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Skin Inflammation Arising from Cutaneous Regulatory T Cell Deficiency Leads to Impaired Viral Immune Responses

Eva-Jasmin Freyschmidt,* Clinton B. Mathias,* Natalia Diaz,* Daniel H. MacArthur,* Amale Laouar,† Narasimhaswamy Manjunath,‡ Matthias D. Hofer,§ Marc-Andre Wurbel,¶ James J. Campbell,‖ Talal A. Chatila,§ and Hans C. Oettgen*

Individuals with atopic dermatitis immunized with the smallpox vaccine, vaccinia virus (VV), are susceptible to eczema vaccinatum (EV), a potentially fatal disseminated infection. Dysfunction of Forkhead box P3 (FoxP3)-positive regulatory T cells (Treg) has been implicated in the pathogenesis of atopic dermatitis. To test whether Treg deficiency predisposes to EV, we percutaneously VV infected FoxP3-deficient (FoxP3KO) mice, which completely lack FoxP3+ Treg. These animals generated both fewer VV-specific CD8+ effector T cells and IFN-γ–producing CD8+ T cells than controls, had higher viral loads, and exhibited abnormal Th2-polarized responses to the virus. To focus on the consequences of Treg deficiency confined to the skin, we generated mixed CCR4-deficient and FoxP3-deficient (CCR4/FoxP3) chimeras in which skin, but not other tissues or central lymphoid organs, lack Treg. Like FoxP3KO mice, the chimeras had impaired VV-specific effector T cell responses and higher viral loads. Skin cytokine expression was significantly altered in infected chimeras compared with controls. Levels of the antiviral cytokines, type I and II IFNs and IL-12, were reduced, whereas expression of the proinflammatory cytokines, IL-6, IL-10, TGF-β, and IL-23, was increased. Importantly, infection of CCR4/FoxP3 chimeras by a noncutaneous route (i.p.) induced immune responses comparable to controls. Our findings implicate allergic skin inflammation resulting from local Treg deficiency in the pathogenesis of EV. The Journal of Immunology, 2010, 185: 1295–1302.

Concerns regarding the potential use of smallpox as a biological weapon have led to resumption of immunization with vaccinia virus (VV). Although immunization is effective, it is associated with significant side effects. The most serious complication, eczema vaccinatum (EV), a localized or potentially lethal systemic dissemination of the virus, occurs in patients with atopic dermatitis (AD) (1). The basis of enhanced susceptibility to VV in AD patients is not well understood. AD is a chronic inflammatory skin disease associated with elevated IgE and blood eosinophilia. Acute AD lesions are infiltrated with CD4+ Th2 cells, which secrete predominantly IL-4, IL-5, and IL-13 (2). In chronic lesions, IFN-γ–producing Th1 cells dominate (3). Several lines of evidence indicate that regulatory T cell (Treg) dysfunction in the skin underlies AD. Mutations in the Forkhead Box P3 (FOXP3) gene result in the immune dysregulation, polyendocrinopathy, enteropathy X-linked syndrome characterized by severe eczema and allergies (4), and single-nucleotide polymorphisms of FOXP3 have previously been reported to be associated with atopy (5). Diminished CD4+CD25+FoxP3+ Tregs have been reported in patients with AD and asthma, and the number of Treg is inversely correlated with IgE, eosinophilia, and IFN-γ levels (6–8). Mice bearing loss of function FoxP3 mutations display severe dermatitis and lymphoproliferation, along with allergic airway and gut inflammation, blood and tissue eosinophilia, and elevated IgE levels (9–11). Recently, it has been shown that tissue-targeted reduction in Treg leads to localized inflammation (12, 13).

