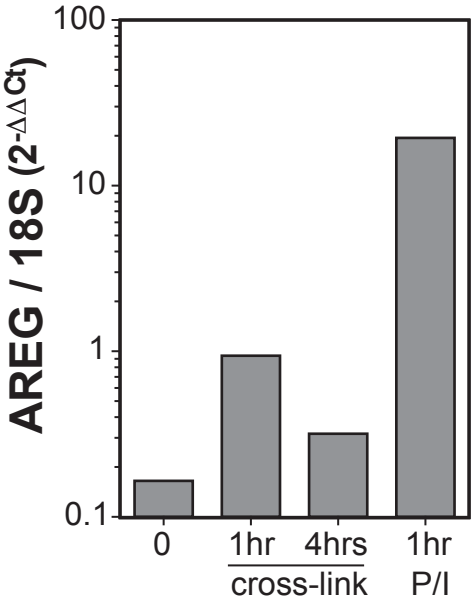

Fig. S1: Activated mouse basophils express Amphiregulin.

Basophils were in vitro differentiated from BM cells in the presence of recombinant IL3. Differentiated cells were stained with fluorescence labeled IgE antibodies and with antibodies to detect CD49b and c-kit expression, and IgE-positive, CD49b positive, c-kit negative cells (the basophil population) were purified by FACS-sort. Cells were then activated by FcεR cross linking or with PMA/Ionomycin. AREG expression was measured by real time-PCR. scDNA levels were equalized to 18S expression.