We hypothesized that allergic inflammation arising in the setting of Treg deficiency might result in ineffective immune responses to VV delivered via the skin and that this might underlie the pathogenesis of EV. To test this hypothesis, we studied VV responses in both FoxP3-deficient (FoxP3KO) mice (11), which lack Treg in all tissues, and in chimeras in which only the skin was deficient in Treg. The chimeras were constructed using a mixture of bone marrow (BM) from CCR4-deficient and FoxP3-deficient mice. In these animals, the only lymphocytes capable of homing to skin (CCR4+) arise from progenitors of FoxP3-deficient origin resulting in a skin-restricted inflammatory phenotype. Evaluation of the VV response in mice with global or skin-restricted Treg deficiency revealed more intense local and systemic infection, a phenotype reminiscent of EV. We characterize the specific abnormalities in the VV immune responses in these mice that predispose to severe VV infection and show that bypassing the skin altogether, by i.p. injection of the virus, normal responses can be elicited. These findings implicate dysfunction of cutaneous FoxP3+ Treg in VV immunity.
Materials and Methods

Virus source and expansion

The VV Western Reserve strain was obtained from American Type Culture Collection (Manassas, VA; ATCC VR-1354) and expanded and tiered in CV1 cells (ATCC CCL-70) by standard procedures (14).

Animals and virus application

FoxP3-deficient mice on a C57BL/6 background were generated as described previously (11). Male CCR4−/− (B6;129P-Ccr4tm1Pwr/J; referred to in the paper as CCR4KO) mice and male Rag1KO mice (B6.129S7-Rag1tm1Mom/J) were purchased from The Jackson Laboratory (Bar Harbor, ME). All experiments were carried out in accordance with Children’s Hospital policies and procedures and were reviewed by the Institutional Animal Care and Use Committee. For epicutaneous VV infection (via skin scarification), mice were anesthetized using avertin (2,2,2-tribromoethanol and tertiary amyl alcohol), and 10 μl VV (1 × 107 PFU) was inoculated with 30 superficial scratches in the skin of the shaven back of the mice using a 27.5-gauge needle. Alternatively, 100 μl VV (2 × 106 PFU) was injected i.p. Lesion sizes were analyzed by using NIH Image software ImageJ (National Institutes of Health, Bethesda, MD).

VV-specific quantitative real-time PCR

DNA was prepared using the Qiagen DNeasy Kit (Qiagen, Valencia, CA), according to the manufacturer’s guidelines. Viral genomes were quantified by real-time PCR using primers specific for vaccinia ribonucleotide reductase (VrHR1 and VrHR2) and an ABI 7700 Sequence Detection System (Applied Biosystems, Foster City, CA) (15). To quantitatively determine the VV genome copies, a standard curve was generated using DNA from purified VV stock with known PFU (determined by plaque assay). Viral copies determined using a standard curve were normalized to the amount of total DNA.

Flow cytometry

Single-cell suspensions of splenocytes were prepared 8 d post infection (p.i.). Splenocytes (1 × 107) were stained according to manufacturer’s instructions. Cells were incubated with 5 μl B8R20–27 (TSYKFESV [16]) MHC class I Pro5TM pentamers conjugated to PE (ProImmune, Oxford, U.K.). Then cell surface staining was performed using allophycocyanin-conjugated anti-mouse CD8a (Ly-2, clone 53-6.7) (eBioscience, Oxford, U.K.). For intracellular IFN-γ, cells were incubated with 5 μl B8R20–27 (TSYKFESV [16]) MHC class I Pro5TM pentamers conjugated to PE, cells were fixed and permeabilized using BD Cytofix/CytopermTM Plus Kit (BD Biosciences Pharmingen) and stained with allophycocyanin-conjugated anti-mouse CD8a and FITC-labeled anti-mouse IFN-γ Ab (BD Biosciences Pharmingen). Samples were analyzed on a BD Biosciences FACSCalibur flow cytometer (BD Biosciences, San Jose, CA). Analysis was performed using CellQuest software (BD Biosciences Pharmingen). Lymphocytes were gated on B220 negative cells when stained with B8R20–27 MHC class I pentamers.

In vitro cytokine synthesis by splenocytes

Single-cell suspensions of splenocytes were prepared and stimulated as reported previously (15). Supernatants were collected at 24 h (for IFN-γ), 48 h (for IL-4), and 72 h (for IL-2). Cytokine levels in supernatants were determined by ELISA following the manufacturer’s instructions (BD Biosciences Pharmingen).

BM chimeras

Femurs and tibiae were flushed with cold sterile PBS. Cells were incubated in hemolytic buffer for 5 min at room temperature, washed, and counted. A total of 2 × 106 cells (1.5 × 106 derived from FoxP3KO or wild-type [WT] mice and 0.5 × 106 from CCR4KO mice) were injected retro-orbitally into anesthetized and irradiated (two doses of 450 rad) Rag1KO mice. AD-like characteristics of the skin were evident starting 3–6 wk after reconstitution. BM chimeras were VV-infected between 4 and 6 wk after reconstitution.

Tissue histology

Skin and colon were fixed in 10% neutral-buffered formalin, paraffin embedded, cut into 5-μm sections, and stained with H&E. Sections were examined for inflammatory infiltrates using standard light microscopy by an investigator unaware of the identity of individual samples. For quantification, infiltrating

![FIGURE 1](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org/)

**FIGURE 1.** More intense skin lesions in VV-infected FoxP3KO mice. A, Primary lesions 7 d after inoculation of VV via skin scarification. B, Number of satellite lesions (contiguous and distant from the inoculation site) per mouse (n = 20). C, Viral genome copy numbers per microgram total DNA in skin other than primary lesion (n = 20). *p < 0.05

![FIGURE 2](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org/)

**FIGURE 2.** VV-specific immune responses in FoxP3KO mice. Splenocytes of cutaneously VV-infected FoxP3KO and WT control mice were analyzed 8 d p.i. Data were compiled from three independent experiments. A, Splenocytes were stained for CD8, VV-specific TCR (using B8R20–27 MHC class I pentamers), and CD45R/B220 and analyzed by flow cytometry. Representative dot plots are shown (VV-infected FoxP3KO mice, n = 8; WT, n = 8). Uninfected mice did not have any VV-specific CD8+ T cells (Supplemental Fig. 3). B, Splenocytes were stimulated in vitro with the VV peptide B8R20–27. Intracellular IFN-γ as well as CD8 and CD4 surface staining were performed, and cells were analyzed by flow cytometry gating on CD8+ cells. Representative dot plots are shown (n = 7). C, Cytokine levels in supernatants of splenocytes from VV-infected FoxP3KO and WT control mice stimulated with irradiated VV-infected (●) or uninfected (○) splenocytes from naive WT mice. Cytokines were determined by ELISA 24 h (IFN-γ), 48 h (IL-4), and 72 h (IL-2) after stimulation (VV-infected FoxP3KO mice, n = 9; all others, n = 7). **p < 0.005; ***p < 0.0005.
cells were counted per 0.01 mm² of two sections per mouse, five mice per group.

ELISA
Total IgE levels in the sera were quantified using a sandwich ELISA with paired Abs from BD Pharmingen (San Diego, CA).

Skin cytokines
Total RNA was isolated from homogenized skin tissue using TRIzol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. cDNA was generated with iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). Quantitative real-time PCR was done using TaqMan Gene Expression Assay probes, TaqMan PCR master mix, and the ABI Prism 7300 sequence detection system, all from Applied Biosystems. Expression of each cytokine transcript was determined relative to a reference gene transcript, GAPDH, calculated as $2^{\Delta\Delta CT}$.

Statistical analyses
Differences in values between experimental groups were examined for significance with GraphPad Prism software using unpaired two-tailed Student t test. Values are presented as means ± SEM.

Results
More severe skin pox lesions in cutaneously VV-infected FoxP3KO mice
Following skin scarification with VV to mimic smallpox immunization, WT mice developed confined pox lesions similar to those observed in vaccinated humans starting 4 d p.i. (average lesion area, 15.7 ± 1.5 mm²) (Supplemental Fig. 1). In contrast, FoxP3KO mice developed both extensive primary lesions (35.0 ± 4.9 mm²) (Supplemental Fig. 1) and numerous satellites in contiguous and distant skin (Fig. 1A,1B). Similar results were obtained even with a 10-fold lower virus dose (data not shown). Viral load assays confirmed that infection was indeed confined to the inoculation site of WT mice, whereas variable amounts of viral genome ($1.5 \times 10^6 \pm 1.3 \times 10^6$ copies/µg DNA) were recovered from contiguous or distant skin in FoxP3KO mice (Fig. 1C), a phenotype reminiscent of EV. Primary lesion sizes in FoxP3 KO mice were positively correlated with viral genome numbers contained within the lesions ($r^2 = 0.934$). These observations indicate defective cutaneous clearance of VV in the setting of Treg deficiency.

Absence of VV-specific CD8+ effector T cells in cutaneously infected FoxP3KO mice
We hypothesized that exposure to virus in the setting of allergic inflammation might fail to induce effective immune responses.
IFN-γ production was corroborated by a lack of IgG2a responses in the FoxP3KO mice (Supplemental Fig. 2). To investigate whether the impaired immune responses in FoxP3KO mice resulted not only in enhanced cutaneous spread of the virus but also in greater systemic dissemination, we assayed the presence of viral DNA 8 d after inoculation. VV was consistently detectable in the organs of FoxP3KO mice (70–100% positive) while only occasionally recoverable from the organs of WT mice (0–37% positive) (Supplemental Fig. 4). These findings suggest that VV infections established in the setting of skin inflammation might go unchecked by normal adaptive immune defenses.

**Impaired Th1 cytokine expression, but enhanced IL-4 production by VV-specific splenocytes from cutaneously VV-infected FoxP3KO mice**

The Th1 cytokine, IFN-γ, is critical in VV immunity, driving expansion of virus-specific CD8⁺ T cell populations (17–19) and mediating early control of VV replication (20, 21). In contrast, Th2 cytokines impair VV clearance (22, 23). In this study, WT splenocytes stimulated with VV displayed robust IFN-γ responses (50.2 ± 9.6 ng/ml), whereas splenocytes from VV-infected FoxP3KO mice produced background levels (15.6 ± 2.4 ng/ml; p < 0.0001) (Fig. 2C). IL-2 responses were similarly impaired in FoxP3KO mice. In striking contrast to the diminished production of Th1 cytokines, Th2 responses were amplified in FoxP3KO mice as evidenced by markedly increased production of IL-4 (0.5 ± 0.1 ng/ml, compared with 0.1 ± 0.0 ng/ml in WT; p < 0.005).

Collectively, these results demonstrate that cutaneous VV infection of FoxP3KO results in impaired Th1 effector immune responses along with a paradoxical induction of Th2 immunity.

**Generation and characterization of CCR4/FoxP3 chimeras**

Because FoxP3KO mice exhibit multisystem inflammation, studies with these animals did not allow us to distinguish effects of skin inflammation on the EV phenotype from those of generalized immune abnormalities resulting from global FoxP3 deficiency. Therefore, additional studies were performed using BM chimeras constructed by infusing mixed marrow from CCR4KO and FoxP3KO mice into Rag1KO in which only FoxP3KO progenitors in the FoxP3 KO mice (Supplemental Fig. 2). To investigate (black column, to GAPDH. Relative expression of IL-4 is represented in relation to GAPDH. *p < 0.05; **p < 0.0001.
primary lesions (45.9 ± 4.1 mm$^2$) than control chimeras (17.1 ± 1.5 mm$^2$) (Supplemental Fig. 5). In addition, satellite lesions arose at a higher frequency in the CCR4/FoxP3 chimeras (16 lesions per 10 mice) compared with controls (3 lesions per 10 mice). Significantly higher viral loads were present in the primary lesions of CCR4/FoxP3 chimeras (1.8 × 10$^7$ ± 0.2 × 10$^7$) than in those of CCR4/WT chimeras (0.7 × 10$^7$ ± 0.2 × 10$^7$; p < 0.0001) along with more abundant VV copies in contiguous and distant skin (1.8 × 10$^8$ ± 1.4 × 10$^8$) in CCR4/FoxP3 chimeras compared with 181 ± 141 in CCR4/WT chimeras (Fig. 6B). In addition, we found significantly higher numbers of CCR4/FoxP3 chimeras with detectable VV in kidney (72.7 ± 3.9%) and testis/ovary (70.2 ± 13.1%) compared with control CCR4/WT chimeras (Fig. 6C). A trend toward higher viral burdens was also suggested for heart and lung. The occasional presence of viral genome copies in the skin and organs of CCR4/WT control chimeras might have resulted from incomplete BM reconstitution with resultant partial immune deficiency in some of these mice.

Analysis of cytokine expression in the skin revealed distinct expression patterns for CCR4/FoxP3 versus CCR4/WT chimeras (Fig. 7). Transcripts for type I and II IFNs and IL-12, all important in viral immunity, were more strongly expressed in cutaneously infected CCR4/WT control chimeras than in controls. In contrast, mRNA for the proinflammatory mediators IL-6, TGF-β, IL-10, and IL-23 was more abundant in the skin of VV-infected CCR4/FoxP3 chimeras than controls. IFN-γ induction in VV-infected CCR4/FoxP3 chimeras was significantly impaired compared with control chimeras. Although we were not able to detect statistically significant differences in IL-17A and IL-17F expression, we observed a trend toward increased levels in CCR4/FoxP3 chimeras. These findings demonstrate impaired local expression of type I IFNs and Th1 cytokines in mice lacking skin Treg accompanied by unchecked viral spread from the primary lesion to contiguous skin and viscera.

Discussion

Recently, we and others have used mouse models to investigate the immunological basis underlying EV. RelB$^{-/-}$ mice (15), epicutaneously OVA-sensitized BALB/c mice (24), mice lacking cathelicidin antimicrobial peptide (25), and NC/Nga mice bearing eczematous skin lesions (26) all exhibit abnormal VV immunity and viral clearance. However, the cellular immune mechanisms of EV have not been directly addressed.

Several observations implicate important roles for FoxP3-expressing Tregs in maintenance of normal skin homeostasis and prevention of AD. Verhagen et al. (6) have recently reported a relative deficiency of Treg and their cytokine products in AD lesional skin. Conflicting data exist regarding associations between the numbers of Tregs in peripheral blood and AD. Reeker et al. (27) as well as Leung and coworkers (28) observed increased Treg numbers in peripheral blood of AD patients with numbers linked to disease severity, whereas Orihara et al. (7) reported the opposite—an inverse correlation between peripheral Treg numbers and IgE, eosinophilia, and IFN-γ levels in patients with AD and asthma. In mice, FoxP3 deficiency results in severe allergic dysregulation with skin as a primary target (11). In addition to being implicated in

![FIGURE 6.](http://www.jimmunol.org/)

**FIGURE 6.** VV infection of CCR4/FoxP3 chimeras. CCR4/FoxP3 and CCR4/WT control chimeras were cutaneously or i.p. infected with VV and analyzed 8 d.p.i. Data were compiled from three independent experiments. A. Splenocytes from cutaneously (n = 11) or i.p. (n = 11) infected (+) or uninfected (−) CCR4/FoxP3 and CCR4/WT chimeras were stained for CD8, VV-specific TCR (using B8R20–27 MHC class I pentamers) and for CD45R/B220. B, VV genome numbers were assessed by quantitative real-time PCR for skin from primary lesions and contiguous/distant sites (satellite lesions in CCR4/FoxP3 chimeras) of cutaneously infected chimeras and shown as VV genome copy numbers per microgram total DNA (n = 10/group, 16 samples of contiguous/distant skin were collected from 10 mice). DNA from uninfected controls was assayed to confirm the specificity of quantitative real-time PCR and did not contain any viral genome copies (data not shown). C, VV genome numbers were assessed by quantitative real-time PCR for kidney, testis/ovary, heart, and lung of cutaneously infected chimeras. Data were compiled from three independent experiments (n = 5/group per experiment). DNA from uninfected controls was assayed to confirm the specificity of quantitative real-time PCR and did not contain any viral genome copies (data not shown). *p < 0.05.

![FIGURE 7.](http://www.jimmunol.org/)

**FIGURE 7.** Expression of cytokines in the skin of CCR4/FoxP3 chimeras after VV infection. Cytokine expression in the skin contiguous to the primary infection site of VV-infected and uninfected CCR4/FoxP3 and CCR4/WT control chimeras were assessed by quantitative real-time PCR 8 d.p.i. (n = 10–18/group). *p ≤ 0.05; **p < 0.005; and ***p < 0.0001.
skin homeostasis, Tregs have been reported to suppress immunity to viral and other infections (29–32). An opposite, protective role for Treg has recently been suggested in a model of HSV infection (33).

To assess whether the absence of FoxP3+ Tregs contributes to increased susceptibility to VV in AD patients, we mimicked smallpox immunization by infecting FoxP3KO mice with VV via skin scarification. Cutaneous infection resulted in viral spread from the inoculation site to contiguous skin and distant satellite lesions in FoxP3KO mice reminiscent of EV. We considered that skin inflammation might impair the induction of protective adaptive immune responses and first focused on the generation of IFN-γ–producing, virus-specific CD8+ effector T cells. IFN-γ has a pivotal role in immune responses to VV. It inhibits VV replication via NO induction in macrophages (20, 21) and exerts pleiotropic effects on immune cells including activation of dendritic cells and macrophages, induction of MHC class I and II expression (34), and expansion of CD8+ T cells (19). CD8+ T cells play a decisive role in protection against VV (35, 36). In our infection model, introduction of VV via the inflamed skin of FoxP3KO animals was met with a complete lack of the VV-specific IFN-γ–producing CD8+ effector cell responses characteristically observed in WT animals, suggesting that impaired early adaptive immune responses might contribute to unchecked viral spread. Furthermore, VV-stimulated splenocytes from cutaneously infected FoxP3KO mice failed to produce IFN-γ and IL-2 but produced elevated amounts of IL-4, indicating markedly dysregulated Th differentiation in these animals following infection.

Our studies using CCR4/FoxP3 chimeras confirm that mice with a FoxP3+ T cell compartment incapable of skin migration spontaneously develop a cutaneous inflammatory disease with red, scaly skin, hyperkeratosis, lymphocytic and eosinophilic infiltration, and elevated local levels of IL-4, TGF-β, and IL-23 and decreased level of IFN-γ as well as high systemic IgE. In addition, we have observed an inverse correlation between the ratio of CCR4KO/FoxP3KO cells and the severity and tempo of appearance of skin inflammation. Importantly, the non-skin-homing T cell compartment in these chimeras includes a FoxP3+CD25+CD4+ Treg subset, and the animals displayed neither splenomegaly nor gut inflammation, consistent with the presence of functional central Treg. Recently, it was reported that both epicutaneous and i.p. VV immunization generate T cell-mediated immunity in mice but that immunization via skin scarification is the more immunogenic route (37). In this study, we show that this is only true when the infection occurs through normal but not through inflamed skin. Epicutaneous but not i.p. VV infection of CCR4/FoxP3 chimeras resulted in significantly reduced development of VV-specific CD8+ effector T cells compared with control chimeras confirming that infection occurring through Treg-deficient inflamed skin causes impaired adaptive immune responses. As expected, we observed impaired viral clearance in CCR4/FoxP3 chimeras as demonstrated by increased numbers of satellite lesions and higher viral loads in skin and organs compared with controls. Unlike the FoxP3KO mice, which were absolutely incapable of generating VV-specific effector T cells after cutaneous infection, the CCR4/FoxP3 chimeras exhibited a partial, albeit markedly impaired, T cell response. We believe this most likely relates both to the much less severe skin inflammation observed in the chimeras and/or to the absence of systemic infection. CCR4/FoxP3 chimeras expressed elevated IL-4 and TGF-β levels in the skin. Infection with IL-4 expressing VV has been demonstrated to result in downregulation of Th1 cytokines, impairment of cytolytic activity, and diminishing of viral clearance (22, 38). Furthermore, IL-4 and IL-13 have been reported to enhance VV replication and suppress antimicrobial peptide expression in a Stat6-dependent manner in human skin (39). In addition, mice exhibiting a Th2 bias and elevated IL-4 expression because of T-bet deficiency are more susceptible to primary VV infection (40).

Upon infection, CCR4/FoxP3 chimeras expressed only modest levels of IFN-α and IFN-γ. The critical role of type I IFNs in innate immunity and antiviral effects has been established. However, type I IFNs are also key players in adaptive immunity to viral infections. They are shown to promote cross-priming after viral infection (41) and effector function of virus-specific CD8+ T cells by activating IFN-γ production in a STAT4-dependent manner (42), enhance the survival of activated T cells (43, 44), and are required for clonal expansion in response to viral infection (45).

IL-12 is a key regulator of cell-mediated immunity through its potent stimulation of IFN-γ production (46). Its cutaneous expression was significantly downregulated in CCR4/FoxP3 chimeras. The enhanced cutaneous expression of IL-23 in CCR4/FoxP3 chimeras mirrors a scenario reported for VV-IL-23 (VV expressing IL-23) infection in IL-12/23p40–deficient mice (47). However, VV-IL-23–infected, IL-12/23p40–deficient mice expressed high levels of IFN-γ and exhibited no viral spread in complete contrast to our CCR4/FoxP3 chimeras. Taken together with our own data, this suggests that the absence of cutaneous IL-12 along with the impaired expression of IFN-α results in the defective IFN-γ response and therefore results in the impaired T effector cell responses in our chimeras.

In contrast to the impaired expression of IL-12 and type I and II IFNs, VV-infected CCR4/FoxP3 chimeras produced significantly elevated levels of the proinflammatory cytokines IL-6, IL-10, TGF-β, and IL-23. IL-10 is highly expressed in AD skin (48) and is known to inhibit Th1-mediated enhancement of CTL activity, NK cell activation, and IFN-γ production (49, 50). Combined with IL-4, it has been shown to be an effective inhibitor of VV clearance (23). IL-10 is not only produced by keratinocytes upon VV infection (51) but is also known to be produced by Th9 cells induced by TGF-β and IL-4 (52) and by Th17 cells induced by TGF-β and IL-6 (53), which additionally require IL-23 for expansion and acquiring full effector function (54). All these cytokines are highly expressed in the skin lesions of CCR4/FoxP3 chimeras. We have observed that IL-17A levels promote VV virulence in BALB/c mice with Ag-induced eczematous skin lesions (55), and similar findings have been reported for NC/Nga mice (26). Although we detected only slightly increased levels of IL-17A and IL-17F upon infection, this may be an issue with assay sensitivity and the relatively low Th17 responses in C57BL/6 versus BALB/c mice (56).

Taken together, these data show that Treg deficiency in the skin leads to inflammation and alterations in cytokine expression, which secondarily affect adaptive immune responses to VV. In light of the reported aberrations in Treg function in atopic individuals, this suggests that Treg dysfunction may, in part, underline the pathogenesis of EV.

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Disclosures
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